475

THE EXPRESSION OF TYPE X COLLAGEN mRNA BUT NOT ALKALINE PHOSPHATASE mRNA INCREASES AS GROWTH PLATE CHONDROCYTES HYPERTROPHY D.E. Carey and X. Liu. Depts. of Pediatrics, University of Connecticut Health Center, Farmington, CT 06032 and Schneider Children's Hospital, New Hyde Park, NY 11042.

In order to study the regulation of the process of growth plate chondrocyte hypertrophy, freshly isolated newborn calf rib growth plate chondrocytes were separated using Percoll density gradient centrifugation. Five subpopulations of chondrocytes of different size were obtained. Alkaline phosphatase activity/ugDNA was lowest in the smallest cells and increased with increasing cell size. mRNA isolated from the five subpopulations was analyzed by northern blot using radiolabeled cDNAs encoding the genes for type II and X collagen, alkaline phosphatase and G-6-PD. The type II collagen gene was highly expressed in all subpopulations. The expression of the type X collagen gene was lowest in the smallest cells and relative expression increased with increasing cell size. By contrast, the expression of the alkaline phosphatase gene did not vary with cell size. These data suggest that while type X collagen synthesis in maturing growth plate chondrocytes may be regulated at the gene level, the increase in alkaline phosphatase activity observed in maturing growth plate chondroctyes may be post-transcriptionally regulated.

476

Gs-UNIT ACTIVITY IN PSEUDOHYPOPARATHYROIDISM SYNDROME : RESULTS OF A PEDIATRIC MULTICENTER STUDY. C. Marguet, D. Martin, J.-P.Basuyau, M. Leroy, Ph. Brunelle, E. Mallet. Groupe de biologie du développement et Service de pédiatrie, CHU, Rouen, France.

Pseudohypoparathyroidism (PsHP) is a rare disorder that might be caused by a defect in the stimulatory G-proteine (Gs), which transduce the PTH receptor signal to adenylate cyclase. We investigate 35 patients (29 children and 6 adults) with PsHP syndrome. Clinical features, Ellsworth-Howard test, serum level of intact PTH (PTHi) and Gs biological activity are studied. Gs activity is measured with a technic adapted from that of LEVINE we optimized to applicate in children (Assay variation : 10 %). 24 patients (68 %) have a PsHP type Ia with a decrease of Gs-unit activity, but we don't find any relation between Albright's osteodystrophy (AHO) and Gs-unit defect : 4 children are AHO - and 20 are AHO +, and 10 of them presented an associate hypothyroidism. 2 patients (6 %) have no response to infusion of exogenous PTH with an increased of Gs-unit activity and could be PsHP type Ib. Only one patient is PsHP type II with normal Gs-unit, serum PTHi level are increased in all patients (mean = 218 pg/ml, range 65 - 571) without difference between PsHP groups, or relation with Gs-unit activity. Other patients would be classified in pseudo-PsHP with decrease Gs - biological activity. In other hand, we studied 10 families, in 9 Gs-unit activity defect is found at least in 1 member (7 times mother, 1 both mother and father, 1 father). In conclusion, Gs-unit activity appears to be of interest in the investigation of PsHP syndrome.

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477

MASSIVE VIRGINAL BREAST HYPERTROPHY AS A CAUSE OF RECURRENT HUMORAL HYPERCALCEMIA DUE TO PARATHYROID HORMONE-RELATED PROTEIN (PTHrP) SECRETION. F. Barry, F.A. Conte, M.M. Grumbach. Department of Pediatrics, University of California San Francisco, San Francisco, CA 94143-0106, USA

Humoral hypercalcemia associated with certain neoplasms is caused by their secretion of a parathyroid-like 16-18 kd peptide, PTHrP. PTHrP also is expressed by keratinocytes, placenta, fetal parathyroids, and lactating mouse mammary tissue. We report a 15 y old female who presented with massive virginal breast hypertrophy and symptoms of urinary frequency, fatigue, and personality changes attributable to hypercalcemia. At 13 1/2 y she had a successful liver transplant for α_1-anti-trypsin deficiency. This was followed by rapid breast growth; 6 months later she had menarche and then regular menses. At 15 y symptomatic hypercalcemia was documented - serum Ca 16 mg/dl, P 2.7 mg/dl, alk phosphatase 151 U/l, PTH 5 pg/ml (n=10-65), and PTHrP 9.4 pmol/L (n=<1.5); $1,25(OH)_2D$ 28 pg/ml (20-76) unlike the usually elevated level with PTH excess. The hypercalcemia responded promptly to saline diuresis. Imaging studies failed to show a parathyroid abnormality or tumor. She had three subsequent episodes of symptomatic hypercalcemia 4-5 days prior to menses; PTHrP levels were undetectable between episodes. Following an extensive reduction mammoplasty, the hypercalcemia has not recurred. Studies of PTHrP mRNA in the tissue are in progress. In summary, benign breast hypertrophy can cause secretion of PTHrP which results in hypercalcemia. The cyclical occurrence and its temporal relationship to the menstrual cycle suggests that changes in circulating ovarian sex steroids may have affected PTHrP synthesis.

