Population Genetics and Pharmacogenetics

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There has been much to report from genetic epidemiology over the last 12 months. The use of common genetic variants (>1% allele frequency) to understand mechanisms of disease continues to make rapid progress through advances in high-throughput genotyping capacity (see Klein et al. below) and huge study sizes (see Guo et al.). There are also some advances in analytical methodology, such as the concept of Mendelian Randomization (see Davey Smith et al.). However, it is still unclear how best to analyze the mountains of genotype data that can now be rapidly generated. Conservative approaches that choose only the most statistically significant results will no doubt throw out many true positive findings. In addition to solely mathematical approaches (to choose the best candidates potentially from all 10 million human single nucleotide polymorphisms (SNPs)), there is increasing debate over statistical methods that include consideration of prior knowledge as to which genes are involved, i.e. a Bayesian approach to analysis [1]. A recent perspective on the genetics of type 2 diabetes showed that knowledge of rare mutations that have substantial effects (e.g. which cause severe insulin resistance, maturity-onset diabetes of the young (MODY) or neonatal diabetes) can support the findings of studies of common genetic variants [2]. The authors concluded that we may not yet be close to understanding the complex polygenic etiology of type 2 diabetes, but at least we may be at the end of the beginning.

While several of these studies may be focused on disease outcomes outside the field of Pediatric Endocrinology, the expected moderate and lifelong effects of common genetic variants on human physiology would predict that many of these genetic effects may be detectable in childhood, and may have much relevance to hormonal activity, growth, and development. From a clinical perspective, the use of DNA analysis for common genetic variants continues to grow, particularly to identify subjects at high risk for drug side effects [3]. There are still no current examples of routine pharmacogenetics in the Pediatric Endocrine Clinic, but if the results of Dos Santos et al. (see below) are replicated, genetic epidemiology may be coming soon to a growth clinic near you!

New genetic associations – general

Gene-gene interaction between PPAR γ 2 and ADR β 3 increases obesity risk in children and adolescents

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Background: Multiple genes are likely to be involved in obesity and the search for gene $\times$ gene interactions is the subject of growing interest. The authors therefore explored the synergistic contribution to obesity risk of the two polymorphisms: Pro12Ala of the peroxisome proliferator-activated receptor γ 2 (PPAR γ 2) gene and Trp64Arg of the β -adrenergic receptor-3 (ADRB3) gene.

Methods: A sex- and age-matched case-control study was performed in Spanish children and adolescents: 185 obese and 185 control children (aged 5–18 years) from the Navarra region were recruited through Departments of Pediatrics. Obesity (case) was defined as a BMI >97th percentile according to Spanish growth reference data. Physical activity was assessed by face-to-face interviews. A validated physical activity questionnaire was used to calculate an 'activity metabolic equivalent index', which is a measure of physical exercise during 1 week. A conditional logistic regression model was used for the analysis, and adjustment was made for sex, age and physical activity.

Results: Obesity risk was higher in PPAR γ 2 Ala allele carriers than in non-carriers (adjusted odds ratio (OR) = 2.18). When family history of obesity was taken into account, the risk of obesity was higher (OR = 2.59). The adjusted OR for obesity linked to both polymorphisms (PPAR γ 2 and ADRB3) was 5.30. The OR for carriers of both polymorphisms was 19.5 after adjustment for family history of obesity.

Conclusion: These results show a synergistic effect between the PPAR γ 2 Pro12Ala and the ADRB3 Trp64Arg polymorphisms for obesity risk in children and adolescents.

It is now generally accepted that the PPAR γ 2 Pro12Ala polymorphism has functional effects and is overall protective for type 2 diabetes [4]. However, among more than 100 publications in humans on the impact of the PPAR γ 2 Pro12Ala polymorphism with various outcomes, including type 2 diabetes, insulin resistance, obesity, cardiovascular diseases and cancer, the directions of the effects are often highly discordant, even for the same outcome. Among other reasons, these discrepancies could be explained by gene–gene or gene–environment interaction. Gene–environment interactions have been described, with dietary polyunsaturated to saturated fat ratio, and also with physical activity [5]. Interaction has been described with the intrauterine environment (birth weight) suggesting that PPAR γ 2 may influence the programming of adult health [6]. The current results from Ochoa et al. are an example of a gene–gene interaction, where the effects of polymorphisms at two different genes taken individually are less important than their combination. The same interaction was shown in an earlier study [7], where PPAR γ 2 Ala carriers were more obese than Pro/Pro subjects, but only if they also carried the ADRB3 Arg63 allele. Because both genes play important regulatory roles in adipocytes, there are several plausible mechanisms by which these variants could interact to enhance obesity risk. Incorporating interactions, whether gene–gene or gene–environment, may enhance our ability to detect genetic effects and our understanding of the etiology of obesity. However, we have to remember that to have sufficient power to reliably detect even the simplest 2 $\times$ 2 factorial interactive effects requires a study size around 4-fold the size of a study to detect single, or main effects [8].

PPAR γ 2 Pro12Ala variant is associated with greater insulin sensitivity in childhood obesity

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Background: Peroxisome proliferator-activated receptor- γ 2 (PPAR γ 2) regulates the transcription of various target genes. Several genetic variants of the PPAR γ 2 gene have been identified, of which the Pro12Ala, a missense mutation in exon 2, is common in Caucasians. Studies of association between this variant and complex traits, such as obesity, insulin sensitivity, and T2DM, are often conflicting.

Methods: The association between the PPAR γ 2 Pro12Ala polymorphism with insulin sensitivity was investigated in 200 obese Italian children (mean age 10.38 $\pm$ 2.8 years). Insulin resistance was assessed by the homeostasis model assessment. Multiple linear regression was used to test the association between Pro12Ala and quantitative traits.

Results: Seventeen percent of all children carried the 12Ala allele. This frequency is similar to that in other Caucasian populations. Fasting insulin levels were lower in 12Ala carriers compared with Pro/Pro (p = 0.008). Consistent with this finding, the x12Ala genotype was associated with lower insulin resistance (p = 0.023).

Conclusion: These results show that in obese children, the 12Ala variant is associated with greater insulin sensitivity. Obesity is one of the most important risk factors for cardiovascular diseases and type 2 diabetes, however obese children who carry the 12Ala allele may be protected by the effect of this allele on insulin resistance.

