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ADRENOCORTICAL TUMORS (ACT) IN CHILDREN: RELATIONSHIP BETWEEN DISEASE STAGE AND OUTCOME. R. Sandrini, L. De Lacerda, G. Sampaio, C. Sabbaga, M.C. Schmit-Lobe, P. Roberson, I. Cat and R. Ribeiro. Department of Pediatrics (DP), Fed. University of Parana, Curitiba, Brazil and St. Jude Children's Research Hospital, Memphis TN

A staging system (SS) for childhood ACT has not been described previously. Based on tumor (tu) resectability and size which have been correlated with outcome, we devised a SS for ACT. Stage I: Tu totally excised and volume (vol) < 200 cm^3; Stage II: Microscopic residual tu, or vol > 200 cm^3, or tu spillage during surgery; Stage III: Gross residual tu; and Stage IV: Distant metastasis. To examine the relationship between SS and outcome, 58 consecutive cases of ACT treated at DP, between 1967 and 1991 were studied. The median age of the 17 boys and 41 girls was 3.6yr. To date, 30 (51%) pts are living disease-free; 24 died from ACT and 4 were lost to follow-up. Univariate analysis which included several relevant clinical and laboratory features disclosed that SS, virilization or mixed clinical types, histology and tu vol were each associated with outcome; also (by Cox regression analysis) that only SS (p=0.0001) and mixed type (p=0.01) were independently associated with outcome. We conclude the SS as used in this study is highly correlated with outcome.

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IMPAIRED GONADAL AND ADRENAL FUNCTIONS IN CHILDREN AND ADOLESCENTS AFTER BONE MARROW TRANSPLANTATION (BMT). C. Vilser, D. Fuchs, F. Zintl, E. Kauf, Children's Hospital, University of Jena, 0-69 Jena, Germany.

Conditioning regimen with BMT may lead to impairment of endocrinologic functions. Therefore, stimulation tests were performed in 22 adolescents (CA 14.5±2.5 yrs) and 5 children (CA 6.2±2.0 yrs) treated with BMT 3.3±2.3 yrs ago for malignant diseases.
Methods: Besides basal hormonal status and diurnal profil of cortisol and ACTH, following stimulation tests were carried out: arginine/insulin, GHRH; GnRH;TRH;CRF;HCG or HMG.
Results: All subjects had low basal levels of DHAS. After HMG, basal E 2 -levels increased significantly ($p<0.05$) less in female pts than in age-matched controls whereas after HCG, testosterone response was only little affected. CRF-stimulation revealed normal ACTH secretion but smaller cortisol peak concentrations leading to lower cortisol/ACTH ratios.

Parameter	Adolescents (males,n=14)	(females,n=8)	children, n=5
DHAS (ng/ml)	974±1253	1498± 958	50±36
	(1780± 962)	(2565±1368)	(541±533
HMG-test:			
Δ- E 2 (fold)		2.3±1.7	1.7±0.3
		(4.3±1.1)	(3.5±2.0)
HCG-test:			
Δ- testost.(fold)	4.7±2.1		4.3±3.2
	(5.7±1.9)		(5.7±4.3)
CRF-test:			
cortisol/ACTH	16.6±8.5	13.5±3.2	7.5±2.1
	(18.1±6.5)	(19.3±5.9)	(16.9±7.1)
cortisol peak	685±379	388±112	395±185
(nmol/l)	(850±230)	(456±227)	(695±196)

Conclusions: Treatment with BMT leads to impaired gonadal and adrenal functions. Induction of puberty seems to be necessary in most girls and substituion with glucocorticoids is recommended in stress conditions, e.g. surgery, infections.

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The adrenal autoantigen in APS I is the side-chain cleavage enzyme

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Autoimmune polyendocrine syndrome type I (Blizzard's syndrome) is an autosomal recessively inherited disease associated with multiple endocrine and non-endocrine manifestations such as autoimmune hypoparathyroidism, adrenalitis, gonadal insufficiency, mucocutaneous candidiasis, alopecia and vitiligo. We have characterised the adrenal autoantigen recognized by autoantibodies in sera from this group of patients. The methods used include indirect immunofluorescence on frozen sections of different tissues, immunoblotting of adrenal subcellular fractions and of proteins expressed in a prokaryotic system and immunoprecipi-ations of labelled lysates of a highly differentiated adrenocortical cell line. In addition, studies on enzyme inhibition were performed. The sera recognized a protein co-migrating in all systems with the rate-limiting enzyme of the steroid biosynthesis, the cholesterol side-chain cleavage enzyme (SCC). Bacterially expressed SCC was recognized by the APS I sera in immunoblotting, whereas no reactivity was found against bacterially expressed 17α-hydroxylase or 21-hydroxylase expressed in a eukaryotic system. SCC-activity in bovine adrenal mitochondria was inhibited by 80% in the presence of APS I sera. Conclusion: SCC is the autoantigen in APS I. This was shown using a variety of different methods, but is in contrast to a recent report. 21-hydroxylase, the autoantigen in idiopathic Addison's disease (ref Winqvist O et al., Lancet 1992: 339;1559-62), is not recognized by APS I sera. These findings illustrate a remarkable specificity in the humoral immune response in the different forms of adrenalitis.

