RECENT ADVANCES

Advances in endocrinology

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New developments in endocrinology, in common with many specialised branches of medicine, have been dominated by advances in molecular genetics. Although these investigations relate to rare single gene disorders, they have resulted in major breakthroughs in many fields of endocrinology, significantly contributing to our understanding of the cellular mechanisms of hormone action. However it is not just the minute detail of single gene disorders and hormone action that has caught the attention of endocrinologists in recent years. This review will also focus on two broader health issues, one epidemiological and the other therapeutic.

The studies of Barker and colleagues have introduced the concept that disease in adult life has its origin in the fetal environment, and that this process can be attributed to changes in the programming of fetal endocrine axes. On the therapeutic agenda, the most dramatic advance in endocrinology occurred over a decade ago with the introduction of recombinant human growth hormone (hGH). This created wide interest in the use of growth hormone treatment in growth disorders with or without associated hypopituitarism. It is appropriate therefore to summarise our long term experiences with hGH as the first generation of these children achieve final height.

Finally the isolation and characterisation of a new hormone is a rare event in the 1990s. Therefore the discovery of the satiety factor, leptin, initially heralded as a potential cure for obesity, has attracted considerable media as well as scientific interest and merits due consideration in this review.

Molecular investigation and diagnosis

The tools to treat endocrine disorders have not changed dramatically in recent years, but what has changed is our understanding of the molecular abnormalities that generate these conditions. Endocrinologists have come to rely heavily on molecular diagnostic techniques. For many disorders, it is insufficient to characterise the disease only on clinical and biochemical grounds. A full appraisal requires a molecular diagnosis. There are many individual examples of significant molecular developments in endocrinology (table 1). A few examples will be discussed.

DISORDERS OF G-PROTEINS AND G-PROTEIN COUPLED RECEPTORS

The majority of polypeptide hormones signal to their target cells through G-protein coupled receptors (GPCRs), which are characterised by seven transmembrane helices connected by extracellular and intracellular loops. The N-terminal extracellular domain and one or more extracellular loops are involved in ligand binding while the intracellular loops and the C-terminal tail are coupled to specific G-protein complexes, composed of α , β , and γ subunits. There are many α subunit genes, whose protein products may be associated with stimulation (G_s) or inhibition (G_i). Activation of a GPCR leads to the dissociation of the G-protein trimer and modulation of the activity of enzymes, such as adenylate cyclase, or ion channels by the α subunit and/or the $\beta\gamma$ dimer. Over the last few years, it has been recognised that either gain or loss of function of G-protein intracellular signalling mechanisms due to mutations either in the GPCR or in the G-proteins can generate a wide spectrum of human endocrine disease.

For instance, both loss and gain of function have been described with mutations of genes encoding the receptors of many hormones (for example adrenocorticotrophic hormone (ACTH), growth hormone releasing hormone (GHRH), vasopressin, luteinising hormone, follicle stimulating hormone, and thyroid stimulating hormone). Mutations of the ACTH receptor cause resistance to ACTH which is characterised by glucocorticoid but not mineralocorticoid deficiency. Some cases are associated with missense, nonsense or frameshift mutations within the ACTH receptor (ACTHR) gene,^{35 53} while others with the features of ACTH resistance (for example the triple A syndrome) have no identified ACTHR mutation,⁵³ implying that postreceptor defects could be responsible. Loss of function in the GHRH receptor is responsible for the "little" mouse,⁵⁴ but has now been linked to familial isolated growth hormone deficiency, with a homozygous nonsense mutation in the GHRH receptor gene.¹ Nephrogenic diabetes insipidus (NDI) is associated with mutations in the V_2 vasopressin receptor³⁹ (X linked) or in the aquaporin-2 gene⁴¹ (autosomal recessive). Rapid identification of these mutations should lead to the early diagnosis and appropriate management of NDI, hopefully avoiding

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repeated episodes of dehydration, which can cause growth failure and mental retardation.

Mutations within the sixth transmembrane domain of the luteinising hormone receptor have recently been linked to male pseudohermaphroditism¹⁷ (female external genitalia, primary amenorrhoea, a short blind ending vagina, and absence of müllerian structures with a 46XY karyotype). It is possible however that other less critical luteinising hormone receptor mutations may account for milder clinical forms of this disorder (for example hypergonadotrophic hypogonadism and micropenis). In contrast activating mutations in the luteinising hormone receptor gene cause familial male precocious puberty which is inherited in an autosomal dominant, male limited pattern.¹⁶ A missense mutation, again in the sixth transmembrane domain of the luteinising hormone receptor, has been found most commonly. Interestingly, no clinical manifestations have been reported in female carriers of the mutant receptor gene.

Endocrine disease is also generated by abnormal G-protein signal transduction. One such example is pseudohypoparathyroidism, a disease with a wide clinical spectrum charac-

terised by insensitivity to parathyroid hormone with or without osteodystrophy and generalised hormone resistance. Defects can occur in the G_{α} protein^{33, 34} or in the association of G-protein subunits with adenylate cyclase or further down the signalling pathway in the intracellular action of cAMP.

