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As in the past four previous Annual Meetings of the Endocrine Society, we will review the 91st Meeting, ENDO 09, which took place in Washington, DC, at the Walter E Washington Convention Center, from Wednesday 10 to Saturday 13, 2009.

This year, the Steering Committee put a line-up featuring 16 plenary lectures, 69 symposia, 128 Meet-the-Professor sessions (Conversations with Clinical and Conversations with Basic Researchers), and 32 Case Management forums. The Program offered new features. The Case Management forum represented one of the new educational opportunities offered by ENDO 09 to clinicians in practice. These forums allowed two experts to take a particular topic and address focused learning objectives through the presentation of illustrative cases. These sessions ran alongside the Meet-the-Professor sessions and had a distinct case management basis allowing opportunities for interactive discussion between experts and the audience. During the poster presentation, select trainee-authored work was presented as part of the Presidential Poster Competition. The meeting also included the presentation of three new clinical practice guidelines from the Endocrine Society. They cover the “Evaluation & Management of Adult Hypoglycemic disorders”, the “Endocrine Treatment of Transsexual Persons” and “Endocrine & Nutritional Management of the Post-Bariatric Surgery Patient”.

The Exhibit Floor offered numerous booths with diverse materials of interest for the 8715 attendees. The web site of the Endocrine Society is “www.endo-society.org“.

Additional information can be gathered by reading the abstracts available in the web page of the Endocrine Society http://www.abstracts2view.com/endo/.

Comments on some of the Plenary Lectures follow.

JL Goldstein. Univ of Texas SW Med Ctr, Dallas, TX

MS Brown. Univ of Texas SW Med Ctr, Dallas, TX
In these two Presidential Plenary lectures, two Nobel Laureates explained their research into how animal cells control cholesterol synthesis and uptake through a feedback mechanism mediated by low-density lipoprotein (LDL) receptors on cells. The scientists, JL Goldstein, MD and S Brown, MD share a laboratory at the Jonsson Center for Molecular Genetics at the University of Texas Southwestern Medical center in Dallas and have been collaborating since 1972. Together, they won the Nobel Prize in Physiology in 1985 for showing that mutations in LDL receptor cause homozygous familial hypercholesterolemia leading to premature myocardial infarction. This finding laid the groundwork for the development of statins. In their studies, they elucidated the mechanism by which the LDL receptor mediates feedback control of cholesterol synthesis.

Cholesterol has a vital function and a lethal function. Its vital function is its role as an essential structural component of plasma membrane cells and in the synthesis of steroid hormone and bile acids. Its lethal aspects come into play when too much cholesterol accumulates in the blood in the form of low-density lipoproteins, which initiates the atherosclerotic process in the artery wall that leads to coronary disease and heart attacks. Because cholesterol is so vital to membrane function, animal cells have evolved a sophisticated feedback mechanism to its concentration so that the lipid composition of plasma membrane can be maintained within very narrow limits. The feedback mechanism operates primarily on the transcriptional level. Key to the feedback are membrane-bound transcription factors known as SREBPs, or sterol regulatory element-binding proteins. SREBPs regulate the synthesis and uptake of cholesterol and fatty acids in animal cells. SREBPs are the master regulators of lipids.

Also involved in the feedback mechanism are a pair of membrane-bound proteins, SCAP and Insig. The polytopic membrane protein SCAP transports SREBPs from the endoplasmic reticulum (ER) to the Golgi, thereby activating cholesterol synthesis. Cholesterol accumulation in the ER membranes changes SCAP to an alternate conformation in which it binds ER-retention proteins, thereby terminating cholesterol synthesis. In the Golgi, which is principally responsible for directing molecular traffic in the cell, sequential proteolysis of SREBPs allow these proteins to enter the cell nucleus where it activates the gene encoding proteins involved in the synthesis and uptake of cholesterol. When excessive amounts of sterols build up in ER membranes, SCAP binds cholesterol and Insig binds oxysterols (i.e. 25-OH-cholesterol). As a consequence of this binding activity, the SCAP and Insig proteins undergo conformational changes resulting in a series of events that block SCAP’s ability to transport SREBPs to the Golgi and terminate cholesterol synthesis and uptake. The pivotal event is the sterol-regulated binding of proteins (Sec23/Sec24) of the complex of COPII coat proteins to a specific sequence in SCAP, named MEDLADL. The result of sterol binding to SCAP and Insig is a conformational change in SCAP that prevents the COPII proteins from gaining access to the MEDLADL sequence.

Dr Brown, the second Nobel Laureate, said that all of the studies that led to this understanding of cholesterol feedback mechanism were conducted in tissue culture cells. He described the research he and his colleague have done in mice livers to confirm their research by manipulating the genes involved in cholesterol synthesis. He said that, as far as synthesis is concerned, the liver is the site of all the action. The results of a series of gene manipulations in the mouse were what it would have been predicted from the tissue culture cells. They made a transgenic mouse that produced a
truncated SREBP which never gets attached to membranes. It doesn’t have to go to the Golgi for processing. It goes directly to the nucleus and it turns on all the genes necessary to make cholesterol and fatty acids.

The truncated SREBP cause the mice livers to become massively enlarged and full of cholesterol and triglycerides. They were able to knock down the Insig gene in these mice, and the livers became stuffed with lipids. They also knocked out the SCAP gene and found that the liver stopped making triglycerides.

Both scientists concluded that they have found the central mechanism that controls cholesterol metabolism by controlling LDL-receptor mediated cholesterol and triglyceride synthesis (Taken from “ENDO daily”, the meeting newspaper, with modifications).

[L2-1] Edwin B Astwood Award Lecture: Ins & Outs of Thyroid Hormone Transporters.
TJ Visser. Internal Med, Erasmus Univ Med Ctr, Rotterdam, Netherlands
ABSTRACT TEXT NOT PROVIDED.

Abstract.
Thyroid hormone metabolism and action are largely intracellular events that require transport of iodothyronines across the plasma membrane. It has been assumed for a long time that this occurs by passive diffusion, but it has become increasingly clear that cellular uptake and efflux of thyroid hormone is mediated by transporter proteins.
Recently, several active and specific thyroid hormone transporters have been identified, including monocarboxylate transporter 8 (MCT8), MCT10, and organic anion transporting polypeptide 1C1 (OATP1C1). The latter is expressed predominantly in brain capillaries and transports preferentially T(4), whereas MCT8 and MCT10 are expressed in multiple tissues and are capable of transporting different iodothyronines. The pathophysiological importance of thyroid hormone transporters has been established by the demonstration of MCT8 mutations in patients with severe psychomotor retardation and elevated serum T(3) levels. MCT8 appears to play an important role in the transport of thyroid hormone in the brain, which is essential for the crucial action of the hormone during brain development. It is expected that more specific thyroid hormone transporters will be discovered in the near future, which will lead to a better understanding of the tissue-specific regulation of thyroid hormone bioavailability. (Heuer H, Visser TJ. Endocrinology. 2009 150:1078-83).

Comments.
The abstract is self explanatory.

[L2-2] Selective Modulation of the Thyroid Hormone Receptor To Attack Atherosclerosis, Obesity & Diabetes
JD Baxter. Diabetes Inst, Methodist Hosp Res Inst, Houston, TX
ABSTRACT TEXT NOT PROVIDED

Review Abstract.
Thyroid hormones influence heart rate, serum lipids, metabolic rate, body weight and multiple aspects of lipid, carbohydrate, protein and mineral metabolism. Although increased thyroid hormone levels can improve serum lipid profiles and reduce fat, these positive effects are counterbalanced by harmful effects on the heart, muscle and bone. Thus, attempts to use thyroid hormones for cholesterol-lowering and weight loss
purposes have so far been limited. However, over the past decade, thyroid hormone analogues that are capable of uncoupling beneficial effects from deleterious effects have been developed. Such drugs could serve as powerful new tools to address two of the largest medical problems in developed countries--atherosclerosis and obesity. (Baxter JD, Webb P. Nat Rev Drug Discov. 2009 Apr;8(4):308-20.)

Comments.
Selective thyroid hormone receptor subtype-beta (TRbeta) agonists have received attention as potential treatments for hypercholesterolemia and obesity, as well as treatments for diabetes. The TRbeta selective agonist KB-141 induces 5-10% increases in metabolic rate and lowering of plasma cholesterol levels without tachycardia in lean rats, unlike the major active thyroid hormone, T3. In experimental studies, KB-141 promotes weight loss in obese animals and anti-diabetogenic effects. In ob/ob mice, KB-141 lowered serum cholesterol, triacylglycerols and both serum and hepatic free fatty acids without tachycardia. Treatment of ob/ob mice with KB-141 improves glucose tolerance and insulin sensitivity in a dose-dependent manner with no effect on heart rate. Thus, KB-141 elicits anti-obesity, lipid lowering and anti-diabetic effects without tachycardia suggesting that selective TRbeta activation may be useful strategy to attenuate features of the metabolic syndrome (Bryzgalova G, J Steroid Biochem Mol Biol. 2008 111:262-7).