This paper shows that in obese children, carriers of the PPAR γ 2 Pro12Ala variant may be protected against insulin resistance. Together with the paper of Ochoa et al. described above, these results raise the intriguing issue that this polymorphism could favor obesity, and yet be associated with a reduced risk of type 2 diabetes. This discrepancy could be partly explained by the modifying effects of both environment and other genetic factors on this polymorphism, as discussed above. However, there is

an alternative explanation for the apparent dissociation between obesity and type 2 diabetes risks in carriers of the Pro12Ala variant. These subjects are generally considered to have genetically increased PPAR γ 2 activity. Similarly, treatment with thiazolidinediones (TZDs), which specifically bind to and activate PPAR γ s, improves insulin sensitivity but increases weight gain and fat deposition at the same time. A recent mouse model shows that PPAR γ 2 regulates the link between nutrition and insulin sensitivity; PPAR γ 2 null mice fed normal diets were more insulin resistant despite similar weight, body composition, food intake, energy expenditure, and adipose tissue morphology to controls [9]. Finally, these results highlight the inequity of obese children with regard to adverse metabolic consequences, and suggest that it may be possible one day to genetically identify those obese children with particularly high predisposition to insulin resistance and type 2 diabetes.

Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene

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Background: Androgen receptor function is crucial for normal male sexual differentiation. Polymorphic CAG and GGN segments in the androgen receptor gene have been shown to regulate androgen receptor function. These 2 polymorphisms were investigated for possible association with the two most common male congenital malformations, hypospadias and cryptorchidism.

Methods: CAG and GGN genotypes were identified by direct sequencing of DNA from patients diagnosed with hypospadias ($n = 51$) and cryptorchidism ($n = 23$) and controls ($n = 210$). The subjects with hypospadias were divided into subgroups of glanular ($n = 21$), penile ($n = 13$), and penoscrotal ($n = 17$) hypospadias.

Results: Median GGN lengths were significantly higher (24 vs. 23 repeats) between both subjects with cryptorchidism vs. controls ($p = 0.001$), and between those with penile hypospadias vs. either controls ($p = 0.003$) or vs. glanular and penoscrotal hypospadias combined ($p = 0.018$). The frequency of cases with GGN 24+ vs. GGN = 23 or less, differed significantly between those with cryptorchidism (65/35%) vs. controls (31/54%; $p = 0.012$), and between subjects with penile hypospadias (69/31%) vs. either controls ($p = 0.035$) or vs. glanular or penoscrotal hypospadias combined (32/55%; $p = 0.056$). There were no significant differences in CAG lengths between the cases and controls.

Conclusions: Longer androgen receptor gene GGN length was associated with increased risks of cryptorchidism and penile hypospadias, both of which are conditions of low androgen activity.

Rare deleterious mutations in the androgen receptor gene (on chromosome Xq11), which severely or moderately impair the receptor function, result in the complete or partial androgen insensitivity syndrome. More common genetic variants in the androgen receptor gene have been shown to encode variable receptor activity, and these polymorphisms have been used in a large number of studies for association with other androgen-related phenotypes, such as prostate cancer, bone mineral density and male pattern baldness [10]. The current study found consistent results in 2 separate groups of subjects with hypospadias and cryptorchidism. However not all studies have found consistent results. The current study could not confirm a previous finding associating the CAG genotype to moderately severe undermasculinization [11]. In a recent meta-analysis, both CAG and GGN segments were significantly associated with risk for prostate cancer, but the overall difference was small [12]. As usual, common genetic variants are likely to be only one factor contributing to these outcomes, and their effects will be clarified by consideration of other potential factors, such as other rare genetic mutations [11], or possibly environmental exposure to androgen-inhibiting chemicals [13].

Genetic regulation of birth weight and fasting glucose by a common polymorphism in the islet cell promoter of the glucokinase gene

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Background: Deleterious mutations in the glucokinase gene impair insulin secretion and result in fasting hyperglycemia. Mothers and offspring with these rare mutations have altered birth weights; however the role of common genetic variation in glucokinase is not known. The G-30A polymorphism in the glucokinase gene β -cell-specific promoter is present in 30% of the population, and is variably associated with type 2 diabetes risks.

Methods: The following healthy UK Caucasian groups were studied: 1,763 adult subjects and 755 pregnant women with fasting plasma glucose; and 2,689 mother/child pairs with birth weight data.

Results: The –30A allele was associated with a 0.061-mmol/l higher fasting glucose in non-pregnant adults ($p = 0.003$), and 0.075-mmol/l higher fasting glucose in pregnant women ($p = 0.003$). Mothers –30A allele was associated with a 64-g (25–102 g) increase in offspring birth weight ($p = 0.001$). No effect of fetal glucokinase genotype was detected.

Conclusion: The increase in offspring birth weight observed in the 30% of mothers carrying an –30A allele could be explained by an elevated fasting glucose during pregnancy. This study shows that common genetic variation in mothers can affect both glucose levels and birth weight.

This study illustrates that the multifactorial etiology of normal birth weight includes not only fetal genes and environmental factors, but also maternal genes and potentially their mutual interactions. Rare mutations in the glucokinase gene cause maturity-onset diabetes of the young type 2 (MODY2) by reducing insulin secretion. Mothers with MODY2 have elevated glucose levels and larger birth weight offspring, but conversely mutation-affected offspring have lower birth weights [14]. This present study shows for the first time that a common genetic variation in *glucokinase* affects both fasting plasma glucose in the mother and fetal growth. Increased fetal growth in variant carrying mothers is likely to be the result of the increase in maternal glucose. Indeed, when they adjusted for the glucose concentration, the effect of the mother's genotype on birth weight was no longer significant. This is a nice example of a common genetic variant in the mother that alters the fetal, or intrauterine, environment and thereby influences fetal growth. Unfortunately, in contrast to the observations with MODY2, they did not see any effect of fetal genotype on fetal growth, even after allowing for the effects of the mother's genotype. The authors did not find any other common variants ($>5\%$) in the *glucokinase* coding region on direct sequencing of 100 subjects. In insulinoma cells, previous studies have shown that mutagenesis of a 10-bp sequence including the *glucokinase* gene (–30) site reduced GCK transcription by 22%. Important functional genetic variations may lie outside, and even distant to, the gene coding regions [15]. Any such variants which influence glucose homeostasis will be candidates for association with several clinical outcomes, including type 2 diabetes, gestational diabetes, and size at birth.

Is glutamate decarboxylase-2 (GAD2) a genetic link between low birth weight and subsequent development of obesity in children?

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Background: Low birth weight is associated with increased risk of obesity and type 2 diabetes. As suggested by the fetal insulin hypothesis, low birth weight could be partly due to genetic factors that impair insulin secretion/sensitivity during fetal development, as shown for glucokinase, the ATP-sensitive K^+ channel subunit Kir6.2, and the small heterodimer partner genes. Glutamic acid decarboxylase-2

(GAD2) gene overexpression impairs insulin secretion in animals. Polymorphisms in the GAD2 gene were recently associated with morbid obesity in adults.

