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For the fifth time in our short website history, we are undertaking the task of presenting a brief report of an Annual Meeting of the Endocrine Society, presently the 92nd, which convened at the Convention Center in San Diego, CA, June 19-22, 2010.

As in previous similar events, the Annual Meeting Steering Committee of the Endocrine Society designed an education program worthy of a meeting known as one of the highest quality endocrinology events in the world. Indeed, they put together a line-up featuring 16 plenary lectures, 80 symposia, 106 Meet-the-Professor sessions, 32 Case Management Forums, as well as, “Year In” and Endocrine Debate sessions, and Practice Management and Career Development workshops. In all, ENDO 2010 features more than 350 presentations from the world leaders in endocrine research and care (ENDO daily). Furthermore, 2364 Free Communications were accepted, 162 as Oral Presentation and 2202 as Poster Presentation. At any given time, there were six or seven things happening simultaneously. It was a feast of information and offered something for everyone. As always the Plenary Lectures were the cornerstone of the meeting, with top-quality speakers and great breath of topics.

It was also announced that, beginning in 2015, when it will return to San Diego, the Endocrine Society will host its annual meetings in March, not June. This is to avoid the Endocrine Society meeting’s close proximity to the American Diabetes Association meeting, which has the two organizations competing to present the most up-to-date science in diabetes, lipids and obesity, at the same time each year.

As usual, The Exhibit Floor offered numerous booths with diverse materials of interest for the more than 8000 attendees. Additional information can be gathered at the web site of the Endocrine Society, “www.endo-society.org”.

Abstracts and Comments on some of the Plenary Lectures and Symposia follow.


Pancreas and pancreatic islet transplantation have been used for beta cell replacement for type I diabetic patients for over 40 years. Pancreas has been used successfully for over 30 years, mostly using the technique of simultaneous pancreas and kidney transplantation. Islets have been used successfully with reproducibility for 10 years using the technique of islet infusion via a catheter into the intrahepatic site. Both
procedures require immunosuppression. Success is primarily judged by discontinuation of exogenous insulin-based treatment and maintenance of normal or nearly normal HbA1c. Using these criteria, pancreas transplantation has a success rate of 70-80% three years post-transplant and islet transplantation has approximately a 50% success 15 months post-transplant. However, even when islet transplantation is only partially successful it is associated with use of less exogenous insulin and relief from hypoglycemia. Successful pancreas transplantation has been shown repeatedly to have beneficial effects on retinopathy, nephropathy, neuropathy, macrovascular disease, and quality of life. Such findings have been suggested for islet transplantation, but insufficient information is available to draw firm conclusions. Directions for future research for both procedures include a search for immunosuppressive drugs that are less toxic to beta cells. For islet transplantation, other goals are to discover means of isolating islets that are less damaging to beta cells and consideration of alternative non-hepatic sites that can afford more protection against problems inherent to the hepatic site, such as exposure to high drug concentrations and ingested environmental toxins. Ultimately, because of the paucity of pancreas that are donated annually, a search for human beta cell surrogates is essential to provide a transplantation approach to therapy for a greater number of recipients.

Extracts from a publication by the speaker (Diabetes 59:1285–1291, 2010). Even though not nearly enough pancreas donors exist to treat patients with type 1 diabetes, let alone all people with type 1 and type 2 diabetes, especially in the face of the current diabetes epidemic, there will always be diabetic patients who need β-cell replacement by transplantation of islets or the pancreas. The experiences with islet transplantation have taught lessons that will be important for the use of β-cell surrogates, be they stem cell derivatives or modified cell lines. We have learned about culturing cells, isolating islets and β-cells, identifying safe and physiological sites for transplantation, avoiding immunosuppressive drugs that are toxic to β-cells, meeting environmental needs for physiological β-cell function, and selecting appropriate patients for β-cell replacement.


The predominant cause of childhood diabetes and approximately 10% of adult onset diabetes is autoimmune destruction of islet beta cells (Type 1A diabetes). We can now predict Type 1A diabetes in man, prevent in animal models, and multiple trials are underway to either prevention the disease or limit beta cell loss post-onset. The disease is predictable because development is almost always slow, requiring years of islet autoimmunity, marked by the presence of islet autoantibodies targeting insulin, GAD65, IA-2, and/or ZnT8. The major genetic locus determining risk of appearance of islet autoantibodies and subsequent development of diabetes are genes within the major histocompatibility complex, and in particular class II HLA molecules. The next most important determinants are polymorphisms of the insulin gene and the T cell signaling molecule PTPN22. More than 40 other loci contribute but with very low odds ratios, such that their influence on disease prediction is minimal. Studies in the NOD mouse model are helping to refine our understanding of pathogenesis with considerable evidence that insulin, and in particular insulin peptide B:9-23 is the primary target autoantigen, such that mutating one amino acid of this peptide prevents all diabetes. Multiple therapies in man including anti-CD20 and anti-CD3, and potentially oral insulin delay but do not halt progression. Combined therapies, earlier treatment, and more targeted therapies will hopefully lead to permanent arrest of beta cell autoimmunity.
Extracts from a publication by the speaker. (Diabetes 59:759-774, 2010). Studies of the non-obese diabetic (NOD) mouse model suggest that the root cause of type 1 diabetes involves germline-encoded sequences forming trimolecular complexes consisting of the insulin peptide B:9-23 presented by the class II major histocompatibility complex (MHC) molecule I-A\textsuperscript{g7} and recognized by T cell receptors having a specific germline-encoded α-chain sequence (TRAV-5D-4*04 V\textalpha). Utilizing genetic, autoantibody, and metabolic parameters it is now possible to predict type 1A diabetes in humans, and immune therapy can delay, but not permanently prevent, destruction of β-cells. With an increasing incidence and an estimated 1 million individuals in the U.S. developing type 1A diabetes, safe prevention has become a major international goal. Achieving this goal may come from incremental modification of immune therapies currently being tested and/or may involve a deeper understanding of the autoimmune trimolecular complexes underlying the disorder’s pathogenesis. Type 1A diabetes is associated with both devastating chronic complications and acute life-threatening ketoacidosis and hypoglycemia. There are multiple pathways being pursued to “cure” this disease or at least dramatically ameliorate the burden it imposes on patients and their families. Continuous glucose monitoring is already improving the lives of many patients by providing “real time” information with alarms for hypo- and hyperglycemia. Multiple groups are now studying devices that will control insulin pumps, in particular turning off insulin delivery to prevent hypoglycemia. In developed countries, such devices will hopefully rapidly become the standard of care for patients with insulin-dependent diabetes. Though many patients do not consider such mechanical devices, especially the current “first” generation of devices, as a true cure, these therapies will set the bar in evaluating immunologic therapies considered for prevention of diabetes and β-cell replacement. Thus, the bar will be high and hopefully ever higher over the next decade. At present, pancreatic (long term) as well as islet transplantation (short term) can cure type 1 diabetes but, for most patients, with unacceptable risks associated with immune suppression. It is likely that autoimmunity, in addition to alloimmunity, limits the therapeutic potential of either of these forms of transplantation. The field addressing the immunology of type 1 diabetes has grown rapidly, with thousands of relevant publications. This review can only recognize a portion of that literature and will emphasize a relatively simple hypothesis that hopefully allows presentation with a clear focus: Autoimmune type 1 diabetes results from specific β-cell destruction due to chronic T cell targeting of insulin, and the major molecular determinants of such targeting are hardwired in the genome. Though there are clear phenotypic differences, it is remarkable at a molecular level how similar the NOD mouse and human type 1 diabetes may be. I will first review the pathogenesis of disease in the NOD mouse (where it is easier to attempt to disprove the above specific hypothesis) and then in type 1 diabetes of humans, ending with an outline of the status of clinical trials. I believe the root cause of type 1 diabetes of the NOD mouse is three genome encoded sequences. The relevant sequences are thought to be: The insulin peptide B:9-23 sequence; The susceptible MHC I-A\textsuperscript{g7} sequence; A specific T cell receptor (TCR) V\textalpha sequence.


