underappreciated manifestations of MAS such as hepatobiliary disease and early death. Identification of constitutively activating mutations of Gs-α as the cause of most if not all of the manifestations of MAS has important implications for our understanding of cAMP regulation of cell function, and ultimately, for treatment of MAS.

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GROWTH HORMONE INSENSITIVITY SYNDROME. U. Francke, Howard Hughes Medical Institute and Departments of Genetics and Pediatrics, Stanford University, Stanford, CA 94305, USA

Growth hormone insensitivity syndrome (GHIS or Laron syndrome) is an autosomal recessive disorder caused by mutations in the gene for the growth hormone receptor (GHR). This gene, located on chromosome region 5p13.1-p12 (1) encodes a 620 amino acid protein with a centrally located hydrophobic transmembrane domain. Proteolytic cleavage of the extracellular domain that includes the growth hormone binding site generates the circulating GHBP. One GH molecule binds to two GHBP molecules resulting in dimerization of the receptors, possibly an essential step for signal transduction.

In order to detect GHR mutations in patients with GHIS, a screening procedure has been devised (2,3). All nine exons and surrounding splice junctions of the GHR gene are amplified by PCR with primers designed from the published sequence. Each PCR product is analysed for altered melting behavior by denaturing gradient gel electrophoresis (DGGE). A GC-clamp is added to one primer to ensure complete analysis of the entire exon and splice site regions. PCR products that are positive by DGGE screening are sequenced either directly or after subcloning. In our laboratory, Dr. Mary Anne Berg has identified two nonsense mutations (R43X and R217X), two splice junction mutations (189-1 G-to-T and 71+1 G-to-A) and two frameshift mutations leading to translational stop codons (46 del TT and 230 del TA) all of which involve either exon 4 or exon 7 (3). The most unusual mutation - that she has detected in over 50 affected individuals from Ecuador - involves a single nucleotide substitution in codon 180 that does not change the amino acid encoded (E180E). The A-to-G transversion, however, creates a new 5' splice site which is exclusively used and leads to an in-frame deletion of 24 nucleotides from exon 6 in the patients' mRNA (2). Since the GH binding and receptor dimerization sites would remain intact but no response to GH has been reported in these patients, the mutant receptor protein is postulated to undergo abnormal folding and intracellular degradation. The E180splice mutation has not been detected in any patients outside of Ecuador.

In general, the mutations are unique to particular families or geographic areas. Only R43X has recurred on different haplotype backgrounds. All mutations we have identified so far are predicted to result in complete absence of a functional receptor protein. Thus, it is not surprising that affected individuals who are homozygotes for particular mutations or compound heterozygotes for two different mutations are clinically indistinguishable. The variable GHBP levels, by indirect immunochemistry, that have been reported among individuals homozygous for the same mutation, remain unexplained. Curiously, all reported mutations involve the extracellular domain. The intracellular domain encoded by one small and one very large exon may present a less favorable target for mutations, or such mutations could cause a different phenotype.

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MOLECULAR GENETICS OF EARLY-ONSET NON-INSULIN-DEPENDENT (TYPE 2) DIABETES MELLITUS. G.I. Bell, Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637, USA

NIDDM is one of the most common metabolic diseases affecting ~5% of the world population. However despite much research, the genetic and nongenetic factors that contribute to its development remain largely unknown. Careful clinical studies of subjects with NIDDM whose onset occurred during childhood or adolescence have identified a familial form of diabetes termed maturity-onset diabetes of the young or MODY. Its early age of onset, autosomal dominant mode of inheritance and availability of multigenerational pedigrees make MODY an attractive model for identifying diabetes-susceptibility genes using genetic approaches. Such studies have shown tight linkage between DNA markers on chromosome 20 and MODY in a large Michigan family of German origin as well as with DNA polymorphisms in the glucokinase gene on chromosome 7 in French and British families. Although the diabetes-susceptibility gene on chromosome 20 has not been identified, mutations in the glucokinase gene have been shown to be the cause of MODY in ~60% of French subjects with this form of NIDDM. The demonstration that mutations in the glucokinase gene can cause diabetes suggests that NIDDM may be, at least in part, a disorder of glucose metabolism. This implies that genes encoding other glycolytic and gluconeogenic enzymes, especially those that control rate-limiting steps in these pathways, are candidates for contributing to the development of this genetically heterogeneous disorder. Genetic studies of early-onset NIDDM have provided a better understanding of its causes and have pointed to cellular pathways that are important for the maintenance of glucose homeostasis and whose perturbation may contribute to the development of the more common late-onset forms of NIDDM.

Fetal and Placental Endocrinology

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THE ROLE OF THE INSULIN-LIKE GROWTH FACTORS IN NORMAL AND ABNORMAL FETAL GROWTH. P.D. Gluckman, Research Centre for Developmental Medicine and Biology, University of Auckland, Auckland, New Zealand.