Methods: The association between the GAD2 gene A-243G functional polymorphism with fetal growth, insulin secretion, food intake, and risk of obesity was investigated in 635 severely obese French Caucasian children from three medical centers.

Results: Firstly, the reported adult obesity risk was confirmed with the -243G variant in this childhood case-control study (odds ratio = 1.25; $p = 0.04$). Moreover, GG children had 270 g lower birth weight ($p = 0.009$) and 1.5 cm lower birth length ($p = 0.013$) than AA children. There was a linear relationship between birth weight and childhood BMI in AA genotype ($p = 0.00001$), whereas this relation was quadratic (U-shaped) in AG/GG genotypes ($p = 0.0009$). G allele carriers had a trend towards lower insulinogenic index, with a 25% reduction in insulin secretion in response to glucose load, compared with A allele carriers ($p = 0.09$). Eighteen percent of GG obese children reported a binge-eating phenotype vs. 5.7% of AA children ($p = 0.04$).

Conclusion: These results confirm the association between the -243G allele variant and increased obesity risk, and suggest that GAD2 may play a role in the complex mechanisms linking birth weight to later metabolic diseases risk. It could involve the pleiotropic effect of insulin on fetal growth and on feeding behavior in postnatal life.

In a previous study in morbidly obese adults (reviewed in last year's *Yearbook*), the authors showed that this -243G variant was functional (it increased GAD2 promoter activity 6-fold), and was associated with obesity, increased hunger scores, and also reduced insulin secretion [16]. They now confirm the association of the variant allele with obesity and binge-eating behavior in a population of obese children. Interestingly, the obesity-related variant was also associated with lower birth weight and shorter birth length. This statistically and clinically significant effect on birth size could reflect impaired insulin secretion during fetal life. Glutamic acid decarboxylase catalyses the formation of γ -aminobutyric acid (GABA), which modulates insulin release, and also acts centrally to stimulate appetite. This study provides an example of a gene having a pleiotropic effect on growth-related phenotypes, yielding to opposite phenotypes at birth and later in life, in this case due to different mechanisms of action during prenatal and postnatal life. If true, this gene could not only represent a genetic link between low birth weight and subsequent obesity, but would also be expected to convey a major risk for type 2 diabetes, by conferring both obesity and reduced insulin secretion.

The effects of the ACE gene insertion/deletion polymorphism on glucose tolerance and insulin secretion in elderly people are modified by birth weight

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Background: The I allele of an insertion/deletion (I/D) polymorphism in the angiotensin-converting enzyme (ACE) gene has been associated with reduced risk of micro- and macrovascular complications of type 2 diabetes. The authors examined whether the ACE I/D polymorphism could explain or modify the association between low birth weight and adulthood glucose tolerance.

Methods: Plasma glucose and insulin levels were measured after a standard oral glucose tolerance test in a group of 423 men and women, ages 65–75 years, with measurements at birth recorded.

Results: Subjects with the I allele (69 homozygotes; 210 heterozygotes) were born at an earlier gestational age (p -trend = 0.006) and, adjusted for gestational age, had higher birth weight (p -trend = 0.008) and birth length (p -trend = 0.02). At age 65–75 years, subjects with the I allele had lower 120 min glucose levels at (p -trend = 0.04) and a greater insulin response (p -trend = 0.03 for 30 min insulin; $p = 0.06$ for AUC insulin). However, the associations between the ACE genotype and adulthood insulin secretion were only present in people with low birth weight (p for interaction birth weight * ACE genotype on insulin at 30 min = 0.003 and on insulin area under the curve = 0.05).

Conclusions: The ACE I allele was associated with shorter gestational age and higher birth weight. The association between the ACE I allele and increased adult insulin secretion was confined to subjects with

the lowest tertile of birth weight. Interactions between genotype and intrauterine environment may result in changes in gene expression.

Angiotensinogen is expressed in adipose tissue, and patients with morbid central obesity have high levels of ACE, plasma renin activity, and aldosterone, sodium retention and potassium loss [17]. Furthermore, the beneficial effects of ACE inhibition and angiotensin-receptor blockade on the development of type 2 diabetes in large clinical trials suggest a pathophysiological role of the adipose-tissue renin-angiotensin system in the metabolic syndrome. The *ACE* gene insertion/deletion (I/D) polymorphism is due to the presence or absence of a 287-bp fragment inside intron 16. The I allele is associated with lower circulating and tissue ACE levels, and genetic association studies show it has beneficial renal and cardiovascular-protective effects, as would be predicted by the clinical effects of ACE inhibition [18]. In addition, ACE is active in the placenta where it may redistribute placental circulation and possibly reduce nutrient transfer to the fetus. Indeed the maternal D allele is associated with preeclampsia, which is characterized by restricted uteroplacental circulation and fetal growth restriction. Future studies should examine whether it is the maternal or the fetal ACE genotype, or both, that influences placental function and birth weight. This study confirmed the results of an early study of young adults that reported the I allele was associated with greater 30-min insulin levels, but only in those born small, and not in those born appropriate for gestational age [19]. However the relevance of these findings to risks for type 2 diabetes and its complications is unclear, as low birth weight was also associated with higher 30-min insulin levels. Genetic association studies for type 2 diabetes should ideally take account of potential confounding factors; not only current diet and physical activity, but also on fetal and early postnatal environment and growth.

Late life metabolic syndrome, early growth, and common polymorphism in the growth hormone and placental lactogen gene cluster

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Background: Growth patterns in fetal life and infancy are associated with the metabolic syndrome and cardiovascular disease in later life. The gene cluster on chromosome 17q23 encoding growth hormone (GH), placental lactogens (chorionic somatomammotropins, CSH), and placental GH, is a potential candidate.

Methods: Common genetic variation in GH-CSH was investigated in relation to weight at birth and at 1 year, and features of the metabolic syndrome in 594 men and 409 women, aged 59–72 years, from Hertfordshire (UK). At the CSH1.01 locus the following allele groups were observed: T, D1, and D2.

Results: Male carriers of the T allele group had the same birth weight but were significantly lighter at 1 year than non-carriers. As adults, these men were shorter, and had higher blood pressures, fasting insulin (T/T 66% higher than D2/D2) and triglyceride levels, and insulin and glucose levels during a glucose tolerance test. The associations of birth weight and 1-year weight with metabolic syndrome traits were independent of the CSH1.01 effects.