With the aging of the population, there is increasing recognition that osteoporosis in men is a significant public health problem. Optimal strategies to prevent and treat this disorder depend, however, on a better understanding of the pathogenesis of bone loss in men. Using direct interventional studies, our group demonstrated that similar to women, estrogen was the dominant regulator of bone resorption in men. However, both estrogen and testosterone contributed to the maintenance of bone formation. We and others have extended these observations to population studies demonstrating that serum estradiol levels are more robust predictors of bone density, rates of bone loss, and fracture risk in
men than serum testosterone levels. Moreover, there appears to be a threshold estradiol level below which rates of bone loss and fracture risk increase in men. Insights from changes in bone turnover markers following acute sex steroid deficiency in men, where bone resorption markers increase whereas bone formation markers decrease, have led to further mechanistic studies examining the production of osteoblast-stimulating factors by osteoclasts. These studies have found that, compared to conditioned medium from pre-osteoclastic cells, medium from mature osteoclasts stimulates mineralization of osteoblastic cells. In the mature osteoclasts, this is accompanied by suppression of the potent inhibitor of bone formation, sclerostin, and increased production of osteoblast-stimulating factors, including Wnt 10b and BMP-6. At a clinical level, our findings (a) point to the need to use standardized serum estradiol measurements (instead of, or in addition to, testosterone measurements) in the evaluation of male osteoporosis; (b) have stimulated studies on the use of selective estrogen receptor modulators in the prevention of bone loss in men (e.g., in patients with prostate cancer undergoing androgen deprivation therapy); and (c) sound a cautionary note regarding the utility of non-aromatizable selective androgen receptor modulators or aromatase inhibitors (to increase endogenous testosterone levels at the expense of estrogen production) in preventing bone loss in men. Overall, using a combination of clinical-investigative, epidemiological, and basic studies, our work provides a better understanding of the mechanisms of sex steroid action on bone in men, with potentially important clinical implications for the diagnosis and treatment of male osteoporosis.


Bone metastases are the most common skeletal complication of malignancy. Tumor cells disrupt normal bone remodeling to promote bone destruction and its associated morbidity. Current therapy can improve skeletal morbidity, but once housed in the skeleton, tumors are incurable. Bone provides a unique microenvironment whose local interactions with tumor cells offer novel targets for therapeutic interventions. Tumor and bone interact in a vicious cycle, where tumor-secreted factors stimulate bone cells, which in turn release growth factors and cytokines that act back on the tumor cells. Within these interactions are many potential therapeutic targets for novel treatment of bone metastatic disease. Therapeutic strategies can be oriented to inhibit osteoclasts and/or osteoblasts or tumor responses to factors enriched in the bone microenvironment. Preclinical models, show that this approach, especially combination treatments, can reduce tumor burden and tumor-derived bone lesions. A novel paradigm in which tumor growth can be effectively inhibited by targeting the bone and its microenvironment, rather than the tumor alone, will be discussed. In an animal model, hypoxia (via hypoxia inducible factor [HIF]-1alpha) and TGF-beta signaling in parallel drive tumor bone metastases and regulate a common set of tumor genes. In contrast, small molecule inhibitors, by acting on both tumor cells and the bone microenvironment, additively decrease tumor burden, while improving skeletal quality. We inhibited HIF-1alpha and TGF-beta pathways in tumor cells by shRNA and dominant negative receptor approaches. Inhibition of either pathway decreased bone metastasis, with no further effect of double blockade. We tested pharmacologic inhibitors of the pathways, which target both the tumor and the bone microenvironment. Unlike molecular blockade, combined drug treatment decreased bone metastases more than either alone, with effects on bone to decrease osteoclastic bone resorption and increase osteoblast activity, in addition to actions on tumor cells. Our studies suggest that inhibitors of HIF-1alpha and
TGF-beta may improve treatment of bone metastases and increase survival. Drugs to act on specific targets, such as transforming growth factor beta, and receptor activator of NF-kappa B ligand (RANKL), have entered the clinic.

[L3-1] Roy O Greep Award Lecture: Function & Dysfunction of the Mammalian Reproductive Tract.
MM Matzuk. Baylor Coll of Med, Houston, TX

My laboratory is interested in defining the essential factors that regulate the hypothalamic-pituitary-gonadal axis in women and men. To approach this axis, we have been using multiple in vitro and in vivo strategies. In particular, we have created over 60 knockout models and have begun to study over a dozen conditional knockout mouse lines. In the process, we have uncovered interesting genes and non-coding RNAs in germ cells and somatic cells of the ovary and testis as well as key factors in the oviduct and uterus that have important implications for fertility and cancer. Our approaches have lead us down exciting paths and in fascinating directions, allowing us to identify candidate targets for contraception and cancer therapy as well as infertility treatments. My Roy O. Greep Award Lecture will focus on the studies that we have been performing on TGF-beta superfamily signaling pathways in the female reproductive tract. I thank the National Institutes of Health for their continuous and generous support of our research programs since 1991, and I appreciate my many colleagues at Baylor College of Medicine and elsewhere who have contributed invaluably to our scientific endeavors.

Transforming growth factor β (TGF-β) superfamily members are critical in maintaining cell growth and differentiation in the ovary. Although signaling of activins, TGF-βs, growth differentiation factor 9, and nodal converge preferentially to SMAD2 and SMAD3, the in vivo functions and redundancy of these SMADs in the ovary and female reproduction remain largely unidentified. To circumvent the deleterious phenotypic aspects of ubiquitous deletion of Smad2 and Smad3, a conditional knockout strategy was formulated to selectively inactivate Smad2, Smad3, or both Smad2 and Smad3 in ovarian granulosa cells. While granulosa cell ablation of individual Smad2 or Smad3 caused insignificant changes in female fertility, deletion of both Smad2 and Smad3 led to dramatically reduced female fertility and fecundity. These defects were associated with the disruption of multiple ovarian processes, including follicular development, ovulation, and cumulus cell expansion. Furthermore, the impaired expansion of cumulus cells may be partially associated with altered cumulus expansion-related transcripts that are regulated by SMAD2/3 signaling. Our results indicate that SMAD2 and SMAD3 function redundantly in vivo to maintain normal female fertility and further support the involvement of an intraovarian SMAD2/3 pathway in mediating oocyte-produced signals essential for coordinating key events of the ovulatory process.

[L4-1] Thyroid Gland Organogenesis: Genes, Mechanisms & Evolution
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The thyroid gland is a very important organ for the development of vertebrates as it synthesizes hormones that are essential for growth, development and survival such as tetraiodothyronine (thyroxine or T4) and triiodothyronine (T3). Thyroglobulin (Tg), thyroperoxidase (TPO), sodium/iodide symporter (NIS), and TSH receptor (TSHr) are
genes necessary for the synthesis of such hormones which takes place in the fully differentiated thyroid cell, called the thyrocite. Indeed, some of these genes mark a differentiated thyroid cell; in particular, thyroglobulin and thyroperoxidase are genes exclusively expressed in thyroid cells. The promoters of these two genes have been extensively studied and three transcription factors, namely TTF-1 (also named Titf1/Nkx2-1), Foxe1 and Pax8, have been demonstrated to be involved in the activation of these genes. During development and in the adult life, these factors are also present in other tissues, but the three of them are co-expressed only in the thyroid. It has been shown that their expression is required for the early stages of thyroid morphogenesis and is crucial for normal thyroid function. Indeed, for all its life a thyroid cell will be hallmarked by the simultaneous presence of TTF-1, Foxe1 and Pax8. Interestingly, these thyroid-enriched transcription factors are likely linked in a regulatory network such that each of them can be involved in the initiation or maintenance of the others.

The information summarized in an article (Endocrine Reviews 25:722-746, 2004) shows that disturbances of thyroid morphogenesis, leading to a group of conditions collectively called thyroid dysgenesis (TD), are due, in some cases, to disturbances in the function of genes that regulate various aspects of thyroid development. Thus TD can be a heritable genetic disease. However, a search for mutations in the genes indicated in this review gave no results in the majority of patients. Furthermore, even in the cases in which mutations in known genes are clearly associated with the disease, a great variability in the phenotype has been observed, even in individuals with the same mutation. Last, but not least, monozygotic twins are mostly discordant for TD. Thus, future studies should address some of the following points: 1. The mutations scored in known candidate genes (TITF1/NKX2-1, FOXE1, PAX8, and TSHR) in TD patients are certainly an underestimate, considering that mutations have been searched mostly in the coding region. Thus, mutations in introns or in regulatory regions may have gone unnoticed. A recent study (BMC Genomics 2010, 11:306) reveals the presence of a thyroid-specific regulatory element in the 5' upstream region of the Pax8 gene. The identification of this regulatory element represents the first step in the investigation of upstream regulatory mechanisms that control Pax8 transcription during thyroid differentiation and are relevant to further studies on Pax8 as a candidate gene for thyroid dysgenesis. 2. Titf1, Foxe1, Pax8, and Hhex are transcription factors regulating the expression of downstream genes that ultimately actuate the organogenesis of the gland. It is possible that other cases of TD could be due to mutations in the genes controlled by these transcription factors. 3. Genes responsible for the initial differentiation events causing thyroid anlage formation have not been identified. Some of these genes could be responsible for true thyroid agenesis. Perhaps the promoter region of genes expressed early in the anlage could be instrumental in searching for such genes. 4. The apparent sporadic appearance of CH associated with TD could suggest that, at least in some cases, this disease could be of polygenic origin. This has been shown to be the case, for example, for genes involved in the establishment of diabetes. Phenotypic variability observed in patients affected by mutations in either PAX8 or TITF1/NKX2-1 genes supports the possibility that other interacting genes may modulate the phenotype. It has been reported that CH may develop as a result of mutations at different loci acting simultaneously and in a synergistic manner. Indeed, double-heterozygous Titf1/Nkx2-1+/−, Pax8+/− mice in a specific genetic background show impaired thyroid function as assessed by high TSH and low T4 hormone levels in blood. 5. At early stages of thyroid morphogenesis, somatic mutations in all the genes already described could affect gland organogenesis. The creation of animal models with a thyroid-specific, conditional
inactivation of these genes will offer a tool to elucidate the possibility that such nongermline mutations result in TD. Finally, the discordance for TD in monozygotic twins suggests that epigenetic mechanisms might be involved. However, as in the case of Beckwith-Wiedemann syndrome, a genetic condition characterized by a few familial and a majority of sporadic cases, it is conceivable that the genes involved are the same but their inactivation may derive from different mechanisms.