[L3-1] Gerald D Aurbach Award Lecture: Calcium-Regulating Hormones: New Roles for Old Hormones
D Goltzman. Med, McGill Univ/Royal Victoria Hosp, Montreal, Canada

Abstract.
Calcium homeostasis is modulated in part by the skeletal catabolic actions of parathyroid hormone (PTH) and 1,25 dihydroxyvitamin D [1,25(OH)2D]. Furthermore, PTH related peptide (PTHrP), when overproduced by malignancy, can induce osteolysis. Consequently all 3 calcium regulating factors are classically believed to induce bone breakdown. Using genetically modified mouse models, we found that endogenous PTH is essential for trabecular bone formation in the fetus and that this anabolic effect may persist into later life. From studies in mice null for intact PTHrP, and studies in mice which were engineered to express a truncated form, we found that PTHrP is required for normal development of the cartilaginous growth plate, mainly via the action of its amino-terminal domain, but also in part via a domain consisting of a nuclear localizing sequence and the carboxyl terminus (nuclear localizing domain). In later life, endogenous PTHrP appears to be anabolic for bone, also due to a dual mechanism. From studies with targeted deletion of the cyp27b1 gene, which encodes the enzyme synthesizing 1,25(OH)2D, we found that 1,25(OH)2D is essential for baseline bone formation. Furthermore exogenous 1,25(OH)2D could be shown to have bone forming properties and at least part of the anabolic effect of exogenous PTH appears to be due to its capacity to stimulate endogenous 1,25(OH)2D production. Although PTHrP has been associated primarily with its action on the skeleton, PTHrP knockout animals die in the neonatal period, and deletion of the nuclear localizing domain produces growth retardation and early lethality, due to effects on multiple tissues derived from neural, hematopoietic and mesenchymal stem cells. Although vitamin D action has also been primarily linked to the skeleton, several studies in humans show that vitamin D intake is associated with reduced mortality, and beneficial effects on the cardiovascular and immune systems, as well on cancer have been
observed. Overall these studies demonstrate anabolic as well as catabolic actions of PTH, PTHrP and 1,25(OH)\textsubscript{2}D in mediating skeletal homeostasis and also implicate PTHrP and vitamin D in regulating important extra-skeletal events.

Comments.
The abstract is self explanatory.

[L3-2] The Novel Endocrinology of Bone
G Karsenty. Columbia Univ Med Ctr, New York, NY
ABSTRACT TEXT NOT PROVIDED

Review Abstract.
Bone remodeling, the process whereby bones renew themselves, is regulated by multiple hormones. The clinical observation that obesity protects from osteoporosis led us to propose that bone remodeling and energy metabolism could be regulated by the same hormone(s). We showed that leptin, an adipocyte-derived hormone, is a major regulator of bone remodeling by acting on osteoblasts through two different neural pathways. On the one hand, sympathetic signaling in osteoblasts favors osteoclast differentiation by inducing RANKL expression; on the other hand, through CART (cocaine amphetamine regulated transcript) leptin inhibits RANKL expression. The notion that the brain regulate bone mass has now been verified experimentally in multiple laboratories. These studies immediately raised a second question: if fat and brain regulates bone remodeling by acting on osteoblasts, are osteoblasts in turn regulating any aspect of energy metabolism? In other words is the skeleton, in addition to its well-known functions, an endocrine organ? In the search for a bone-derived hormone regulating energy metabolism we generated Osteocalcin-/- mice which display a high bone mass phenotype. While analyzing these mutant mice we also noticed that they had an abnormal amount of visceral fat (P. Ducy and G. Karsenty, unpublished observation). This was the first evidence suggesting that the skeleton regulates energy metabolism and it prompted us to study this question. To identify osteoblast-enriched genes affecting energy metabolism, we generated mutant mouse strains lacking genes expressed only or preferentially in osteoblasts. Through this effort we inactivated, via classical means and in an osteoblast-specific manner, \textit{Esp}, a gene expressed in osteoblasts and sertoli cells that encodes a receptor-like protein tyrosine phosphatase termed OST-PTP4. \textit{Esp} -/- mice are hypoglycemic, protected from obesity and glucose intolerant because of an increase in beta-cell proliferation, insulin secretion and sensitivity, whereas mice lacking \textit{Osteocalcin} display glucose intolerance and decreased beta-cell proliferation. Genetic, cell-based and biochemical analyses show that osteoblasts via osteocalcin stimulate beta-cell proliferation and expression of \textit{Insulin} and \textit{Adiponectin}, an insulin-sensitizing adipokine, that \textit{Esp}-deficient mice metabolic phenotype is caused by a gain of osteocalcin bioactivity and that OST-PTP regulates indirectly osteocalcin post-translational modification. By revealing that the skeleton exerts an endocrine regulation of sugar homeostasis this study expands our understanding of energy metabolism and its disorders. (N.K. Lee and G. Karsenty J Musculoskeletal Neuronal Interact 2008; 8:351).

[L4-1] New Tricks by an Old Dogma: Cellular Mechanisms of Brain Sexual Differentiation
**MM McCarthy. Dept of Physiology, Univ of Maryland-Baltimore, Baltimore, MD**

**ABSTRACT TEXT NOT PROVIDED**

**Review Abstract.**

The brain has been known to be a sensitive target organ for the permanent organizational effects of gonadal steroids for close to 50 years. Recent advances have revealed a variety of unexpected cellular mechanisms by which steroids impact on the synaptic profile of hypothalamic nuclei critical to the control of reproduction. This review focuses on three in particular: 1) prostaglandins in the masculinization of the preoptic area and control of male sexual behaviour; 2) GABA in the arcuate nucleus and potential control of the anterior pituitary; and 3) non-genomic activation of phosphotydolinositol 3 (PI3) kinase and glutamate in the ventromedial nucleus, which is relevant to the control of female reproductive behaviour. The importance of cell-to-cell communication, be it between neurones or between neurones and astrocytes, is highlighted as an essential principle for expanding the impact of steroids beyond those cells that express nuclear receptors.

Brain development is impacted by a multitude of endogenous and exogenous factors ranging from genetics to the environment. Variations as subtle as the phenotype of neighbouring cells, the intensity of sensory input and the amount of maternal attention received shortly after birth can permanently imprint upon on the developing neuronal network. Gonadal steroids constitute a particularly unique signal for regulating brain development in that levels differ between males and females, are endogenously derived but can be exogenously mimicked, and act during a perinatal sensitive period in specific parts of the brain to exert epigenetic and largely permanent modifications that direct adult brain function. The focus of this review is on one gonadal steroid in particular, oestradiol. The reason for this emphasis is twofold; (i) in the rodent brain oestradiol is a primary determinant of masculinisation following the aromatisation of testicular androgens, and (ii) accumulating evidence suggests that oestradiol can be synthesised entirely within the brain. We shall highlight emerging principles of oestrogen action both for purposes of achieving sex differences in the brain and as a general trophic and possibly endogenous neuroprotective factor. Main important points: 1) Estradiol effects on developing neurons are not cell-autologous. 2) Immature astrocytes are responsive to estradiol and important to establishment of sex differences in the synaptic profile. The signaling molecule mediating the effect of estradiol on astrocyte morphology in the arcuate nucleus is GABA. 3) Moreover, estradiol-induced masculinization is also mediated by a prostaglandin (prostaglandin E2, PGE2). Estradiol up-regulates COX-2 mRNA and protein, an enzyme necessary for prostaglandin synthesis in the pre-optic area (POA) of developing males. PGE2 induces calcium-dependent glutamate release from astrocytes and promotes the formation of dendritic spines to coordinate transcellular changes. A similar effect is seen in the mediobasal nucleus of the hypothalamus by both genomic and non-genomic mechanisms. In summary, the mechanisms of estradiol action are distinct and regionally specific. (McCarthy MM, Schwarz JM, Wright CL, Dean SL. J Neuroendocrinol. 2008 20:777-83).
The central actions of leptin are essential for homeostatic control of adipose tissue mass, glucose metabolism, and many autonomic and neuroendocrine systems. In the brain, leptin acts on numerous different cell types via the long-form leptin receptor (LepRb) to elicit its effects. The precise identification of leptin's cellular targets is fundamental to understanding the mechanism of its pleiotropic central actions. We have systematically characterized LepRb distribution in the mouse brain using in situ hybridization in wildtype mice as well as by EYFP immunoreactivity in a novel LepRb-IRES-Cre EYFP reporter mouse line showing high levels of LepRb mRNA/EYFP coexpression. We found substantial LepRb mRNA and EYFP expression in hypothalamic and extrahypothalamic sites described before, including the dorsomedial nucleus of the hypothalamus, ventral premammillary nucleus, ventral tegmental area, parabrachial nucleus, and the dorsal vagal complex. Expression in insular cortex, lateral septal nucleus, medial preoptic area, rostral linear nucleus, and in the Edinger-Westphal nucleus was also observed and had been previously unreported. The LepRb-IRES-Cre reporter line was used to chemically characterize a population of leptin receptor-expressing neurons in the midbrain. Tyrosine hydroxylase and Cre reporter were found to be coexpressed in the ventral tegmental area and in other midbrain dopaminergic neurons. Lastly, the LepRb-IRES-Cre reporter line was used to map the extent of peripheral leptin sensing by central nervous system (CNS) LepRb neurons. Thus, we provide data supporting the use of the LepRb-IRES-Cre line for the assessment of the anatomic and functional characteristics of neurons expressing leptin receptor. (Scott MM, Lachey JL, Sternson SM, Lee CE, Elias CF, Friedman JM, Elmquist JK. J Comp Neurol. 2009 514:518-32).