Conclusion: Common diversity in GH-CSH correlates with low 1-year weight and with features of the metabolic syndrome in adulthood. GH-CSH genotype may add to but does not explain the associations between low body weight at birth and in infancy, and the metabolic syndrome.

Polymorphism in the growth hormone gene, weight in infancy, and adult bone mass

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Background: Gene-environment interactions during early life could play an important role in the etiology of osteoporosis and fracture in adulthood.

Methods: Common single nucleotide polymorphisms (SNPs) in the human GH (GH1) gene were studied for association with weight in infancy, adult bone mass and bone loss rates, and circulating GH profiles. From the Hertfordshire Cohort Study, 205 men and 132 women, aged 61–73 years, were included, and over 4 years had measurement of bone mineral density by dual energy X-ray absorptiometry. Twenty-four-hour circulating GH profiles were measured in a subset of 71 subjects. Two SNPs in the GH gene (one in the promoter region and one in intron 4) were examined.

Results: Rare allele homozygotes at loci GH1 A5157G and T6331A had lower baseline bone density and accelerated bone loss. There was a significant interaction between the effects of genotype and weight at 1 year on bone loss ($p = 0.04$). The rare alleles at both loci were also additively associated with lower circulating GH levels in men.

Conclusion: Common diversity in the GH1 region could predispose to osteoporosis via lower levels of GH expression. The interaction with infant weight implies that early growth patterns may play a role in the effect of GH1 genotype on bone loss.

In the context of growing interest in the early origins of adult health, more studies are investigating the role of genetic factors in the associations of birth weight and early growth to chronic disease in adulthood, such as these studies from the same research group. Day et al. showed in men that a common variant in this region was associated to low weight at 1 year and also to increased adverse metabolic syndrome features in adulthood. Dennison et al. studied another variant, which was associated with adult bone density and bone loss, and seemed to interact with the effect of weight at 1 year. Indeed, individuals who carried the unfavorable homozygous genotype, and who also grew poorly in fetal life and infancy, could have accelerated bone loss and increased osteoporosis risk. As expected, only weight at 1 year and not birth weight showed association or interaction with the genetic variants in these two studies. Neither of the studies showed that the genotype was responsible for the association between early growth and adult phenotypes; in particular, the genotypes and early weight gain were associated with different features of the adult metabolic syndrome. These studies do not therefore provide any more support for the ‘thrifty genotype’ theory that states that the link between early growth and later disease would be due to genes that influence both phenotypes.

New mechanisms

Characterization of an adrenocorticotropin (ACTH) receptor promoter polymorphism leading to decreased adrenal responsiveness to ACTH

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Background: The ACTH receptor regulates adrenal cortisol secretion.

Methods: Sequencing of a BAC clone obtained from the German Resource Center and Primary Database revealed a base exchange from the consensus sequence at position –2 of exon 1 of the human ACTH receptor gene (from CTC to CCC). The genotype frequency in 1,266 unrelated healthy men was 80.2% for CTC/CTC, 19.0% for CTC/CCC, and 0.8% for CCC/CCC. The clinical significance was investigated by a 6-hour ACTH stimulation test with increasing ACTH(1–24) doses in normal subjects.

Results: In vitro studies showed that the variant was associated with a lower promoter activity in both basal state (CCC, $73 \pm 4\%$; CTC, $100 \pm 5\%$; $p = 0.02$) and with forskolin-stimulation (CCC,

143 ± 13%; CTC, 194 ± 15%; $p = 0.0008$). In vivo, CCC/CCC subjects showed a blunted cortisol response compared with individuals (cortisol AUC in CCC/CCC: 12,176 ± 966 vs. CTC/CTC: 16,334 ± 1051 nmol/l · min; $p < 0.03$). CCC/CCC subjects also showed a higher ACTH/cortisol ratio ($p < 0.05$) after CRH stimulation, suggesting decreased adrenal responsiveness to endogenous ACTH.

Conclusions: The ACTH receptor promoter polymorphism results in a lower promoter activity in vitro, and is associated with a lower cortisol secretion to prolonged ACTH stimulation in vivo. This polymorphism might influence cortisol homeostasis under stress conditions.

The ACTH receptor is a G protein-coupled receptor, also known as melanocortin receptor type 2. Its gene *MCR2* maps to chromosome 18p11.2 and comprises two exons, of which exon 1 is not translated but contains the transcriptional start site. The upstream promoter region contains response elements for steroidogenic factor 1 and other cAMP response elements. Rare deleterious mutations in the coding region of the *MC2R* gene result in familial glucocorticoid deficiency (FGD). Other common variants in the gene have been described, but are not known to be functional. The authors found a variant 2 bp upstream of the transcriptional start site, showed it had a minor allele frequency of 20% in a large population study, and characterized it in detail through both in vitro based on transfection experiments, as well as in vivo as demonstrated by ACTH and CRH stimulation tests. They found consistently that the CCC variant encoded for a receptor that was less sensitive to ACTH. Other than possibly explaining some of the inter-individual variance in cortisol levels in response to ACTH administration, the clinical relevance of this finding is yet unknown. Because glucocorticoids are not stored in the adrenal cortex, cortisol secretion is dependent on acute regulation of adrenal steroidogenesis. Common genetic variations in CRF, ACTH and their receptors, could therefore influence both altered responsiveness to acute stress and also longer-term risks for insulin resistance and cardiovascular disease [20].

A polymorphism in type-I deiodinase is associated with circulating free insulin-like growth factor I levels and body composition in humans

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Background: There is a complex interaction between the thyroid and GH/IGF-1 axes. The authors recently reported thyroid hormone level association with two genetic variants in type I deiodinase (D1a and D1b), which converts T4 to T3. Haplotype 2 (aT-bA) was associated with lower type I deiodinase activity; haplotype 3 (aC-bG) was associated with higher type I deiodinase activity. Whether these type I deiodinase genetic variations were associated with the IGF-I system was investigated.

Methods: In 156 anonymous blood donors and 350 elderly men, the type I deiodinase haplotypes were analyzed for association with circulating IGF-I and free IGF-I levels, and with muscle strength and body composition in the elderly population.

Results: In blood donors, haplotype 2 (lower type I deiodinase activity) was associated with higher levels of free IGF-I. In elderly men, haplotype 2 also showed an allele dose increase in free IGF-I levels and an allele dose decrease in serum T3 levels, independent of age; haplotype 2 carriers also had higher lean body mass and muscle strength. In blood donors, total IGF-I levels were negatively related to T4 and free T4 levels; and positively related to T3:T4 and T3:rT3 ratios. Free IGF-I was negatively correlated with T4 and T4-binding globulin, and was positively correlated with the T3:T4 ratio.