[L4-2] Molecular Genetics & Molecular Diagnostics of Thyroid Tumors
YE Nikiforov. Univ of Pittsburgh, Pittsburgh, PA

Our understanding of the molecular mechanisms of thyroid cancer development has expended dramatically in the recent years. Thyroid carcinogenesis appears to be triggered and propelled by discrete mutational events as well as by disregulation of gene expression via epigenetic mechanisms and alteration in microRNA expression. A number of new mutations has been discovered and characterized, most of which affect the MAPK and PI3K/PTEN/AKT signaling pathways. The role of activation of these pathways in tumor initiation and in the process of dedifferentiation and anaplastic transformation is now better understood. The nature of a mechanism of gene mutation, i.e. point mutation vs. chromosomal rearrangement, appears to be influenced by specific etiological factors involved in thyroid carcinogenesis. The advances in molecular genetics of thyroid cancer have started to translate into clinical practice, offering significant improvement in cancer diagnosis in thyroid nodules and better tumor prognostication. Four mutation types constitute the majority of mutations known to occur in papillary and follicular types of thyroid cancer. They are BRAF and RAS point mutations and RET/PTC and PAX8/PPARγ chromosomal rearrangements. Recent studies have shown that these genetic markers have significant diagnostic utility, and some of them can be used for tumor prognostication and also serve as potential therapeutic targets. The diagnostic utility of these markers appears to be of particular importance in thyroid fine-needle aspiration (FNA) samples, especially in thyroid nodules with indeterminate cytology. Some molecular markers, such as BRAF, offer significant help in determining more aggressive tumor behavior and can be used to optimize the surgical and post-surgical management of patients with thyroid cancer.


BB Kahn. Beth Israel Hosp/Harvard Med Sch, Boston, MA

The adipose cell functions as an endocrine organ in addition to its role in energy storage. Hormones, cytokines and other factors secreted from adipose cells influence energy balance, glucose homeostasis, insulin sensitivity and cardiovascular biology through actions in the central nervous system and peripheral tissues. Since insulin resistant humans with obesity and type 2 diabetes show down-regulation of the Glut4 glucose transporter selectively in adipose tissue, we knocked out or overexpressed Glut4 selectively in adipocytes in mice. Adipose-Glut4 knockout mice are insulin resistant and adipose-Glut4 overexpressing mice have increased glucose tolerance. Gene array analysis of adipose tissue from these mice reveals novel pathways that regulate glucose homeostasis and adiposity. One reciprocally regulated, secreted protein is Retinol Binding Protein 4 (RBP4), the major vitamin A carrier in blood. We found that
RBP4 levels correlate highly with insulin resistance, intra-abdominal adipose mass and other cardiovascular risk factors in many human populations and mechanistic studies indicate elevated RBP4 may contribute to insulin resistance and hypertension. We also discovered non-secreted proteins in adipose tissue that alter systemic fuel utilization such as Carbohydrate Response Element Binding Protein (ChREBP), a transcription factor that regulates lipogenesis and glycolysis. Our data show that ChREBP controls a transcriptional pathway in adipocytes that regulates whole body fuel homeostasis and insulin sensitivity. In parallel, we found that altered adipocyte glucose metabolism coordinately regulates enzymes that oxidize branched chain amino acids (BCAAs) and that normal adipose tissue can regulate circulating BCAA levels. These findings may have clinical implications since obesity and insulin resistance are associated with downregulation of enzymes controlling BCAA metabolism in adipose tissue. Furthermore, we find downregulation of ChREBP expression in fat of obese humans and this correlates highly with the degree of insulin resistance. These findings indicate that adipocytes sense and respond to changes in glucose availability by altering pathways that regulate BCAA and lipid metabolism. These novel pathways reveal the intricate function of adipocytes in regulating systemic fuel metabolism and insulin sensitivity. These pathways could provide new biomarkers and targets for prevention and treatment of type 2 diabetes and the metabolic syndrome.

[1] Non-Classical ERα Signaling Mechanisms in Neuroendocrine Systems

JE Levine. Northwestern Univ, Evanston, IL
Ovarian estrogens exert critically important actions in hypothalamic neurons to regulate ovulatory cyclicity and energy homeostasis. Estrogen receptor alpha (ERα) appears to mediate most of these effects, as disruption of ERα signaling leads to infertility and metabolic syndrome. ERα signaling mechanisms may include “classical genotropic” effects mediated by direct binding of receptor dimers to DNA, “non-classical genotropic” effects involving tethering of ERs to other transcription factors, and “non-classical non-genotropic” actions mediated by cytoplasmic ERs coupled to membrane-initiated signal transduction pathways. Our studies make use of novel ER mutant mouse models to ascertain the cellular mechanisms by which ERα mediates E2 effects on these physiological and behavioral processes. We have utilized a novel mutant ER knock-in mouse model, which confers non-classical genotropic and non-genotropic signaling in the absence of classical signaling, to determine that non-classical ERα signaling can convey E2 effects integral to homeostatic feedback control of reproductive hormone secretions, as well as E2 actions governing energy homeostasis, adiposity, and insulin sensitivity. Non-classical ERα signaling has also been found to mediate suppressive actions of estradiol on expression of the neuropeptide, kisspeptin, in arcuate nucleus neurons. Conditional targeting of the ERα gene in kisspeptin neurons has revealed that ERα signaling in kisspeptin neurons mediates feedback actions of estradiol in the reproductive axis. Thus, disruption of ERα signaling recapitulates a subset of the reproductive and metabolic abnormalities observed in the complete ERα null mutant: peripubertal LH excess, irregular or absent estrous cyclicity, ovarian follicular cysts, increased body weight, and insulin resistance. Since these abnormalities are also common features of polycystic ovarian syndrome, we have proposed that cell-specific resistance in non-classical ERα signaling pathways may be one common mechanism for the development of major symptoms of the disorder.

[6-2] Sex Battles in the Brain
C Dulac. Harvard Univ/Howard Hughes Med Inst, Cambridge, MA
Genomic imprinting results in preferential gene expression from paternally versus maternally inherited chromosomes. We used a genome-wide approach to uncover sex-specific parent-of-origin allelic effects in the adult mouse brain. Our study identified preferential selection of the maternally inherited X chromosome in glutamatergic neurons of the female cortex. Moreover, analysis of the cortex and hypothalamus identified 347 autosomal genes with sex-specific imprinting features. In the hypothalamus, sex-specific imprinted genes were mostly found in females, suggesting parental influence over the hypothalamic function of daughters. We show that Interleukin 18, a gene linked to diseases with sex-specific prevalence, is subject to complex, regional, and sex-specific parental effects in the brain. Parent-of-origin effects thus provide new avenues for investigation of sexual dimorphism in brain function and disease.

[6-1] Edwin B Astwood Award Lecture: Steroid Hormone Action from Genome to Cistrome
M Brown. Dana-Farber Cancer Inst, Boston, MA
Steroid hormone receptors act as ligand-regulated transcription factors that play critical roles in normal physiology and pathologic functions in diseases including breast and prostate cancers. Sequencing of the human genome has allowed the near complete identification of the expressed regions of protein-coding genes as well as increasing
numbers of non-coding RNA genes, however, little is known on a genomic scale concerning the organization of the cis-regulatory elements. Elucidation of the regulatory networks controlled by these receptors is fundamental to the understanding of steroid hormone biology. We have taken unbiased genome-wide approaches to identify the complete collection of steroid receptor regulatory sites. We coined the term “Cistrome” to define the set of cis-regulatory targets of trans-acting factors across the entire genome. The analyses of steroid receptor cistromes combined with gene expression profiling data have revealed new insights into the organization of hormone-responsive genes and the existence of a transcription factor and epigenomic code that dictates cellular responses to steroid hormones.