G_{α} mutations may generate simultaneously loss of function in one tissue (pseudohypoparathyroidism) and gain in another (gonadotrophin independent precocious puberty), as described in two unrelated boys.⁵⁵ Expression of the mutant protein showed that it was rapidly degraded at 37°C (that is a loss of function mutation) explaining the pseudohypoparathyroidism phenotype, but at 32°C (compatible with the temperature in the testis) the protein was constitutively activated causing the precocious puberty by stimulating Leydig cell cAMP formation. Another example of disease associated with an activating mutation of the G_{α} gene is the McCune-Albright syndrome,⁵⁶ classically defined by polyostotic fibrous dysplasia, café-au-lait spots, sexual precocity, and other hyperfunctional endocrinopathies. Activating mutations of the G_{α} gene have been found in various tissues, which appear to cause

Table 1 Examples of a wide range of conditions, relevant to endocrine practice, where the target gene has recently been identified, are shown. References are given for each disorder

System	Mutated target gene	Endocrine disorder
Growth	GHRH receptor GH-1 Pit-1 GH receptor IGF-I	Familial GH deficiency ¹ Short stature with bioinactive GH ² Combined pituitary hormone deficiency ³⁻⁵ GH insensitivity in familial short stature ⁶ Intrauterine and postnatal growth retardation with IGF-I deficiency ⁷
Sex differentiation, puberty, and fertility	SHOX SRY DAX1 SOX9 AMH receptor KAL LH receptor: Activation Inactivation FSH receptor: Activation Inactivation LH FSH Oestrogen receptor Aromatase P450 (CYP19) Gs-protein	Turner's syndrome, idiopathic short stature ⁸ XY female with gonadal dysgenesis ⁹⁻¹¹ Hypogonadotropic hypogonadism ¹² Campomelic dysplasia with sex reversal ¹³ Persistent müllerian structures in a male ¹⁴ X linked Kallmann's syndrome ¹⁵ Familial male precocious puberty ¹⁶ Male pseudohermaphroditism ¹⁷ Sustained spermatogenesis in a male with treated hypopituitarism ¹⁸ Hypergonadotropic ovarian dysgenesis ¹⁹ Male hypogonadism ²⁰ Female infertility ²¹ Tall stature in male ²² Tall stature in a male and female pseudohermaphroditism ²³ McCune-Albright syndrome ²⁴
Thyroid	TSH receptor: Inactivation Activation	Familial hypothyroidism ²⁵ Non-autoimmune hyperthyroidism ^{26, 27} Hyperfunctioning thyroid adenomas ²⁸ Hypoparathyroidism ^{29, 30}
Calcium homeostasis	PTH Calcium sensing receptor: Inactivation Activation Gs-protein	Familial hypocalciuric hypercalcaemia and severe neonatal primary hyperparathyroidism ³¹ Familial hypocalcaemia ³² Pseudohypoparathyroidism ^{33, 34} Familial ACTH resistance ^{35, 36} X linked congenital adrenal hypoplasia ¹²
Adrenal axis	ACTH receptor DAX1	Autosomal dominant neurogenic diabetes insipidus ^{37, 38} X linked nephrogenic diabetes insipidus ³⁹ Autosomal recessive nephrogenic diabetes insipidus ^{40, 41}
Water balance	Prepro-AVP-NP11 V ₂ vasopressin receptor Aquaporin-2	Achondroplasia ^{42, 43} Thanatophoric dysplasia ⁴⁴ Hypochondroplasia ⁴⁵ Diastrophic dysplasia ⁴⁶ Achondrogenesis type 1B ⁴⁷ Atelosteogenesis type II ⁴⁸ X linked hypophosphatemic rickets ⁴⁹ Jansen's metaphyseal chondrodysplasia ⁵⁰ Persistent hyperinsulinaemic hypoglycaemia of infancy ^{51, 52}
Bones	FGFR-3 Diastrophic dysplasia sulphate transporter	
Pancreas	PEX PTH receptor Sulphonylurea receptor and K _{ATP} channel	

ACTH=adrenocorticotrophic hormone; AMH = anti-müllerian hormone; AVP = arginine vasopressin; FGFR = fibroblast growth factor receptor; FSH=follicle stimulating hormone; GH=growth hormone; GHRH=growth hormone releasing hormone; IGF=insulin-like growth factor; LH=luteinising hormone; NP = neurophysin; PTH=parathyroid hormone; TSH=thyroid stimulating hormone.

widespread disease manifestations if they occur early in embryogenesis, but result in focal disease if activated later in development.

ABNORMALITIES IN GROWTH HORMONE SIGNAL TRANSDUCTION

Interest in the wider therapeutic applications of growth hormone has paralleled a rapid advance in our understanding of the cellular mechanisms of growth hormone action. In contrast to the G-protein coupled receptors listed above, the growth hormone receptor (GHR) belongs to the class I cytokine receptor superfamily, whose members possess extracellular, transmembrane and intracellular domains, but lack any intrinsic tyrosine kinase activity. Signalling molecules are recruited from the cytoplasm on activation of the receptor to associate with the intracellular domain. In the case of the GHR, the pivotal signalling molecule is the Janus kinase, Jak 2. Its activation causes a sequence of phosphorylation events down distinct signalling pathways, leading to phosphorylation of mitogen activated protein kinase, signal transducers and activators of transcription, and insulin receptor substrate and eventually changes in nuclear events.⁵⁷

It has been recognised for many years that mutations within the GHR gene,⁵⁸ that prevent expression of GHR, can generate growth hormone insensitivity, characterised by extreme short stature and a phenotype similar to that of severe growth hormone deficiency. This condition (Laron's syndrome) is very rare, usually follows an autosomal recessive inheritance pattern but has been identified throughout the world. An increasing number of mutations within the GHR are being recognised,⁵⁹ including instances where the GHR is expressed but dysfunctional⁶⁰ or where the GHR appears to be functioning normally but the signal transduction pathway is defective.⁶¹

Most importantly this rare disorder has led to the recognition that lesser degrees of GHR dysfunction may be relevant to some cases of idiopathic short stature,⁶² where clear endocrine deficits have not been identified. Although screening has revealed a low incidence of heterozygous GHR mutations in idiopathic short stature (ISS), the concept of a range of growth hormone sensitivity has been introduced. Such data demonstrate how rapidly the molecular characterisation of a candidate disease, in this case growth hormone insensitivity, can then be used to assess another common disorder, namely ISS.