Conclusion: A genetic variant (haplotype 2) related to decreased type I deiodinase activity was associated with higher free IGF-I levels. The relevance of this association is supported by increased muscle strength and muscle mass in carriers of haplotype 2 in the elderly population. The association of haplotype 2 with serum T3 levels in this population suggests a relative increase in its contribution to circulating T3 in old age.

It is known that GH therapy increases serum T3 levels and decreases T4 and rT3, suggesting that GH, or IGF-1, may stimulate type I deiodinase activity. Conversely, T4 therapy stimulates both GH secretion and IGF-1 production. Genetic variants in either pathway may therefore have multiple effects on hormone levels and body composition. Accordingly, this study tested a large and potentially confusing

Fig. 1. Schematic diagram of the biochemical inter-relationships between the thyroid and IGF-1 axes.

number of hormones and their ratios, but made a number of consistent findings: type I deiodinase haplotype associations with IGF-1 levels were supported by consistent associations with lean body mass and muscle strength; and haplotype 2 and haplotype 3 showed opposite associations, in keeping with their opposite effects on type I deiodinase activity. However, the genetic and biochemical associations disagreed. Genetically lower type I deiodinase activity, and subsequently expected *higher* T4:T3 ratio, was associated with higher free IGF-1. In contrast, higher free and total IGF-1 levels were strongly associated with *lower* T4:T3 ratio. The authors suggest that the latter observation reflects the effect of IGF-1 on type I deiodinase activity. In addition, the balance between effects of type I deiodinase haplotype and IGF-1 on each other's axis could vary with age. We look forward to performing studies when validated genetic markers for each of the hormone pathways are available.

New paradigms

Possible genomic imprinting of three human obesity-related genetic loci

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Background: Parent-of-origin effects of genetic factors on obesity have been investigated only recently, with inconsistent results. The authors aimed to detect potentially imprinted genetic loci that influence obesity.

Method: A genome-wide parent-of-origin linkage analysis was performed under an allele-sharing model for discrete traits and under a family regression model for obesity-related quantitative traits. The study included a European-American sample of 1,297 individuals from 260 families, and 391 microsatellite markers were analyzed. Two smaller independent samples were used for replication (370 German subjects from 89 families, and 277 African-American subjects from 52 families).

Results: For discrete-trait analysis, a maternal effect in chromosome region 10p12 was found across the three samples. For the pooled sample, the LOD scores were 5.69 (single-point) and 4.52 (multi-point). For quantitative-trait analysis, the strongest evidence for a maternal effect was found in region 12q24 (single-point LOD of 2.85; multipoint LOD of 4.01 for BMI and 3.69 for waist circumference) and for a paternal effect in region 13q32 (single-point LOD of 4.79; multipoint LOD of 3.72 for BMI), in the European American population.

Conclusion: Parent specific inheritance of certain genetic factors appears to influence human obesity risk, perhaps due to imprinted genes.

This is the third genome-wide parent-of-origin linkage analysis for obesity-related traits, and it stresses the importance of taking account of potential imprinting of candidate genetic factors or regions in family-based linkage studies. While only around 1% of genes are affected by imprinting, this mechanism linking gene expression to maternal or paternal inheritance commonly affects genes

that influence growth and behavior. Imprinted genes may therefore be good candidates for obesity risk. The current results suggest in particular that a maternally expressed genetic factor in chromosome region 10p12 may influence obesity risk. This 10p12 region has previously been linked to obesity risk in several independent linkage studies, and it includes the GAD2 gene which appears to regulate appetite [16]. This gene is not known to be imprinted, but this study suggests that GAD2 could be imprinted, at least in some tissues and/or at certain times during development. The ability to include parent-of-origin effects within linkage analysis of quantitative traits will significantly increase the power of the study if imprinting is indeed present. Differences between mother and fathers genetic effects should more systematically be taken into account, and especially when the phenotype of interest is related to growth.

Association of C-reactive protein with blood pressure and hypertension

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Background: Many studies have shown that C-reactive protein (CRP) levels are positively associated with blood pressure and hypertension, but it is unclear whether this association is due to a causal link.

Methods: The cross-sectional associations of CRP levels with systolic blood pressure, pulse pressure, and prevalent hypertension in a representative sample of >3,500 British women aged 60–79 years were investigated. Evidence for causality was sought using a Mendelian randomization approach: the G1059C polymorphism in the human CRP gene was analyzed in association with CRP levels, arterial blood pressure, pulse pressure, and hypertension.

Results: Substantial associations were observed between CRP levels and all blood pressure-related outcomes. However, these associations were seriously weakened by adjustment for many confounding factors acting over the life course. Homozygotes for the common CRP G1059 allele had much higher CRP levels than subjects with the 1059C allele. However, in contrast to what would be expected from a causal link, GG homozygotes did not have any difference in blood pressure, pulse pressure, or hypertension risk. The predicted causal effects of CRP on blood pressure, pulse pressure, and hypertension were close to zero, although with wide confidence intervals.

Conclusion: CRP levels are associated with blood pressure, pulse pressure, and hypertension, but adjustment for life course confounding and a genetic Mendelian randomization approach suggest that this link is not causal.

Mendelian randomization is a genetic tool that has been recently taken up by epidemiologists in order to tackle the thorny issue of causation in the exposure-disease association [21]. It may not be a totally new concept for geneticists, who have long used the phenotypic characterization of functional genetic variants and rare mutations in humans and knock-out animal models to establish molecular contributions to pathogenesis. However, broader acceptance of its use by epidemiologists can help to separate causal from non-causal associations. For example, the well-established low risk of cardiovascular disease in menopausal women observed to use hormone replacement therapy was not supported by subsequent huge randomized trials [22]; the women originally observed to use hormone replacement therapy may have been somehow clinically selected for lower risk of cardiovascular disease (e.g. their physicians avoided giving hormone replacement therapy to women with risk of thrombosis), or possibly they were more likely to make other healthy lifestyle choices. Mendelian randomization is based on Mendel's second law that inheritance of one trait is independent of inheritance of other traits. Associations between genetic variants and outcome are therefore not confounded by behavioral or environmental exposures, i.e. genetic variant association studies have similar properties to randomized controlled trials. This method has already led to confirmation of several causal links, for example: between maternal folate deficiency and offspring neural tube defect; and between homocystine levels and stroke [23]. Conversely it can demonstrate the absence of causality, as in this study. The lack of association between CRP genotype and blood pressure or hypertension suggests that the cross-sectional association between CRP protein level and hypertension is due to confounding, or reverse causality (i.e. higher blood pressure leads to higher CRP levels). This is important, as it argues strongly against setting up large trials to lower CRP levels to treat or

prevent hypertension. There is obviously a very wide scope for the use of common genetic factors in the investigation of multifactorial diseases; the rate of progress will depend on identifying and validating other genetic variants that are clearly representative for protein differences in the candidate pathways.