Abstract of a publication by the speaker. (Endocr Relat Cancer 2009 16:381-9)
Alterations in transcription programs are a fundamental feature of cancer. Nuclear receptors, such as the estrogen receptor alpha (ERalpha) and androgen receptors (ARs), are central in this process as they can directly impact gene expression through interaction with the chromatin and subsequent association with coregulators and the transcriptional machinery. Thousands of putative ER-binding sites are found across the human genome. This includes over 60,000 estrogen-responsive elements (EREs) and a number of regions recruiting ER through a tethering mechanism. However, lineage-specific ER recruitment, as reported between breast and osteosarcoma cancer cell lines, is central to the unique transcriptional program generated in each cell type following estrogen (E2) treatment. Unbiased genome-wide investigations have demonstrated the predominant recruitment of both ERalpha and AR to distant (non-promoter)-regulatory elements. Furthermore, these studies revealed a clear relationship between sites of transcription factor recruitment and gene regulation. Indeed, expression profiles from AR-positive primary prostate tumors and cell lines directly relate to the AR cistrome in prostate cancer cells, while the ERalpha cistrome in breast cancer cells relates to expression profiles from ERalpha-positive primary breast tumors. Additionally, cell-type-specific ERalpha cistromes are linked to lineage-specific estrogen-induced expression profiles in different cell types, for example osteosarcoma and breast cancer cells. The pioneer factor forkhead box A1 (FoxA1/HNF3alpha) plays a central role in AR and ERalpha signaling. It is recruited in a lineage-specific manner translating the epigenetic signature consisting of mono- and dimethylated histone H3 on lysine 4 (H3K4me1/me2) into functional regulatory elements. Hence, through the interplay between the pioneer factor, namely FoxA1, and epigenetic events, the transcriptional potential of a given cell lineage is predefined. Since this directly impacts signaling through nuclear receptors, these discoveries should significantly impact the development of novel therapeutic strategies directed against multiple types of cancer.

[85-2] Insulin & Insulin-Like Growth Factors in Cancer Risk & Therapy
D Yee. Masonic Cancer Ctr, Univ of Minnesota, Minneapolis, MN
Population and laboratory data show that insulin and the IGFs stimulate cancer cell biology. This growth factor system has been linked to both cancer risk and progression. Because of these findings, numerous drugs are in clinical trial examining the utility of disrupting IGF signaling in cancer cells. Several monoclonal antibodies directed against the type I IGF receptor (IGF1R) have been created. These antibodies effectively inhibit signaling by downregulating the IGF1R, but have minimal effects on insulin receptor. In contrast, tyrosine kinase inhibitors disrupt the biochemical function of the receptor, do not downregulate receptor levels, and are not selective; they inhibit IGF1R and insulin receptor. To address the relevance of insulin receptor signaling, we created cells (MDA-435/LCC6 and T47D) that have downregulated insulin receptor. These cells
have reduced tumor growth and metastasis compared to their parental cells. Insulin signaling induces expression of several genes including HIF1alpha, VEGF-A, and VEGF-D resulting in decreased angiogenesis and lymphangiogenesis. These data show that inhibition of both insulin and IGF1R may be necessary to disrupt the malignant phenotype. In addition to identifying relevant receptor targets, we have also demonstrated that the insulin receptor substrate (IRS) proteins link IGF/insulin signaling to specific cancer phenotypes. Recently, we examined gene expression profiling downstream of IGF1R activation in T47D cells and have found that the gene expression profile patterns are different between IRS-1 and -2. Furthermore, IRS-derived gene expression profiles identify tumors with more aggressive biological behavior. Finally, we have found that a single chain chimeric antibody that downregulates both IGF1R and IRS-2 results in more profound inhibition of cancer cell motility than just downregulating the receptor. Thus, the insulin and IGF signaling pathway undoubtedly regulate cancer cell biology. Disrupting the signaling pathway of these receptors shows promise as cancer therapy and additional modeling in preclinical systems should guide the development of appropriate clinical applications.

SYMPOSIA: TRANSLATIONAL - Premature Adrenarche: Is It Benign?

[S7-1] Steroid Biosynthetic Pathways in Normal & Premature Adrenarche

WE Rainey. Med Coll of Georgia, Augusta, GA

The mechanisms causing the rise in adrenal androgen production during the course of adrenarche remain to be defined. However, the increase in steroid release is clearly associated with a series of intra-adrenal changes in the expression of steroidogenic enzymes needed for androgen production, as well as an expansion of the adrenal zona reticularis (ZR). We and others have defined the adrenal expression pattern of key steroidogenic enzymes during adrenarche and in adult adrenals. As adrenarche proceeds, the expanding ZR expresses greater levels of 17β hydroxysteroid dehydrogenase type 5 (HSD17B5), cytochrome b5 (CYB5) and steroid sulfotransferase (SULT2A1) than the adjacent fasciculata. ZR enhanced expression of CYB5 acts to increase conversion of 17α hydroxypregnenolone to DHEA through its effects on the 17,20 lyase activity of CYP17. The increase in SULT2A1 is responsible for the conversion of DHEA to its sulfated form. The role of HSD17B5 is currently under investigation, but this enzyme is likely responsible for the small amount of adrenal testosterone production that occurs during and after adrenarche through its conversion of androstenedione to testosterone. The growing ZR, in contrast, is deficient in the enzyme 3β-hydroxysteroid dehydrogenase type 2 (HSD3B2). The lack of HSD3B2 allows precursors (pregnenolone and 17α hydroxyprogrenenolone) to flow toward DHEA. With the above in mind, the resulting profile of steroidogenic enzymes in the post-adrenarche ZR lends itself toward the production of adrenal androgens as opposed to glucocorticoids. My presentation will focus on the intra-adrenal changes that provide the ZR with the ability to secrete its unique set of adrenal androgens.


Age-related morphologic development of human adrenal zona reticularis (ZR) has not been well examined. Therefore, in this study, 44 human young adrenal autopsy specimens retrieved from large archival files (n=252) were examined for immunohistochemical and morphometric analyses. Results demonstrated that ZR became discernible around 4 years of age, and both thickness and ratio per total cortex of ZR increased in an age-dependent fashion thereafter, although there was no significant increment in total thickness of developing adrenal cortex. We further
evaluated immunoreactivity of both KI67 and BCL2 in order to clarify the equilibrium between cell proliferation and apoptosis in the homeostasis of developing human adrenals. Results demonstrated that proliferative adrenocortical cells were predominantly detected in the zona glomerulosa and partly in outer zona fasciculata (ZF) before 4 years of age and in ZR after 4 years of age, but the number of these cells markedly decreased around 20 years of age. The number of BCL2-positive cells increased in ZR and decreased in ZF during development. Adrenal androgen synthesizing type 5 17beta-hydroxysteroid dehydrogenase (HSD17B5 or AKR1C3 as listed in the Hugo Database) was almost confined to ZR of human adrenals throughout development. HSD17B5 immunoreactivity in ZR became discernible and increased from around 9 years of age. Results of our present study support the theory of age-dependent adrenocortical cell migration and also indicated that ZR development is not only associated with adrenarche, but may play important roles in an initiation of puberty.

[S7-2] IGF System, Insulin Sensitivity & Estrogen: Regulators of Adrenal Androgens?
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Adrenarche is a process of postnatal sexual maturation occurring in higher primates, in which there is an increase in the secretion of adrenal androgens. It is the consequence of a process of postnatal organogenesis characterized by the development of a new zone in the adrenal cortex, the zona reticularis (ZR). The mechanism of the phenomenon remains poorly understood, suggesting that it might be a multifactorial event. A relationship between circulating IGF-I, insulin sensitivity, and adrenal androgens has been postulated. Boys and girls have different patterns of changes in insulin sensitivity at puberty, perhaps secondary to differences in the estrogen milieu. Peripheral or local IGF-1 actions could regulate adrenal progenitor cell proliferation and migration. Since adrenal progenitor cells, as well as IGF-1 and the IGF-R1, are located in the outer zone of the adrenal cortex during childhood and adolescence, this peripheral cell layer, below the capsule, may contain undifferentiated progenitor cells. Therefore, the IGF-R1 signaling pathway might positively modulate the proliferation and migration of adrenal progenitor cell to stimulate the development of adrenal zones, including ZR. However, no evidence of a direct action of IGF-1 on ZR was found. In addition, a role for estrogens in the ontogenesis of ZR is suggested by the presence of aromatase (CYP19) and GPR-30 in the subcapsular zona glomerulosa and in the adrenal medulla. Estrogens produced locally could act on ZR by interacting with estrogen receptor beta, but not alpha and membrane estrogen receptor GPR-30, regulating adrenal androgen production. After adrenarche, there is a progressive increase in circulating DHEA and DHEA-S of adrenal origin. Functionally, adrenarche could result from an increase in 17,20-lyase activity of P450c17 secondary to high levels of cytochrome b5 expression, and from a decrease in 3βHSD2 expression along with an increase in the expression of SULT2A1, in the ZR. The GH-IGF system and insulin, among other factors, might also modulate adrenal androgen production. In summary, several lines of evidence point out to the action of multiple factors, such as local adrenal maturational changes, as well as peripheral metabolic signals, on postnatal human adrenal gland ZR formation and function.

