Editor's choice

Jean-Claude Carel and Ze'ev Hochberg

New mechanisms: of mice and men

Mouse model of Noonan syndrome reveals cell type – and gene dosage – dependent effects of *Ptpn11* mutation

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Introduction: Noonan syndrome is an autosomal dominant birth defect, characterized by short stature, facial abnormalities, heart defects and possibly increased risk of leukemia. Mutations of *Ptpn11* (also known as *Shp2*), which encodes the protein-tyrosine phosphatase Shp2, occur in ~50% of individuals with Noonan syndrome.

Methods and Results: Generation of mice expressing the Noonan syndrome-associated mutant D61G. When homozygous, the D61G mutant was embryon lethal, whereas heterozygotes have decreased viability. Surviving *Ptpn11*^{D61G/+} embryos (~50%) have short stature, craniofacial abnormalities similar to those in Noonan syndrome, and myeloproliferative disease. Severely affected *Ptpn11*^{D61G/+} embryos (~50%) have multiple cardiac defects similar to those in mice lacking the Ras-GAP protein neurofibromin. Their endocardial cushions have increased Erk activation, but Erk hyperactivation is cell and pathway specific.

Conclusions: The study shows that a single *Ptpn11* gain-of-function mutation evokes all major features of Noonan syndrome, including a tendency for leukemia, by acting on multiple developmental lineages in a gene dosage-dependent and pathway-selective manner.

Noonan syndrome is the most common non-chromosomal cause of congenital heart disease, mostly pulmonary stenosis. Cryptorchidism, lymphatic abnormalities, skeletal defects, mild mental retardation, hearing difficulties, and bleeding problems occur variably. A small number develop myeloproliferative disease, which usually resolves but can develop into leukemia. Germline gain-of-function mutations of human *PTPN11* occur in ~50% of individuals with Noonan syndrome, but poor genotype-phenotype correlation was reported. The protein product, Shp2, is a ubiquitously expressed, non-transmembrane protein-tyrosine phosphatase. In fact, Shp2 is essential for normal activation of the Ras-Erk cascade in most, if not all, receptor tyrosine kinases and cytokine receptor signaling pathways. This paper shows that a single Shp2 mutation associated with Noonan syndrome, acting predominantly if not exclusively as a hypermorph, has gene dosage-dependent and cell/tissue-specific effects on mouse development. Abnormal cardiac valve development and the myeloproliferative disease reflect Shp2 gain of function, leading to increased proliferation and decreased apoptosis. This animal model is opting to reveal other signaling pathways deregulated in these mice and the mechanisms by which these cause Noonan syndrome-associated phenotypes.

Thyroid hormone receptor- α is a molecular switch of cardiac function between fetal and postnatal life

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Introduction: Thyroid hormones regulate gene transcription by binding to their nuclear receptors TR α and TR β . In the absence of triiodothyronine (T3), the unliganded receptors (aporeceptors) do bind DNA and repress the transcription of target genes. The role of thyroid hormone aporeceptors as repressors was observed in hypothyroid adult mice, but its physiological relevance in nonpathological hypothyroid conditions remain to be determined.

Results: This paper shows that in the normal mouse fetus, TR α aporeceptors repress heart rate as well as the expression of TR β and several genes encoding ion channels involved in cardiac contractile activity. Right after birth, when the T3 concentration sharply increases, liganded TR α (holoreceptors) turn on the expression of some of these same genes concomitantly with heart rate increase.

Conclusions: Conversion of TR α from aporeceptors into holoreceptors, upon changes in T3 availability, plays a determinant role in the developmental process.

Thyroid receptors are transcription factors with ligand-regulated activity. In the absence of T3, the unliganded receptors (aporeceptors) recruit co-repressors and repress the transcription of target genes. Upon hormone binding, the receptors (holoreceptors) exchange co-repressors for co-activators and activate transcription. This repressive effect of aporeceptors has been clearly documented in hypothyroid mice. This suggested that a change in T3 concentration might act as a molecular switch under physiological conditions. One situation in which the T3 level naturally changes is during the transition from fetal to neonatal life. In mice, fetal circulating levels of T3 are quite low and highly increase at birth. This study shows that all tested genes in the heart are controlled by TR α in opposite ways before and after birth. At the end of the fetal development and mainly during the early post-natal period, the availability of T3, mostly produced by the fetus itself, releases this blockage.

Clinical trials: guard their heart

Efficacy and safety of statin therapy in children with familial hypercholesterolemia: a randomized controlled trial

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Introduction: Children with familial hypercholesterolemia have endothelial dysfunction and premature atherosclerotic disease. The long-term efficacy and safety of cholesterol-lowering medications have not been evaluated in children.

Design: Randomized, double-blind, placebo-controlled trial to determine the 2-year efficacy and safety of pravastatin therapy in 214 children aged 8–18 years with familial hypercholesterolemia.

Results: Carotid thickness showed a trend toward regression with pravastatin ($p = 0.02$). Also, pravastatin significantly reduced mean low-density lipoprotein cholesterol ($p < 0.001$). No differences were observed for growth, muscle or liver enzymes, endocrine function parameters, Tanner staging scores, onset of menses, or testicular volume between the 2 groups.

Conclusions: Two years of pravastatin therapy induced a significant regression of carotid atherosclerosis in children with familial hypercholesterolemia, with no adverse effects on growth, sexual maturation, hormone levels, or liver or muscle tissue.

