# 338

ZADIK A, ZUNG A, LEIBERMAN E, CHALEW S, KOWARSKI A

PEDIATRIC ENDOCRINE UNITS: KAPLAN, SOROKA ISRAEL AND UNIV. OF MARYLAND BALTIMORE USA

THE EFFECT OF AGE ON BONE MATURATION IN GROWTH HORMONE (GH) TREATED BOYS

GROWTH ACCELERATION DURING GH THERAPY IS ACCOMPANIED BY ACCELERATION OF BONE AGE (BA). IN THIS STUDY WE COMPARED THE EFFECT OF GH THERAPY ON BONE MATURATION OF CHILDREN WITH CLASSICAL GH DEFICIENCY (GHD), MAX STIMULATED GH (GH-MAX) <10 mcg/L, n= 20, TO THAT OF NON GHD CHILDREN GH-MAX >10 mcg/L n=24 (SS GROUP). FORTY FOUR BOYS, 4.2-16 YRS, GROWTH RATE < 4.5 cm/yr BA < 2SD FOR CHRONOLOGICAL AGE (CA) WERE TREATED WITH GH (0.3 mg/kg/week) FOR 3.3-7 YEARS. ANALYSIS OF CHILDREN > 12 YEARS OF AGE WAS DONE SEPARATELY. (7 GHD, 10 SS). THE CHANGE IN CA-BA OVER THE YEARS WAS ANALYZED. IN BOYS <12 YRS THERE WAS NO DECREASE IN CA-BA DURING THE FIRST TREATMENT YEAR WITH A DECLINE OF 0.7±0.1 AND 0.9±0.1 YEARLY (IN GH AND SS GROUPS RESPECTIVELY) WHILE IN THE OLDER GROUPS THE DECLINE WAS 1.2±0.1 AND 1.4±0.1 YEARLY. THE DIFFERENCE BETWEEN THE YOUNG AND OLD GROUPS WAS SIGNIFICANT AT THE 0.01 LEVEL. THE DECLINE IN THE CA-BA IS IMPORTANT SINCE IT REDUCES PREDICTED FINAL HEIGHT. WE CONCLUDE THAT THERE IS NO DIFFERENCE IN THE EFFECT OF GH THERAPY ON BA MATURATION OF THE GHD AND SS GROUPS.

# * 339

MOLECULAR ANALYSIS OF TWO FAMILIES WITH LARON SYNDROME AND POSITIVE GROWTH HORMONE BINDING PROTEIN. P. Duquesnoy[1], M-L. Sobrier[1], C.R. Buchanan[2], B. Duriez[1], H.G. Maheshwari[2], M.O. Savage[3], M-C. Postel-Vinay[4], M. Norman[2], A.M. Cotterill[3], M.A. Preece[2], M. Goossens[1] and S. Amselem[1]. [1]INSERM U91, Créteil, France; [2]Inst. of Child Health, London, England; [3]Bart's Hosp. London, England; [4]INSERM U344, Paris, France.

Using linkage analysis, we previously showed that the growth hormone receptor (GHR) was involved in Laron dwarfism, an autosomal recessive GH resistant disorder. Interestingly, the extracellular domain of the GHR is found in the serum in the form of a hormone binding protein (GHBP), which binds GH with approximately the same affinity as the native receptor and which is undetectable, in terms of binding activity, in the serum of patients with Laron syndrome. We have analyzed the GHR gene in two unrelated Asian families in which patients displayed the classical features of Laron phenotype except for the presence of serum GH binding activity. To evaluate the involvement of the GHR in this new phenotype, we first performed linkage studies: in one family, the GHR markers cosegregated with the disease phenotype whereas the other family was not informative. Analysis of the coding region of the GHR gene revealed, in both families, a missense defect (Asp152->His) within the exoplasmic domain of the receptor. Expression of the corresponding mutated cDNA into eukaryotic cells led to the synthesis of a receptor that displayed normal binding kinetics. We expect that this mutation is deleterious since it is linked to the disease phenotype and involves a highly conserved amino acid. In addition, previous studies have shown that the complex between GH and the extracellular domain of its receptor (GHBP) consists of one molecule of GH per two molecules of GHBP (I and II) and that hormone-induced receptor dimerization is relevant to the signal transduction mechanism. Interestingly, since the mutation described is located at the GHBPI / GHBPII interface, it is very probable that it plays a critical role in growth signal transduction.