New hopes

Complement factor H polymorphism in age-related macular degeneration

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Background: Age-related macular degeneration is a major cause of blindness in the elderly. Earlier linkage studies mapped the age-related macular degeneration locus to the 1q31 chromosomal region.

Methods: A genome-wide screen of 96 cases and 50 controls for polymorphisms associated with age-related macular degeneration using 100,000 single-nucleotide polymorphism (SNP) gene chips from Affymetrix Corporation was performed.

Results: Among 116,204 SNPs genotyped, 103,611 SNPs were autosomal, informative and passed the quality-control checks. Only one SNP, an intronic and common variant in the complement factor H gene on chromosome 1q31, was strongly significantly associated with age-related macular degeneration (Bonferroni-corrected $p = 0.0043$). In individuals homozygous for the risk allele, the likelihood of age-related macular degeneration increased by a factor of 7.4 (95% confidence interval 2.9–19). Resequencing revealed a polymorphism in linkage disequilibrium with the risk allele representing a tyrosine-histidine change at amino acid 402.

Conclusions: The complement factor H gene is located on chromosome 1 in a region repeatedly linked to age-related macular degeneration in family-based studies. This polymorphism is in a region of complement factor H gene that binds heparin and C-reactive protein, and explained 20–50% of the risk of age-related macular degeneration.

This paper is clearly outside the remit of Pediatric Endocrinology. However it is the best demonstration yet of the new extremely large-scale gene chips that can genotype 100,000 or more SNPs at once, and will hopefully greatly speed up the discovery of genetic associations. The authors used a genome-wide 100,000 SNP chip and found that the strongest signal mapped to chromosome 1q31, which had been previously linked to age-related macular degeneration. They then resequenced this gene to identify and genotype additional SNPs. Two other papers in this edition of *Science* also reported genetic association with the complement factor H gene. Genetic researchers have been buoyed by these results using the latest genome-wide technology, which may direct therapeutic approaches aimed at primary or secondary prevention. Affymetrix now offer gene chips that include 600,000 SNPs, which represents approximately 5% of all the expected SNPs in the entire human genome, at a cost of around USD 1,000 per sample. The main problem encountered was to differentiate true findings from false-positive results due to the large number of loci tested. Two approaches were used: firstly they increased the genetic contribution to age-related macular degeneration in their study population by matching for known risk factors for age-related macular degeneration: sex and smoking, by carefully selecting cases by quantitative photographic assessment, and by choosing older controls who were more likely to be genetically 'age-related macular degeneration-free'. They also selected cases and controls all of whom were 'white, not of Hispanic origin'. Secondly, they used the very conservative Bonferroni correction to account for multiple testing, and considered significant only those SNPs for which $p < 0.05/103,611$ (i.e. $p < 4.8 \times 10^{-7}$). It may be frustrating to think how many other true-positive associations did not survive the stringent Bonferroni correction. Identifying these slightly weaker true positives from the many false-positive associations may require advances in statistical analyses that somehow include our prior knowledge of the biological basis of disease [1, 24].

Single nucleotide variants in the β 2-adrenergic and β 3-adrenergic receptor genes explained 18.3% of adolescent obesity variation

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Background: Candidate genes for associations with obesity include: the β -adrenergic receptor genes (ADRBs), peroxisome proliferator-activated receptor- γ (PPAR γ), and uncoupling proteins (UCPs).

Methods: Three hundred and twenty-nine Korean teenagers were analyzed for the obesity-related phenotypes body mass index (BMI), percentage of body fat, plasma leptin and insulin levels, fasting glucose concentration, and plasma lipid profile for association with the following seven single nucleotide variants: 252G/A, 523C/A and 1053G/C in ADRB2; Trp64Arg in ADRB3; 161C/T in PPAR γ ; Ala55Val in UCP2, and 210C/T in UCP3.

Results: The 1053G/C polymorphism ($p < 0.05$) in the ADRB2 gene and the Trp64Arg polymorphism ($p < 0.01$) in the ADRB3 gene were associated with BMI after adjustment for dietary energy intake. Trp64Arg polymorphism also influenced percentage body fat ($p < 0.01$) and plasma leptin level ($p < 0.05$). Furthermore, significant interaction effects between the 1053G/C and Trp64Arg polymorphisms were observed on BMI ($p < 0.01$). The polymorphisms of the ADRB2 and ADRB3 genes explained 4.3% and 10.1% of the variation on BMI, and the two loci effect, including their epistasis, explained 18.3%.

Conclusions: The ADRB2 1053G/C and the ADRB3 Trp64Arg polymorphisms additively and interactively contributed substantially to the variation of complex adolescent obesity.

This paper reports an astonishingly large contribution of two candidate obesity SNPs to the variation in BMI in 329 unrelated adolescents aged 11–19 years. The β -adrenergic receptors mediate the action of catecholamines on multiple human tissues including adipose tissue, and their genes are therefore good candidates for obesity risk, independently of dietary factors. However the results are much stronger than others previously reported. Earlier meta-analyses of the ADRB3 Trp64Arg polymorphism found an overall significant but modest effect on BMI. So what makes these results different to other studies? The findings could have arisen by chance (which is possible in a relatively small sample size), or the subjects could somehow have been over-selected in favor of increased ADRB3 genetic effects. The ADRB3 Trp64Arg variant has higher frequencies in Japanese and other populations, which increases the study power. Also the subjects had a very wide range in BMI (12.9–38.0 kg/m²), which again increases power. The authors sensibly excluded subjects with endocrine disorders, or intentional weight reduction during the preceding 6 months, or those treated with any anti-obesity agent or insulin. Lastly, the study included assessment for dietary energy intake using a semiquantitative food frequency questionnaire, which itself had a remarkably large contribution to BMI and body fat. Indeed the associations with ADRB2 and ADRB3 were only significant after adjustment for diet. We are sure there will still be some scepticism over the representativeness of these results; for example age and puberty stage were not included in the analytical models. However, it shows that genetic contributions may be highlighted by consideration of potentially confounding factors.

New concerns

A common polymorphism of the growth hormone receptor is associated with increased responsiveness to growth hormone

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Background: Growth hormone is used to increase height in short children who are not deficient in growth hormone, but response to treatment is variable across individuals. The genetic factors that influence this response are still unknown.

