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

culture modulation of EGFR expression by hormones such as parathyroid-related peptide and retinoic acid or toxic substances such as smoke derived products interferes with placental endocrine functions. Interestingly, in microvilli purified from placenta of intrauterine growth retardation (IUGR) a decrease or absence of the EGFR tyrosine kinase activity is observed. This can be related to a decrease in EGFR expression in placenta with IUGR related to maternal toxemia. In some placentas with idiopathic IUGR a truncated form of EGFR lacking in tyrosine kinase activity, is observed. This suggests that an alteration of EGFR biological activity might interfere with the fetoplacental unit development.

IDDM: Etiology, Genetics and Environmental Factors

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IMMUNOGENETICS OF TYPE I DIABETES MELLITUS. M. Trucco, Department of Pediatrics, University of Pittsburgh, Pittsburgh, PA 15213, USA

Type I (insulin-dependent) diabetes mellitus (IDDM), is generally considered to be an autoimmune disease based on: 1) the finding of numerous inflammatory cells in the islets of Langerhans at the onset of the disease; 2) the presence of circulating autoantibodies directed against the islet cells; and 3) the fact that immunosuppressive agents, such as cyclosporin A, are able to transiently block the course of the disease. Moreover, the observation that certain HLA class II alleles are associated with an increased risk for the disease, supports an autoimmune etiology for IDDM. In particular, the absence of aspartic acid in position 57 of the HLA-DQβ and the presence of arginine in position 52 of the DQα chain together, have been found to be strongly associated with IDDM in population studies. These heterodimers have been thought to play an important role in the pathogenesis of IDDM. In fact, the HLA class II molecule participates in a complex interaction between the processed foreign antigen and the T cell receptor (TCR) molecule, which starts the immune response. As the result of a stochastic rearrangement among variable (V), diversity (D), joining (J) and constant (C) gene segments encoding both α and β chains, the TCR may assume 10^{10} different configurations. If IDDM is provoked by a single antigen, a restricted set of T cell clones should emerge during the destruction of the insulin producing β cells. However, this phenomenon is difficult to study because T cells from the pancreas of patients with IDDM, at the onset of the disease, are required to characterize this process. We had the opportunity to study the pancreas of a newly diagnosed child with IDDM who unfortunately died of brain swelling during treatment of diabetic ketoacidosis. T cells from isolated islets were characterized based on their expression of different TCR Vβ regions. The presence of a remarkably restricted TCR Vβ repertoire of the T cells infiltrating the pancreatic islets of this patient strongly suggests the involvement of superantigens in the etiology of IDDM

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POPULATION SCREENING FOR RISK OF IDDM. E.A.M. Gale, Department of Diabetes & Metabolism, St. Bartholomew's Hospital, London EC1, UK.

Risk of progression to IDDM has been assessed extensively in first degree relatives of patients with IDDM and highly specific prediction is possible within a small subset of this population. Since around 90% of future cases will come from those who have no close relative with IDDM, prediction and intervention within the general population will become the main priority for the future. Risk of progression to IDDM can be assessed by means of a decision tree analysis. This highlights the different prognosis of markers when applied to those with and without a family history of the disease, and provides a logical approach to disease prediction. Large numbers of first degree relatives must be screened in order to recruit sufficient numbers of high risk individuals for prospective study or controlled trials of intervention. This implies the need for careful standardization and multicentre collaborative studies. This approach should allow new predictive markers and models to be evaluated, and strategies of intervention to be tested, with maximum efficiency and minimal delay.

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INCIDENCE VARIATION AND ETIOLOGY OF TYPE I DIABETES

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Variations in incidence rates of IDDM have been documented for the last 20 years in terms of geographical heterogeneity, seasonality, age and secular trend. None of these variations can fit an explanatory model for the etiology of the disease but speculations involving "environmental factors" -in contrast with the genetic determinants- originate from these observations.

The geographical variations in IDDM incidence in children across Europe are known since the '70s. They have been claimed to figure a north-south gradient of incidence along the continent, where Finland represented the top and France the bottom. But not in all European countries was IDDM incidence evaluated and the methodology was so different that it did not allow for a strict comparison. This was the aim of the Eurodiab ACE program, a concerted action by the European Community, to measure IDDM incidence rates in children (0-14yr) with a common, prospective and exhaustive protocol over 2 years (1989-90) to allow for comparisons between geographical area. More than 15 countries are involved in the project and the results demonstrate large (tenfold) variations in incidence rates across Europe: Finland maintains the highest rate worldwide (≈$40/10^5$/yr), followed by the other Nordic countries. There is a trend for the rates to decrease from north to south, but also from west to east: Greece and Romania exhibit the lowest rates in Europe (<$6/10^5$/yr). There is the puzzling cluster of Sardinia in the middle of the mediterranean basin with a value as high as in Finland.