[L5-1] Roy O Greep Award Lecture: New Insights into Old Theories for How Stress Inhibits Reproduction -- A Role for Glucocorticoids
FJ Karsch. Molecular and Integrative Physiology, Univ of Michigan, Ann Arbor, MI
ABSTRACT TEXT NOT PROVIDED

Publication Abstract.
Stress-like elevations in plasma glucocorticoids rapidly inhibit pulsatile LH secretion in ovariectomized sheep by reducing pituitary responsiveness to GnRH. This effect can be blocked by a nonspecific antagonist of the type II glucocorticoid receptor (GR) RU486. A series of experiments was conducted to strengthen the evidence for a mediatory role of the type II GR and to investigate the neuroendocrine site and cellular mechanism underlying this inhibitory effect of cortisol. First, we demonstrated that a specific agonist of the type II GR, dexamethasone, mimics the suppressive action of cortisol on pituitary responsiveness to GnRH pulses in ovariectomized ewes. This effect, which became evident within 30 min, documents mediation via the type II GR. We next determined that exposure of cultured ovine pituitary cells to cortisol reduced the LH response to pulse-like delivery of GnRH by 50% within 30 min, indicating a pituitary site of action. Finally, we tested the hypothesis that suppression of pituitary responsiveness to GnRH in ovariectomized ewes is due to reduced tissue concentrations of GnRH receptor. Although cortisol blunted the amplitude of GnRH-induced LH pulses within 1-2 h, the amount of GnRH receptor mRNA or protein was not affected over this time frame. Collectively, these observations provide evidence that cortisol acts via the type II GR within the pituitary gland to elicit a rapid decrease in responsiveness to GnRH, independent of changes in expression of the GnRH receptor. (Breen KM, Davis TL, Doro LC, Nett TM, Oakley AE, Padmanabhan V, Rispoli LA, Wagenmaker ER, Karsch FJ. Endocrinology. 2008 149:767-73).
Steroidogenic Factor 1: Regulating This Master Regulator of the Endocrine System

HA Ingraham. Univ of California-San Francisco, San Francisco, CA

Publication Abstract.
Despite the fact that many nuclear receptors are ligand dependent, the existence of obligate regulatory ligands is debated for some receptors, including steroidogenic factor 1 (SF-1). Although fortuitously bound bacterial phospholipids were discovered in the structures of the SF-1 ligand-binding domain (LBD), these lipids might serve merely as structural ligands. Thus, we examined whether exogenously added phospholipids would exchange for these bacterial lipids and bind to SF-1. Here, we report the first crystal structure of the SF-1 LBD bound by the exchanged phosphatidylcholine. Although the bound phosphatidylcholine phospholipid mimics the conformation of bound bacterial phospholipids, two surface loops, L2-3 and L11-12, surrounding the entrance to the pocket vary significantly between different SF-1 LBD structures. Based on this observation, we hypothesized that a bound ligand might control the conformations of loops L2-3 and L11-12, and that conserved residues in these dynamic loops could influence ligand binding and the receptor function. Consistent with this hypothesis, impaired phospholipid exchange and diminished transcriptional activity were observed for loop L11-12 SF-1 mutants and for the loop L2-3 human mutant R255L. The endocrine disease associated with this L2-3 mutation coupled with our cellular and biochemical data suggest that critical residues at the mouth of the ligand-binding pocket have evolved for efficient binding of phospholipid ligands and for achieving optimal SF-1 activity. (Sabin EP, Blind RD, Krylova IN, Ingraham JG, Cai F, Williams JD, Fletterick RJ, Ingraham HA. Mol Endocrinol. 2009 23:25-34).

Nuclear Receptors & Atopic Diseases

P Chambon. Functional Genomic, Inst de Genetique et de Biol Moleculaire, Illkirch, France

Publication Abstract.
Atopic dermatitis (AD) is often the initial step in the "atopic march," given that more than half of AD patients with moderate to severe AD develop asthma later in life. Both AD and asthma share a similar "atopy" phenotype that includes T helper type 2 inflammation with eosinophilia and hyper-IgE immunoglobulinemia, but the molecular mechanisms underlying the "atopic march" remain elusive. In the present study, we show that induced expression of thymic stromal lymphopoietin (TSLP) in mouse epidermal keratinocytes upon topical application of MC903 (a low calcemic analogue of vitamin D3) not only triggers AD as we previously reported but also aggravates experimental allergic asthma induced by ovalbumin sensitization and challenge. Our study, which provides a mouse model to study human "atopic march," indicates that keratinocyte-produced TSLP may represent an important factor in the link of atopic dermatitis to asthma. (Zhang Z, Hener P, Frossard N, Kato S, Metzger D, Li M, Chambon P. Proc Natl Acad Sci U S A. 2009 106:1536-41).
Publication Abstracts.
This article describes the origins and evolution of "antiestrogenic" medicines for the treatment and prevention of breast cancer. Developing drugs that target the estrogen receptor (ER) either directly (tamoxifen) or indirectly (aromatase inhibitors) has improved the prognosis of breast cancer and significantly advanced healthcare. The development of the principles for treatment and the success of the concept, in practice, has become a model for molecular medicine and presaged the current testing of numerous targeted therapies for all forms of cancer. The translational research with tamoxifen to target the ER with the appropriate duration (5 years) of adjuvant therapy has contributed to the falling national death rates from breast cancer. Additionally, exploration of the endocrine pharmacology of tamoxifen and related nonsteroidal antiestrogen (e.g. keoxifene now known as raloxifene) resulted in the laboratory recognition of selective ER modulation and the translation of the concept to use raloxifene for the prevention of osteoporosis and breast cancer. However, the extensive evaluation of tamoxifen treatment revealed small but significant side effects such as endometrial cancer, blood clots and the development of acquired resistance. The solution was to develop drugs that targeted the aromatase enzyme specifically to prevent the conversion of androstenedione to estrone and subsequently estradiol. The successful translational research with the suicide inhibitor 4-hydroxyandrostenedione (known as formestane) pioneered the development of a range of oral aromatase inhibitors that are either suicide inhibitors (exemestane) or competitive inhibitors (letrozole and anastrozole) of the aromatase enzyme. Treatment with aromatase inhibitors is proving effective and is associated with reduction in the incidence of endometrial cancer and blood clots when compared with tamoxifen and there is also limited cross resistance so treatment can be sequential. Current clinical trials are addressing the value of aromatase inhibitors as chemopreventive agents for postmenopausal women. (Jordan VC, Brodie AM Steroids. 2007 72:7-25).
Using the intratumoral aromatase xenograft model, we have observed that despite long-lasting growth inhibition, tumors eventually begin to grow during continued letrozole treatment. In cells isolated from these long-term letrozole-treated tumors (LTLT-Ca), estrogen receptor-alpha (ERalpha) levels were decreased, whereas signaling proteins in the mitogen-activated protein kinase cascade were up-regulated along with human epidermal growth factor receptor 2 (Her-2). In the current study, we evaluated the effect of discontinuing letrozole treatment on the growth of letrozole-resistant cells and tumors. The cells formed tumors equally well in the absence or presence of letrozole and had similar growth rates. After treatment was discontinued for 6 weeks, letrozole was administered again. Marked tumor regression was observed with this second course of letrozole treatment. Similarly, in MCF-7Ca xenografts, a 6-week break in letrozole treatment prolonged the responsiveness of the tumors to letrozole. To understand the mechanisms of this effect, LTLT-Ca cells were cultured in the absence of letrozole and had similar growth rates. After treatment was discontinued for 6 weeks, letrozole was administered again. Marked tumor regression was observed with this second course of letrozole treatment. Similarly, in MCF-7Ca xenografts, a 6-week break in letrozole treatment prolonged the responsiveness of the tumors to letrozole. To understand the mechanisms of this effect, LTLT-Ca cells were cultured in the absence of letrozole for 16 weeks. The resulting cell line (RLT-Ca) exhibited properties similar to MCF-7Ca cells. The cell growth was inhibited by letrozole and stimulated by estradiol. The expression of phosphorylated mitogen-activated protein kinase (MAPK) was reduced and ERalpha and aromatase levels increased compared with LTLT-Ca cells and were similar to levels in MCF-7Ca cells. These results indicate that discontinuing treatment can reverse letrozole resistance. This could be a beneficial strategy to prolong

[L7-1] Clinical Investigator Award Lecture: Glucagon-Like Peptides –Mechanisms of Action & Therapeutic Relevance
DJ Drucker. SLRI, Mt Sinai Hosp, Toronto, Canada
ABSTRACT TEXT NOT PROVIDED

Publication Abstracts.
This review focuses on the mechanisms regulating the synthesis, secretion, biological actions, and therapeutic relevance of the incretin peptides glucose-dependent insulinitropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1). The published literature was reviewed, with emphasis on recent advances in our understanding of the biology of GIP and GLP-1. GIP and GLP-1 are both secreted within minutes of nutrient ingestion and facilitate the rapid disposal of ingested nutrients. Both peptides share common actions on islet beta-cells acting through structurally distinct yet related receptors. Incretin-receptor activation leads to glucose-dependent insulin secretion, induction of beta-cell proliferation, and enhanced resistance to apoptosis. GIP also promotes energy storage via direct actions on adipose tissue, and enhances bone formation via stimulation of osteoblast proliferation and inhibition of apoptosis. In contrast, GLP-1 exerts glucoregulatory actions via slowing of gastric emptying and glucose-dependent inhibition of glucagon secretion. GLP-1 also promotes satiety and sustained GLP-1-receptor activation is associated with weight loss in both preclinical and clinical studies. The rapid degradation of both GIP and GLP-1 by the enzyme dipeptidyl peptidase-4 has led to the development of degradation-resistant GLP-1-receptor agonists and dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes. These agents decrease haemoglobin A1c (HbA1c) safely without weight gain in subjects with type 2 diabetes. GLP-1 and GIP integrate nutrient-derived signals to control food intake, energy absorption, and assimilation. Recently approved therapeutic agents based on potentiation of incretin action provide new physiologically based approaches for the treatment of type 2 diabetes. (Baggio LL, Drucker DJ. Gastroenterology. 2007 132:2131-57).