Methods: Two cohorts of 76 and 96 short children treated with growth hormone were genotyped for the growth hormone receptor gene isoform that lacks exon 3 (d3-GHR). Increases in growth rate in response to growth hormone administration were compared by genotype.

Results: The d3 genotype was associated with 1.7–2 times faster growth acceleration induced by growth hormone compared to the full-length GHR isoform ($p < 0.0001$). In vitro transfection experiments showed that the d3 homo- or heterodimers were associated with approximately 30% higher transduction of growth hormone signaling than through full-length GH receptor homodimers ($p < 0.0001$).

Conclusion: Half of all Europeans are hetero- or homozygous for the allele encoding the d3 isoform, which is dominant over the full-length isoform. These observations suggest that the polymorphism in exon 3 of the GH receptor may be important in growth hormone pharmacogenetics.

This difference in drug response is a striking example of the potential important use of pharmacogenetics. The authors showed that short children who carried the shorter variant of the growth hormone (GH) receptor (GHR) gene grew 1.7–2 times faster in response to GH therapy than did short children with a longer form of the receptor. In vitro studies showed that transduction of GH signaling through d3 homo- or heterodimers was approximately 30% higher than through full-length GHR homodimers. These children were not GH deficient, but both groups had a combination of small-for-gestational age and ‘idiopathic’ short children. No differences were detectable between genotype in GH or IGF-1 levels at baseline, and the findings have yet to be reproduced in other studies. Hundreds of thousands of children in the US and Europe may be eligible for GH prescription and half of them carry this variant. If the effects are confirmed, pharmacogenetic testing could be used in the future to provide more appropriate GH therapy. From the currently fixed weight-related dosing, we could move to more personalized doses. However, the concept of pharmacogenetics also raises ethical issues. To date, most of the clinical uses of pre-medication genetic testing are to avoid drug side effects. For example, for HIV therapy routine HLA region typing has markedly reduced the incidence of a potentially life-threatening hypersensitivity syndrome due to the reverse-transcriptase inhibitor abacavir [3]. Similarly it could be advantageous to be able to identify children with genetic high risk of GH-induced adverse effects, such as type 2 diabetes or cancer. However, if we can now start to identify children who are genetically predisposed to respond poorly or need more expensive larger doses, will these children be prejudiced against when deciding who should receive treatment?

A functional variant of SUMO4, a new I κ B α modifier, is associated with type 1 diabetes

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Background: More than 20 genetic regions, including *IDDM5*, have been linked to susceptibility to type 1 diabetes (T1D). However identification of specific genes within such regions has been challenging.

Methods: The *IDDM5* region was genotyped using 50 flanking markers (average of 100 kb/SNP) initially in 703 cases with T1D and 916 matched controls from multiple ethnic groups. Initial positive findings were confirmed in an intrafamilial study with 944 multiethnic families.

Results: The G variant of the 001Msp SNP was more common in cases from US (57.4%) than in controls (45.6%; $p = 0.0006$), and also showed excess transmission in families with T1D ($p = 1.6 \cdot 10^{-5}$). In this region a new gene encoding small ubiquitin-like modifier 4 protein (SUMO4) was cloned. A substitution SNP (M55V) at an evolutionarily conserved residue of SUMO4 was strongly associated with T1D ($p = 1.9 \times 10^{-7}$). Functional studies showed that SUMO4 conjugates to I κ B α and negatively regulates NF κ B transcription. The M55V substitution was associated with 5.5 times greater NF κ B transcriptional activity and 2 times greater expression of IL12B, an NF κ B-dependent gene.

Conclusions: These findings identify a novel candidate gene for *IDDM5*, and suggest a new pathway that may be implicated in the pathogenesis of T1D.

The recent papers on the possible association between common genetic variation in *SUMO4* and type 1 diabetes are highly educational with regard to the current debates in genetic epidemiology. Although in this study the authors confirmed their seemingly consistent positive findings by case-control association and allele transmission using reasonably large sample sizes, and also by functional studies, the findings were quickly refuted by negative findings in subsequent much larger association studies. Notably, Smyth et al. [25] found no convincing evidence for the association of the M55V substitution with T1D in a massive study of 18,132 individuals from various countries (3,007 transmissions and 7,230 cases and controls). They also point out that while functional studies of common genetic variants are essential, they do not solve the problem of false positives. On reflection there was already evidence of heterogeneity (different directions of effects) in the original findings (between UK and other families), and between other studies using UK populations [26]. Hundreds of laboratories may be testing thousands of candidate SNPs for disease association, and availability of data for pooling in meta-analyses may help to identify the true-positive effects. However, sometimes there may be true discrepancies between results from different populations or studies; reasons for this include genetic heterogeneity or population differences in gene – gene and gene – environment interactions.

Food for thought

A common inversion under selection in Europeans

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Background: Large chromosomal rearrangements, such as deletions, duplications and rearrangements contribute substantially to genomic variation among humans and account for much of the genomic difference between humans and other primates.

Methods: By genotyping RP11 clones from 17q21.31 for 60 microsatellite markers and assembling the clones into chromosome-specific BAC contigs, the H2 haplotype was found to be structurally different from the Build 34 assembly.

Results: The refined physical map of chromosome 17q21.31 uncovered a 900-kb inversion polymorphism that contains several genes, including those encoding corticotropin releasing hormone receptor 1 (CRHR1) and microtubule-associated protein tau (MAPT). Chromosomes with the inverted segment in different orientations represent two distinct lineages, H1 and H2, which have diverged for as much as 3 million years. The H2 lineage is rare in Africans, almost absent in East Asians but has a frequency of 20% in Europeans. By genotyping 29,137 Icelanders, 16,959 women and 12,178 men, born between 1925 and 1965, carriers of the H2 haplotype were observed to be more fertile, having 3.2% more children per generation than non-carriers. The rate of genetic recombination increased by 0.472 Morgans per copy of H2 (two sided $p = 0.0002$), or 1.0% of the average female genetic length for the genome.

Conclusions: The H2 lineage is undergoing positive selection in the Icelandic population, such that carrier females have more children and have higher recombination rates than non-carriers.

This paper forces us to reconsider the use of large-scale SNP genotyping alone to map common genetic variation for association with common disease or phenotypes. The human genome sequence is extremely rich in segmental duplications of unknown function [27]. Other large-scale genomic variations have been thought to be on the whole deleterious, and therefore rare. However, without painstaking study, such variations may be difficult to identify. The MAPT gene region has been associated with Parkinson disease, and previous studies identified strong linkage disequilibrium across this region, with two highly divergent MAPT haplotypes, H1 and H2. However, none of those earlier studies detected the inversion. Remarkably, the common H2 inversion was significantly associated with both higher birth rates in women, and also with a higher rate of genetic recombination. Such

active selection is supported by worldwide studies that show a wide variation in frequencies of the H2 inversion, with high frequencies among individuals of European ancestry. Characterization of other common large-scale genomic variations will lead to a new generation of genetic association studies with human disease.