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TESTICULAR HISTOPATHOLOGY IN CONGENITAL LIPOID ADRENAL HYPERPLASIA: A LIGHT AND ELECTRON MICROSCOPIC STUDY. T. Orata, N. Matsuo, M. Aya, and A. Prader†. Department of Pediatrics, Keio University, Japan; and Department of Pediatrics, University of Zurich, Switzerland†

This paper reports on testicular histopathology in 2 Japanese 46,XY patients with congenital lipoid adrenal hyperplasia who underwent gonadectomy at ages 12 and 17 years, respectively. Both patients were completely feminized, although hCG stimulated serum testosterone levels differed (case 1, <0.1→<0.1 ng/ml; case 2, <0.5→ 1.3 ng/ml). Case 1: The interstitium contained increased number of grossly swollen Leydig cells filled with numerous lipid droplets. Seminiferous tubules were normal in diameter (115.9±5.9 μm) with age-appropriate number of spermatogonia (0-11/tubule) and well developed Sertoli cells. There were no spermatocytes, spermatids, or sperms. Case 2: The interstitium contained mildly enlarged Leydig cells with dispersed lipid droplets. Seminiferous tubules were normal in diameter (88.7±18.7 μm) and contained age-appropriate number of spermatogonia (0-9/tubule) and well developed Sertoli cells. A small number of spermatocytes were found, although no spermatids or sperms were identified. We conclude that the defect is characteristically addressed in the testis, consisting of increased number and fatty metamorphosis of Leydig cells, hampered germ cell maturation, and apparently spared Sertoli cells.

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ANDROGEN RECEPTOR (AR) GENE MUTATIONS IN 6 FAMILIES WITH ANDROGEN INSENSITIVITY SYNDROME.

Introduction. AR belongs to a family of ligand-induced transcription factors, and its normal function is to control the differentiation, development and maintenance of male reproductive function. Complete (CAIS) and partial (PAIS) androgen insensitivity syndrome are X-linked disorders occurring in 46,XY patients and leading respectively to a female phenotype and an undermasculinized male. We have determined the AR binding capacity on genital skin fibroblasts and studied the AR gene in the DNA of 5 patients with CAIS and one patient with PAIS. **Families.** All patients with CAIS were characterized by a female phenotype. Four of them were familial cases (indicated by * in the table). The patient with PAIS was referred to our clinic for genital bud, cryptorchidism and perineal hypospadias. Two maternal uncles had ambiguous genitalia. **Methods.** AR binding capacity was determined on genital skin fibroblasts (GSF) using [3H]-R1881. Point mutations were detected by SSCP analysis of exons 2-7 of the AR gene on DNA from white blood cells and identified after direct DNA sequencing. **Results.** AR binding capacity (Bmax), dissociation constant (Kd) and detected mutations are reported in the table below. Normal Bmax = 650 ± 200 fmol/mg DNA. Normal Kd = 0.6 ± 0.3 nM.

Family	Diagnosis	AR characteristics		Genetic alterations	
		Bmax	Kd	amino acid substitution	Exon
1.*	PAIS	300	0.7	Gly568→Phe	2
2.*	CAIS	450	0.5	Val581→Phe◊	2
3.	CAIS	< 80	-	Gly743→Val†	5
4.*	CAIS	< 80	-	Phe754→Val†	5
5.	CAIS	< 80	-	Asp767→Glu◊	5
6.*	CAIS	ND	-	Arg855→Cys	7

Discussion. In these families, we detected only amino acid substitution. In two cases (indicated by †), a new restriction site was created which made possible the carrier diagnosis of patients' sisters. Patient 3 presented a *de novo* mutation. In 2 cases (indicated by ◊) a restriction site was abolished and carrier diagnosis was possible. In family 6, we found a mutation reported threefold indicating a hot-spot amino acid. As expected, the mutation described within the DNA-binding domain did not alter the AR binding capacity (patients 1 and 2). Further receptor studies are currently underway to support the potential androgen-AR and DNA-AR affinity changes.

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KLINEFELTER'S SYNDROME AND MICROPENIS : PARTIAL ANDROGEN INSENSITIVITY SYNDROME (PAIS) ?

Introduction. Genital abnormalities such as micropenis, hypospadias, and cryptorchidism have been reported in Klinefelter's syndrome. We studied the biochemical and molecular characteristics of the androgen receptor (AR) in 5 patients with Klinefelter's syndrome (47,XXY) and a severe micropenis. **Patients.** Clinical, biochemical and molecular data are reported in the table below.

Patient	External genitalia	AR		AR gene
		Bmax	Kd	(androgen binding domain)
1. (11 yr)	cryptorchidism, micropenis	204	0.7	+
2. (11 yr)	cryptorchidism, micropenis	270	1.0	+
3. (30 d)	cryptorchidism, micropenis	306	0.4	+
4. (fetus 20 wk)	absence of external genitalia	260	0.8	+
5. (8 m)	hypospadias, micropenis	251	0.8	+

Methods. AR binding capacity was studied on genital skin fibroblasts and SSCP analyses were performed in exons 4-8 in order o detect any alterations within the androgen binding domain of the AR gene. **Results.** The 5 patients exhibited a decreased amount of AR (mean = 258±36 fmol/mg DNA vs 650±200 fmol/mg DNA for N) compatible with the diagnosis of PAIS while the Kd of the AR were in normal range (mean = 0.7±0.2 nM vs 0.6±0.3 nM for N). Furthermore, no band shifts, characteristic of point mutations, were found by PCR coupled with SSCP. Known AR mutated exons detected by SSCP were used as control. **Discussion.** AR gene mutations have been reported in patients with partial androgen insensitivity syndrome and diminished receptor binding capacity. These mutations have been located within the androgen-binding domain. In these patients with Klinefelter's syndrome and severe micropenis, the decrease of AR binding capacity is in favor of PAIS. However, a diminution of AR gene expression responsible for the low amount of AR cannot be ruled out.