80

GENITAL DUCT DEVELOPMENT ON STREAK GONAD SIDE IN FIFTEEN CHILDREN WITH 45,X AND Y-CONTAINING CHROMOSOME MOSAICISM. T.Tanaka, I.Hibi, Y.Kakizawa, R.Ito, A.Tanae and K.Shimizu, Divisions of Endocrinol. & Metabolism, Urology and Pathology, National Children's Hospital, Tokyo 154, Japan

Genital duct development were studied in 15 children with 45,X and Y-containing chromosome mosaicism by laparotomy. Group 1:One patient showed bilateral intra-abdominal testes with an epididymis, a vas deferens, and a follopian tube. Group 2: Eight patients showed an unilateral scrotal testis and a contralateral streak gonad. On streak gonad side, an epididymis or vas deferens or both were found in 5 patients. Group 3: Four patients showed a unilateral intra-abdominal testis and a contralateral streak gonad. On the streak gonad side, a rudimentary vas deferens was found in 2 patients and a uterus were found in all 4 patients. Group 4: Two patients showed bilateral streak gonads associated with fallopian tube and a uterus. A rudimentary epididymis and vas deferens accompanied 1 out of 4 streak gonads. Twelve patients of Groups 2 and 3 were compatible with a diagnosis of mixed gonadal dysgenesis (MGD). Wolffian duct remnants accompanied 7 (58%) out of 12 streak gonads in 12 patients with MGD. and 8 (50%) out of 16 streak gonads in 14 patients of groups 2, 3 and 4.

These findings indicated that the streak gonads in children with 45,X and Y-containing chromosome mosaicism frequently had functioning Leydig cells at least at the time of differentiation of the genital ducts, although none had properly functioning Sertoli cells at that time.

81

DETECTION OF THE SRY AND ZFY SEQUENCES IN PATIENTS WITH ABNORMAL GONADAL DIFFERENTIATION. B.B.Mendonça; I.J.P. Arnhold; W. Bloise; M.Y. Nishi; A.S. Barbosa; W. Nicolau; B.L. Wajchenberg; C.A. Moreira-Filho.

Gonads and Intersex Unit - Division of Endocrinology, Department of Immunology, B.S.I. University of São Paulo. P.O.Box 8091-01065-970 - São Paulo, SP., Brasil.

Thirteen patients with abnormal gonadal differentiation confirmed by pathology were studied. Seven with 46,XY karyotype (six with dysgenetic gonads and one with gonadal agenesis) and six with 46,XX karyotype: three 46,XX true hermaphrodites, two 46,XX males (one with ambiguous genitalia and the other with normal external genitalia and gynecomastia) and one 46,XX patient with primary gonadal failure, born from a consanguineous marriage and sister of the 46,XY patient with gonadal agenesis. The SRY sequence was amplified by PCR with the primers EA and EB located within the SRY conserved sequence, amplifying a 317-bp fragment. The Y-specific DNA sequence ZFY was detected by Southern hybridizations using the pDP1007 probe, which corresponds to a Y-chromosome segment mapping close to the testis determining factor region. The ZFY sequence was analysed in 8 cases (three 46,XX and five 46,XY patients) and found to be present in all 46,XY patients being absent in the 46,XX patients. The SRY sequence was analysed by PCR in 8 cases (four 46,XX patients and four 46,XY patients) being present in one 46,XX patient (a true hermaphrodite) and in all 46,XY (gonadal dysgenesis patients) and absent in three 46,XX patients. It was concluded that: a)-testicular differentiation can occur in the absence of the Y-chromosome sequences SRY and ZFY; b)-gonadal dysgenesis in SRY and ZFY-positive patients could be caused by mutations, out of the SRY and ZFY loci; c)-46,XX and 46,XY gonadal agenesis present in two sisters born from consanguineous marriage suggest a role for autosomal loci in gonadal differentiation.

* 82

PARTIAL ANDROGEN INSENSITIVITY DUE TO *VAL → LEU* SUBSTITUTION IN CODON 866 OF THE ANDROGEN RECEPTOR CAN BE TREATED WITH HIGH DOSES OF TESTOSTERONE. G.H.G. Sinnecker, O. Hiort, C. Brack[1], E. Nitsche, K. Kruse, Depts. of Pediatrics, Medical University of Lübeck and [1]University of Bonn, Germany

Androgen insensitivity syndromes have different phenotypes, depending on severity of the receptor defect, which in turn depends on the type of androgen receptor gene mutation. A molecular genetic based subclassification may help to define those, who are responsive to high dose androgen therapy. We report two brothers who were successfully treated with high doses of testosterone. ***Patients:*** Two brothers, $11^{5}/_{12}$ (A) and $15^{3}/_{12}$ (B) years, had micropenis, penoscrotal hypospadias and chordee. Penis size was 3 and 3.5 cm, resp. ***Results:*** Basal serum hormone levels (LH, FSH, testosterone, DHT) were prepubertal in (A). LH was increased in (B) (13,3 U/L). The androgen sensitivity test revealed a partial defect of androgen action, as evidenced by a decreased SHBG response to the anabolic steroid stanozolol (nadir 71% and 66 %, resp.). DNA analysis revealed a single base substitution (T to G) in the hormone-binding region (exon 7) of the androgen receptor gene which causes a *Val → Leu* substitution in codon 866. Treatment was initiated with 500 mg testosterone enanthate, im, every 2 weeks. Penis size increased to 8.5 (A) and 9 (B) cm, pubic hair reached Tanner stage 5, voice broke, a moustache, axillary hair and gynecomastia appeared. Erections and ejaculations were reported. Under treatment serum levels of testosterone (71,1 (A), 147,3 (B) nmol/L) and estradiol (191 (A), 396 (B) pmol/L) were increased (2 weeks after the last injection). ***Conclusion:*** High dose androgen treatment produced pubertal virilization and phallic growth in both patients. The partial impairment of androgen receptor function may be overcome by supraphysiological serum concentrations of testosterone. Thus, such treatment seems to be indicated in certain patients with partial androgen insensitivity, in particular in patients with the *Val → Leu* substitution in codon 866 of the hormone-binding domain of the androgen receptor.