[S7-3] Premature Adrenarche (PA) as a Precursor of the Polycystic Ovary Syndrome (PCOS)
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Premature adrenarche (PA), a developmentally programmed increase in the production of C-19 adrenal steroids late in pre-puberty, manifests clinically as early appearance of pubic hair. PA has been proposed as a harbinger of PCOS, sharing many of its characteristic biochemical and physical features, including hyperandrogenism, hyperinsulinism, dyslipidemia, increased LH:FSH ratios, increased AMH concentrations, and increased CYP17 activity. Despite these similarities, not all girls with PA develop PCOS. Prenatal, environmental, and genetic factors, including family history of PCOS, intrauterine growth retardation (IUGR), excessive childhood and peri-pubertal weight gain, hyperinsulinism, insulin resistance (IR), and fluctuations in steroidogenic enzyme activities are proposed to govern outcomes in girls with PA.

Ibanez and colleagues have elegantly annotated the progression to PCOS among a cohort of pre-adolescent girls with PA. The incidence of PCOS in this population is 15-fold that of the control population. Across the breadth of their pubertal maturation, girls with PA evidence significant hyperinsulinemia and IR that has been highly correlated with their degree of androgen excess (both ovarian and adrenal) and with their IUGR status. Buttressing the argument that insulin resistance underlies both PCOS and PA, insulin sensitization of pre-pubertal girls with PA prevented the PCOS outcome. Prospective, longitudinal studies to illuminate the causal relationship between PA and emergence of PCOS are lacking, hindered both by the long interval between presentation with PA and definitive outcomes and human subject concerns regarding the conduct of research in children. An early longitudinal study estimated the risk of PCOS among girls with PA at <20%. In a more recent prospective outcome study, lower SHBG and insulin resistance, excessive peri-pubertal weight gain and allelic differences for steroidogenic (e.g., CYP21), Insulin Receptor Substrate (IRS), and PPAR-g genes correlated with greater proclivity to PCOS among PA girls. Recently, several groups have demonstrated early metabolic derangements, aberrant adipocytokines, and altered AMH levels among the peri-pubertal daughters of women with PCOS; proposed as early signals of PCOS, these remain to be studied in girls with PA. Thus, the pathophysiological mechanisms linking PA with PCOS remain obscure. Their elucidation should prove invaluable for decoding the ontogeny of PCOS.

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SYMPOSIA: CLINICAL - Concepts & Controversies in the Management of Short Stature

[S31-1] What Dictates Referral for Short Stature?
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Background: Candidates for specialty drugs, the fastest growing and costliest pharmaceuticals, typically originate with primary care referrals. However, little is known about what drives such referrals - especially for large populations such as short, otherwise normal children (idiopathic short stature; ISS). Expansions of Food and Drug Administration approvals for growth hormone (GH) makes more than 400,000 U.S. children potentially eligible for such treatment.

Methods: To assess the relative impact of patient physiological indicators, physician characteristics, and consumer preferences on referrals to endocrinologists (and potential access to GH) for short children, a national study of 1268 randomly selected U.S. pediatricians was conducted, based on a full factorial experimental design in a
structured survey.

Results: While patient indicators (height, growth pattern) significantly influenced referrals, consumer drivers (family concern) and physician attitudes had almost as great an impact—especially for children with less severe growth impairment. Physician belief that short stature impairs emotional well-being and physician characteristics also influenced referrals—indeed independent of growth parameters.

Conclusions: Referral recommendations that create the pool of candidates for the specialty drug GH are heavily swayed by physician characteristics and consumer preferences, particularly in the absence of compelling physiological evidence. This makes the majority of children with short stature highly susceptible to non-physiological influences on referrals that render them candidates for this specialty drug.

Abstract of a Publication by the speaker. Arch Pediatr Adolesc Med. 2002 156:230-40. (Effect of growth hormone therapy on height in children with idiopathic short stature: a meta-analysis). Finkelstein BS et al. CONTEXT: Use of growth hormone (GH) therapy to promote growth in children with idiopathic short stature is controversial. A fundamental issue underlying the controversy is uncertainty about the magnitude of effectiveness of GH for this condition. OBJECTIVE: To determine the effect of GH on short- and long-term growth in idiopathic short stature. STUDY DESIGN: Systematic review of controlled and uncontrolled studies. DATA SOURCES: MEDLINE (1985-2000), key journals, cross-referencing of bibliographies, abstract booklets, and experts. STUDY SELECTION AND DATA EXTRACTION: We performed a meta-analysis of all studies satisfying the inclusion criteria for idiopathic short stature: initial height below the 10th percentile, normal stimulated GH levels (>10 microg/L), absence of comorbid conditions, no previous GH therapy, treatment with biosynthetic GH, and inclusion of major outcome measures. PRIMARY OUTCOME MEASURES: Growth velocity and height SD score (number of SDs from mean height for age and sex) at baseline and after 1 year to evaluate the short-term effect of GH. Adult height was analyzed to evaluate the long-term effect of GH. DATA SYNTHESIS: Ten controlled trials (434 patients) and 28 uncontrolled trials (655 patients) met the inclusion criteria. While baseline growth velocities were equivalent at baseline, 1-year growth velocity of the GH-treated group significantly exceeded that of controls by 2.86 cm/y. Similarly, in uncontrolled trials, growth velocity increased after 1 year, and height SD score increased from -2.72 at baseline to -2.19. In controlled studies, the adult height of the GH-treated group significantly exceeded controls by 0.84 SD, and in uncontrolled trials the adult height attained after GH treatment (-1.62 SDs) exceeded that predicted at baseline (-2.18 SDs). These results suggest an average gain in adult height of approximately 4 to 6 cm (range, 2.3-8.7 cm) with GH therapy. Given current treatment costs, this corresponds to more than $35 000 per inch (2.54 cm) gained in adult height in idiopathic short stature. CONCLUSIONS: Treatment with GH results in short-term increases in growth for children with idiopathic short stature, and long-term GH can increase adult height. These results are fundamental to decisions about GH use and raise questions about the goals of treatment. Use of GH for idiopathic short stature in clinical practice will depend on its efficacy in promoting growth and the value of this effect to families, physicians, and third-party payers.

[S31-2] Efficacy of Growth-Promoting Agents: An Overview

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Abstract of a publication by the speaker. J Clin Endocrinol Metab. 2008 93:823-31. (Anastrozole increases predicted adult height of short adolescent males treated with
growth hormone: a randomized, placebo-controlled, multicenter trial for one to three years)

**CONTEXT:** The process of epiphyseal fusion during puberty is regulated by estrogen, even in males. **OBJECTIVE:** Our objective was to investigate whether anastrozole, a potent aromatase inhibitor, could delay bone age acceleration and increase predicted adult height in adolescent boys with GH deficiency. **METHODS:** Fifty-two adolescent males with GH deficiency treated with GH were randomized to cotreatment with anastrozole or placebo daily for up to 36 months. **RESULTS:** Fifty subjects completed 12 months, 41 completed 24 months, and 28 completed 36 months. Linear growth was comparable between groups; however, there was a significantly slower increase in bone age advancement from baseline in the anastrozole group vs. placebo group after 2 yr (+1.8+/−0.1 vs. +2.7+/−0.1 yr, P<0.0001) and after 3 yr (+2.5+/−0.2 vs. +4.1+/−0.1 yr, P<0.0001). This resulted in a net increase in predicted adult height of +4.5+/−1.2 cm in the anastrozole group at 24 months and +6.7+/−1.4 cm at 36 months as compared with a 1-cm gain at both time points in the placebo group. Estradiol and estrone concentrations increased less in the anastrozole group compared with placebo group. All boys on the aromatase inhibitor had normal tempo of virilization. Safety data, including glucose, and plasma lipid concentrations were comparable between groups. **CONCLUSIONS:** Anastrozole increases adult height potential of adolescent boys on GH therapy while maintaining normal pubertal progression after 2-3 yr. This treatment offers an alternative in promoting growth in GH-deficient boys in puberty. Long-term follow up is needed to elucidate fully the safety and efficacy of this approach.