Overall these developments in our understanding of the molecular basis for defects in hormone receptor signalling have provided: (i) the potential for prenatal diagnosis in affected families, (ii) the opportunity to make an accurate molecular diagnosis where clinical/biochemical uncertainty exists, and most importantly (iii) the recognition of the key receptor sequences or signalling molecules relevant to human disease and therefore the potential to design specific treatments.

Fetal origin of adult disease

Restricted fetal and early infant growth has been associated with the later development of significant risk factors for cardiovascular disease, in particular hypertension.⁶³ These relationships were initially demonstrated using retrospective data on cohorts born earlier this century in the UK, on whom detailed obstetric and perinatal growth data were available (reviewed in Barker⁶⁴). The association between low birth weight and postnatal development of hypertension has been demonstrated prospectively in rat models.⁶⁵ The relationship has not been confirmed in UK adolescents.⁶⁶ The timing of the growth restriction and hence the body phenotype generated are proposed to influence the risk of other conditions in addition to raised blood pressure. Thus syndrome X (hypertension, impaired glucose tolerance, non-insulin-dependent diabetes mellitus (NIDDM), hypertriglyceridaemia, low high density lipoprotein-cholesterol, and central obesity) is more likely to occur in those with mid-trimester growth retardation, who are thin at birth but regain normal weight by one year, while abnormal lipid and haemostatic profiles occur in those with poor growth in late pregnancy, who have reduced abdominal circumference, are short at birth and remain so postnatally.⁶⁴ Although many studies support the fetal origin hypothesis, it has been suggested that selection bias, confounding variables, and inconsistency between studies have not been fully eliminated.^{67 68} It is important therefore to continue to rigorously test the hypothesis, and to unravel the mechanisms that generate these associations.

It is postulated that alterations in fetal nutrition lead to permanent changes in hormone sensitivity.⁶⁹ Evidence that programming within the insulin and growth hormone-insulin-like growth factor (IGF) axes to generate a multihormone resistant syndrome is accumulating both from studies in animals^{70 71} and in children who have suffered intrauterine growth retardation (IUGR).^{72 73} Such studies have demonstrated that growth retarded neonates can develop altered insulin:glucose ratios, indicative of insulin resistance, raised growth hormone and IGF-I concentrations, suggesting insensitivity within the growth axis, and raised IGF binding protein-1 levels, that may reduce IGF-I bioavailability. Persistence of these abnormalities may permanently reset homeostatic control of hormonal axes, leading to a clinically significant insulin resistant syndrome in adulthood. In both UK and Indian children, serum IGF-I concentrations in early to mid-childhood were higher in taller and heavier children, but also inversely related to birth weight.⁷⁴ Thus children with low birth weight had higher IGF-I levels than would be expected for their size. The development of NIDDM in subjects, who had disproportionate IUGR but became obese adults, provides further evidence for a link between prenatal and postnatal growth.⁷⁵ Additionally there is some evidence that the adrenal axis may be programmed: adrenal androgen and glucocorticoid excretion, assessed in mid-childhood,

was raised in those with low birth weights.⁷⁶ One caveat to these data is that all these common health problems will usually occur in those without a history of IUGR; fetal environment is but one of many other factors (lifestyle, activity, diet, and smoking) that contribute.

This is an area with considerable implications not only to obstetricians and neonatologists but to all paediatricians. An understanding of the key events involved in reprogramming an endocrine axis offers the possibility that therapeutic manipulation of the prenatal or postnatal environment could modify the incidence of some of the most common disorders of Western society.

Efficacy of long term growth hormone treatment

Although molecular investigation has dominated most specialised areas of paediatrics, accumulating knowledge on practical therapeutic issues should not be overlooked. This is of particular relevance to the field of growth hormone treatment. It is just over a decade since the first recombinant (r)hGH was issued a license in the UK for the treatment of growth hormone deficiency. Since that time, many other conditions associated with growth failure have been treated with growth hormone. As many of these children are now approaching final height, it is pertinent to review our experiences with growth hormone and decide just how effective such treatment has been.

The final height prognosis of those with isolated growth hormone deficiency or multiple pituitary hormone deficiency has improved over the last decade, in part related to the optimisation of growth hormone administration schedules, including adjustment of growth hormone dose according to body size. Most will achieve a height within the normal range, but still below that predicted from parental target range.⁷⁷⁻⁷⁹ In a study of children who received rhGH from the prepubertal years to near adult height, factors that influenced the adult height achieved included the duration of growth hormone treatment, age at starting treatment (the younger the child at the start, the greater the adult height), height at the start and growth rate in the first year.⁷⁹ These data clearly indicate that prompt diagnosis and initiation of growth hormone treatment are the principal factors that can be modified in order to improve further the efficacy of growth hormone in growth hormone deficiency.

Growth hormone has been licensed to promote growth in Turner's syndrome since 1989; this indication accounts for up to 25% of the caseload in growth clinics. Studies from the USA, Canada, and Europe are now reporting final heights after four to 10 years of growth hormone treatment⁸⁰⁻⁸⁴: the parameter used to judge success has been the gain in height over that projected from disease specific growth charts at the start of treatment, a technique that can be compromised by secular trends and application of national standards to a different population. Nevertheless growth hormone treatment would appear to promote an average gain in height above that predicted of 3-8cm,

with individuals gaining at best >15 cm of extra height. Factors influencing this gain include the timing of pubertal induction and the length of growth hormone treatment. The adjuvant use of oxandrolone would appear to provide only a small additional benefit to final height. Despite these studies, the optimal times for starting growth hormone treatment and for the induction of puberty have not been defined, nor has the wide range of individual response to growth hormone been adequately explained. Coordinated treatment policies across the growth centres in the UK may help to address these issues over the next decade.