Whereas statins have been used off-label in children heterozygous for familial hypercholesterolemia, we can now say that this is evidence-based. Statins lower serum cholesterol and prevent atherosclerosis. Despite rumors to the contrary, the safety profile of this therapy was unaffected. This is a 2-year study, long enough to show an effect, but too short to ascertain long-term safety and efficacy. We can only hope that these and other investigators will keep monitoring safety and efficacy. This study does not provide evidence for what it has not evaluated. We do not know if statin therapy is effective in the hypercholesterolemia of diabetes type-2 or the metabolic syndrome.

Important observations for clinical practice: run safely

Effects of oral contraceptives on body composition and physical performance in female athletes

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Introduction: Menstrual disturbances are common among female athletes, and oral contraceptives are often recommended as estrogen substitution. The aim of this study was to investigate the effects of contraceptive use on body composition and physical performance in female athletes.

Methods: Twenty-six endurance athletes (13 with oligo-/amenorrhea and 13 regularly menstruating athletes) and 12 sedentary controls were examined before and after 10 months of treatment with a low-dose, monophasic, combined pill.

Results: Significant changes in body composition were recorded in the athletes, but not in the controls. There was an increase in weight and fat mass only in athletes with oligo-/amenorrhea. These changes were associated with a decrease in ovarian androgens. Estrogen treatment also increased bone mineral density, with the largest increase in athletes with a low bone mineral density at baseline. Despite significant changes in body composition, little impact on physical performance was recorded.

Conclusions: Contraceptive treatment in female athletes has predominantly beneficial effects on body composition without adverse effects on physical performance and could be used for the prevention of osteoporosis in athletic amenorrhea. However, it cannot be excluded that a marked increase in fat mass might have unfavorable effects for athletic performance in individual women.

Amenorrhea and an hypogonadal state are common among female endurance athletes. Increased bone resorption, in particular, loss of trabecular bone, constitutes a serious consequence of this condition. Menstrual disturbances in athletes are also related to an increase in musculoskeletal injuries and a 2- to 4-fold higher risk of stress fractures. In clinical practice, contraceptives are often recommended to prevent bone loss during conditions of estrogen deficiency, but evidence has been lacking. This study evaluated the effect of oral contraceptives on body composition and physical performance in female athletes before and after 10 months of treatment. The researchers noted significant changes in body composition in the athletes, but not in the controls. Also, athletes without menstruation experienced an increase in weight and fat mass while taking the oral contraceptives. The researchers associated this change with a decrease in ovarian androgens. Oral contraceptives also resulted in an increase in bone mineral density in all women, but larger in the athletes who had a low bone mineral density at baseline. Thus, oral contraceptives can help women athletes regulate their menstrual cycle and at the same time increase their body composition. But why use a gonadotroph-suppressive contraceptive when the aim is to give hormone replacement therapy? A low-dose estrogen may do the job.

Deletion of *Bax* eliminates sex differences in the mouse forebrain

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Introduction: Several of the best-studied sex differences in the mammalian brain are ascribed to hormonal control of cell death. The molecular mechanisms of hormone-regulated, sexually dimorphic cell death are unknown. *Bax* is a member of the Bcl-2 family of proteins that is required for cell death in many developing neurons, might it be essential for sex differences in neuron number.

Methods and Results: Comparing *Bax* knockout mice and their WT siblings, this study focused on two regions of the mouse forebrain that show opposite patterns of sexual differentiation: the principal nucleus of the bed nucleus of the stria terminalis, in which males have more neurons than do females, and the anteroventral periventricular nucleus (AVPV), where females have more neurons overall and many more dopaminergic neurons than do males. Testosterone, or its metabolites, is responsible for the sex differences in both nuclei. A null mutation of the *Bax* gene completely eliminated sex differences in overall cell number in both the principal nucleus of the bed nucleus of the stria terminalis and AVPV.

Conclusions: *Bax*-dependent cell death is required for sexual differentiation of cell number, regardless of whether testosterone decreases or increases cell death. In contrast, the sex difference in AVPV dopaminergic cell number was not affected by *Bax* gene deletion, demonstrating heterogeneity of mechanisms controlling cell number within a single nucleus.

Cell death is a fundamental process in brain development capable of dictating functional relationships between populations of neurons. It plays a particularly important role in the differentiation of areas involved in sexually dimorphic functions, as several of the best-known sex differences in the mammalian nervous system involve differences in neuron number. For example, male rats have many more neurons than do females in the sexually dimorphic nucleus of the preoptic area. Conversely, in the anteroventral periventricular nucleus, implicated in the control of gonadotrophin secretion, overall volume, cell density, and the number of dopaminergic neurons are greater in females than in males. For pediatric endocrinologists it is not news that male and female brains are different, but the processes controlling certain neuroanatomical disparities between them are unclear. It is thought that hormones, such as testosterone, help to control the survival of sex-specific neurons during development. This paper implicates one of the main players in programmed cell death in controlling events in specific regions. Mice lacking the BCL2-associated X protein (*Bax*) gene, the proapoptotic protein that induces cell death affects the male-specific but not the female-specific region. These observations raise the question of whether sex differences in neuroendocrine function or behavior are affected by *Bax* gene deletion. For example, the ability of estrogens to activate gonadotrophin-releasing hormone neurons in the forebrain and trigger a LH surge is sexually differentiated in rodents. Might the 'feminization' of overall cell number allow *Bax*^{-/-} males to mount a LH surge? It seems that *Bax* can override hormonal signals, and may be the target gene regulated by testosterone.