We report the efficacy of a new peptide with agonism at the glucagon and GLP-1 receptors that has potent, sustained satiation-inducing and lipolytic effects. Selective chemical modification to glucagon resulted in a loss of specificity, with minimal change to inherent activity. The structural basis for the co-agonism appears to be a combination of local positional interactions and a change in secondary structure. Two co-agonist peptides differing from each other only in their level of glucagon receptor agonism were studied in rodent obesity models. Administration of PEGylated peptides once per week normalized adiposity and glucose tolerance in diet-induced obese mice. Reduction of body weight was achieved by a loss of body fat resulting from decreased food intake and increased energy expenditure. These preclinical studies indicate that when full GLP-1 agonism is augmented with an appropriate degree of glucagon receptor activation, body fat reduction can be substantially enhanced without any overt adverse effects. (Day JW, Ottaway N, Patterson JT, Gelfanov V, Smiley D, Gidda J, Findeisen H, Brueemmer D, Drucker DJ, Chaudhary N, Holland J, Hembree J, Abplanalp W, Grant E, Ruehl J, Wilson H, Kirchner H, Lockie SH, Hofmann S, Woods SC, Nogueiras R, Pfluger PT, Perez-Tilve D, Dimarchi R, Tschöp MH. Nat Chem Biol. 2009)
Publication Abstract.

Western medicine is in crisis. Continually increasing resources are being expended to combat the age-related diseases that include diabetes and metabolic syndrome, Alzheimer's disease, Parkinson's disease, cardiovascular disease, and cancer. Yet the causes of these diseases remain a mystery, while their incidence and morbidity either remain constant or are increasing.

Huge investments in biomedical research in the recent past have resulted in some striking accomplishments, including the sequencing of the human chromosomal DNA, the identification of hundreds of thousands of human chromosomal single nucleotide polymorphisms (SNPs), and the identification of regional clusters of chromosomal SNPs (the HapMap). However, these accomplishments have failed to reveal the anticipated genetic causes for the common age-related diseases. For example, a series of “whole-genome scans” encompassing hundreds of thousands of chromosomal SNPs and >32,000 subjects has revealed nine polymorphic loci associated with type II diabetes, yet the aggregate risk for all nine loci accounts for only a small proportion of the overall diabetes risk. Thomas Kuhn, in his book *The Structure of Scientific Revolutions*, argued that when the scientific effort expended on a problem increases—yet productivity declines—then the difficulty may lie with the assumptions (paradigms) on which the research is based. For the past 100 years, Western biomedical science has stood on two philosophical pillars: the anatomical paradigm of medicine and the Mendelian paradigm of genetics. The anatomical paradigm of medicine has at its foundation the work of Vesalius, who first described the organs of the human body 450 years ago. Since then, physicians and medical scientists have specialized in individual organs and their associated disease manifestations, leading to the fields of neurology, ophthalmology, nephrology, cardiology, endocrinology, etc. The organ-specific compartmentalization of medicine has also led to several generally accepted corollaries: organ-associated symptoms are the result of organ-specific problems, organ-specific problems are the result of tissue-specific protein and gene defects, and tissue-specific protein defects should be treated with chemicals that specifically interact with the defective tissue-specific protein.

The Mendelian paradigm of genetics argues that genetic traits are transmitted across generations according to the laws of Gregor Mendel. The associated medical corollary is that if a clinical trait is transmitted in a Mendelian fashion, it is genetic, but if it is not, then the trait must be the consequence of environmental factors. This corollary is formalized through the estimation of heritability by dividing the frequency that a phenotypic trait is shared by identical twins with the frequency that it is shared by fraternal twins. However, since Mendelian genetics is the result of chromosomal dynamics, the Mendelian paradigm is specific for nuclear DNA (nDNA) genes.

While the anatomical paradigm of medicine and the Mendelian paradigm of genetics have been powerful predictors of medical relationships for the past century, they are failing to direct us toward solutions for the common age-related diseases. To resolve the crisis and return to productive “normal science,” a new paradigm must be generated that encompasses the strengths of the previous paradigm but adds new elements that address the current problems being confronted. What could be the missing components of the
anatomical and Mendelian paradigms necessary for understanding the age-related diseases?

The first suggestion of an answer to this question came with the publication of three articles in 1988—20 years ago. The first article reported that deletions in the extra-nuclear mitochondrial DNA (mtDNA) could be associated with a characteristic muscle pathology involving ragged red muscle fibers and abnormal mitochondria, designated mitochondrial myopathy. Mitochondrial DNA deletions and mitochondrial myopathy have subsequently been associated with the spontaneously occurring chronic external progressive ophthalmoplegia. The second article reported that a missense mutation at nucleotide (nt) 11,778 (G > A) in the mtDNA ND4 polypeptide (R340H) was the cause of maternally inherited Leber hereditary optic neuropathy (LHON). The third article used maternal inheritance to link a familial brain and muscle disease called myoclonic epilepsy and ragged red fiber to the mtDNA, a conclusion that was subsequently confirmed by the identification of the causal mutation in the mtDNA tRNA\textsubscript{Lys} gene at nt 8344 (A > G). Since the mtDNA encodes genes for proteins of mitochondrial energy metabolism, these articles had two major implications. First, human diseases affecting a wide range of organs could result from systemic defects in energy metabolism and, second, hereditary human diseases could result from mutations in the non-Mendelian mtDNA. Consequently, mitochondrial biology and genetics become excellent candidates for expanding the anatomical and Mendelian paradigms to address the complexities of the age-related diseases, aging, and cancer.

Life involves the interplay between structure and energy. For the eukaryotic cell, this duality was cemented ~2 billion years ago by the symbiosis of what appears to have been a glycolytic motile cell, which gave rise to the nucleus–cytosol, and an oxidative \(\alpha\)-proteobacterium, which evolved into the mitochondrion. Initially, each organism was free living and contained all of the genes for an independent life form. However, over the subsequent 1.2 billion years, the single-cell descendants of the initial symbiosis experimented with many alternative arrangements of biochemical interdependence and genomic reorganization. Ultimately, however, an arrangement was achieved in which the mitochondrion became specialized in energy production and the nucleus–cytosol became specialized in structure. This final design provided the impetus for the development of multicellularity and the evolution of higher plants and animals, including humans.

The restructuring of the proto-mitochondrial genome included the transfer of virtually all of the genes of the mitochondrial genome, ~1500, into the chromosomal nDNA. Yet the mtDNA persisted and today still retains 13 polypeptide-encoding genes plus a small and large rRNA gene and 22 tRNA genes. All of the mtDNA-encoded polypeptides are core subunits of the enzyme complexes of the mitochondrial energy-generating apparatus, oxidative phosphorylation (OXPHOS). In OXPHOS, reducing equivalents (electrons) derived from the calories of our diet are transferred down a series of redox enzyme complexes located within the mitochondrial inner membrane, collectively known as the electron transport chain. The electrons enter at either complex I or II and are transferred through coenzyme Q to complex III, then to cytochrome c, on to complex IV, and finally to oxygen to generate \(\text{H}_2\text{O}\). The energy that is released as the electrons traverse complexes I, III, and IV is used to pump protons out of the mitochondrial matrix across the inner membrane, resulting in an electrochemical gradient, the biological equivalent of a capacitor. This capacitance is used as a source of potential energy to drive a variety of activities. For example, the protons can flow back across the inner membrane into the matrix through a proton channel in complex V, the ATP synthase. In the process, potential energy is converted into the high-energy \(\gamma\)-
phosphate bond of ATP, which can be used to drive chemical work. If mitochondrial OXPHOS is efficient in converting caloric energy to ATP, it is said to be tightly coupled, and these mitochondria will generate the maximum ATP and thus work for the minimum calories burned. However, if the mitochondria are less efficient at generating ATP, partially uncoupled, then more calories must be burned to generate the same amount of ATP. The energetic difference is dissipated as heat. Thus, in endotherms such as humans, changes in the mitochondrial coupling efficiency determine the relative allocation of calories between ATP for work and heat to maintain the body temperature. Another product of OXPHOS is reactive oxygen species (ROS). Mitochondrial ROS provides a signaling system from the mitochondrion to the nucleus. However, when mitochondrial ROS production becomes excessive, the mitochondria and mtDNAs can be damaged. While each cell contains hundreds of mitochondria and thousands of mtDNAs, as the cellular mtDNAs become mutated by oxidative damage, the mtDNA information necessary for repairing damaged mitochondria is depleted and the mitochondrial energy output declines. Ultimately, there is insufficient mitochondrial energy for the cell to carry out its normal function and it malfunctions. The malfunctioning cell can then disrupt normal tissue function and integrity. To resolve this deleterious state, the mitochondria-deficient cell must be removed by apoptosis. This is achieved by the activation of the mitochondrial permeability transition pore (mtPTP), which senses increased oxidative stress, reduced electrochemical potential, reduced high-energy phosphates, and the mitochondrial uptake of excessive calcium. The 13 polypeptides of the mtDNA include 7 of the ∼45 polypeptides of complex I (ND1, -2, -3, -4L, -4, -5, -6), 1 of the 11 polypeptides of complex III (cytochrome b), 3 of the 13 polypeptides of complex IV (COI, -II, -III), and 2 of the ∼15 polypeptides of complex V (ATP6 and -8). All of the other genes of the mitochondrial genome are dispersed across the chromosomes and include the mitochondrial DNA polymerase γ (POLG), RNA polymerase, ribosomal proteins, metabolic enzymes, etc. If it was beneficial for the first 1500 mitochondrial genes to be transferred to the nucleus, why not the last 13? After all, transfer of the final 13 proteins would have permitted the elimination of an entire redundant mitochondrial genetic information system. Yet every oxidative organism retains an mtDNA and virtually all organisms of the fungal–animal lineage retain the same mtDNA genes. Hence, the retention of these genes in the mtDNA must be important. For those mtDNA-encoded proteins for which the function is known (cytochrome b, COI, COII, COIII, and ATP6), the protein is either an electron or a proton carrier of OXPHOS. Moreover, all of these charge carriers interact in the generation, maintenance, or utilization of the same entity, the mitochondrial inner membrane electrochemical gradient. Thus the polypeptide genes of the mtDNA encode the wiring diagram for the mitochondrial capacitor in a single integrated mitochondrial circuit. As a consequence, a mutation in any one of the mtDNA polypeptides within a mtDNA will have physiological consequences for all of the other polypeptides in that mtDNA, effectively shifting the energetic balance of the entire circuit. The new aggregate metabolic state will then be tested for local genetic fitness by natural selection. The accrual of mtDNA mutations over many generations will then result in the divergence of mtDNA sequences and the development of new metabolic strategies for coping with changing environments. Because of the functional coevolution of the genes of an individual mtDNA, all of the genetic polymorphisms for the proteins of that mtDNA must be intercompatible; i.e., they must match. Therefore, the random mixing of the protein polymorphisms between two different mtDNA lineages could result in combining incompatible genetic elements, thus shorting the capacitor and resulting in
energetic failure. To prevent such random mixing of divergent circuit elements, the genes of different mtDNA lineages must be prohibited from undergoing recombination. This is accomplished by having the mtDNA inherited from only one parent—the mother in the case of humans and most other species. Because mitochondrial OXPHOS impinges on many cellular functions, including energy allocation, ROS generation, redox control, calcium homeostasis, and programmed cell death, different mitochondrial energy circuits can be beneficial in a wide spectrum of environmental contexts. For example, mtDNA polymorphisms that produced tightly coupled mitochondria could be advantageous in the tropics where calories would produce maximum ATP and minimum heat. By contrast, more loosely coupled mitochondria could be advantageous in the arctic where the oxidation of additional calories to generate heat would increase the resistance to cold. Because the energetic demands of the environment can change rapidly, it is advantageous for an endothermal species to maintain a diverse array of mtDNA genotypes and thus energetic solutions. This would ensure that some individuals can survive a sudden environmental energetic change. However, the lack of recombination limits the ability of the mitochondrial system to generate an array of genetic combinations and thus energetic solutions. This dilemma is resolved by the mtDNA having a high mutation rate, such that new mitochondrial energy solutions are generated de novo each generation. Presumably, the mtDNA mutation rate is regulated by modulating mitochondrial ROS production and detoxification rates as well as by mtDNA repair. Rapid segregation of variant mtDNAs within the female germline results in maternal lineages that approach homoplasmic (purely mutant) for variant mtDNAs. Individuals harboring these variant mtDNA genotypes can differ in mitochondrial physiologies. This provides the needed variation among the individuals within the population to increase the probability that some individuals may survive if the environment changes suddenly.