Final height data after growth hormone are also being reported in those children, variably classified as short normal, normal variant, or idiopathic short stature.⁸⁵⁻⁸⁷ These cohorts are not growth hormone deficient by classical criteria. Their early response to growth hormone was encouraging, but in some studies this has not been maintained to final height, with insignificant increments in height above that predicted (for example mean 2.5 cm in girls and 2.8 cm in boys⁸⁷). Those with chronic renal failure⁸⁸ and with IUGR⁸⁹ have also been targeted for growth hormone treatment, but as yet final height data are not available in significant numbers. Certainly growth rate can be improved by supraphysiological doses of growth hormone, but experience with short normal children might suggest that we should be cautious in our predictions for final height.

Biosynthetic hGH would appear to be a safe agent with undisputed benefit in congenital and acquired growth hormone deficiency. In fact the lifelong metabolic consequences of growth hormone deficiency have led to its introduction as replacement therapy in the adult growth hormone deficient population. In all non-growth hormone deficient growth disorders, there would appear to be a range of sensitivity to growth hormone with potential benefits to many of the recipients. It remains our task to work out which *individuals* with which conditions are the most appropriate targets for treatment.

Regulation of leptin

In 1994 a new hormone, exclusively expressed in adipose tissue, was characterised and cloned from the genetically obese (ob/ob) mouse.⁹⁰ The hormone, named leptin (leptos = thin), was rapidly identified as a potent modulator of appetite and thermogenesis. Its administration to the ob/ob mouse resulted in a marked reduction in food intake and hence weight loss.⁹¹⁻⁹⁴ The leptin receptor was soon cloned,⁹⁵ and mutations in this gene result in the obese phenotype of the db/db mouse.^{96,97} It had been postulated for many years that a factor produced in fat may exist, which would be capable of signalling to central mechanisms governing appetite. Leptin appears to be a candidate for such a role, but its actions may be even more significant in that it also has an additional direct effect on the reproductive system.⁹⁸ It is also present in significant amounts in amniotic fluid and cord blood,⁹⁹ the

level in the latter correlating with birth and placental weight, suggesting that it may have a role in fetal growth and metabolism.

Leptin mRNA is expressed exclusively in adipose tissue, while the leptin receptor (a member of class I cytokine family) is found in a range of tissues, which include the hypothalamus, choroid plexus but also lung and gonadal tissues.¹⁰⁰ Circulating concentrations of leptin in humans are most closely correlated to body fat mass^{101 102} and are acutely regulated by starvation, insulin, and glucocorticoids (reviewed in Saladin *et al.*¹⁰³). It is postulated that leptin exerts its central effects on appetite through a long feedback loop from the periphery to the central nervous system to decrease the expression of neuropeptide-Y and hence appetite¹⁰⁴ (reviewed in Campfield *et al.*¹⁰⁵ and Rohrer-Jeanraud *et al.*¹⁰⁶). Its potential therapeutic role as an anorectic factor, however, is confounded by the fact that the majority of cases of human obesity appear to be associated with a leptin resistant state. Nevertheless it is thought that 5% of those with obesity may have a leptin concentration lower than expected for their fat mass.¹⁰⁷ Of particular interest to paediatricians is the recent discovery of the first cases of the Ob gene mutation, analogous to that found in the ob/ob mouse, in two massively obese children from a consanguineous family.¹⁰⁸ Although very rare, this diagnosis should be considered in children, who develop progressive severe obesity in infancy.

The ob/ob mouse is recognised to be sterile, but it was found that parenteral administration of leptin not only induced weight loss but also stimulated the reproductive system¹⁰⁹ and restored fertility.^{98 110} A series of experiments in normal rats and mice have demonstrated conclusively that leptin administration is also able to bring forward the age at which reproductive ability is achieved, despite lowering body weight.^{111–113} It will also partially reverse the relative hypopituitarism induced by starvation of mice.¹¹⁴ These effects are likely to be mediated through actions on the gonadotrophin releasing hormone pulse generator, but could also involve stimulation directly of the gonads. In humans, hypothalamic hypogonadism can be induced by weight loss and anorexia, while the obese child can proceed into a relatively early puberty. We have recently shown that leptin increases through the prepubertal years in both sexes, a rise that is related to body mass index and to increasing age.¹¹⁵ Leptin concentrations peak in early puberty in both sexes, then decline to adulthood in boys, but show a late rise in girls. It is possible therefore that leptin may play a part in facilitating progress into puberty. In a more general context, it may act as a marker of the interaction between nutrition and maturation.

Summary

Molecular genetics will continue to help us to make precise diagnoses. At present, the expertise to achieve this for a specific disease is often exclusive to one unit with a research interest. It will be important to establish a coordinated approach at a supraregional level to provide

molecular diagnosis for rare disorders as a fast reliable clinical service. In addition understanding the molecular mechanisms of disease is likely to direct a search for new treatments. For instance, calcium channel blockers have been used in nesidioblastosis to reduce the hypersecretion of insulin, as a result of the recognition of the role that calcium has in the function of the β -cell ATP sensitive K^+ channel.¹¹⁶

Although the potential benefits of hGH are now being clearly defined in a range of growth disorders, the treatment is invasive and expensive. It is likely that future endocrine therapeutic developments could include slow release growth hormone preparations, orally active growth hormone mimetics, or even hormone production from an ectopic viral cDNA vector. The next "advances in endocrinology" will also reveal whether leptin will have a therapeutic role in appetite control or even the modulation of pubertal development.