Inactivation of TGF β signaling in neural crest stem cells leads to multiple defects reminiscent of DiGeorge syndrome

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Introduction: Specific inactivation of TGF β signaling in neural crest stem cells (NCSCs) results in cardiovascular defects and thymic, parathyroid, and craniofacial anomalies. All these malformations characterize DiGeorge syndrome, the most common microdeletion syndrome in humans.

Results: Consistent with a role of TGF β in promoting non-neural lineages in NCSCs, mutant neural crest cells migrate into the pharyngeal apparatus but are unable to acquire non-neural cell fates. Moreover, in neural crest cells, TGF β signaling is both sufficient and required for phosphorylation of CrkL, a signal adaptor protein implicated in the development of DiGeorge syndrome.

Conclusions: TGF β signal modulation in neural crest differentiation might play a crucial role in the etiology of DiGeorge syndrome.

Patients with DiGeorge syndrome have an array of facial, heart, endocrine and immune abnormalities. This paper shows how problems with signaling by TGF β may contribute to this condition. The researchers examined the role of TGF β in neural-crest cells which migrate from the neural tube in the growing embryo to various locations that are affected by DiGeorge syndrome, including the face, heart and thymus. They found that mice genetically engineered to lack TGF β in their neural-crest cells mimic many of the defects seen in DiGeorge syndrome patients. Their observations suggest that the cells are unable to adopt the fates of smooth muscle or other tissues. The microdeletion on chromosome 22 (del22q11) found in most DiGeorge syndrome patients encompasses ~30 genes but does not include genes encoding either TGF β receptors or its ligands. They propose that TGF β partly controls the activity of CrkL, a gene product which lies in a segment of chromosome 22 that is missing in DiGeorge patients. Thus, they speculate that at least part of the syndrome is caused when loss of this gene disrupts TGF β signaling.

New mechanisms: Refinements in glucose homeostasis

When we learned the principles of glucose regulation in high school, it looked like a simple loop, with blood glucose being sensed by the β -cell, therefore producing insulin to maintain it at a preset level. Indeed, several monogenic disorders such as MODY2 or neonatal hypoglycemia have exemplified that disturbances in this loop result in derangements of glucose homeostasis. This year, we learn, if this was needed, that regulation of plasma glucose is a lot more complex than this initial scheme. Central integration, entero-insular axis, parental imprinting and microRNAs influencing insulin secretion are in this year's new mechanisms in the long march to decipher glucose metabolism.

Hypothalamic K(ATP) channels control hepatic glucose production

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Introduction: The medial hypothalamus is a major integrator of nutritional and hormonal signals, which play pivotal roles not only in the regulation of energy balance but also in the modulation of liver glucose output. Bidirectional changes in hypothalamic insulin signaling therefore result in parallel changes in both energy balance and glucose metabolism.

Results: This paper shows that activation of ATP-sensitive potassium (K_{ATP}) channels in the mediobasal hypothalamus is sufficient to lower blood glucose levels through inhibition of hepatic gluconeogenesis. The infusion of a K_{ATP} blocker within the mediobasal hypothalamus, or the surgical resection of the hepatic branch of the vagus nerve, negates the effects of central insulin and halves the effects of systemic insulin on hepatic glucose production. Consistent with these results, mice lacking the SUR1 subunit of the K_{ATP} channel are resistant to the inhibitory action of insulin on gluconeogenesis.

Conclusions: Activation of hypothalamic K_{ATP} channels normally restrains hepatic gluconeogenesis. Any alteration within this central nervous system/liver circuit can contribute to diabetic hyperglycemia.

Insulin targets the liver directly, but Pocai et al. have identified an additional pathway by which the hormone, acting on the brain, sends messages via the nervous system to exert a higher level of control on glucose output from the liver. In recent years, the idea that glucose metabolism throughout the body is coordinated by the brain has gained growing support. It is increasingly evident, for example, that the liver receives control signals from the hypothalamus, and we now learn that insulin acts on K_{ATP} channels in the hypothalamus to control glucose production. These channels, which lie in the outer membranes of hypothalamic neurons, were named for their ability to release potassium ions from cells in response to a drop in ATP. The authors' results link two findings that had previously been viewed in isolation: that insulin can modulate liver glucose output through an unknown action in the hypothalamus, and that it opens K_{ATP} channels in an unidentified set of neurons in a part of the hypothalamus that monitors blood metabolites and hormones. K_{ATP} channels are generally known for their ability to convert metabolic signals into changes in electrical activity in the β cells of the pancreas. It seems contradictory that insulin, the hormonal indicator of nutrient excess, should have the same effect as glucose deprivation on K_{ATP} channel activity and neuronal excitability. However, this paper shows convincingly in rats that opening K_{ATP} channels in the hypothalamus by infusing the K_{ATP} channel opener diazoxide causes a neurally mediated reduction in liver glucose production, typical of the response to high rather than low nutrient availability. There are several inherited insulin-secretion disorders that are caused by altered K_{ATP} channel activity in pancreatic β cells, and assessment of liver glucose handling in such disorders would be of great interest. K_{ATP} channel inhibitors are used routinely to increase insulin secretion in type-2 diabetes, and it may be that, if these oral hypoglycemic agents gain access to and inhibit hypothalamic K_{ATP} channels, they might adversely affect glucose handling in the liver. The role of K_{ATP} channels in the central control of glucose homeostasis is also demonstrated in the paper by Lam et al. [1], showing that free fatty acids are sensed by the hypothalamus.

Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120

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Background: Diabetes, a disease in which the body does not produce or use insulin properly, is a serious global health problem. Gut polypeptides secreted in response to food intake, such as glucagon-like peptide-1, are potent incretin hormones that enhance the glucose-dependent secretion of insulin from pancreatic β cells. Free fatty acids provide an important energy source and also act as signaling molecules in various cellular processes, including the secretion of gut incretin peptides.

Results: A G-protein-coupled receptor, GPR120, which is abundantly expressed in the intestine, functions as a receptor for unsaturated long-chain free fatty acids. Furthermore, the stimulation of GPR120 by free fatty acids promotes the secretion of glucagon-like peptide-1 in vitro and in vivo, and increases circulating insulin.

Conclusion: Because glucagon-like peptide-1 is the most potent insulinotropic incretin, GPR120-mediated glucagon-like peptide-1 secretion induced by dietary free fatty acids is important in the treatment of diabetes.

Insulin secretion is primarily regulated by glucose, but it has long been known that insulin is invoked before glucose reaches the β cell by signals involving the interaction between nutrients and the digestive tract. The role of glucagon-like peptide-1 in this incretin effect is well known and glucagon-like peptide-1 is actively developed as a drug for type-2 diabetes. Glucagon-like peptide-1 release by the intestinal tract is stimulated by free fatty acids but the mechanism of this was unknown. The authors started with the observation that GPR120, a so far orphan nuclear G-protein-coupled receptor, was abundantly expressed in the gut and in intestinal endocrine cell lines. They found that GPR120 was important for long-chain free fatty acid-mediated release of glucagon-like peptide-1 in vitro, using various approaches. In vivo, indirect approaches suggest that GPR120 plays an important role in the incretin effect. In addition, GPR40 has been shown to be activated by free fatty acids and to play a role in the incretin effect and in the metabolic syndrome resulting from a high fat diet [2]. Altogether, these findings identify a new series of receptors expressed in the gut that represent promising targets for nutritional disorders such as type-2 diabetes, obesity or eating disorders.

Micro-RNA and insulin secretion: small but efficient

A pancreatic islet-specific microRNA regulates insulin secretion

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Nature 2004;432:226–230

Background: MicroRNAs (miRNAs) constitute a growing class of non-coding RNAs that are thought to regulate gene expression by translational repression. Several microRNAs in animals exhibit tissue-specific or developmental-stage-specific expression, indicating that they could play important roles in many biological processes.

Results: To study the role of microRNAs in pancreatic endocrine cells the authors cloned and identified a novel, evolutionarily conserved and islet-specific microRNA (miR-375). They show that overexpression of miR-375 suppressed glucose-induced insulin secretion, and conversely, inhibition of endogenous miR-375 function enhanced insulin secretion. The mechanism by which secretion is modified by

miR-375 is independent of changes in glucose metabolism or intracellular Ca^{2+} -signaling but correlated with a direct effect on insulin exocytosis. Myotrophin was predicted to be and validated as a target of miR-375. Inhibition of myotrophin by small interfering (si)RNA mimicked the effects of miR-375 on glucose-stimulated insulin secretion and exocytosis.

Conclusion: MiR-375 is a regulator of insulin secretion and may thereby constitute a novel pharmacological target for the treatment of diabetes.

MicroRNAs are 21- to 23-nucleotide non-coding RNAs processed from double-stranded hairpin precursors and have been identified in the genomes of a wide range of multicellular life forms, including plants and animals. The function of microRNAs in vertebrates and mammals is largely unknown, but microRNAs can bind to target sites in messenger RNAs with imperfect base pairing and, by unknown mechanisms, significantly reduce translational efficiency. Furthermore, genetic studies have identified important functions of specific microRNAs in the coordination of cell proliferation and cell death during development and in fat metabolism. The authors have cloned microRNAs from a pancreatic β cell line and identified miR-375 as the most abundant one. They show that overexpression of miR-375 decreases insulin release, mostly by impairing exocytosis and have then searched the target gene of this microRNA. They identified in silico V-1/myotrophin as a putative target and found that overexpression of miR-375 decreased V-1/myotrophin expression. They then silenced V-1/myotrophin using siRNAs and recapitulated the effect of miR-375. Altogether, this is a new and unexpected piece in the complex puzzle of glucose homeostasis. Additional targets of miR-375 are likely to contribute to the regulation of insulin secretion and other microRNA could also play a role in endocrine pancreas development. The analysis of temporal and spatial expression and the generation of loss-of-function mutations of islet microRNAs will shed light on this new class of genes. Tissue-specific microRNAs have the potential to become novel targets for therapeutic intervention in diabetes mellitus and other endocrine diseases.

Transient and mysterious: neonatal diabetes

Impaired glucose homeostasis in transgenic mice expressing the human transient neonatal diabetes mellitus locus, TNDM

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J Clin Invest 2004;114:339–348

Background: Transient neonatal diabetes mellitus is a rare inherited diabetic syndrome apparent in the first weeks of life and again during early adulthood. The relative contributions of reduced islet β cell number and impaired β cell function to the observed hypoinsulinemia are unclear. The inheritance pattern of this imprinted disorder implicates overexpression of one or both genes within the TNDM locus: ZAC, which encodes a proapoptotic zinc finger protein, and HYMAI, which encodes an untranslated mRNA.