The large number of newly diagnosed cases (>3000) included in the first phase of the Eurodiab ACE program allows for a detailed specific analysis of age and seasonality. Altogether there is an over-representation of boys vs girls (B/G =1.1). The age distribution at diagnosis was 18%, 34% and 48% in the 0-4yr, 5-9yr and 10-14 yr old groups respectively. There is no significant effect of sex, age group and country by themselves on specific incidence rates, but there is a significant interaction between age group and geographical area indicating that incidence rates for the 0-4 and 5-9 yr groups seem to vary from country to country in a similar fashion but not in the pubertal age group (10-14yr). The observed data for seasonal variation fit to a sinusoidal model with a peak at wintertime, amplitude of which can afford up to 40% of variation and tends to increase with age and with the incidence level of the country.

In the countries with long-term incidence registries it is clear that a secular increase (20-25%) in incidence rates has occured between the '70s and the late '80s. This is the strongest evidence for the so-called environmental factors to play an important role in the etiology of IDDM. For instance, cow milk consumption has been involved in both geographical and secular variations in incidence of the disease. Large case-control surveys in Sweden have also suggested the role of nitrosamine consumption and of peri-natal events

Although not largely documented, the geographical distribution of IDDM incidence does not overlap the distribution of the known genetic susceptibility markers linked to HLA DR or DQ in the background populations. Islet cells antibodies prevalence rates in schoolchildren have been measured in several European countries and seem to parallel the incidence of the disease. This type of results needs to be confirmed by very large scaled studies of uniform methodology. This is now the scientific goal of Eurodiab ACE to set up large epidemiological programs across Europe to determine the respective roles of immunogenetic determinants and environmental factors in the etiology of IDDM in children.

Growth Hormone

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MOLECULAR BASIS OF GROWTH HORMONE ACTION. C. Carter-Su, X. Wang, G. S. Campbell, L. S. Argetsinger, N. Billestrup, G. Norstedt, D. Meyer and J. Schwartz, Department of Physiology, University of Michigan Medical School, Ann Arbor, MI 48109, USA; Hagedorn Research Laboratory, DK-2820 Gentofte, Denmark; Center for Biotechnology, Karolinska Institute, Novum, 141 57, Huddinge, Sweden.

The intracellular pathways by which binding of GH to its receptor elicits its diverse effects on growth, differentiation and metabolism have eluded investigators for many years. We have hypothesized that activation by GH of a GH receptor (GHR)-associated tyrosine kinase is an important early, and perhaps, initiating step in signal transduction by GH. This was suggested by initial studies showing that GH stimulates the tyrosyl phosphorylation of the GHR and that highly purified GH-GHR complexes have tyrosine kinase activity. These findings were consistent with GHR itself being a ligand-activated tyrosine kinase like the receptors for many growth factors. However, more recent studies using cells transfected with the cloned liver GHR cDNA lead to the hypothesis that the GHR forms a complex with a non-receptor tyrosine kinase, the amount of which may vary with cell type. Experiments using truncated GHRs expressed in Chinese hamster ovary (CHO) and rat insulinoma (RIN) cells indicate that the kinase is likely to be a ~120-kDa protein. The physiological importance of the GHR-associated kinase is attested to by experiments using anti-phosphotyrosine antibodies, tyrosine kinase inhibitors, and/or truncated and mutated GHRs. The results of these experiments indicate that the GHR-associated tyrosine kinase: 1) is stimulated very rapidly following binding of GH to its receptor (<30 sec); 2) is stimulated by very low GH concentrations (0.5 ng/ml); and 3) is likely to play a role in stimulation by GH of a variety of cellular responses, including MAP (mitogen activated protein) kinase activity and c-*fos* gene expression. Furthermore, phosphorylation of specific tyrosyl residues in GHR appears to be necessary for some responses to GH, including stimulation of MAP kinase activity. These results suggest that the GHR-associated tyrosine kinase has at least two roles. The first is to phosphorylate and thereby activate other proteins. The second is to phosphorylate tyrosyl residues in itself and the GHR. These phosphorylated tyrosines may serve as docking sites for proteins in other signalling pathways. This new vision of how GH functions should lead to the identification of new cellular actions for GH and thereby increase our understanding of how GH regulates growth, differentiation and metabolism.