- 1 Wajnrach MP, Gertner JM, Harbison MD, *et al.* Nonsense mutation in the human growth hormone-releasing hormone receptor causes growth failure analogous to the little (lit) mouse. *Nat Genet* 1996;12:88–90.
- 2 Takahashi Y, Hidesuke K, Okumura Y, *et al.* Short stature caused by a mutant growth hormone. *N Engl J Med* 1996;334:432–6.
- 3 Tatsumi K, Miyai K, Notomi T, *et al.* Cretinism with combined hormone deficiency caused by a mutation in the PIT1 gene. *Nat Genet* 1992;1:56–8.
- 4 Radovick S, Nations M, Du Y, *et al.* A mutation in the POU-homeodomain of Pit-1 responsible for combined pituitary hormone deficiency. *Science* 1992;257:1115–8.
- 5 Pfäffle RW, DiMattia GE, Parks JS, *et al.* Mutation of the POU-specific domain of Pit-1 and hypopituitarism without pituitary hypoplasia. *Science* 1992;257:1118–21.
- 6 Ayling RM, Ross R, Townner P, *et al.* A dominant-negative mutation of the growth hormone receptor causes familial short stature. *Nat Genet* 1997;16:13–4.
- 7 Woods KA, Camacho-Hübner C, Savage MO, *et al.* Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *N Engl J Med* 1996;335:1363–7.
- 8 Rao E, Weiss B, Fukami M, *et al.* Pseudoautosomal deletions encompassing a novel homeobox gene cause growth failure in idiopathic short stature and Turner syndrome. *Nat Genet* 1997;15:54–63.
- 9 Pivnick EK, Wachtel S, Woods D, *et al.* Mutations in the conserved domain of SRY are uncommon in XY gonadal dysgenesis. *Hum Genet* 1992;90:308–10.
- 10 Jager RJ, Harley VR, Pfeiffer RA, *et al.* A familial mutation in the testis determining gene SRY shared by both sexes. *Hum Genet* 1992;90:350–5.
- 11 Hawkins JR, Taylor A, Goodfellow PN, *et al.* Evidence for increased prevalence of SRY mutations in XY females with complete rather than partial gonadal dysgenesis. *Am J Hum Genet* 1992;51:979–84.
- 12 Muscatelli F, Strom TM, Walker AP, *et al.* Mutations in the DAX-1 gene give rise to both X-linked adrenal hypoplasia congenita and hypogonadotropic hypogonadism. *Nature* 1994;372:672–6.
- 13 Foster JW, Dominguez-Steglich MA, Guioli S, *et al.* Campomelic dysplasia and autosomal sex reversal caused by mutations in a SRY-related gene. *Nature* 1994;372:525–9.
- 14 Imbeaud S, Faure E, Lamarre I, *et al.* Insensitivity to anti-Müllerian hormone due to a mutation in the human anti-Müllerian hormone receptor. *Nat Genet* 1995;11:382–8.
- 15 Hardelin JP, Leveilliers J, Young J, *et al.* Xp22.3 deletions in isolated familial Kallmann's syndrome. *J Clin Endocrinol Metab* 1993;76:827–31.
- 16 Shenker A, Laue L, Kosugi S, *et al.* A constitutively activating mutation of the luteinizing hormone receptor in familial male precocious puberty. *Nature* 1993;365:652–4.
- 17 Kremer H, Kraaij R, Toledo SP, *et al.* Male pseudohermaphroditism due to a homozygous missense mutation of the luteinizing hormone receptor gene. *Nat Genet* 1995;9:160–4.
- 18 Gromoll J, Simoni M, Nieschlag E. An activating mutation of the follicle-stimulating hormone receptor autonomously sustains spermatogenesis in a hypophysectomized man. *J Clin Endocrinol Metab* 1996;81:1367–70.
- 19 Aittomäki K, Lucena JLD, Pakarinen P, *et al.* Mutation in the follicle-stimulating hormone receptor gene causes hereditary hypergonadotropic ovarian failure. *Cell* 1995;82:959–68.