478

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SEXUAL DEVELOPMENT OF TURKISH BOYS DURING ADOLESCENCE

In order to evaluate the sexual maturation rating of Turkish boys, 879 healthy male adolescents, aged between 11-19 years,were included in the study. Their pubic hair stages (PHS), testicular lengths and volumes were correlated with their chronological ages (CA) and skletal ages (SA). Mean testicular lengths and volumes were figured for every PHS, CA and SA. At PHS 2, the mean CA and SA of adolescents were found to be 12.04 ± 1.74 and 11.28 ± 1.74 years and at PHS 5, 15.97 ± 3.15 and 16.41 ± 3.00 respectively.At PHS 1, the mean testicular volume was calculated to be 3.11 ±1.70 ml and at PHS 5, 22.27 ± 4.55 ml.

Comparing our results with those of literature, Turkish boys have been seen to reach PHS 2,3 and 4 earlier than other groups. At PHS 5, this correlation seemed to be reversed. Testicular volumes with respect to the PHS, CA and SA showed that testicular maturation was also earlier and greater than the others.

The differences between the results of our study and of others could be explained by racial differences;genetic, socioeconomic and environmental factors and the influence of secular trend. This study shows us the importance of local antropometrical values for various racial groups.

479

SERUM SEX HORMONE BINDING GLOBULIN (SHBG) CONCENTRATIONS ARE NOT AFFECTED BY THE ESTROGEN MILIEU IN FEMALE CENTRAL PRECOCIOUS PUBERTY (CPP). L.R.Garibaldi, T.Aceto Jr., C. Weber and D. Clifton, Dept. of Pediatrics, St.Louis University & Cardinal Glennon Children Hosp, St. Louis, MO 63104, USA

Although exogenous estrogens stimulate SHBG synthesis *in vivo* and *in vitro*, the effect of endogenous estradiol (E2) secretion on SHBG in female puberty is unknown, due to the confounding age-related decline of serum SHBG levels. The increased E2 secretion associated with CPP, and its suppression by GnRH agonists (GnRHa) provide a unique *in vivo* model to single out the effect of estrogens on SHBG. We thus measured serum SHBG (Farmos IRMA), LH (IRMA) and E2 (RIA) levels in 12 girls with CPP, Breast stage II-IV, before and during treatment (Tx) with depot leuprolide acetate (7.5 mg every 4 wks for 0.5-2.2 years); and in 12 age-matched, prepubertal girls. Values are Mean±SD. * p<0.005

	Age (yrs)	LH (IU/l) baseline	LH (IU/l) Post GnRH	E2 (pmol/l)	SHBG (nmol/l)
Controls	6.6±2.2	<0.4	-	<18	80±18
CPP PreTx	6.7±1.9	3.7±1.5 *	61±80 *	74±42 *	48±18 *
CPP Tx	7.7±1.8	1.3±0.9 *	2 ±1.4 *	21±4 *	52±23

Thus, despite pubertal E2 concentrations, serum SHBG levels were not increased in CPP and were paradoxically lower than in controls. Moreover, SHBG levels were not modified by GnRHa-induced suppression of E2 secretion. These data suggest that estrogens, at the concentrations observed in early-mid puberty, do not participate in the regulation of serum SHBG levels.

480

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DEPOT LEUPROLIDE ACETATE IN GIRLS WITH CENTRAL PRECOCIOUS PUBERTY

Ten girls (age 3-7 years) with central precocious puberty (CPP) were treated for at least 12 months (range 12-36) with a depot formulation of a GnRH analogue (leuprolide acetate). The diagnosis of CPP was based upon the following criteria: premature (< 8 years) breast development, increased height velocity, advanced bone age, a pubertal pattern of gonadotropin release by exogenous GnRH. LA was administered intramuscularly every 4 weeks (dose: 7.5 mg). All of the LA doses were given by health care providers to ensure compliance. Overnight pulses (sampling every 15 minutes from 2200-0800), GnRH test and E2 were evaluated every 3 months in the first year and every 6 months thereafter. Bone age was performed every 6 months using the Greulich and Pyle atlas. Ultrasound evaluation of the uterus and the ovaries was part of the follow-up. Classification of pubertal stage was made by the same observer every 3 months in the first year and every 6 months thereafter. Only in three girls CPP was well suppressed (nocturnal and GnRH-stimulated peak LH: mean 2.8 ± 0.2 IU/L; area under the curve (AUC): 1680 ± 74 IU/L min; frequency (F): 2.0 ± 1.7 pulses/10h; mean pulse amplitude (MPA): 4.3 ± 1.0 IU/L; peak LH: 3.7 ± 1.1 IU/L); in seven patients non-complete suppression of CPP was evident ((mean: 5.7 ± 0.3 IU/L; AUC: 3030 ± 284 IU/L min; F: 7.6 ± 0.8 pulses/10h; MPA: 7.1 ± 1.0 IU/L; peak LH: 7.5 ± 1.1 IU/L). The difference between suppressed and non-suppressed girls was statistically significant for mean, AUC and F ($p < 0.025$). Girls with non-suppressed CPP had higher skeletal maturation index (Δ bone age/Δ chronological age) than patients with well-suppressed CPP. Depot LA (7.5 mg, im, every 4 weeks) does not produce complete desentitization in all girls with CPP; studies comparing different dosages are necessary for the assessment of depot LA dose-adequacy.