# * 340

SPECTRUM OF GROWTH HORMONE RECEPTOR MUTATIONS AND ASSOCIATED HAPLOTYPES IN LARON SYNDROME. S. Amselem[1], F. Dastot[1], P. Duquesnoy[1], M-L. Sobrier[1], S. Vallex[1], B. Duriez[1], M. Goossens[1] and the Kabi collaborative study group[2]. [1]INSERM U91, Créteil, France; [2]Kabi, Stockholm, Sweden.

Laron syndrome is an autosomal recessive growth resistance condition genetically linked to the growth hormone receptor (GHR) gene. In the course of an IGF-1 therapeutic trial, we have analyzed 15 unrelated patients from various ethnic origins by means of denaturing gradient gel electrophoresis of PCR-amplified GHR fragments; in each of them, we have determined the nucleotide sequence of 6 intragenic polymorphic sites defining 8 GHR frameworks and examined all coding exons along with the splice junctions of this gene. This procedure allowed us to identify mutations in the 30 chromosomes studied and to characterize the GHR frameworks associated with each GHR mutation. Direct sequencing of PCR products that showed an altered electrophoretic behavior has led to the characterization of 12 different point mutations including 2 nonsense mutations, 3 splicing defects and 7 missense mutations associated with various GHR frameworks. Two of these defects were found to be recurrent. These results further illustrate the allelic heterogeneity of this syndrome and confirm the independent origin of the molecular defects. We expect all the missense mutations to be deleterious since 1) they all involved conserved codons among GHR from different species and 2) those mutations which are located within the exoplasmic domain were found in patients who lacked plasma GH binding activity; examination of corresponding mutated cDNAs expressed in eukaryotic cells, currently underway, should explain the GH resistance phenotype observed *in vivo* and provide insights into the structure-function relationships of the GHR and related receptors. In addition, such studies may be helpful to genetic counselling of affected families.

# 341

XO/XY MOSAICISM: SHORT STATURE AND RESPONSE TO GROWTH HORMONE (GH) TREATMENT. B.L. Silverman and the National Cooperative Growth Study. Children's Memorial Hospital, Chicago, IL and Genentech, Inc., So. San Francisco, CA.

Children with 45,X/46,XY mosaicism are frequently reported to have mixed gonadal dysgenesis (MGD), a variant form of Turner syndrome (TS). However, surveys of prenatal chromosomal analyses find that 90-95% of newborns with XO/XY mosaicism have normal male genitalia. We report an 11 yr old boy with short stature, normal genitalia and XO/XY mosaicism. Since growth velocity and adult height are both improved in girls with TS treated with GH, we compared baseline characteristics and response to GH in TS, Turner mosaicism (XO/XX) (TM) and MGD in the National Cooperative Growth Study (NCGS) database. The NCGS contained data on 949 TS girls, 142 TM, 20 MGD females and 10 MGD males. At the time that GH therapy was begun, no significant differences were found for age, bone age, parental heights, or GH response to provocative stimuli. Boys with MGD were taller than the three groups of girls at initiation of GH therapy. In response to GH, growth velocity increased from 3.8±2.1 cm/yr (mean±SD) to 7.4±2.0 in TS, 4.0±2.1 to 7.5±2.2 in TM, and 3.9±2.0 to 7.5±1.5 in MGD after 1 yr of treatment. After 2 yrs of treatment, Bayley-Pinneau predicted adult heights increased by 0.6, 0.5 and 0.5 SD respectively. For the NCGS population, we conclude: Boys with MGD are taller at the time of treatment than girls with MGD, TS or TM; this may represent a sex based treatment bias, or a true biological difference. Otherwise, baseline characteristics are remarkably similar among these four groups of subjects. The short term increases in growth velocity and predicted adult height are similar in these four groups. Unrecognized 45,X/46,XY mosaicism in boys with short stature may be more common than currently appreciated. As GH treatment may be beneficial, the clinical spectrum of this disorder needs to be better defined.