The mtDNA mutation rate is high not only in the female germline, but also in the body's somatic tissues. Consequently, mtDNA rearrangement and base substitution mutations have been found to accumulate with age in multiple tissues. As a result, the age-related accumulation of mtDNA mutations with an associated decline in mitochondrial function is thought to be an important factor in the aging clock. Two lines of evidence suggest that the accumulation of somatic mutations is a cause of aging in mammals. Introduction of an error-prone POLG into the mouse resulted in an increased mtDNA mutation rate and a premature aging phenotype. Also the generation of a transgenic mouse in which the peroxisomal antioxidant enzyme catalase was redirected into the mitochondrial matrix resulted in an extended life span in conjunction with reduced mtDNA oxidative damage and somatic mutation levels.

A synthesis of these specific mitochondrial genomic concepts provides a plausible model for the predisposition toward and development of age-related diseases. In this scenario, an individual is born with an initial mitochondrial energetic capacity based on inherited variation in nDNA- and mtDNA-encoded mitochondrial genes. Relevant nDNA variation could affect mitochondrial energetic output, antioxidant defenses, apoptotic thresholds, mitochondrial biogenesis and turnover, etc. As the individual ages, somatic mtDNA mutations arise in post-mitotic cells and stem cells, and the individual somatic mtDNA mutations become clonally amplified within each affected cell, both post-mitotic and stem. The clonal amplification within the cell either is the result of intracellular genetic drift during the turnover of mitochondria during successive cell divisions or, alternatively, is the result of the selective amplification of the mutant mtDNA. As the mutant mtDNAs accumulate, they progressively erode the individual
cell's energetic capacity. Ultimately, cellular energetics drops below the minimal output necessary for normal cellular and tissue function and survival. This leads to a decline in organ function, loss of cell numbers, tissue failure, and an aging phenotype. While mitochondrial defects are systemic, the clinical manifestations are often organ specific. This is because different organs and tissues in the body have different needs and roles in energy homeostasis, the body's energy anatomy. Certain tissues require high levels of mitochondrial ATP, such as the retina of the eye, the cochlea of the ear, the other components of the central nervous system, the heart, the muscles, and the renal system. These organs are preferentially affected as mitochondrial energy production declines. Other tissues store energy in fat. The white adipose tissues store fat for later ATP production while the brown adipose tissues store fat for later thermal regulation. The liver is an energy homeostasis tissue, maintaining the serum glucose level within acceptable limits. The pancreatic \( \alpha \)- and \( \beta \)-cells are energy-sensing tissues. They monitor calorie type and availability and send the appropriate signals, glucagon or insulin, to the energy-utilizing, storage, and homeostasis tissues.

The eukaryotic cell nucleus–cytosol and mitochondrial duality now completes the human biomedical paradigm. The nucleus–cytosol organism specializes in elaborating new structures by changing the expression of tissue and organ-specific genes and proteins through developmental regulators. The nuclear genes are biparental, permitting recombination between the mother's and the father's alleles to maximize their capacity to generate structural diversity. The two copies for each gene also result in quantized biochemical and phenotypic manifestations of biallelic loci (+/+, +/−, and −/−), facilitating the expression of new genetic combinations. In contradistinction, the mitochondrial organism specializes in energetics. Its mtDNA genes are uniparental, present in thousands of copies per cell, and have a high sequence evolution rate. Thus, a continuous distribution of percentages of mutant mtDNAs are possible for a biallelic mtDNA locus, resulting in graded biochemical changes and a quantitative genetics advantageous for coping with graded changes in the environment. Therefore, the structural adaptation of the nDNA genes and the energetic adaptation of the mtDNA genes provide the animal with a multidimensional capacity for adapting to new environments, providing a coherent evolutionary medicine. Similarly, the quantized nDNA and statistical mtDNA genetics generate both stepwise and graded genotypic and consequent phenotypic changes, providing a coherent medical genetics. Acting together, these two systems provide all of the parameters necessary for understanding the biology and genetics of the common age-related diseases.

While the bipartite eukaryotic cell provides an explanation for many of the unexplained phenomena of age-related diseases, will it be useful for making predictions that result in effective new treatments for these diseases? Unfortunately, very limited resources have been invested in understanding mitochondrial biology and genetics. Still, a few drugs have been shown to increase mitochondrial function. These include those that upregulate the transcription of nDNA-encoded mitochondrial genes through acting as agonists of the transcription factor peroxisome-proliferator-activated receptor \( \gamma \) (PPAR\( \gamma \)) (e.g., rosiglitazone) or by the activation through deacetylation of the mitochondrial PPAR\( \gamma \) transcriptional coactivator-1\( \alpha \) (PGC-1\( \alpha \)) via agonist-activated SIRT1 (e.g., resveratrol and derivatives). Mitochondrial fatty acid oxidation has been enhanced using bezafibrate and attempts have been made to ameliorate the adverse effects of mitochondrial ROS production using combinations of natural antioxidants (lipoic acid, vitamins A and C, CoQ\( \text{_{10}} \), etc.) or synthetic catalytic antioxidants (MnTDEIP, EUK134, etc.). Apoptosis is being modulated by regulating the mtPTP
using cyclosporin A and its analogs (e.g., N-methyl-4-isoleucine cyclosporine) and efforts are being directed toward regulating mitochondrial turnover by autophagy. Still, the number of therapeutic approaches of relevance to the mitochondrion are severely limited by our lack of basic knowledge about mitochondrial biology. How might we jump-start the search for mitochondrially active backbone compounds to treat the age-related diseases? One promising approach might be traditional Asian herbal medications. Unlike drug development in Western pharmacology, which requires a known drug target around which the drug is developed, traditional Asian pharmaceuticals were discovered by trial and error, on the basis of what made the patient better. This trial-and-error approach to drug development is inefficient but it is paradigm blind. If mitochondrial dysfunction is as important a factor in age-related diseases as proposed in this essay, then Asian herbal medications should be as likely to have targeted a mitochondrial energetic function as a tissue-specific structural function. If so, we might be able to identify active mitochondrial backbone drugs by screening traditional Asian therapeutics for those that modulate mitochondrial function. To detect mitochondrially active compounds, we have assembled a mitochondrial cDNA expression array, the MITOCHIP, which interrogates ~1000 genes involved in mitochondrial energy production, ROS biology, and apoptosis. We have tested the veracity of our hypothesis that Asian medications might target mitochondrial function by testing the effects of Ginkgo biloba leaf extract on mitochondrial function in cultured cells. G. biloba did indeed alter mitochondrial gene expression, appearing to modulate mitochondrially associated apoptosis. The association between Asian herbal medications and mitochondria has been further enhanced by the discovery that the potent antimalarial Artemisinin (qinghaosu) acts on the mitochondrion and the observation that, after screening 2490 compounds for the effects on mitochondrial gene expression and physiology, the Chinese herbal derivative deoxysppanone B was found to act through microtubules to increase OXPHOS and decrease mitochondrial ROS. Perhaps, then, a systematic survey of Asian herbal medications using a variety of mitochondrial functional readouts may reveal previously unrecognized mitochondrial pathways and new therapeutic strategies to manipulate them. These could then be applied to treating the common age-related diseases. If this strategy proves successful, then it may have been prescient that a major concept in the parlance of traditional Asian medicine is “chi,” which loosely translates as “vital force or energy.” (Wallace DC Genetics. 2008 June; 179:727–735)

[406-51] Genomics, Proteomics & Other "omics" in Cardiovascular Medicine
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ABSTRACT TEXT NOT PROVIDED

Publication abstracts.