[S31-3] **Endocrine Management of Short Stature: Assumptions & Evidence Regarding the Quality of Life Benefits**

**DE Sandberg. Univ of Michigan Med Sch, Ann Arbor, MI**

Growth velocity in childhood and adolescence helps inform healthcare providers about overall health. Atypical growth deceleration or acceleration can trigger diagnostic evaluation and treatment of underlying disease. Increasingly, however, *height* itself, (in particular short stature; SS), has become a target of endocrine interventions. Recombinant human growth hormone (rhGH), alone or in combination with other growth-promoting agents, is now prescribed to physically healthy youths (as in idiopathic short stature) or to those with complex medical conditions in which SS is one feature (e.g., Turner syndrome). Unlimited availability of rhGH and other growth-promoting agents has contributed to the disassociation of the treatment of SS from its causes. Treatment of short children and adolescents (with or without accompanying medical conditions) is predicated on beliefs that (1) SS is an undesirable physical characteristic associated with psychosocial adaptation problems and a diminished quality of life (QoL) and (2) hormonally-induced height increases will improve QoL. This presentation summarizes what is known about psychosocial aspects of SS and QoL benefits of medical intervention. Stereotypes and assumptions about SS are evaluated in light of empirical findings. Problems of psychosocial adjustment are relatively common in the general population. Because of the salience of SS, and its potential to serve as a lightning rod to divert attention from other factors interfering with healthy psychosocial adaptation, clinicians must be watchful of misattributions for ongoing problems and unrealistic predictions of the benefits of taller stature. For these reasons, healthcare providers should consider incorporating a psychosocial component in the diagnostic evaluation of children with SS to broaden potential treatment options and recommendations.
[S39-1] Structural & Pituitary Abnormalities in Congenital GH Deficiency  
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Only small advances were made in the field of pituitary imaging until the advent of magnetic resonance imaging (MRI), which led to an enormous increase in our detailed knowledge of pituitary morphology, thus improving the differential diagnosis of hypopituitarism. Indeed, MRI represents the examination method of choice for evaluating hypothalamic-pituitary-related endocrine diseases thanks to its ability to provide strongly-contrasted high-resolution multi-planar and spatial images. Specifically, MRI allow a detailed and precise anatomical study of the pituitary gland by differentiating between the anterior and posterior pituitary lobes. The MRI identification of pituitary hyperintensity in the posterior part of the *sella*, now considered a marker of neurohypophyseal functional integrity, has been the most striking finding contributing to the diagnosis and understanding of some forms of “idiopathic” and permanent growth hormone deficiency (GHD).

Published data show a varying correlation between pituitary abnormalities as observed on the MRI scan and a patient's endocrine profile. Indeed several trends have emerged and have been confirmed: 1) normal MRI scan or anterior pituitary hypoplasia mainly indicates isolated GHD that is generally transient and not confirmed after adult height achievement; 2) Patients with combined pituitary hormone deficiency (CPHD) seldom have a normal pituitary gland; 3) The classic triad of ectopic posterior pituitary gland, pituitary stalk hypoplasia/agenesis and anterior pituitary gland hypoplasia is more frequently reported in CPHD patients and its generally associated with permanent GHD. Pituitary abnormalities have been reported in patients with GHD carrying mutations in several genes encoding transcription factors such as POU1F1, PROP1, HESX1, LHX3, LHX4, GLI2, PITX1, PITX2, SOX3, SOX2 and OTX2. Establishing endocrine and MRI phenotypes is extremely helpful in the selection and management of patients with hypopituitarism, both in terms of possible genetic counseling and of early diagnosis of evolving anterior pituitary hormone deficiencies.

[S39-2] Consequences of Lifetime Isolated GH Deficiency  
R Salvatori. Johns Hopkins Univ Sch of Med, Baltimore, MD

Several years ago we identified a kindred with approximately 100 subjects with severe isolated GH deficiency (IGHD) residing in or around Itabaianinha, a rural area of the Brazilian state of Sergipe. Their IGHD is caused by a homozygous inactivating mutation in the GHRH receptor gene. None of the adult individuals has received GH therapy during childhood, and therefore they represent a unique opportunity to study the consequences of lifetime untreated IGHD. These individuals go through life with very low levels of GH and IGF-I. As result of this, they present severe but proportionate short stature, with adult height standard deviation score ranging from -9.6 to -5.1. The reduction of the cephalic perimeter is less accentuated than the reduction in facial height, causing a disproportion between the calvarium and the face, resulting in a “doll” or cherubim angel facies, which, together to the underdevelopment of the larynx, leads to a characteristic high-pitched voice, more marked in males than in other forms of short stature. When corrected by body surface, they have reduced size of the pituitary (likely due to reduced somatotroph cell mass), thyroid, heart, uterus, and spleen, and relatively large liver, pancreas and kidney, suggesting that the growth of the latter organs depends less from an intact GH-IGF-I axis. Puberty is delayed, but reproductive life and quality of life are normal. Beginning of climacteric is anticipated, but menopausal symptoms
are not different from local controls. Bone density is somewhat reduced, without apparent increase in fractures. These IGHD subjects also exhibit an increase in total and LDL cholesterol, together with high levels of C reactive protein, and increased blood pressure. Body composition is markedly abnormal, with reduction in fat free mass with a mirror increase in percent fat mass. Despite these body composition changes, they have an increase in insulin sensitivity. Furthermore, despite the unfavorable cardiovascular risk profile, they do not exhibit premature atherosclerosis, and their carotid intima media thickness (IMT) is normal. Surprisingly, carotid IMT was actually increased by a 6 month sub maximal treatment with depot GH. Finally, despite an apparent increase in death rate in young (<20 yrs) IGHD females, once adulthood is reached, the longevity of IGHD subjects is similar to their normal-stature relatives, suggesting that the lifetime lack of GH is not a risk factor for cardiovascular mortality.

[S39-3] GH Replacement & Cardiovascular & Metabolic Risk in GH Deficiency
AF Attanasio. Cascina del Rosone, Agliano Terme, Italy

The adult GHD hypothesis predicts that GH replacement will improve metabolic status and reduce cardiovascular risk (CVD) in hypopituitary GHD patients. This assumption has been based on a multitude of controlled studies which have consistently shown improvement in markers of metabolic and CVD risk with GH administration in GHD patients as well as in obese, but otherwise normal adult subjects. In addition, some evidence that these changes may translate in improved outcomes has been provided by epidemiological studies performed predominantly in Northern Europe. However, preexisting risk factors independent of the GHD condition will also influence outcomes. The adult GHD phenotype shares features such as abdominal obesity, dyslipidemia, and insulin resistance with the metabolic syndrome (MetS), a cluster of risk factors for cardiovascular disease and type 2 diabetes. Accordingly, in overweight or obese adult patients with GHD, the metabolic abnormalities could be associated with GHD but could also exist independently of GHD. In such a composite situation, the GH-induced effects may not be sufficient to reduce risk below established thresholds. In a recent analysis on a large cohort of patients from the Hypopituitary Control and Complication Study (HyppoCCS) prevalence of MetS was unaffected by GH replacement, and baseline MetS status and obesity were strong predictors of MetS [1]. This suggests that GH intervention alone may not be sufficient to improve metabolic and CVD risk outcomes in adult GHD subjects in the presence of other non-GHD factors. Thus, appropriate treatment of non-GHD-related conditions will be a prerequisite to achieve and document measurable metabolic benefits of GH replacement.

SYMPOSIA: TRANSLATIONAL - Molecular Defects Affecting IGF-I Production, Transport & Action

[S58-1] STAT5b Gene Defects in Insulin-Like Growth Factor-I Deficiency (IGFD)
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Insulin-like growth factor (IGF)-I is a growth factor known to exert endocrine, paracrine and autocrine effects on the proliferation, differentiation, and metabolic functions of cells. In humans, an IGF-I deficiency (IGFD) from any etiology results in growth failure. When IGFD begins in utero, e.g. mutations in the IGFI gene, both prenatal and postnatal growth are impacted; when IGFD begins near term or after birth, only postnatal growth is affected. Postnatal production of circulating IGF is most dependent on growth hormone (GH), as clinical conditions of congenital GH deficiency (GHD) and GH insensitivity (GHI) results in IGFD with accompanying growth retardation
GH regulates IGF-I production primarily through activation of the GH receptor (GHR)-signal transducer and activator of transcription (STAT)-5b signaling cascade. One of four STAT proteins (STAT1, -3, -5a and -5b) activated by the GH-GHR system, the critical importance of STAT5b in IGF-I production came with the identification, in 2003, of the first homozygous STAT5b mutation, a missense mutation (A630P), reported in a young patient of normal birth size who presented with severe postnatal growth failure (height of -7.5 SD at age 16.5) associated with elevated serum concentrations of GH, normal GHR, and markedly reduced serum IGF-I (1). Exogenous GH treatment did not increase serum IGF-I or induce catch-up growth. At the cellular level, the mutant STAT5b protein was demonstrated to be poorly expressed, and presence of the closely-related STAT5a could not compensate for the loss of STAT5b as GH-induced regulation of IGF-I expression was abrogated in the STAT5b deficient cells. To date, a total of 7 unique homozygous STAT5b mutations, all autosomal recessive, have been identified in 10 case reports of IGFD associated with GHI and severe short stature. Unlike GHR mutations, these homozygous STAT5b mutations (with one exception) also conferred a phenotype of immune dysregulation, with T-cell homeostasis perturbed, and evidence of chronic pulmonary diseases. Interestingly, mutations in the STAT1 and the STAT3 genes, identified in immuno-compromised patients, were not associated with postnatal growth failure. Deciphering the molecular mechanisms of STAT5b-mediated IGF-I production in humans will further our understanding of GH action and will facilitate better diagnosis and management of children presenting with abnormal growth and development.