Results: To investigate the consequences for pancreatic function, a high-copy transgenic mouse line, TNDM29, was generated carrying the human transient neonatal diabetes mellitus locus. TNDM29 neonates display hyperglycemia, and older adults, impaired glucose tolerance. Neonatal hyperglycemia occurs only on paternal transmission, analogous to paternal dependence of transient neonatal diabetes mellitus in humans. Embryonic pancreata of TNDM29 mice showed reductions in expression of endocrine differentiation factors and numbers of insulin-staining structures. By contrast, β cell mass was normal or elevated at all postnatal stages, whereas pancreatic insulin content in neonates and peak serum insulin levels after glucose infusion in adults were reduced.

Conclusion: Expression of human ZAC and HYMAI in these transgenic mice thus recapitulates key features of transient neonatal diabetes mellitus and implicates impaired development of the endocrine pancreas and β cell function in disease pathogenesis.

Transient neonatal diabetes mellitus is characterized by intrauterine growth retardation and dehydration and hyperglycemia in the first weeks of life. Remission occurs at an average of 3 months and

the majority of affected individuals develops type-2 diabetes in adolescence or early adult life. Three genetic anomalies have been described: paternal uniparental isodisomy of chromosome 6, unbalanced paternal duplication of 6q24, and maternal methylation anomalies. The fact that inheritance of the duplication from the father causes transient neonatal diabetes mellitus suggests that the disease is a manifestation of overexpression of an imprinted gene(s). Here the authors have directly tested this hypothesis by generating a mouse model with the human genomic sequence encompassing the two candidate genes, ZAC and HYMAI. They found that the transgenic mice who inherit the transgene from their father, but not from their mother, displayed a mild neonatal hyperglycemia phenotype reminiscent of the human disease. The parallelism and differences between the mouse model and the human disease are nicely analyzed in an accompanying editorial [3]. More importantly, the model gives an indication that the candidate genes are indeed involved and an opportunity to evaluate the mechanisms linking their overexpression to β -cell function. β -Cell mass was increased, mainly in the neonates and expression of all islet hormones (insulin, glucagon, somatostatin and PP) was decreased. Expression of differentiation markers was also reduced in embryonic pancreata. This model will certainly help further explore the mechanism of this mysterious neonatal disease and will expand our understanding of a key mechanism in the regulation of β -cell function, of relevance for more common conditions such as type-2 diabetes. In addition, the respective roles of the two candidate genes (ZAC and HYMAI) will have to be established.

Growth regulation: from a rare disease to common variants

Regulatory variation at glypican-3 underlies a major growth QTL in mice

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Background: The genetic basis of variation in complex traits remains poorly understood, and few genes underlying variation have been identified. Previous work identified a quantitative trait locus (QTL) responsible for much of the response to selection on growth in mice, effecting a change in body mass of approximately 20%.

Results: By fine-mapping, the location of this QTL was resolved to a 660-kb region containing only two genes of known function, *Gpc3* and *Gpc4*, and two other putative genes of unknown function. There are no non-synonymous polymorphisms in any of these genes, indicating that the QTL affects gene regulation. Mice carrying the high-growth QTL allele have an approximately 15% lower *Gpc3* mRNA expression in the kidney and liver, whereas expression differences at *Gpc4* are not significant. Expression profiles of the two other genes within the region are inconsistent with a factor responsible for a general effect on growth. Polymorphisms in the 3' untranslated region of *Gpc3* are strong candidates for the causal sequence variation. *Gpc3* loss-of-function mutations in humans and mice cause overgrowth and developmental abnormalities. However, no deleterious side effects were detected in the mice, indicating that genes involved in Mendelian diseases also contribute to complex trait variation.

Conclusion: *Gpc3* polymorphism contributes to growth variation in mice. Small changes in gene expression can have substantial phenotypic effects.

Simpson-Golabi syndrome results from loss of function mutations in the glypican-3 gene and is characterized by tall stature and developmental delay. In this mouse model, variation of statural growth was mapped to a quantitative trait locus on chromosome X, accounting for a 20% variation in body mass and containing two genes, glypican-3 and 4. The difference was seen both in hemizygous males and in heterozygous females. The paper describes a detailed analysis of the genetic region involved and gives convincing evidence that glypican-3 gene polymorphisms are causally related to the variation in growth. Not surprisingly, the tall mice carrying one high-line allele had lower glypican-3 expression in various tissues but none of the developmental defects associated with loss of function mutations. None of the polymorphisms identified concerned the coding sequence, leaving open the exact mechanism of the association. This paper is a good example of the power of rare monogenic

disorders to identify candidate genes associated with normal variation of a phenotypic trait and makes glypican-3 an attractive candidate for genetic analyses of human growth.

Familial glucocorticoid deficiency type n: waiting for type n+1

Mutations in MRAP, encoding a new interacting partner of the ACTH receptor, cause familial glucocorticoid deficiency type 2

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Nat Genet 2005;37:166–170

Background: Familial glucocorticoid deficiency, or hereditary unresponsiveness to adrenocorticotropin (ACTH; OMIM 202200), is an autosomal recessive disorder resulting from resistance to the action of ACTH on the adrenal cortex, which stimulates glucocorticoid production. Affected individuals are deficient in cortisol and, if untreated, are likely to succumb to hypoglycemia or overwhelming infection in infancy or childhood. Mutations of the ACTH receptor (melanocortin-2 receptor, MC2R) account for approximately 25% of cases of familial glucocorticoid deficiency. Familial glucocorticoid deficiency without mutations of MC2R is called familial glucocorticoid deficiency type 2.