Heart failure is a considerable social and economic burden to society and is increasing in prevalence. Despite considerable advances in recent years, the management of heart failure remains challenging. The diagnosis of heart failure is conventionally made on the basis of a combination of history, physical examination and assessment of left ventricular function. However, these standard approaches may be inadequate as many of the cardinal symptoms and signs are non-specific. Even with recent substantial therapeutic advances, patients with heart failure continue to experience progressive symptoms and ultimately a shorter life span. There is a real need for simple biomarkers to help differentiate the diagnosis, determine prognosis and aid management of this
increasingly complex syndrome. A biomarker may be defined as a measurable event in a biological system that indicates an alteration in physiology from normal. The ideal biomarker should meet several stringent standards. In addition to being biologically plausible, it should be diagnostically sensitive and specific and ideally be applicable across all sub-groups irrespective of age, gender, racial background or indeed aetiology. The assay should be relatively easy to perform and be readily reproducible between different clinical laboratories. Finally, an ideal biomarker should demonstrate a temporal relationship, accurately reflecting changes in clinical status. Clinical application should complement and offer incremental value to clinical acumen in terms of diagnosis, prognosis or measuring response to therapy. As our understanding of the pathophysiology of heart failure has been progressively unravelled and expanded from a simple haemodynamic model to an ever more complex multi-system syndrome, the portfolio of candidate molecules has expanded concordantly. As such, a myriad of potential biomarkers have been proposed ranging from routinely measured laboratory parameters to novel peptides. The most considerable challenge is translating this to achieve tangible improvements in clinical care and the targeting of intensified and often costly therapies to those most at risk. (J. P. Rocchiccioli, J. J. V. McMurray, A. F. Dominiczak, Biomarkers in heart failure: a clinical review, Heart Fail Rev. 2008).

Recent advances in genotyping technology and insights into disease mechanisms have increased interest in the genetics of cardiovascular disease. Several candidate genes involved in cardiovascular diseases were identified from studies using animal models, and the translation of these findings to human disease is an exciting challenge. There is a trend towards large-scale genome-wide association studies that are subject to strict quality criteria with regard to both genotyping and phenotyping. Here, we review some of the strategies that have been developed to translate findings from experimental models to human disease and outline the need for optimizing global approaches to analyze such results. Findings from ongoing studies are interpreted in the context of disease pathways instead of the more traditional focus on single genetic variants. (Delles C, McBride MW, Padmanabhan S, Dominiczak AF. Trends Endocrinol Metab. 2008 19:309-16).

Among the common complex diseases, hypertension has been particularly unlucky in the recent surge of positive results from genome-wide association studies. We summarize the evidence that would support continuing the effort in the hunt for a genetic basis for hypertension. The problems facing the genetic studies for hypertension are not unique, but phenotypic characterization, heterogeneity and high prevalence make it a special case requiring a more individualized approach. We argue that, even in the presence of a strong environmental component to hypertension risk, the common disease/common variant model is relevant for hypertension and discuss the issues involved in designing a genome-wide association study for hypertension. It is likely that the individual odds ratios for disease variants will be less than 1.3 and, although individually these effect sizes are minor, the combination of even a few such common polymorphisms can have substantial population attributable risks. The identification of hypertension gene variants should provide new insight into the disease susceptibility, progression and severity. This will lead to the identification of potential targets for lifestyle and pharmacological interventions, with the ultimate goal of improving prevention, diagnosis and treatment. (Padmanabhan S, Melander O, Hastie C, Menni C, Delles C, Connell JM, Dominiczak AF. J Hypertens. 2008 26:1275-81).
Primary aldosteronism is a leading cause of secondary hypertension (HTN), but the mechanisms underlying the characteristic renin-independent secretion of aldosterone remain unknown in most patients. They report a new familial form of aldosteronism in a father and two daughters. All were diagnosed with severe HTN refractory to medical treatment by age 7 yr. They performed a variety of clinical, biochemical, and genetic studies to attempt to clarify the underlying molecular defect. Biochemical studies revealed hyporeninemia, hyperaldosteronism, and very high levels of 18-oxocortisol and 18-hydroxy cortisol, steroids that reflect oxidation by both steroid 17-alpha hydroxylase and aldosterone synthase. These enzymes are normally compartmentalized in the adrenal fasciculata and glomerulosa, respectively. Administration of dexamethasone failed to suppress either aldosterone or cortisol secretion; these findings distinguish this clinical syndrome from glucocorticoid-remediable aldosteronism, another autosomal dominant form of HTN, and suggest a global defect in the regulation of adrenal steroid production. Genetic studies excluded mutation at the aldosterone synthase locus, further distinguishing this disorder from glucocorticoid-remediable aldosteronism. Because of unrelenting HTN, all three subjects underwent bilateral adrenalectomy, which in each case corrected the HTN. Adrenal glands showed dramatic enlargement, with paired adrenal weights as high as 82 g. Histology revealed massive hyperplasia and cellular hypertrophy of a single cortical compartment that had features of adrenal fasciculata or a transitional zone, with an atrophic glomerulosa. These findings define a new inherited form of aldosteronism and suggest that identification of the underlying defect will provide insight into normal mechanisms regulating adrenal steroid biosynthesis. (Geller DS, Zhang J, Wisgerhof MV, Shackleton C, Kashgarian M, Lifton RP. A novel form of human mendelian hypertension featuring nonglucocorticoid-remediable aldosteronism. J Clin Endocrinol Metab. 2008 93:3117-23).

Two familial forms of primary aldosteronism [familial hyperaldosteronism (FH)] had been described so far, referred to as FH-I, also known as glucocorticoid-remediable aldosteronism (GRA), and FH-II. This new familial form of PA, which could be called familial hyperaldosteronism type III (FH-III), is characterized by severe hypertension in early childhood associated with marked aldosteronism, hypokalemia, and significant target organ damage, which were resistant to aggressive antihypertensive therapy including spironolactone and amiloride, thus requiring bilateral adrenalectomy. The authors could not identify the genetic alteration responsible for the disease.

FH-I/GRA is transmitted as an autosomal dominant disease and is characterized by hypertension, elevated ACTH-dependent aldosterone secretion, renin suppression, and high levels of the hybrid steroids, 18OHcortisol (18OHF) and 18oxo-cortisol (18oxoF). The genetic defect leading to FH-I/GRA is an unequal genetic recombination between CYP11B1 (11β-hydroxylase) and CYP11B2 (aldosterone synthase), generating a chimeric CYP11B gene containing CYP11B1 sequences (including the promoter) at its 5’ end and CYP11B2 sequences at its 3’ end. Because CYP11B1 expression is regulated by ACTH, the hybrid gene encodes a chimeric enzyme with aldosterone synthase activity and ACTH-dependent expression throughout the adrenal cortex. Hence, in FH-I/GRA patients, aldosterone levels are persistently suppressed by glucocorticoid administration. Most affected individuals develop severe hypertension in early life, but
patients with mild hypertension or blood pressure in the normal range are described in many families.

FH-II is a nonglucocorticoid remediable familial form of PA. Patients affected by FH-II present a family history of PA caused by either an adrenal adenoma or hyperplasia. Each single case of FH-II is clinically, biochemically, and morphologically indistinguishable from apparently sporadic forms of PA. In most families, a vertical transmission suggests an autosomal dominant inheritance. The diagnosis of FH-II is based on the demonstration of PA in at least two members from the same family. Unfortunately, the genetic background of FH-II remains unknown, and thus, the diagnosis is made by the finding of a consistently high Aldosterone Renin Ratio (ARR) (without interfering medications) with a positive confirmatory test (saline load ± fludrocortisone) and lack of the hybrid gene responsible for FH-I/GRA.

This new familial form of PA (FH-III) is characterized by severe hypertension in early childhood associated with marked aldosteronism, hypokalemia, and significant target organ damage, which were resistant to aggressive antihypertensive therapy including spironolactone and amiloride, thus requiring bilateral adrenalectomy.