- 20 Weiss J, Axelrod L, Whitcomb RW, *et al.* Hypogonadism caused by a single amino acid substitution in the beta subunit of luteinizing hormone. *N Engl J Med* 1992;326:179–83.
- 21 Matthews CH, Borgato S, Beck Peccoz P, *et al.* Primary amenorrhoea and infertility due to a mutation in the beta-subunit of follicle-stimulating hormone. *Nat Genet* 1993;5:83–6.
- 22 Smith EP, Boyd J, Frank GR, *et al.* Estrogen resistance caused by a mutation in the estrogen receptor gene in a man. *N Engl J Med* 1994;331:1056–61.
- 23 Morishima A, Grumbach MM, Simpson ER, *et al.* Aromatase deficiency in male and female siblings caused by a novel mutation and the physiological role of estrogens. *J Clin Endocrinol Metab* 1995;80:3689–98.
- 24 Weinstein LS, Shenker A, Gejman PV, *et al.* Activating mutations of the stimulatory G protein in the McCune-Albright syndrome. *N Engl J Med* 1991;325:1688–95.
- 25 Sunthornthepvarakul T, Gottschalk ME, Hayashi Y, *et al.* Resistance to thyrotropin caused by mutations in the thyrotropin-receptor gene. *N Engl J Med* 1995;332:155–60.
- 26 Duprez L, Parma J, Van Sande J, *et al.* Germline mutations in the thyrotropin receptor gene cause non-autoimmune autosomal dominant hyperthyroidism. *Nat Genet* 1994;7:396–401.
- 27 Kopp P, Van Sande J, Parma J, *et al.* Brief report: congenital hyperthyroidism caused by a mutation in the thyrotropin-receptor gene. *N Engl J Med* 1995;332:150–4.
- 28 Parma J, Duprez L, Van Sande J, *et al.* Somatic mutations in the thyrotropin receptor gene cause hyperfunctioning thyroid adenomas. *Nature* 1993;365:649–51.
- 29 Arnold A, Horst SA, Gardella TJ, *et al.* Mutations of the signal peptide encoding region of preproparathyroid hormone gene in isolated hypoparathyroidism. *J Clin Invest* 1990;86:1084–7.
- 30 Parkinson DB, Thakker RV. A donor splice site mutation in the parathyroid hormone gene is associated with autosomal recessive hypoparathyroidism. *Nat Genet* 1992;1:149–52.
- 31 Pollak MR, Brown EM, Wu Chou Y-H, *et al.* Mutations in the human Ca^{2+} -sensing receptor gene cause familial hypocalcemic hypercalciuria and neonatal severe hyperparathyroidism. *Cell* 1993;75:1297–303.
- 32 Pollak MR, Brown EM, Estep HL, *et al.* Autosomal dominant hypocalcemia caused by a calcium-sensing receptor gene mutation. *Nat Genet* 1994;8:303–7.
- 33 Patten JL, Johns DR, Valle D, *et al.* Mutations in the gene encoding the stimulatory G protein of adenylate cyclase in Albright's hereditary osteodystrophy. *N Engl J Med* 1990;322:1412–9.
- 34 Weinstein LS, Gejman DV, Freidman E, *et al.* Mutations of the Gs alpha subunit gene in Albright's hereditary osteodystrophy detected by denaturing gradient gel electrophoresis. *Proc Natl Acad Sci U S A* 1990;87:8287–90.
- 35 Tsigos C, Arai K, Hung W, *et al.* Hereditary isolated glucocorticoid deficiency is associated with abnormalities of the adrenocorticotropin receptor gene. *J Clin Invest* 1993;92:2458–61.
- 36 Weber A, Toppari J, Harvey RD, *et al.* Adrenocorticotropin receptor gene mutations in familial glucocorticoid deficiency: relationships with clinical features in four families. *J Clin Endocrinol Metab* 1995;80:65–71.
- 37 Ito M, Mori Y, Oiso Y, *et al.* A single base substitution in the coding region for neurophysin II associated with familial central diabetes insipidus. *J Clin Invest* 1991;87:725–28.
- 38 Bahnsen U, Oosting P, Swaab DF, *et al.* A missense mutation in the vasopressin-neurophysin precursor gene co-segregates with human autosomal dominant neurohypophyseal diabetes insipidus. *EMBO J* 1992;11:19–23.
- 39 Bichet DG, Arthus MF, Loneragan M, *et al.* X-linked nephrogenic diabetes insipidus mutations in North America and the Hopewell hypothesis. *J Clin Invest* 1993;92:1262–8.
- 40 Deen PMT, Verdijk MAJ, Knoers NVAM, *et al.* Requirement of human renal water channel aquaporin-2 for vasopressin-dependent concentration of urine. *Science* 1994;264:92–5.
- 41 Van Lieburg AF, Verdijk MAJ, Knoers NVAM, *et al.* Patients with autosomal nephrogenic diabetes insipidus: Homozygous for mutations in the aquaporin 2 water-channel gene. *Am J Hum Genet* 1994;55:648–52.
- 42 Rousseau F, Bonaventure J, Legeal-Mallet L, *et al.* Mutations in the gene encoding fibroblast growth factor receptor-3 in achondroplasia. *Nature* 1994;371:252–4.
- 43 Shiang R, Thompson LM, Zhu YZ, *et al.* Mutations in the transmembrane domain of FGFR-3 cause the most common genetic form of dwarfism, achondroplasia. *Cell* 1994;78:335–42.
- 44 Tavormina PL, Shiang R, Thompson LM, *et al.* Thanatophoric dysplasia (types I and II) caused by distinct mutations in fibroblast growth factor receptor 3. *Nat Genet* 1995;9:321–8.
- 45 Bellus GA, McIntosh I, Smith EA, *et al.* A recurrent mutation in the tyrosine kinase domain of fibroblast growth factor receptor 3 causes hypochondroplasia. *Nat Genet* 1995;10:357–9.
- 46 Hästbacka J, de la Chapelle A, Mahtani MM, *et al.* The diastrophic dysplasia gene encodes a novel sulfate transporter: positional cloning by fine-structure linkage disequilibrium mapping. *Cell* 1994;78:1073–87.
- 47 Superti-Furga A, Hästbacka J, Wilcox WR, *et al.* Achondrogenesis type IB is caused by mutations in the diastrophic dysplasia sulphate transporter gene. *Nat Genet* 1996;12:100–2.