Results: Using SNP array genotyping, a locus involved in familial glucocorticoid deficiency type 2 was mapped to chromosome 21q22.1. Mutations in a gene encoding a 19-kDa single-transmembrane domain protein, now known as melanocortin-2 receptor accessory protein were identified. Melanocortin-2 receptor accessory protein interacts with MC2R and may play a role in the trafficking of MC2R from the endoplasmic reticulum to the cell surface.

Conclusion: Mutations in the melanocortin-2 receptor accessory protein gene are a cause of familial glucocorticoid deficiency.

This paper is a good example of how rare diseases in pediatric endocrinology are rapidly being dissected and assigned to a number of genetic etiologies. Here, two consanguineous families gave enough power to locate the gene, and expression studies identified melanocortin-2 receptor accessory protein among 30 candidate genes. Deleterious homozygous mutations were found in affected members of the family and in 21 of 104 families with familial glucocorticoid deficiency and no mutation in the ACTH receptor. Rare diseases lead to identification of important biochemical partners, here in the regulation of glucocorticoid secretion. It also shows that we will be waiting for at least one additional cause of familial glucocorticoid deficiency, so far mapped to chromosome 8.

Childhood obesity: early causes, late consequences

Early life risk factors for obesity in childhood: cohort study

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BMJ 2005;330:1357

Background: To identify risk factors in early life (up to 3 years of age) for obesity in children in the United Kingdom.

Methods: Prospective cohort study. Avon longitudinal study of parents and children, UK. Participants: 8,234 children in cohort aged 7 years and a sub-sample of 909 children (children in focus) with data on additional early growth-related risk factors for obesity. Main outcome measures: Obesity at age 7 years, defined as a body mass index $\geq$ 95th centile relative to reference data for the UK population in 1990.

Results: Eight of 25 putative risk factors were associated with a risk of obesity in the final models: parental obesity (both parents: adjusted odds ratio, 10.44), very early (by 43 months) body mass index or adiposity rebound (15.00), more than 8 h spent watching television per week at age 3 years (1.55), catch-up growth (2.60), standard deviation score for weight at age 8 months (3.13) and 18 months (2.65); weight gain in first year (1.06 per 100 g increase); birth weight, per 100 g (1.05); and short (<10.5 h) sleep duration at age 3 years (1.45).

Conclusion: Eight factors in early life are associated with an increased risk of obesity in childhood.

Adult socioeconomic, educational, social, and psychological outcomes of childhood obesity: a national birth cohort study

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Background: To assess adult socioeconomic, educational, social, and psychological outcomes of childhood obesity by using nationally representative data.

Methods: 1970 British birth cohort. 16,567 babies born in Great Britain 5–11 April 1970 and followed up at 5, 10, and 29–30 years. Main outcome measures: obesity at age 10 and 30 years, self-reported socioeconomic, educational, psychological, and social outcomes at 30 years. Odds ratios were calculated for the risk of each adult outcome associated with obesity in childhood only, obesity in adulthood only, and persistent child and adult obesity, compared with those obese at either period.

Results: Of the 8,490 participants with data on body mass index at 10 and 30 years, 4.3% were obese at 10 years and 16.3% at 30 years. Obesity in childhood only was not associated with adult social class, income, years of schooling, educational attainment, relationships, or psychological morbidity in either sex after adjustment for confounding factors. Persistent obesity was not associated with any adverse adult outcomes in men, though it was associated among women with a higher risk of never having been gainfully employed (odds ratio 1.9) and not having a current partner (2.0).

Conclusion: Obesity limited to childhood has little impact on adult outcomes. Persistent obesity in women is associated with poorer employment and relationship outcomes. Efforts to reduce the socioeconomic and psychosocial burden of obesity in adult life should focus on prevention of the persistence of obesity from childhood into adulthood.

Childhood obesity is clearly a hot topic and several new aspects are analyzed in papers selected in Martin Wabitch's chapter. These two papers show the power of carefully designed studies to detect the causes (or associated factors) and consequences of childhood obesity. In the study by Reilly et al., 8.5% of a cohort of more than 8,000 children were found to be obese at age 7 years. Among 25 putative risk factors in early life, 8 were identified that were independent predictors. Of note, some of them, such as parental weight are already well-known. The originality of the study was to focus on early life determinants of later obesity. In particular, television watching and sleep duration at age 3 years were independent predictors of obesity at age 7 years, pinpointing targets for intervention. However, for most of the other factors detected, the usual question of the relative roles of nature and nurture remains. In the study by Viner and Cole, long-term outcome of childhood obesity was assessed on psychosocial parameters in a national cohort. Several adjustments were made to take into account parental BMI, education and social class. Of interest, individuals with childhood obesity only had no adverse psychosocial outcomes at age 30. In contrast, women with childhood and adulthood obesity were more likely to have never been employed and to have no current partner. No similar observations were made in men. Importantly, negative findings included the lack of effect of obesity on education and on psychological morbidity. Since these results contrast with previous studies, one might question whether obesity is slowly becoming a more acceptable social standard, therefore limiting its psychosocial influence. Nevertheless, this powerful and well-designed study is important to us pediatricians, suggesting that it is not too late to intervene on obesity at the age of 10 years and that those who do not remain obese in adulthood have no long-lasting psychosocial consequences at age 30. However, it remains to be determined whether health consequences of childhood obesity can appear later in life.