83

FUNCTIONAL ASSESSMENT OF ANDROGEN RECEPTOR MUTANTS IN VIVO. O. Hiort and G.H.G. Sinnecker, Department of Pediatrics, Medical University of Lübeck, Lübeck, Germany

Germinal point mutations within the androgen receptor (AR) gene cause a heterogenous group of androgen insensitivity syndromes (AIS). Assessment of residual function of AR mutants is important for gender assignment and prediction of pubertal development in patients with AIS. We have used an in vivo test involving the sex hormone-binding globulin (SHBG) decline in response to the anabolic steroid stanozolol (1) to characterize androgen sensitivity in subjects with partial AIS in whom a distinct mutation of the AR gene has been defined (2). **Patients:** Subject 1 has a female phenotype with subtle narrowing of the vaginal introitus without clitoral enlargement. Subjects 2 and 3 are brothers who initially presented with micropenis and hypospadias, and at the time of puberty developed gynecomastia. **Results:** A G→A transversion causing an arginine to histidine exchange in position 840 of the AR gene was characterized in patient 1. SHBG fell to 81% of initial value. In patients 2 and 3 a G→T substitution leads to a valine to leucine exchange in codon 866. Patient 2 had a SHBG decline to 66%, while his brother had a decline to 71% of initial value. **Conclusion:** Studies of patients carrying AR mutants using the SHBG test in vivo may provide physiologically relevant functional information about the AR and may permit clinically valuable genotype-phenotype prediction. Further tests in patients with AR mutations are in progress.

1. Sinnecker GHG, Köhler S: J Clin Endocrinol Metab 1989; 68, 1195
2. Hiort et al.: Am J Hum Genet 1992; 51 (4), A 170

84

PREDICTION OF CORTISOL PRODUCTION RATES (FPR) WITH STABLE ISOTOPES AND GAS CHROMATOGRAPHY-MASS SPECTROSCOPY (GCMS) GM Bright, D Darmaun, MW Haymond. Nemours Children's Clinic, Jacksonville, FL 32207 USA

Estimates of FPR have been performed indirectly with radiotracer infusions and prolonged, quantitative urine collections; techniques that are difficult or unethical in infants, children and pregnant women. Utilizing 9,9,12-$^{2}H_{3}$ Cortisol (D3F) and gas chromatography-mass spectroscopy, we have developed a straight forward method for the determination of D3F enrichment of cortisol (F). The method requires 4 extraction steps but is easily accomplished with 1 mL plasma. To determine whether such methods could accurately predict entry of F into the plasma space, five dexamethasone suppressed, post-absorptive adults were infused with precisely known quantities of F and D3F. We tested whether the method was able to predict the rate of F infusion. On each of two study days, the subjects received a continuous, nonprimed 12 hr infusion of D3F. From hrs 4-8 and 8-12 on each day, F infusions were used to achieve F concentration plateaus of 7.8, 17.4, 25.8 and 35.7 µg/dL, respectively. Near isotope and hormone steady state (variance <10%) was achieved by hr 3 of each plateau. The isotope dilution method predicted FPR with a mean error insignificantly different from that expected by propagation of error analysis. We conclude that our methods employing stable isotopes can accurately predict the entry of F into the plasma space during steady state conditions and are suitable for exploring F production in humans of all ages.

85

X-LINKED ADRENOLEUKODYSTROPHY (ALD): HORMONAL AND CLINICAL FINDINGS DURING DIETARY ERUCIC-TRIOLEATE THERAPY. M.Cappa, P.del Balzo, P.Cambiaso, E.Bertini, P.Borrelli,

Departments of Endocrinology and Neurology,Bambino Gesù Children's Hospital, IRCCS, Rome, Italy.

X-linked ALD is characterized by primary adrenal insufficiency, demyelination and accumulation of very long chain fatty acids (VLCFA). VLCFA have a toxic effect on adrenocortical cell by increasing cell membrane microviscosity, leading to decreased ability to respond to ACTH (Whitcomb RW et al. J Clin Invest 81:185-188,1988). The regimen of VLCFA restricted diet supplemented with a mixture of oleic and erucic acid (GTEO) determined, for the first time, a normalisation of plasma VLCFA levels in ALD patients. Thus the data suggest that the pathological changes affecting the nervous and endocrine system may be delayed, or even arrested, in these patients. We now report the effects on the clinical course of ALD of this therapeutic approach in 18 patients treated with dietary-GTOE supplementation up to 5 years. There was no effect on the clinical course in patients with childhood and adolescent ALD assessed by neurological, neuropsycological and neuroradiological (NMI) evaluations. Patients with adrenomyeloneropathy and presymptomatic did not exhibit progression of the disease. All but 3 patients had hypoadrenalism. Plasma aldosterone levels were reduced and PRA was increased. Interestingly, normalisation of VLCFA levels in these patients resulted in an increase of aldosterone (M±SD: 112.3±94.6 vs 379.2±12.1 pg/ml, $p<0.05$) and a reduction of PRA levels from 10.2±7.6 to 5.1±2.8 ng/ml/h, confirming that VLCFA excess has a direct toxic effect on the adrenals whereas other factors are more likely involved in the determination of the pathological changes in the nervous system.

(Ricerca corrente 91/02/P/245, Ministero della Sanità Italiano)