- 48 Hästbacka J, Superti-Furga A, Wilcox WR, *et al.* Atelosteogenesis type II is caused by mutations in the diastrophic dysplasia sulfate-transporter gene (DTDST): evidence for a phenotypic series involving three chondrodysplasias. *Am J Hum Genet* 1996;58:255–62.
- 49 The HYP Consortium. A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. *Nat Genet* 1995;11:130–6.
- 50 Schipani E, Kruse K, Juppner H. A constitutively active mutant PTH-PTHrP receptor in Jansen-type metaphyseal chondrodysplasia. *Science* 1995;268:98–100.
- 51 Thomas PM, Cote GT, Wohlk N, *et al.* Mutations in the sulfonyleurea receptor gene in familial persistent hyperinsulinemic hypoglycemia of infancy. *Science* 1995;268:426–9.
- 52 Kane C, Shepherd RM, Squires PE, *et al.* Loss of functional K_{ATP} channels in pancreatic beta cells causes persistent hyperinsulinemic hypoglycemia of infancy. *Nat Med* 1996;2:1344–7.
- 53 Tsigos C, Arai K, Latronico AC, *et al.* A novel mutation of the adrenocorticotropin receptor (ACTH-R) gene in a family with the syndrome of isolated glucocorticoid deficiency, but no ACTH-R abnormalities in two families with the triple A syndrome. *J Clin Endocrinol Metab* 1995;80:2186–9.
- 54 Godfrey P, Rahal JO, Beamer WG, *et al.* GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. *Nat Genet* 1993;4:227–32.
- 55 Iiri T, Herzmark P, Nakamoto JM, *et al.* Rapid GDP release from G_q in patients with gain and loss of endocrine function. *Nature* 1994;371:164–8.
- 56 Shenker A, Weinstein LS, Moran A, *et al.* Severe endocrine and non-endocrine manifestations of the McCune-Albright syndrome associated with activating mutations of the stimulatory G protein, Gs. *J Pediatr* 1993;123:509–18.
- 57 Carter-Su C, Schwartz J, Smith LS. Molecular mechanism of growth hormone action. *Annu Rev Physiol* 1996;58:187–207.
- 58 Rosenfeld RG, Rosenbloom AL, Guevara-Aguirre J. Growth hormone insensitivity (GHI) due to primary GH receptor deficiency. *Endocr Rev* 1994;15:369–90.
- 59 Sobrier M-L, Dastot F, Duquesnoy P, *et al.* Nine novel growth hormone receptor gene mutations in patients with Laron Syndrome. *J Clin Endocrinol Metab* 1997;82:435–7.
- 60 Duquesnoy P, Sobrier M-L, Duriez B, *et al.* A single amino acid substitution in the extracellular domain of the human growth hormone (GH) receptor confers familial GH resistance (Laron syndrome) with positive GH-binding activity by abolishing receptor homodimerization. *EMBO J* 1994;13:1386–95.
- 61 Freeth JS, Ayling RM, Whatmore AJ, *et al.* Human skin fibroblasts as a model of growth hormone (GH) action in GH receptor-positive Laron's syndrome. *Endocrinology* 1997;138:55–61.
- 62 Goddard AD, Covello R, Luoh SM, *et al.* Mutations of the growth hormone receptor in children with idiopathic short stature. The Growth Hormone Insensitivity Study Group. *N Engl J Med* 1995;333:1093–8.
- 63 Barker DJP. *Fetal and infant origins of adult disease*. London: BMJ Publishing Group, 1992.
- 64 Barker DJP. Fetal origins of coronary heart disease. *BMJ* 1995;311:171–4.
- 65 Langley SC, Jackson AA. Increased systolic blood pressure in adult rats induced by fetal exposure to maternal low protein diets. *Clin Sci* 1994;86:217–22.
- 66 Matthes JWA, Lewis PA, Davies DP, *et al.* Relation between birth weight at term and systolic blood pressure in adolescence. *BMJ* 1994;308:1074–7.
- 67 Paneth N, Susser M. Early origin of coronary heart disease (the "Barker" hypothesis). *BMJ* 1994;310:411–2.
- 68 Kramer MS, Joseph KS. Enigma of fetal/infant-origins hypothesis. *Lancet* 1996;348:1254–5.
- 69 Barker DJP, Gluckman PD, Godfrey KM, *et al.* Fetal nutrition and cardiovascular disease in adult life. *Lancet* 1993;341:938–41.
- 70 Woodall SM, Breier BH, Johnston BM, *et al.* A model of intrauterine growth retardation caused by chronic maternal undernutrition in the rat: effects on the somatotrophic axis and postnatal growth. *J Endocrinol* 1996;150:231–42.
- 71 Frampton RJ, Jonas HA, MacMahon RA, *et al.* Failure of IGF-I to affect protein turnover in muscle from growth-retarded neonatal rats. *J Dev Physiol* 1990;13:125–33.
- 72 Gluckman PD, Cutfield W, Harding JE, *et al.* Metabolic consequences of intrauterine growth retardation. *Acta Paediatr Suppl* 1996;417:3–6.
- 73 Hofman PL, Cutfield WS, Robinson EM, *et al.* Insulin resistance in short children with intrauterine growth retardation. *J Clin Endocrinol Metab* 1997;82:402–6.
- 74 Fall CH, Pandit AN, Law CM, *et al.* Size at birth and plasma insulin-like growth factor-1 concentrations. *Arch Dis Child* 1995;73:287–93.
- 75 Phillips DI, Barker DJ, Hales CN, *et al.* Thinness at birth and insulin resistance in adult life. *Diabetologia* 1994;37:150–4.
- 76 Clark PM, Hindmarsh PC, Shiell AW, *et al.* Size at birth and adrenocortical function in childhood. *Clin Endocrinol (Oxf)* 1996;45:721–6.
- 77 Rikken B, Massa GG, Wit JM. Final height in a large cohort of Dutch patients with growth hormone deficiency treated with growth hormone. The Dutch Growth Hormone Working Group. *Horm Res* 1995;43:135–7.
- 78 Price DA, Ranke MB. Final height following growth hormone treatment. In: Ranke MB, Gunnarsson R, eds.