**SYMPOSIUM SESSION: BASIC - GnRH: An Out of Body Experience.**

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**Abstract.**  
Although the onset of puberty in primates is delayed for several years after birth, GnRH pulse generation during infancy is robust, and in the infantile boy and male monkey the endocrine activity of the pituitary-testicular axis is similar to that seen post-pubertally. Within a few months after birth GnRH pulsatility is restrained and thereby the quiescence of the prepubertal gonad is guaranteed. This “up-down-up” pattern of GnRH pulsatility during postnatal development unfolds in the absence of the gonad, and conceptually may be viewed to result from the application of a neurobiological brake that restrains the activity of the GnRH neuronal network during the intervening childhood/juvenile phase of development. Interestingly, expression of the gene coding for GnRH does not appear to be subjected to major developmental regulation, and an adult pattern of pulsatile GnRH release may be induced immediately and with surprising ease from the juvenile hypothalamus with application of an intermittent neurochemical stimulus. Evidence will be presented that kisspeptin immunoactivity in the mediobasal hypothalamus (MBH) changes in parallel with that of GnRH release from birth to puberty, and that repetitive stimulation of the kisspeptin receptor (GPR54) in the juvenile hypothalamus induces a sustained precocious pubertal pattern of GnRH release. Kisspeptin perikarya in the primate brain appear to be located primarily in the region of the arcuate nucleus in the MBH, while GnRH cell bodies are located more laterally. Although kisspeptin neurons appear to communicate with GnRH neurons via axo-somatic and axo-dendritic contacts, extensive and intimate association between kisspeptin and GnRH axonal projections in the internal layer of the monkey median eminence raises the possibility that regulation of the developmental pattern of GnRH release by kisspeptin may be exerted, at least in part, at the level of the median eminence. It remains to be determined, however, whether the kisspeptin neurons of the arcuate nucleus are a component of a pubertal clock or growth tracking device, or simply serve as a link that relays information from such a puberty control center to the GnRH neuronal network. Similarly, there is an urgent need to integrate kisspeptin
signaling with that of other pathways (neurokinin B, GABA, neuropeptide Y, glutamate, and glial derived growth factors) implicated in the developmental regulation of GnRH release in primates.

SYMPOSIUM SESSION: CLINICAL - Fetal & Maternal Hypothyroidism

[S14-1] Non-Mendelian Mechanisms of Thyroid Dysgenesis.
*JY Deladoey. CHU Sainte-Justine, Montreal, Canada*

Permanent primary hypothyroidism is the most common congenital endocrine disorder. In up to 85% of cases, it results from thyroid dysgenesis, a condition comprising defects in the differentiation, migration or growth of thyroid tissue. Of these defects, incomplete migration resulting in ectopic thyroid tissue ((sub) lingual thyroid) is the most common (up to 80%). Indeed, congenital hypothyroidism from thyroid dysgenesis (CHTD) is a heterogeneous disease, which exists in familial (2%) and non-familial (sporadic, 98%) forms. Moreover, the results of a survey of monozygotic twins, which yielded a discordance rate of 92% and the documented sexual dimorphism in CHTD suggest that complex non-Mendelian mechanisms underlie this condition. On the other hand, environmental causes are unlikely because: (i) no temporal or seasonal trends for CHTD have been observed and (ii) MZ twins who are discordant for CHTD are concordant for birth weight (unpublished observations). Consistent with a complex non-Mendelian genetic contribution, germinal mutations in thyroid related transcription factors TTF-1, TTF-2, PAX-8, and NKX2.5 have been identified in only 3% of patients with sporadic CHTD and linkage analysis excluded these genes in families with CHTD. This suggests the involvement of novel genes and underlines the need for an original approach. Therefore, they propose a multiple-hit model that would be compatible with the usual sporadicity and the evidence for a complex non-Mendelian genetic contribution in the etiology of CHTD. In this model, a combination of multiple genetic hits in threshold-sensitive genes involved in thyroid development would lead to thyroid dysgenesis (i.e. multiple rare variants hypothesis). These mutational hits can occur either in the germline, in tissue or both. They currently focus on the role of somatic (epi)-mutations in CHTD. To assess whether specific genes have relevant somatic genetic or epigenetic alterations, they use a combined analysis of transcriptome (confirmed by qRT-PCR), methylome and structural genome variations (Copy Number Variants, CNVs) of ectopic compared with eutopic thyroids. Preliminary results suggest dysregulation of the Wnt/catenin pathway and of genes encoding cytoskeleton proteins in ectopic thyroids, which are independent of promoter methylation. Whether this observation is a cause or a consequence of the ectopic location of the thyroid requires further studies.

[S14-3] TRH Testing in Congenital Hypothyroidism.
*T Vulsma. Pediatric Endocrinology, Emma Children's Hosp AMC, Amsterdam, Netherlands*

The main regulatory system for maintaining euthyroidism is localized in the hypothalamus-pituitary complex. This system, using a negative feedback interaction, develops during early gestation and fully functions at term. The thyroid hormones and the pituitary hormone TSH are secreted in the main circulation, and concentrations can be easily measured. The hypothalamic hormone TRH is secreted in the portal
vasculature between hypothalamus and pituitary gland, and cannot be measured in peripheral blood. The adequacy of the thyroid gland to secrete thyroid hormone can be established by measuring serum (free) T4, and more accurately by measuring serum TSH. Measuring the adequacy of the regulatory system is less easy. Reference values for (free) T4 show such a broad range of variation that an additional diagnostic determinant is needed. The shape of the response curve of serum TSH following intravenous administration of TRH strongly depends on the thyroid hormone state, and also reflects at which 'level' this entity is disturbed. Neonatal screening on congenital hypothyroidism (CH) is meant to prevent brain damage (signs and symptoms of CH are often not recognized at birth). Usually TSH is measured in dried blood samples on filter paper, obtained by a heel puncture a few days after birth. This test is very effective to detect CH due to defects in the thyroid (CH-T), but CH due to defects in hypothalamus or pituitary (CH-C) remains undetected. Since the incidence of CH-C is estimated 1:15,000 neonates, at least 10% of the CH patients will be missed by screening methods based on determination of TSH. Also, CH-C patients often have multiple pituitary hormone deficiencies (MPHD), and especially hypocortisolism is a life-threatening condition. Nevertheless, neonatal screening by measuring TSH is still predominant worldwide. By measuring primarily (free) T4 CH-T and CH-C can both be detected. Yet, it is less easy to verify the diagnosis CH-C than CH-T. To prevent treatment of children with false positive screening results, and to improve detection of CH patients with a risk of MPHD, a TRH test can be performed in newborns with low (free) T4 and low to slightly increased TSH concentrations. According to a Dutch study, also in neonates the TSH response curve after administration of TRH has proven to be a valuable determinant for discrimination between normal and hypothalamic or pituitary deficiencies. To verify incidence figures worldwide, additional studies are required.

SYMPOSIUM SESSION: BASIC - Functional Significance of Rapid Steroid Hormone Action in the Brain

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ABSTRACT TEXT NOT PROVIDED

Publication abstract

Until recently, the idea that oestradiol could affect cellular processes independent of nuclear oestrogen receptors (ERs) was controversial. This was despite the large number of carefully controlled studies performed both within and outside the nervous system demonstrating that oestrogens regulate various intracellular signalling pathways by acting at the membrane surface of cells and/or at biological rates incompatible with the time course of genomic-initiated events. At present, it is far less controversial that oestradiol acts at surface membrane receptors to regulate nervous system function. Recent studies have demonstrated that the classical intracellular ERs, ERalpha and ERbeta, are major players in mediating the actions of oestradiol on the membrane surface. This review focuses on one potential mechanism by which surface-localised ERalpha and ERbeta stimulate intracellular signalling events in cells of the nervous system. After oestradiol treatment, both ERalpha and ERbeta are capable of activating different classes of metabotropic glutamate receptors (mGluRs). Oestradiol activation of mGluRs is independent of glutamate, but requires expression of several different
caveolin proteins to compartmentalise the different ERs with mGluRs into functional signalling microdomains. ER/mGluR signalling is a potential means by which oestrogens can both rapidly and for extended periods, influence a variety of intracellular signalling processes and behaviours. (Mermelstein PG. Membrane-localised oestrogen receptor alpha and beta influence neuronal activity through activation of metabotropic glutamate receptors. J Neuroendocrinol 2009 21:257-62).

SYMPOSIUM SESSION: CLINICAL - Lessons Learned: From Genetic Causes of Hypopituitarism

[S23-1] Congenital ACTH Deficiency: Lessons Learned from Tpit.
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Abstract.
Most cases of congenital ACTH deficiency involve other deficiencies such as in combined pituitary hormone deficiencies. Some cases with severe early-onset obesity have been linked to POMC or PC1 gene mutations. Congenital isolated ACTH deficiency (IAD) is very rare. A few cases have been described with onset from the perinatal period to early teens, but the pathophysiology and clinical description of this condition was poorly defined before we identified TPIT gene mutations as the principal molecular cause of congenital IAD. Tpit is a T-box transcription factor restricted to the pituitary POMC-expressing lineages in mice and humans. We investigated the TPIT gene coding sequences in the largest series of IAD patients (n=73) and found a mutation in 63% of the neonatal cases (n=56) but never in cases of juvenile onset (n=17). The clinical presentation of IAD patients with TPIT mutations remains very homogeneous; neonatal hypoglycemia, very low ACTH and cortisol levels, and normal other pituitary functions were found in all cases, associated with a prolonged cholestatic jaundice in 60% and a neonatal death in 20% of cases. We identified 9 new TPIT gene mutations: a one-bp deletion, 3 splice-site mutations, 3 missense mutations affecting the T-box and one interesting missense mutation affecting the C-terminus of the TPIT protein. Our study revealed a relatively high frequency (40%) of compound heterozygotes, suggestive of a significant frequency of TPIT mutant alleles in the human population. In 3 patients of our series, we only found one mutant TPIT allele and the other allele appeared normal in TPIT coding sequences. In each, the familial genetics is consistent with the proband being compound heterozygote since parents or sibs carrying the mutant TPIT allele are unaffected. The other unexplained allele could be a large deletion of the gene or a mutation in regulatory sequences. Using quantitative-PCR, we identified a large deletion encompassing exon 1 in one patient. We confirmed that TPIT is the principal causal gene of congenital early-onset IAD, a homogeneous recessive disease more frequent than previously expected. We have identified 21 TPIT gene mutations that lead to loss-of-function by different mechanisms, such as non-sense mediated mRNA decay, loss of TPIT DNA binding or protein-protein interactions, and loss of mRNA splicing. Human mutations provide a unique experiment in mutagenesis to reveal important biochemical correlates to gene function.