[S58-2] IGFALS Gene Defects

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The majority of IGFs circulate in the serum as a complex with IGFBP-3 or IGFBP-5, and an acid labile subunit (ALS). The well-established function of ALS is to prolong the half-life of the IGFs-IGFBP-3/IGFBP-5 binary complexes. Human ALS deficiency, caused by inactivating mutations in the IGFALS gene, is characterized by moderate growth retardation and marked reduction of IGF-I and IGFBP-3 levels that remain low after GH stimulation. Since the original report of human ALS deficiency in 2004, at least 21 patients have been diagnosed with this condition, suggesting that complete ALS deficiency could be more prevalent than previously suspected. Of the 21 patients presenting 16 different mutations in the IGFALS gene, 12 were found to be homozygous and nine were compound heterozygous. Consanguinity was present in less than half of the families. Absence of ALS protein results in the disruption of the entire IGF circulating system. Levels of IGF-I and specially IGFBP-3 are markedly reduced. Circulating IGF-II, IGFBP-1, and -2 levels are also reduced and insulin resistance is frequently observed. Despite a profound circulating IGF-I deficiency, only a mild impact on postnatal growth is observed. Perhaps, under the stimulation of normal or even increased GH levels, the local production of IGF-I could be sufficient to maintain linear growth near or within normal limits.

The observation that first degree relatives heterozygous carriers (HC) for IGFALS gene mutations are frequently shorter than wild type (WT) relatives, suggests that heterozygous carriers for IGFALS gene mutations may have a mild growth phenotype. Two independent preliminary reports have shown a relatively high frequency of HC for IGFALS gene mutations in children with idiopathic short stature. HC patients usually exhibit decreased levels of IGF-I, IGFBP-3 and ALS. In families of ISS children, short stature and low levels of members of the IGF system segregate with HC status. These
findings indicate that both gene alleles are required to maintain optimal ALS levels and to fulfill growth potential, suggesting a potential involvement of heterozygosity at the IGFALS gene in the etiology of short stature in a subset of short children presenting reduced levels of IGF-1 and IGFBP-3. These studies expand the spectrum of IGFALS gene defects in children with abnormal growth and confirm the crucial role of ALS in maintaining the integrity of the circulating IGF system.

[S58-3] IGF-I Receptor Gene Defects

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The insulin-like growth factor-I receptor (IGF1R) is a tetrameric \((\alpha_2/\beta_2)\) transmembrane tyrosine kinase. It is a member of the insulin receptor (IR) family consisting of the IGF1R, the IR and the insulin receptor-related receptor (IRR). The IGF1R and the IR show a sequence homology of approximately 60%. Both receptors can bind IGF-1, IGF-2 and insulin. The IGF1R exerts higher affinity for IGF-1 and the IR for insulin. The human IGF1R is transcribed as a single mRNA from its chromosomal location on 15q26.3 and becomes translated as a monomeric preproreceptor. Two IGF1R precursors are assembled by disulfide bonds to a precursor dimer. After dimerization and glycosylation the IGF1R is transported to the Golgi complex where it is cleaved into alpha and beta subunits. The physiological role of the IGF-1/IGF1R system is manifold. It plays an important role in regulation of cell growth and metabolism and has been shown to have an impact on cancer development and lifespan. IGF1R null mutant mice die at birth due to respiratory failure and have a reduction of size and weight by about 55%. Heterozygous knockout mice are born with a similar birth weight as their wild type littermates but develop growth retardation. Newborn patients with IGF1R mutations have been described as small for gestational age. The birth length varies between -5.8 and -0.3 SDS and the birth weight between -3.5 and -1.5 SDS. All patients reported so far did not show spontaneous postnatal catch-up growth and their height ranged between -5.0 and -1.6 SDS. Some of these patients present slight dysmorphic features (like triangular shape of face or small hands and feet) but others are phenotypically normal. Information about the impact of the mutations on mental development is inconsistent. In different developmental tests their scores varied between 60 and 134. Mutations of the human IGF-I receptor gene have been described to result in impaired receptor protein processing, reduced receptor number per cell, decreased receptor phosphorylation and/or disturbed postreceptor signalling. Genotype-phenotype relations and patterns of inheritance are being discussed.

SYMPOSIA: TRANSLATIONAL - Disorders of Puberty: Clinical & Molecular Approaches

S68-1] Mutations in CHD7 in Idiopathic Hypogonadotropic Hypogonadism

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CHARGE and Kallmann syndromes (KS) are two distinct developmental disorders sharing overlapping features of impaired olfaction and hypogonadism. KS is a genetically heterogeneous disorder combining idiopathic hypogonadotropic hypogonadism (IHH) and anosmia, which has been associated with mutations in \(\geq 6\) genes \((KAL1-6)\). CHARGE syndrome, a multisystem autosomal dominant disorder consisting of eye Coloboma, Heart defects, choanal Atresia, Retardation of growth and development, Genito-urinary anomalies, and Ear abnormalities (vestibular and auditory), is caused by CHD7 \((\text{Chromodomain Helicase DNA-binding protein 7})\)
mutations in ∼70% of patients. Mice heterozygous for N-ethyl- N-nitrosourea (ENU)-induced Chd7 mutations had a similar phenotype with cleft palate, choanal atresia, cardiac septal defects, hemorrhage, prenatal death, vulvar and clitoral defects. CHD7 is a nuclear protein belonging to a family of 9 CHD proteins that are able to utilize ATP hydrolysis to alter nucleosome structure. Chromodomains have been suspected to mediate chromatin interactions, and have been found to interact with DNA, RNA, and histone targets. Several studies had implicated the CHD7 gene in IHH/KS, although these CHARGE syndrome patients were not yet of pubertal age. We hypothesized that IHH/KS represents a milder allelic variant of CHARGE syndrome. The large CHD7 gene consists of 38 exons spanning 188 kb on chromosome 8q12.2 with a 10kb transcript encoding 2997 amino acids. Mutation screening of CHD7 was performed in 101 IHH/KS patients without a CHARGE phenotype. In an additional 96 IHH/KS patients, selected exons encoding the conserved chromodomains were sequenced. Heterozygous mutations were identified in sporadic KS and sporadic IHH. Three mutations affected chromodomains critical for proper CHD7 function in chromatin remodeling and transcriptional regulation, while four affected conserved residues, suggesting they are deleterious. CHD7's role was further corroborated by specific expression in IHH/KS-relevant tissues and appropriate developmental expression. Sporadic CHD7 mutations occur in 6% of both IHH and KS patients. CHD7 represents the first identified chromatin remodeling protein with a role in human puberty, and is designated as KAL5. Our findings indicate that IHH/KS are mild allelic variants of CHARGE syndrome caused by CHD7 mutations. These findings also underscore the importance of detailed phenotypic examination of IHH/KS to identify associated CHARGE abnormalities.

[S68-2] The Complex Genetics of Kallman Syndrome

C Dode. Inst Cochin, Paris, France

Kallmann syndrome (KS) typically combines severe congenital hypogonadotropic hypogonadism (HH) with anosmia. The degree of the hypogonadism and that of the smell deficiency can, however, vary significantly, not only between unrelated patients, but also within affected families. Some patients may also have non-reproductive, non-olfactory additional anomalies. Five causal genes have been identified to date, namely, KAL1, FGFR1, FGF8, PROKR2, and PROK2. Mutations (mainly nonsense or frameshift mutations) in KAL1 encoding the extracellular glycoprotein anosmin-1, underlie the X chromosome-linked form, which accounts for roughly 8% of all KS cases. Loss-of-function mutations (mainly missense mutations) in FGFR1 or FGF8 encoding fibroblast growth factor receptor-1 and fibroblast growth factor-8, respectively, underlie autosomal dominant forms with incomplete penetrance, which accounts for approximately 10% of KS cases. Notably, as many as 30% of the FGFR1 mutations found in the patients could be de novo mutations. Putative loss-of-function mutations in PROKR2 and PROK2, encoding prokineticin receptor-2 and prokineticin-2, respectively, have been detected in approximately 9% of the KS patients. Most of these mutations are missense mutations, and many are also present in apparently unaffected individuals, thus initially raising questions regarding their pathogenic role in the disease. For most of these mutations, however, deleterious effects on prokineticin-signalling have been shown. The finding of both heterozygous and homozygous unrelated patients for given PROKR2 and PROK2 mutations is quite remarkable, and raises the question of a possible digenic or oligogenic mode of inheritance in heterozygous patients. Digenic inheritance has indeed been shown in a few patients who carry missense mutations both in PROKR2 and PROK2, KAL1, FGFR1, or genes
responsible for normosmic congenital HH such as GNRHR and KISS1R. Most patients heterozygous for PROKR2 or PROK2 mutations, however, are expected to carry additional mutations in as-yet-undiscovered KS genes. Indeed, mutations in the genes identified so far have been found in less than 30% of all KS patients, indicating that other disease genes remain to be discovered. Finally, a majority of the patients affected by the CHARGE syndrome, a pleiotropic developmental disease that includes KS, carry neomutations in CHD7 encoding the chromodomain helicase DNA-binding protein 7.