- Progress in growth hormone therapy—5 years of KIGS.* J & J Verlag Mannheim, 1994: 129–144.
- 79 Blethen SL, Baptista J, Kuntze J, *et al.* Adult height in growth hormone (GH)-deficient children treated with biosynthetic GH. *J Clin Endocrinol Metab* 1997;**82**:418–20.
 - 80 Rosenfeld RG, Frane J, Attie KM, *et al.* Six-year results of a randomized prospective trial of human growth hormone and oxandrolone in Turner syndrome. *J Pediatr* 1992;**121**: 49–55.
 - 81 Rochiccioli P, Battin J, Bertrand AM, *et al.* Final height in Turner syndrome patients treated with growth hormone. *Horm Res* 1995;**44**:172–6.
 - 82 Van den Broeck J, Massa GG, Attanasio A, *et al.* Final height after long-term growth hormone treatment in Turner syndrome. *J Pediatr* 1995;**127**:729–35.
 - 83 Nilsson KO, Albertsson-Wikland K, Alm J, *et al.* Improved final height in girls with Turner's syndrome treated with growth hormone and oxandrolone. *J Clin Endocrinol Metab* 1996;**81**:635–40.
 - 84 Taback SP, Collu T, Deal CL, *et al.* Does growth-hormone supplementation affect adult height in Turner syndrome? *Lancet* 1996;**348**:25–7.
 - 85 Loche S, Cambiaso P, Setzu S, *et al.* Final height after growth hormone therapy in non-growth-hormone deficient children with short stature. *J Pediatr* 1994;**125**:196–200.
 - 86 Wit JM, Boersma B, de Muinck Keizer-Schrama SM, *et al.* Long-term results of growth hormone therapy in children with short stature, subnormal growth rate and normal growth hormone response to secretagogues. *Clin Endocrinol (Oxf)* 1995;**42**:365–72.
 - 87 Hindmarsh PC, Brook CGD. Final height of short normal children treated with growth hormone. *Lancet* 1996;**348**: 13–6.
 - 88 Tonshoff B, Fine RN. Recombinant human growth hormone for children with renal failure. *Adv Ren Replace Ther* 1996;**3**:37–47.
 - 89 Chernausek SD, Breen TJ, Frank GR. Linear growth in response to growth hormone treatment in children with short stature associated with intrauterine growth retardation: the National Cooperative Growth Study experience. *J Pediatr* 1996;**128**:S22–7.
 - 90 Zhang Y, Proenca R, Maffei M, *et al.* Positional cloning of the mouse obese gene and its human homologue. *Nature* 1994;**372**:425–32.
 - 91 Halaas JL, Gajiwala KS, Maffei M, *et al.* Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* 1995;**269**:543–6.
 - 92 Pelleymounter MA, Cullen MJ, Baker MB, *et al.* Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* 1995;**269**:540–3.
 - 93 Campfield LA, Smith FJ, Guisez Y, *et al.* Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* 1995;**269**: 546–9.
 - 94 Weigle DS, Bukowski TR, Foster DC, *et al.* Recombinant ob protein reduces feeding and body weight in the ob/ob mouse. *J Clin Invest* 1995;**96**:2065–70.
 - 95 Tartaglia LA, Dembski M, Weng X, *et al.* Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995;**83**:1263–71.
 - 96 Chen H, Charlat O, Tartaglia LA, *et al.* Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996;**84**:491–5.
 - 97 Lee G-H, Proenca R, Montez JM, *et al.* Abnormal splicing of the leptin receptor in diabetic mice. *Nature* 1996;**379**: 632–5.
 - 98 Chehab FF, Lim ME, Lu R. Correction of the sterility defect in homozygous obese female mice by treatment with the human recombinant leptin. *Nat Genet* 1996;**12**:318–20.
 - 99 Schubring C, Kiess W, Englarp P, *et al.* Levels of leptin in maternal serum, amniotic fluid, and arterial and venous cord blood: relation to neonatal and placental weight. *J Clin Endocrinol Metab* 1997;**82**:1480–3.
 - 100 Cioffi JA, Shafer AW, Zupancic TJ, *et al.* Novel B219/OB receptor isoforms: possible role of leptin in hematopoiesis and reproduction. *Nat Med* 1997;**2**:585–9.
 - 101 Maffei M, Halaas J, Ravussin E, *et al.* Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* 1995;**1**:1155–61.
 - 102 Considine RV, Sinha MK, Heiman ML, *et al.* Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* 1996;**334**:292–5.
 - 103 Saladin R, Staels B, Auwerx J, *et al.* Regulation of ob gene expression in rodents and humans. *Horm Metab Res* 1996; **28**:638–41.
 - 104 Stephens TW, Basinski M, Bristow PK, *et al.* The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature* 1995;**377**:530–2.
 - 105 Campfield LA, Smith FJ, Burn P. The OB protein (leptin) pathway—a link between adipose tissue mass and central neural networks. *Horm Metab Res* 1996;**28**:619–32.
 - 106 Rohner-Jeanraud F, Cusin I, Sainsbury A, *et al.* The loop system between neuropeptide Y and leptin in normal and obese rodents. *Horm Metab Res* 1996;**28**:642–8.
 - 107 Blum WF, Englarp P, Hanitsch S, *et al.* Clinical studies of serum leptin—an overview. *Proceedings of the European Leptin Symposium*. Giessen, Germany, 1997.
 - 108 Montague CT, Sadaf Farooqi I, Whitehead JP, *et al.* Congenital leptin deficiency is associated with severe early-onset obesity in humans. *Nature* 1997;**387**:903–8.
 - 109 Barash IA, Cheung CC, Weigle DS, *et al.* Leptin is a metabolic signal to the reproductive system. *Endocrinology* 1996;**137**:3144–7.
 - 110 Mounzih K, Lu R, Chehab FF. Leptin treatment rescues the sterility of genetically obese ob/ob males. *Endocrinology* 1997;**137**:1190–3.
 - 111 Chehab FF, Mounzih K, Lu R, *et al.* Early onset of reproductive function in normal female mice treated with leptin. *Science* 1997;**275**:88–90.
 - 112 Ahima RS, Dushay J, Flier SN, *et al.* Leptin accelerates the onset of puberty in normal female mice. *J Clin Invest* 1997; **99**:391–5.
 - 113 Cheung CC, Thornton JE, Kuijper JL, *et al.* Leptin is a metabolic gate for the onset of puberty in the female rat. *Endocrinology* 1997;**138**:855–8.
 - 114 Ahima RS, Prabakaran D, Mantzoros C, *et al.* Role of leptin in the neuroendocrine response to fasting. *Nature* 1996; **382**:250–2.
 - 115 Clayton PE, Gill MS, Hall CM, *et al.* Serum leptin through childhood and adolescence. *Clin Endocrinol (Oxf)* 1997;**46**: 727–33.
 - 116 Lindley KJ, Dunne MJ, Kane C, *et al.* Ionic control of beta cell function in nesidioblastosis. A possible therapeutic role for calcium channel blockade. *Arch Dis Child* 1996;**74**: 373–8.