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Abstract.
LHX4 is an LIM homeodomain transcription factor involved in pituitary ontogenesis.
Only five heterozygous LHX4 mutation have been reported to be responsible for congenital pituitary hormone deficiency (Machinis, 1999; Pfaeffle, 2007; Castinetti, 2008). In a recent multicenter study based on 136 patients (from 133 pedigrees) bearing growth hormone deficiency associated with midline or brain abnormalities, we had reported three allelic variants of LHX4 (c293InsC, pThr90Met, and pGly370Ser). On the basis of functional studies, only one mutation (c293InsC) was responsible for the patients' phenotype, whereas pThr90Met and pGly370Ser were likely polymorphisms. Interestingly, patients bearing the heterozygous c293InsC mutation had variable phenotypes: two brothers presented somatolactotroph and thyrotroph deficiencies, with pituitary hypoplasia; the youngest brother (propositus) also had partial atrophy of corpus callosum and ectopic neurohypophysis; their father only had somatotroph and partial gonadotroph deficiencies without intracranial malformation or pituitary abnormalities. This c293InsC mutation induces a frameshift with a resulting truncated protein. Functional studies showed that the frameshift induced a complete loss of transcriptional activity on PRL, GH, and POU1F1 promoters. Cotransfection of c293InsC mutant and wild-type LHX4 failed to evidence any dominant negative effect, suggesting a mechanism of haploinsufficiency. Comparison with other LHX4 mutations clearly shows a wide phenotypic variability in terms of pituitary deficiencies (inconstant or delayed), MRI abnormalities (pituitary hypo or hyperplasia, inconstant cerebral abnormalities). Interestingly, 50% of the patients present a poorly developed sella turcica. This phenotypic variability exists between mutations, but also between patients of the same family carrying the same mutation. It is thus highly likely that LHX4 interacts with several as yet unknown pituitary transcription factors, activators or repressors, that play a major role in this phenotypic variability. Despite the low number of mutations reported to date, there is a high phenotypic variability that makes it difficult to define a phenotype in which LHX4 gene mutations should be screened. Further studies based on mouse models should help us to better understand the pathophysiological mechanisms of LHX4 induced congenital hypopituitarism, and the involvement of this factor in pituitary development.

**S42-2| Environmental Influences & Puberty Timing**  
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**Abstract**

Timing of puberty is largely determined by genetic factors. Twin studies and other epidemiological studies suggest that delayed puberty follows an autosomal dominant trait of inheritance. However, it is clear that environment influences the timing, too. Secular trends in the onset of menarche indicate the permissive role of sufficient nutrition, whereas obesity may be linked to accelerated onset of puberty, particularly in girls. Frequent occurrence of precocious central puberty in girls moving from developing countries to wealthier areas suggest an environmental trigger for the onset of puberty. It has been hypothesized that withdrawal from an exposure to weak estrogenic endocrine disruptors could be such a trigger. These compounds would first weakly stimulate hypothalamo-pituitary-gonadal axis and at the same time suppress gonadotropin secretion by a negative feed-back. When the exposure stops, negative feedback would cease and puberty could start. Epidemiological evidence for the connection of endocrine disruptors to central precocious puberty is still limited. In contrast, there are plenty of cases and even epidemics of peripheral precocious puberty caused by pharmaceutical estrogens and endocrine disruptors.
Puberty Timing: Is It Changing? Does It Matter?

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Abstract.
Most experts believe that there is a secular trend in the U.S. toward an earlier timing of puberty in girls. There has been significant debate though, about possible ascertainment bias as breast development was judged primarily by inspection. A recent reanalysis of the NHANES III data found that girls with normal body mass indexes attain thelarche and menarche at ages that are not as early as previously suggested and that adiposity and ethnic differences account for much of the trend towards an earlier age of puberty. A few cross-sectional as well as longitudinal studies have also shown that increasing adiposity in girls likely causes an earlier entrance into puberty, with leptin playing a significant role as a permissive factor. The timing of puberty in boys has not been so thoroughly studied and the consequence of adiposity may be the opposite of what is seen in girls. The possible effects of endocrine-disrupting compounds (EDCs) on the timing of puberty are also of significant concern. Animal studies have demonstrated earlier reproductive development following exposure to phytoestrogens, pesticides, fungicides, polychlorinated biphenyls (PCBs) and bisphenol A, to name only a few. How these EDCs affect human reproductive development is less obvious, but increased levels of phthalates and PCBs have been associated with early pubertal development in girls. Possible long-term health implications associated with an earlier age of puberty include both medical and psychosocial problems. Earlier menarche is a known risk factor for breast cancer and subsequent obesity. Fertility has also been shown to be adversely affected as early menarche is associated with limited ovarian reserve. Fewer studies have evaluated the influence of puberty timing on long-term health in boys. While the peak incidence of testicular cancer has been definitively linked to the timing of puberty, whether testicular cancer risk is increased with earlier puberty remains unclear. Data also indicate that early maturing girls are at higher risk for psychopathology, negative body image, substance abuse and early sexual experiences. Interestingly, the same problems are not seen in boys, and a later timing of puberty has seemingly more detrimental effects on self-esteem and psychological well being.

SYMPOSIUM SESSION: TRANSLATIONAL - Unraveling the Complexities of Growth

GH Pharmacogenetics: Predicting Response to GH Treatment

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Recombinant human GH (rhGH) has been used to treat short stature in several different conditions, but considerable interindividual variation in short and long term growth response exists. Pharmacogenomics can provide important insights to rhGH therapy. Growth hormone receptor (GHR) is the first key molecule mediating GH action. In the last 3 years, a common GHR polymorphism, the presence (GHRfl) or absence (GHRd3) of exon-3, has been under intensive investigation regarding its influence on the response to hGH therapy. These two GHR isoforms present a widespread distribution in humans, since the first report, a total of 1,063 Caucasian controls have been genotyped with the frequency of each genotpe being 43% for fl/fl, 45% for fl/d3 and 12% for d3/d3. The 22 residues codified by exon 3 are not located in the GH binding interface. Initial experimental studies demonstrated that GHRd3 and GHRfl present the same binding
capacity to 22 kDa hGH. However, one study showed that cells transiently transfected with GHRd3 induced higher transcription activity after treatment with 22kDa hGH than cells transfected with GHRfl in luciferase reporter assays. Studies that evaluated these two GHR isoforms in children and adults with GH deficiency, girls with Turner syndrome, children born small for gestational age and acromegalic patients showed that patients carrying the GHRd3 allele presented a greater GH sensitivity than patients homozygous for the GHRfl allele. However, other studies presented contradictory results, in a typical pattern observed in association studies. The contradictory data may be caused by confounding factors, such as small sample sizes and differences in experimental design. This GHR-exon 3 genotype is the first identified genetic factor that modulates the individual response to GH therapy.

Recently, our group demonstrated that the -202 A allele of IGFBP3 promoter region polymorphism is associated with increased IGFBP-3 levels and growth velocity during rhGH treatment in prepubertal GHD children. The pharmacogenetic studies published to date in relation to rhGH therapy and new insights about the influence of polymorphisms in other genes will be discussed. The analyses of present and future validation studies may define the use of this and other polymorphisms in clinical practice, moving from pharmacogenetics to routine application and allowing individualization of hGH doses to optimize final outcome.

[S59-3] Epigenetic, Genomic Imprinting and Regulation of Growth
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Epigenetic mechanisms play a key role in regulating gene expression. One of the most studied epigenetic modifications is DNA methylation at CpG dinucleotides. Imprinting refers to an epigenetic marking of genes that results in monoallelic expression depending on their parental origin. There are two critical time periods in epigenetic reprogramming: gametogenesis and early preimplantation development. Major reprogramming takes place in primordial germ cells in which parental imprints are erased and totipotency is restored. Imprint marks are then re-established during spermatogenesis or oogenesis, depending on sex. Upon fertilization, genome-wide demethylation occurs followed by a wave of de novo methylation, both of which are resisted by imprinted loci. Disruption of imprinting causes disorders involving growth defects, such as the overgrowth Beckwith-Wiedemann (BWS) and the intrauterine and postnatal growth retardation Russell-Silver (RSS) syndromes with opposite phenotypes. These growth disorders are caused by abnormal DNA methylation at the 11p15 imprinted region (ICR) encompassing many imprinted genes as IGF II. In BWS, loss of methylation (LOM) at the centromeric ICR2/KCNQ1OT1 region on the maternal allele or gain of methylation at the telomeric ICR1/IGFII/H19 region on the maternal allele have been shown. This latter epigenetic defect is associated with a higher risk of tumor. On the opposite, LOM at the telomeric ICR1 on the paternal allele was demonstrated in RSS. Early embryogenesis is a critical time for epigenetic regulation, and this process is sensitive to environmental factors. An abnormally high prevalence of ART conception among patients with BWS have been observed, suggesting that ART may favour imprinting alterations at the imprinted centromeric 11p15 locus (LOM at the maternally methylated ICR2). The underlying cause of these imprinting defects (following ART or occurring spontaneously) remains unclear. However, recent data have shown that in patients with BWS, including those born following the use of ART, the DNA methylation defect involves imprinted loci other than 11p15. This suggests a dysregulation of a trans-acting regulatory factor following fertilization.