80 IDENTIFICATION OF NOVEL GH AND IGF-1 RESPONSIVE GENES IN HUMAN CHONDROCYTES BY MICROARRAY ANALYSIS


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Longitudinal bone growth is the result of chondrocyte proliferation followed by ossification in the growth plate. Both GH and IGF-1 are known to have direct effect on the growth plate, but their mediating factors are not fully understood. In an attempt to find novel candidate genes for the regulation of longitudinal growth in children, (1) we have selected candidate genes by performing microarray analysis on human chondrocytes, (2) identified sequence variations in the candidates, and (3) will study those sequence variations in short and normal height populations. The microarray analysis was performed on primary cultured human chondrocytes treated with GH or insulin-like growth factor I (IGF-I) or saline (control) in duplicates using the U133A chips containing more than 12,000 genes (Affymetrix). The selection was based on signal intensity and quality. We identified several genes that have changed expression in response to GH (n=25), IGF-I (n=93), and both (n=11) compared to control. Of these 11 genes responding to both GH and IGF-I, CYR61 was particularly interesting for our purpose since it is involved in angiogenesis. A database searches identified 10 SNPs in the CYR61 gene, and a cross species comparison reveals that CYR61 is highly conserved. Interestingly, some of these SNPs are located in conserved regions. Sequence analysis will be used to identify additional SNPs and will also used to study the relationship between height and sequence variations in CYR61 in short and normal height populations.

82 MATERNAL UNIPARENTAL ISODISOMY FOR CHROMOSOME 7 IN A CASE WITH SILVER-RUSSELL SYNDROME

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Maternal uniparental disomy for chromosome 7 (mUPD7) occurs in approximately 7% of Silver-Russell syndrome (SRS), which is characterized by intrauterine and postnatal growth retardation and several clinical features. We report a case with SRS having mUPD7). The one year old boy was born to parents of Japanese father and British mother. His birth weight was 2250 g; length was 46 cm. He has broad forehead, relative macrocephaly, protruded ears without downsloping mouth corners and triangular face. He has been suffering from severe feeding difficulties and excessive sweating from early neonatal period. Repeated massive vomiting had occurred from one month old partly due to progressive gastroesophageal reflux. He showed no appetite and refused to take food orally although his pharyngeal and laryngeal function is normal. To treat his severe growth retardation and malnourishment, we performed fundoplication and gastrostomy at 10 months old, which improved vomiting but did not help stopping extreme discomfort and nausea incurred by feeding. Growth hormone treatment was also started and he showed height increase from -4.3 to -3 SD during six months with rise of IGF-I from 8 ng/ml to 140 ng/ml, however, his weight gain has been still poor. Genotyping was performed on blood DNA with six chromosome 7 specific microsatellite markers. The patient revealed to have maternal complete isodisomy.

mUPD7) is characterized by pre- and postnatal growth retardation, feeding difficulties, hypotonia, clumsiness of motor skill, and verbal retardation. The uniparental disomy on chromosome 7, such as GRB10, PEG1/4/5/6/7, P2X4, are reported to cause growth retardation by doubling expression of growth receptor gene or deficiency of growth promoter gene. The other uniparental disomy genes (EGFR, AUTS1, etc.) may play a role in the other specific character of this case.

81 Two-dimensional gel electrophoresis for insulin like growth factor binding protein-1 isomers

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Impaired postnatal growth in intra-uterine growth retardation (IUGR) is a major clinical challenge and may also serve as a model in studying the mechanisms of growth retardation in general. In cord blood and late gestation maternal serum, the insulin-like growth factor (IGF-I) is positively correlated with birth weight, whereas IGF binding protein-1 (IGFBP-1) is inversely correlated with birth weight. IGFBP-1 has been shown to alter cellular responses to IGF-I. Human IGFBP-1 undergoes serine phosphorylation, and this enhances both its affinity for IGF-I by six to eightfold and its capacity to inhibit IGF-1 actions. Therefore it was necessary to characterize the role of IGFBPs and IGFBP-5, key regulators of fetal and infant growth, during the postnatal period in IUGR infants. But it was difficult to analyze IGFBP-1 isomers because IGFBP-1 level in amniotic fluid and serum was very low and IGFBP-1 isomers have small difference of property. Here I reported that IGFBP-1 from amniotic fluid, were analyzed by a modified two-dimensional gel electrophoresis followed by Western ligand blotting. The samples were subjected to immobilized pH gradient isoelectric focusing in the first dimension, followed by SDS-PAGE in the second dimension and autoradiography after ligand blotting with [125I]IGF-I. The identity of the binding proteins was confirmed by immunoblotting and immunoprecipitation with specific antibodies. Using this method, all IGFBP-1 isomers could be clearly separated from each other according to their isoelectric points (pI).

83 Analysis of suppressor of cytokine signaling-2 (SOCS-2) gene in overgrowth syndrome

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[Introduction] Elaborately orchestrated GH/IGF-1 axis is essential for signal transduction on growth mechanisms. Recently, negative regulator for the GH/IGF-1 axis was reported as a suppressor of cytokine signaling-2 (SOCS-2). The SOCS-2 deficient mice indicated gigantism with activation of Jak2STATS signaling. To identify the etiology of two cases with significant overgrowth independent to GH/IGF-1, SOCS-2 gene analysis was executed.

[Materials and Methods] Case 1: A 16 year old boy with bone dysplasia and pre- and postnatal overgrowth. He was born at 38w gestational age with a weight of 6130g (7.85SD), a height of 64cm(4.84SD). Estrone treatments have failed to epiphyseal closure in spite of inhibiting signs for feminization. Serum GH, IGF-I and GHBP levels were low. He died suddenly in high school due to unknown etiology. Previous study clarified no mutation in FGFR3 and Gypsy1 (GPC3) and no loss of imprinting of IGF-2. Case 2: An 11year old boy with bone dysplasia, mental retardation and hyponatremia. He was born at 37w gestational age with a weight of 3500g(5.45SD), a height of 54cm(2.45SD). Symptomatic G-6-PD deficient syndrome was suspected, however, molecular study has revealed no mutation in G6PC. DNAs were extracted from peripheral blood leukocyte of each case. SOCS-2 coding region was sequenced by PCR and sequenced.

[Result] No SOCS-2 mutations was detected in both cases.

[Discussion] SOCS-2 offers a new mechanism in signal control and crosstalk of GH/IGF-1 signaling. Human SOCS-2 dysfunction may be implicated in GH independent overgrowth. However no SOCS-2 mutation was detected in coding region in this study, further examination would be necessary for a new level of understanding of GH/IGF-1 signal network in human.