# 342

PRETREATMENT WITH SMS 201-995 POTENTIATES GRF RESPONSE IN SHORT CHILDREN. Z. Dickerman, H.J. Guyda, G.S. Tannenbaum. Endocrinology & Neurology, McGill University, Montreal Canada H3HIP3

We tested the hypothesis that prior inhibition of spontaneous GH secretion by administration of the somatostatin analog SMS 201-995 (SMS) will enhance the GH response to a subsequent GRF challenge. Two controlled protocols were employed in 37 short children [M=31, F=6, aged 11.8 ± 1.6 yr (M ± SEM), height SDS -2.25 ± 0.55]. Study 1 determined an optimal regimen: SMS (0.8-2.2 μg/kg, sc) was randomly administered or not at 08:00 h and a GRF bolus (50 μg, iv) was given 4 h later. Study 2 used standard doses of SMS (1 μg/kg, sc) and GRF (1 μg/kg, iv), and a 1 h delay of the GRF injection. GH levels were measured q 20 min until 2 h following the GRF and expressed as the GH area under the curve during the 4-5 h SMS-GRF interval (AUC 1, μg/L/h) and as GH response to GRF for 2 h following GRF (AUC 2, μg/L/h; GH peak, μg/L). *P<0.05 ** P<0.001

| Study 1 | N | AUC 1 | AUC 2 | GH Peak |
|---|---|---|---|---|
| Control | 12 | 6.2 ± 0.9 | 85.0 ± 13.5 | 36.0 ± 6.2 |
| SMS | 12 | 2.2 ± 0.4** | 41.5 ± 7.8** | 17.4 ± 3.1** |
| Study 2 | N | AUC 1 | AUC 2 | GH Peak |
| Control | 25 | 7.9 ± 0.9 | 77.5 ± 6.8 | 30.5 ± 3.0 |
| SMS | 25 | 3.8 ± 0.4** | 103.7 ± 10.3* | 56.7 ± 5.5** |

In study 1, SMS significantly suppressed spontaneous GH secretion and GH response to GRF, compared to control. In study 2, GH secretion was still suppressed during the 5-h SMS-GRF interval, but both the GH area and peak GH response to GRF were significantly increased by pretreatment with SMS. Thus, a 'priming' SMS dose of 1 μg/kg significantly augmented the GH response to GRF given 5 h later, enhancing the diagnostic value of the 'standard' GRF test.

# 343

NUTRITION IS AN IMPORTANT REGULATOR OF THE GROWTH HORMONE BINDING PROTEIN. M.C. Postel-Vinay, C. Saab, M. Gourmelen. INSERM Unité 344, Hôpital Necker, and Laboratoire d'Explorations Endocriniennes, Hôpital Trousseau, Paris, France.

Food intake is known to have a major role in the regulation of hepatic GH receptors in rats. To evaluate effects of nutrition on the GH binding protein (GHBP), a soluble short form of the GH receptor, we have studied children presenting with celiac disease and children with obesity. Eight girls and two boys, aged 3-14 yr, all prebubertal, with short stature (- 4.2 ± 0.2 SD from normal height) had celiac disease. Their plasma $IGF_1$ level was very low (mean ± SEM = 66.3 ± 10.3 ng/ml vs normal value for age = 216 ± 22 ng/ml). GHBP (16.4 ± 2.2% of radioactivity) was significantly lower than the value of prepubertal normal children (24.8 ± 1.7% of radioactivity). In six boys and two girls with obesity (+ 4.2 ± 0.4 SD from normal weight), aged 4-10 yr prepubertal, with normal height, GHBP was very high (56.3 ± 3.5% of radioactivity). The increased GHBP level was related to high binding capacity without change in affinity. The growth defect presented by children with celiac disease is associated with partial GH resistance and low GH receptor level. On the contrary, children with obesity and normal growth, have a high GH receptor level.