[S68-3] Normosmic Isolated Hypogonadotropic Hypogonadism: Gene Mutations & Genotype Phenotype Relationship
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Congenital hypogonadotropic hypogonadism (CHH) results from abnormal gonadotropin secretion and is characterized by a complete or partial lack of pubertal development. The biological hallmark of CHH is a low level of circulating sex steroids, together with low or “normal” levels of FSH and LH.

Identification of genetic abnormalities related to CHH over the last two decades has provided major insights into the pathways critical for the development, and function of the reproductive axis. Mutations of 5 genes have been found specifically in Kallmann syndrome (KS), a disorder in which CHH is related to abnormal GnRH neuron ontogenesis and is usually associated with anosmia or hyposmia.

In complex syndromic CHH, in which gonadotropin deficiency is either incidental or only one aspect of a more complex endocrine disorder (adrenal or multiple pituitary insufficiencies, obesity) or a non endocrine disorder (malformations, neurological disabilities), other mutations affecting gonadotropin secretion have been reported. A subset of patients with syndromic form of CHH or KS may also appear to have isolated CHH, but close clinical, familial and genetic studies can reorient the diagnosis, which is important for genetic counseling and assisted medical procreation.

In isolated (non syndromic) CHH, the phenotype is usually tightly linked to an isolated deficiency of gonadotrophin secretion. These patients, have no associated signs or hormone deficiencies independent of the deficiency in gonadotrophin and sex steroids. Such cases, particularly familial, are due to genetic alterations affecting the GnRH sensitivity of gonadotropic cells (mutations in GNRHR) or the GnRH secretion (mutations in GNRH1, GPR54/KISS1R and TAC3 and TACR3), that can be reversed by pulsatile GnRH administration (hypothalamic isolated CHH). In several patients with TAC3 and TACR3 mutations we observed a dissociation between the very low LH and normal or nearly normal FSH levels, this gonadotropin responding excessively to the GnRH challenge test. This particular hormonal profile, suggest the possibility of a specific neuroendocrine impairment of GnRH secretion in patients with alteration of neurokinin B signaling. In all the patients with hypothalamic CHH, pulsatile GnRH administration normalized circulating sex steroids, LH release and restored fertility.

Published cases of different causes of isolated CHH, its clinical and endocrine features, and genotype-phenotype relationships will be discussed.

SYMPOSIA: CLINICAL - Pediatric Endocrine Screening Programs: Getting the Balance Right

S81-1] Improving Newborn Screening for CAH
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Within the past decade, the United States and 12 other countries have instituted universal newborn screening for the most common form of classic congenital adrenal
hyperplasia (CAH). Screening clearly reduces the time to diagnosis, and consequently prevents morbidity and mortality associated with severe salt-wasting 21-hydroxylase deficiency. Screening was originally performed by radioimmunoassay of blood spot 17-hydroxyprogesterone (17OHP); however the current prevailing technique is automated time-resolved dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA-TM). Immunoassays often have low positive predictive value due to poor antibody specificity coupled with high circulating levels of cross-reacting hormones in newborn blood. Moreover, stress, prematurity, and critical illness all contribute to transient elevations in cortisol and 17OHP secretion. Attempts to improve the predictive value of screening programs have included measuring the ratio of 17OHP to cortisol and stratifying cut-off values by gestational age or birth weight, but false positive screening tests results persist. Genotyping has also been proposed as a second tier test. CYP21A2-specific genotype analysis with a panel of probes or primers for the 10 most common mutant alleles can identify about 90-95% of affected haplotypes. However, this means that without complete CYP21A2 sequence analysis, about 5-10% of affected alleles could go undetected. Recent studies demonstrate a much-improved positive predictive value, as well as improved negative predictive value, when steroid profiling is done with liquid chromatography-tandem mass spectrometry as a second tier screening test. In addition to diagnosing CAH caused by 21-hydroxylase deficiency, it is possible with a 24 hour turn-around time for tandem mass spectrometry to detect other rarer forms of CAH (e.g., 11-hydroxylase deficiency, 3-beta-hydroxysteroid dehydrogenase deficiency, or P450 oxidoreductase deficiency) or congenital forms of adrenal insufficiency (e.g., adrenal hypoplasia congenital). Rapid and accurate CAH diagnosis could alleviate the high financial and emotional burden associated with the large number of false positive results we currently encounter.

[S81-2] Screening for Congenital Hypothyroidism: Controversies & Global Challenges
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The neuropsychological prognosis of patients with congenital hypothyroidism (CH) has been transformed by systematic biochemical screening of newborns: the gain in mean IQ is about 20 points and intellectual deficiency (IQ <70) is no longer observed, which has a major effect at the population level and makes screening for CH the most cost-effective of all neonatal screening procedures. Because primary CH is by far the commonest form of the condition, being identified by screening in one in three to four thousand newborns, and is almost never recognized clinically, TSH screening has been adopted by most jurisdictions. T4 screening will also identify central CH, but this condition is about ten-fold less frequent than primary CH and is almost always associated with multiple pituitary hormone deficiencies: therefore, hypoglycemia, cholestatic jaundice, micropenis and cryptorchidism should lead to the diagnosis on clinical grounds. Regular audits and logistical improvements have decreased the turnaround time of most CH screening programs from four weeks to 10 to 14 days. For patients with the most severe form of CH, who still have a loss of IQ in some recent studies, even earlier treatment may be necessary to ensure a completely normal neuropsychological development. Early discharge of newborns may require adjustments in screening procedures: because of the normal surge in TSH immediately after birth, screening samples are generally taken on day 2 or 3 of life. Aside from striving for the shortest possible turnaround time overall, the first screening sample should be taken at the prescribed time even in sick or premature babies. Whether these require a second screening sample is controversial and likely less important than ensuring that they have
their first sample taken in a timely manner. False negatives of TSH screening may result from Dopamine infusions and from fetal blood mixing between monozygotic twins, who are generally discordant for CH. Lowering the TSH cut-off for recall predictably results in more patients receiving a diagnosis of CH, but the extra cases generally have mild and often transient functional disorders of unknown significance for psychomotor development. Importantly, biochemical screening is only the first step of a process that should include etiological diagnosis (the commonest being thyroid ectopy, which is best documented by sodium pertechnetate scintigraphy), treatment and follow-up of all newborns who test positive.

[S81-3] Screening for Diabetes in Children & Adolescents: To Dream the Impossible Dream (or NOT?)

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Our simplistic picture of diabetes in children has become significantly more complex as has the potential strategies to cost effectively screen for hyperglycemia and/or at risk children. Screening school aged children has lead to a capture rate of less that 0.5% utilizing OGTT in populations at high risk for type 2 diabetes. HLA and antibody screening studies may predict a group at risk children for developing type 1 diabetes. From a variety of TrialNet studies, prospective identification of at risk children has lead to early detection of glucose intolerance, early treatment intervention and avoidance of diabetic ketoacidosis. Genotyping or gene wide screening of all children at birth is attractive for children with type 1 and MODY forms of diabetes, as is screening for antibodies predictive of diabetes. With current methodologies, screening for these parameters would be cost prohibitive but as cost are reduced over time, may become more realistic. Identification of at risk populations would not necessarily predict a diabetes phenotype because of variable penetrance requiring prospective follow up to ascertain the cost effectiveness of this strategy. The acceptance of Hg A1c as a diagnostic test for diabetes opens the potential for its use in screening all children but would only be a major step forward in identifying children who are developing progressive hyperglycemia in the absence of clinical symptoms or signs. The use of Hg A1c for screening would largely depend on establishing an easy, cost effective platform and method which would provide rapid turnaround on a population wide basis. These and other issues will be reviewed and potential strategies proposed as to the future of meaningful screening of children and adolescents for diabetes.