Interindividual variation in posture allocation: possible role in human obesity

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Background: Obesity occurs when energy intake exceeds energy expenditure. Humans expend energy through purposeful exercise and through changes in posture and movement that are associated with the routines of daily life (called nonexercise activity thermogenesis – NEAT).

Results: To examine the role of NEAT in obesity, 10 lean and 10 mildly obese sedentary volunteers were recruited and their body postures and movements every half-second were measured for 10 days. Obese individuals were seated, on average, 2 h longer/day than lean individuals. Posture allocation did not change when the obese individuals lost weight or when lean individuals gained weight, suggesting that it is biologically determined.

Conclusion: If obese individuals adopted the NEAT-enhanced behaviors of their lean counterparts, they might expend an additional 350 cal (kcal)/day.

Weight gain and obesity occur when energy intake exceeds energy expenditure. 20–40% of daily energy expenditure is spent on physical activity, its most variable component. Physical activity can itself be subdivided between exercise and NEAT. NEAT is distinct from purposeful exercise and includes the energy expenditure of daily activities such as sitting, standing, walking, and talking. The authors have previously shown that when humans overeat, activation of NEAT helps to prevent weight gain. Here NEAT is separated into the thermogenesis associated with posture (standing, sitting, and lying) and that associated with movement (ambulation). They show that obese individuals have a markedly reduced NEAT due to the longer time spent seated than lean controls. This results in a 350-cal/day difference in energy expenditure. Although this result might be viewed as expected from daily observation, the study intriguingly shows that even after weight loss, obese individuals retain their previous habits and a decreased NEAT. However, there were marked inter-individual variations in NEAT suggesting that this is only one of the components of overall variation in energy expenditure. Once again the question of nature and nurture stands out. Is this variation genetically determined or is it educational? Can we intervene on this component of energy expenditure in obese individuals or should we advise people to vary their exercise activity thermogenesis? Encouraging endurance running seems to be particularly relevant since the genus *Homo* was found to be well fitted to this activity [4].

The never-ending complexity of GNAS

Deletion of the NESP55 differentially methylated region causes loss of maternal GNAS imprints and pseudohypoparathyroidism type Ib

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Background: Epigenetic defects in the imprinted GNAS cluster are associated with pseudohypoparathyroidism type Ib.

Results: In two kindreds with this disorder, deletions that remove the differentially methylated region encompassing exon NESP55 and exons 3 and 4 of the antisense transcript are reported. When inherited

from a female, either deletion abolishes all maternal GNAS imprints and derepresses maternally silenced transcripts.

Conclusion: The deleted region contains a cis-acting element that controls imprinting of the maternal GNAS allele.

Pseudohypoparathyroidism, first described by Albright et al. [5] is a model for how new layers of biological complexity appear when researchers think they have deciphered mechanisms. We knew that loss-of-function mutations on the maternally inherited GNAS gene explain most cases of pseudohypoparathyroidism type Ia and that most individuals with pseudohypoparathyroidism type Ib (without Albright's osteodystrophy) have a loss of methylation of exon A/B in the differentially methylated region of the GNAS gene. How this occurs is the topic of this paper. When pseudohypoparathyroidism type Ib is dominantly transmitted most individuals carry a deletion of STX16, an upstream regulator of exon A/B methylation. In contrast, in apparently sporadic cases and in some families with dominant inheritance, the basis of the disease has not been established. Here, the authors find a deletion of the differentially methylated region in two kindreds and analyze their consequences in terms of expression of the various transcripts encoded by the GNAS gene. The mechanism leading to PTH resistance in these patients is suggested but not directly demonstrated. Altogether this shows the complexity of the regulation of the GNAS gene and how diverse genetic mechanisms can lead to similar phenotypes.

Growth hormone in Turner syndrome: evidence at last

Impact of growth hormone supplementation on adult height in Turner syndrome: results of the Canadian randomized controlled trial

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Background: A randomized, controlled trial of GH supplementation to adult height in girls with short stature due to Turner syndrome was conducted in Canada.

Methods: One hundred and fifty-four girls with Turner syndrome, aged 7–13 years, were randomly assigned to 1 of 2 groups: (1) GH by s.c. injection 6 times per week (0.30 mg/kg/week), and (2) control, no GH treatment. Both cohorts received standardized sex steroid replacement starting at a chronological age of 13 years. Subjects were followed until protocol completion, defined as height velocity of less than 2 cm/year and bone age 14 years or greater. A subsequent protocol addendum requested follow-up safety and efficacy assessment in all patients at least 1 year after the last core protocol visit.

Results: One hundred and four patients completed the study (61 GH, 43 controls), and 50 withdrew (15 GH, 35 controls). At protocol completion, mean heights were 147.5 ± 6.1 (GH) and 141.0 ± 5.4 cm (controls), respectively ($p < 0.001$). Of those who completed the protocol, 59 (40 GH, 19 controls) had height data at least 1 year after protocol completion; in that group, mean heights were 149.0 ± 6.4 (GH) and 142.2 ± 6.6 cm (controls), respectively ($p < 0.001$). At protocol completion and follow-up, the mean height gain due to GH, estimated by analysis of covariance, was +7.2 and +7.3 cm, respectively (both $p < 0.001$).

Conclusion: This is the first evidence from a randomized, controlled trial to adult height that GH supplementation with induction of puberty at a near physiological age increases the adult height of girls with Turner syndrome.