Fetal growth (FG) in late gestation (LG) is primarily determined by uteroplacental transfer of nutrients. FG is normally constrained by the capacity of this transfer. In LG, fetal IGF-1 is acutely regulated by glucose, but not by amino acid availability, acting via enhanced insulin release; this provides the mechanism of fetal overgrowth induced by hyperinsulinism. Fetal IGF-1 levels correlate with fetal nutritional status and thus FG. Direct evidence for the role of IGF-1 in FG is provided by embryo transfer experiments in mice selected for high or low IGF-1 levels. The infusion of IGF-1 (60μg/kg/hr) to LG fetal sheep increased IGF-1 4 fold without a measurable change in fetal or placental glucose uptake. However placental lactate production fell ($p < 0.03$). The amino-nitrogen concentration fell ($p < 0.01$) in both mother and fetus as did fetal urea production ($p < 0.05$). These observations suggest that IGF-1 inhibits fetal protein catabolism, promotes fetal anabolism, enhances placental amino acid transfer and alters placental metabolism favourably. It is suggested that glucose availability affects fetal IGF-1 production via altered insulin secretion and this in turn has anabolic and anticatabolic consequences. IGF binding proteins are also acutely modulated by nutrient availability. Fetal IGF-2 in late gestation is under lesser nutritional regulation compatible with a more constitutive role in the regulation of FG. Maternal IGF-1 administration throughout pregnancy in rodents abolishes the physiological constraints on FG without affecting placental growth. Maternal IGF-1 administration reduces fetal urea production in sheep. It is suggested that maternal IGF-1, under nutritional and hormonal control,determines nutrient availability and transfer across the placenta. Thus optimal FG depends on coordinate increases in both fetal and maternal IGF-1.

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PLACENTAL GROWTH HORMONE AND RELATED PROTEINS. N.E. Cooke, B.K. Jones, A. Misra-Press, M. Urbanek, J.E. Russell, S.A. Liebhaber, Departments of Medicine and Genetics, University of Pennsylvania, Philadelphia, PA 19104, USA.

The human growth hormone (hGH) gene cluster contains the pituitary hGH-N gene as well as 4 placentally expressed genes: hCS-A, hCS-B, hCS-L, and hGH-V (placental GH). The 4 placental genes are coordinately induced during fetal development. There is a developmentally regulated switch in the relative expression of hCS-A and hCS-B. The hGH-V gene produces a maternally circulating hormone that reaches maximal levels during the 2nd and 3rd trimesters, as well as a larger protein, hGH-V2, that remains associated with the cell. hGH-V maintains the full spectrum of GH-like bioactivity but is 7-fold more purely somatogenic than hGH-N. The hCS-L gene has lost its normal exon 2 splice donor and undergoes extensive alternative splicing. Expression of the growth hormone receptor (GHR) has been detected in all 4 layers of the placenta, suggesting a means of signal transduction for the placental GH isoforms. The placental villi selectively express an alternatively spliced form of the receptor lacking exon 3, hGHRd3, while decidua and chorion predominantly express the full-length receptor. Both forms of hGH receptor possess indistinguishable ligand binding activities when expressed in Xenopus oocytes. To study the developmental and tissue-specific regulation of this gene cluster, 150 kb of DNA including and flanking the cluster have been analyzed for DNase I hypersensitive sites (HSS). A set of HSS were identified in syncytiotrophoblastic nuclei and in the nuclei of a GH-secreting pituitary adenoma about 40 kb upstream and >20 kb downstream of the cluster. These sites were absent from a number of cell types that do not express the GH-related genes. Constructs containing the hGH-N gene plus and minus the HSS were used to generate transgenic mice. When linked to the 5'-HSS the hGH-N gene was specifically expressed in the pituitary with serum levels of 2-10 ng/ml hGH-N. The 5'-HSS reformed in the transgene in nuclei from pituitaries of transgenic mice. The possibility that this region, remote from the structural genes, is critical to their activation in transgenic mice suggests a novel mechanism for the control of the members of this cluster.

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PLACENTAL EPIDERMAL GROWTH FACTOR RECEPTORS: FROM PHYSIOLOGY TO PATHOLOGY. D. Evain-Brion, E. Alsat, S. Roulier, C.Bonneton. C.Fondacci Labo. de Physiopathologie du Développement, ENS, 75005 Paris

Alteration of placental development directly interferes with fetal growth. Epidermal growth factor (EGF) plays a major role in placental implantation, growth and differentiation. EGF acts on its placental target cells, ie the trophoblasts, via a specific receptor which belongs to the tyrosine kinase receptor family. Placental abundant EGF receptors (EGFR) localize in the brush border at the fetomaternal interface. EGFR expression is increased *in vivo* and *in vitro* with the differentiation of the syncytiotrophoblast which is the functional endocrine tissue of the placenta. In trophoblast cells in