References

1. Thomas DC, Clayton DG: Betting odds and genetic associations. *J Natl Cancer Inst* 2004;96:421–423.
2. O'Rahilly S, Barroso I, Wareham NJ: Genetic factors in type 2 diabetes: the end of the beginning? *Science* 2005;307:370–373.
3. Hughes DA, Vilar FJ, Ward CC, Alfirevic A, Park BK, Pirmohamed M: Cost-effectiveness analysis of HLA B*5701 genotyping in preventing abacavir hypersensitivity. *Pharmacogenetics* 2004;14:335–342.
4. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN: Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003;33:177–182.
5. Franks PW, Luan J, Browne PO, Harding AH, O'Rahilly S, Chatterjee VK, et al: Does peroxisome proliferator-activated receptor gamma genotype (Pro12Ala) modify the association of physical activity and dietary fat with fasting insulin level? *Metabolism* 2004;53:11–16.
6. Eriksson JG, Lindi V, Uusitupa M, Forsen TJ, Laakso M, Osmond C, et al: The effects of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-gamma2 gene on insulin sensitivity and insulin metabolism interact with size at birth. *Diabetes* 2002;51(7):2321–2324.
7. Hsueh WC, Cole SA, Shuldiner AR, Beamer BA, Blangero J, Hixson JE, et al: Interactions between variants in the beta3-adrenergic receptor and peroxisome proliferator-activated receptor-gamma2 genes and obesity. *Diabetes Care* 2001;24:672–677.
8. Smith PG, Day NE: The design of case-control studies: the influence of confounding and interaction effects. *Int J Epidemiol* 1984;13:356–365.
9. Medina-Gomez G, Virtue S, Lelliott C, Boiani R, Campbell M, Christodoulides C, et al: The link between nutritional status and insulin sensitivity is dependent on the adipocyte-specific peroxisome proliferator-activated receptor-gamma2 isoform. *Diabetes* 2005;54:1706–1716.
10. Hillmer AM, Hanneken S, Ritzmann S, Becker T, Freudenberg J, Brockschmidt FF, et al: Genetic variation in the human androgen receptor gene is the major determinant of common early-onset androgenetic alopecia. *Am J Hum Genet* 2005;77:140–148.
11. Lim HN, Nixon RM, Chen H, Hughes IA, Hawkins JR: Evidence that longer androgen receptor polyglutamine repeats are a causal factor for genital abnormalities. *J Clin Endocrinol Metab* 2001;86:3207–3210.
12. Zeegers MP, Kiemeny LA, Nieder AM, Ostrer H: How strong is the association between CAG and GGN repeat length polymorphisms in the androgen receptor gene and prostate cancer risk? *Cancer Epidemiol Biomarkers Prev* 2004;13:1765–1771.
13. Swan SH, Main KM, Liu F, Stewart SL, Kruse RL, Calafat AM, et al: Decrease in anogenital distance among male infants with prenatal phthalate exposure: the Study for Future Families Research Team. *Environmental Health Perspectives* 2005;doi:10.1289/ehp.8100.[Online 27 May 2005].
14. Hattersley AT, Beards F, Ballantyne E, Appleton M, Harvey R, Ellard S: Mutations in the glucokinase gene of the fetus result in reduced birth weight. *Nat Genet* 1998;19:268–270.
15. Kleinjan DA, van Heyningen V: Long-range control of gene expression: emerging mechanisms and disruption in disease. *Am J Hum Genet* 2005;76:8–32.
16. Boutin P, Dina C, Vasseur F, Dubois SS, Corset L, Seron K, et al: GAD2 on chromosome 10p12 is a candidate gene for human obesity. *PLoS Biol* 2003;1:E68.
17. Ruano M, Silvestre V, Castro R, Garcia-Lescun MC, Rodriguez A, Marco A, et al: Morbid obesity, hypertensive disease and the renin-angiotensin-aldosterone axis. *Obes Surg* 2005;15:670–676.
18. Ng DP, Tai BC, Koh D, Tan KW, Chia KS: Angiotensin-I converting enzyme insertion/deletion polymorphism and its association with diabetic nephropathy: a meta-analysis of studies reported between 1994 and 2004 and comprising 14,727 subjects. *Diabetologia* 2005;48:1008–1016.
19. Cambien F, Leger J, Mallet C, Collin D, Czernichow P: Angiotensin I-converting enzyme gene polymorphism modulates the consequences of in utero growth retardation on plasma insulin in young adults. *Diabetes* 1998;47:470–475.
20. Tomlinson JW, Walker EA, Bujalska IJ, Draper N, Lavery GG, Cooper MS, et al: 11beta-hydroxysteroid dehydrogenase type 1: a tissue-specific regulator of glucocorticoid response. *Endocr Rev* 2004;25:831–866.
21. Davey Smith G, Ebrahim S: What can Mendelian randomisation tell us about modifiable behavioural and environmental exposures? *BMJ* 2005;330:1076–1079.
22. Gabriel SR, Carmona L, Roque M, Sanchez GL, Bonfill X: Hormone replacement therapy for preventing cardiovascular disease in post-menopausal women. *Cochrane Database Syst Rev* 2005(2):CD002229.
23. Casas JP, Bautista LE, Smeeth L, Sharma P, Hingorani AD: Homocysteine and stroke: evidence on a causal link from mendelian randomisation. *Lancet* 2005;365:224–232.
24. Gill CJ, Sabin L, Schmid CH: Why clinicians are natural bayesians. *BMJ* 2005;330:1080–1083.
25. Smyth DJ, Howson JM, Lowe CE, Walker NM, Lam AC, Nutland S, et al: Assessing the validity of the association between the SUMO4 M55V variant and risk of type 1 diabetes. *Nat Genet* 2005;37:110–111; author reply 112–113.
26. Bohren KM, Nadkarni V, Song JH, Gabbay KH, Owerbach D: A M55V polymorphism in a novel SUMO gene (SUMO-4) differentially activates heat shock transcription factors and is associated with susceptibility to type I diabetes mellitus. *J Biol Chem* 2004;279:27233–27238.
27. Sharp AJ, Locke DP, McGrath SD, Cheng Z, Bailey JA, Vallente RU, et al: Segmental duplications and copy-number variation in the human genome. *Am J Hum Genet* 2005;77:78–88.