The long-awaited Canadian randomized control trial is a major milestone after years of uncertainty. The investigators randomly assigned 154 children to treatment with GH or no treatment and both groups were observed until adult height was reached. They were able to follow 61/76 treated patients and 43/78 controls to adult height and observed a 7.2-cm difference between the 2 groups. The dose used (42 μ g/kg/day) corresponds to 78 and 84% of the upper limit of the dose range

recommended for Turner syndrome in the US and in Europe, respectively. The mean duration of treatment was 5.7 years, and puberty was induced pharmacologically at a chronological age of 13 years. A key issue in the interpretation of long-term results for GH treatment is the identification of bias due to the involuntary selection of good responders, who tend to stay in the trials versus poor responders who tend to be lost to follow-up, resulting in the overestimation of height gain. In the Canadian trial, the proportion of patients followed to adult height was high, and the data were analyzed carefully to rule out such bias. Such a high level of evidence has rarely been achieved for other pediatric GH indications and we are grateful to the Canadian investigators for their patience in carrying this trial through. This will allow clinicians to give evidence-based estimates of GH-induced height gains in Turner syndrome. However, the situation remains complex since we have to put the height gain into perspective, given the lack of evidence for a beneficial psychosocial effect and the need for long-term safety studies.

New hormones: just born

Renalase is a novel, soluble monoamine oxidase that regulates cardiac function and blood pressure

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Introduction: The kidney not only regulates fluid and electrolyte balance but also functions as an endocrine organ producing, among others, circulating erythropoietin and the enzyme renin.

Result: This is a report of a novel flavin adenine dinucleotide-dependent amine oxidase (renalase) that is secreted into the blood by the kidney and metabolizes catecholamines *in vitro*. In humans, renalase gene expression is highest in the kidney but is also detectable in the heart, skeletal muscle, and the small intestine. The plasma concentration of renalase is markedly reduced in patients with end-stage renal disease, as compared with healthy subjects. Renalase infusion in rats caused a decrease in cardiac contractility, heart rate, and blood pressure and prevented a compensatory increase in peripheral vascular tone.

Conclusions: Renalase is a novel amine oxidase that is secreted by the kidney, circulates in blood, and modulates cardiac function and systemic blood pressure.

Renalase is a novel flavin-adenine dinucleotide (FAD)-containing amine oxidase (MAO). It is critically dependent on FAD for oxidase activity, and has weak amino acid similarities to the mitochondrial enzymes MAO-A and MAO-B that metabolize intracellular catecholamines. Renalase metabolizes dopamine most efficiently, followed by epinephrine, and then norepinephrine. Unlike MAO-A and B, renalase is the only MAO secreted into the blood, and oxidase activity has been measured in human plasma and is mediated by vascular adhesion protein-1 (VAP-1). The kidney appears to be the major source of circulating renalase, since the protein is nearly absent in the blood of patients with end-stage renal disease. In that respect renalase is a new enzyme/hormone. Another source is the skeletal muscle, which expresses high renalase levels suggesting that the enzyme may play an important role in regulating local and perhaps systemic catecholamines. Recombinant renalase lowers blood pressure when administered *i.v.* The hemodynamic changes observed with renalase infusion are most likely mediated by circulating catecholamine degradation, which would be expected to decrease cardiac contractility and heart rate. It is also possible that a significant fall in circulating catecholamine levels results in venous dilatation and decreased venous return. The identification of this endocrine enzyme is an important step in developing a more detailed understanding of cardiovascular physiology and perhaps also in the quest for providing optimal treatment for patients with kidney disease.

Mast cells: A unique source of renin

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Introduction: A great deal of evidence favors the existence of numerous independent tissue-specific renin-angiotensin systems.

Methods and Results: Renin-specific antibodies demonstrate that cardiac mast cells contain renin. Extending this observation to the human mast cell line HMC-1, the paper shows that these mast cells also express renin. The HMC-1 renin RT-PCR product is 100% homologous to Homo sapiens renin. HMC-1 cells also contain renin protein. Renin released from HMC-1 cells is active; furthermore, HMC-1 cells are able to synthesize renin.

Discussion: Inasmuch as myocardial interstitium contains angiotensinogen and angiotensin-converting enzyme, renin was detected only in mast cells, it is postulated that the release of renin from cardiac mast cells is the pivotal event triggering local formation of angiotensin II. These findings provide a rationale for targeting mast cells in conjunction with rennin-angiotensin system inhibitors in the management of angiotensin II-related dysfunctions.

The rate-limiting step in the formation of angiotensin II is the proteolytic action of renin, which cleaves angiotensinogen to the intermediate angiotensin I. Traditionally renin is viewed as a circulating factor from the kidneys in response to decreased renal perfusion pressure and decreased delivery of NaCl. In addition to this conventional pathway, many tissues, including heart and brain, are capable of local angiotensin II production via tissue-specific renin-angiotensin systems. These findings indicate that mast cells that are present in many body organs, are a previously undescribed source of renin which, once released, activates local angiotensin. These results represent a unique paradigm for understanding local formation of angiotensin II in all organs. In the heart, mast cells are found in the interstitial space, where both angiotensinogen and ACE are present. With mast cell degranulation, as occurs in myocardial ischemia-reperfusion, renin released from mast cells acts directly on interstitial angiotensinogen which may then be converted to angiotensin II by available ACE. Thus, release of mast cell-derived renin is now suggested to be the pivotal event initiating local activation of this system. This system represents a more widespread principle of tissues harboring complete paracrine-autocrine-intracrine systems, previously thought to be restricted to circulating hormones.

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