Population Genetics and Pharmacogenetics

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The highlight for 2005/6 in population genetics was undoubtedly the publication of HapMap, a freely available public resource nearly of all common (>5% minor allele frequency) genetic differences in humans. Compared to the estimated 10 million single nucleotide polymorphisms (SNPs) that we all share, at the launch of HapMap in 2002 there were fewer than 1.7 million SNPs on the public database dbSNP. Today, largely due to HapMap and related efforts, that number is around 9.2 million. Furthermore, investigators no longer need to tediously resequence their chosen candidate genes to find the common variants, but can rather spend a few minutes on www.hapmap.org. The data can also be downloaded to select only those tagSNPs that efficiently represent all the other variants in your chosen region.

Of course, only a minority of these SNPs will alter gene expression or function. The main limitations of this resource are that it does not cover: (1) rarer although possibly important functional genetic variations; (2) large-scale genomic variations (see paper by Bellanne-Chantelot below), or (3) other populations which may possibly be less well represented by the four populations genotyped by HapMap. However, we may already be seeing the fruit of increasing numbers of larger and more comprehensive genetic association studies. Examples are the remarkably strong and consistent associations between the TCF7L2 gene and adult type 2 diabetes [1], and within Pediatric Endocrinology the growing number of positive associations reported with childhood obesity (see below).

New hope – the era of the HapMap

A haplotype map of the human genome

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Background: Inherited genetic variation has a critical but as yet largely uncharacterized role in human disease.

Results: The authors report a public database of common variation in the human genome: more than 1 million single nucleotide polymorphisms (SNPs) for which accurate and complete genotypes have been obtained in 269 DNA samples from four populations, including ten 500-kb regions in which essentially all information about common DNA variation has been extracted. These data document the generality of recombination hotspots, a block-like structure of linkage disequilibrium and low haplotype diversity, leading to substantial correlations of SNPs with many of their neighbors.

Conclusions: The HapMap resource can guide the design and analysis of genetic association studies, shed light on structural variation and recombination, and identify loci that may have been subject to natural selection during human evolution.

The human haplotype map (HapMap) is a nearly complete catalogue of the common genetic differences among people, produced by the International HapMap project. High-density SNP genotyping was performed across the whole human genome in 90 (30 trios) European-origin Americans in Utah, 90 (30 parent-offspring trios) Yoruba Nigerians in Ibadan, 45 Han Chinese in Beijing, and 45 Japanese in Tokyo. **Phase I HapMap:** The above paper was published in *Nature* on 27th October 2005 and described the Phase I HapMap, which aimed to genotype at least one common SNP every 5 kb across the genome in each of 269 DNA samples, comprising 1.3 million SNPs genotyped in 269 people.

Phase II HapMap: However, at the same time the HapMap Project also released online (www.hapmap.org) the Phase II HapMap, comprising >3.5 million SNPs genotyped in 270 people. This extension

increased the average density of the map from 1 SNP per 3 kb to about 1 SNP every 600 bases, by taking advantage of the rapid, high-throughput genotyping capacity of Perlegen Sciences, Inc., Calif., to test another 4.6 million SNPs from publicly available databases. In non-Africans, Phase II captures an estimated 94% of all common SNPs (minimum allele frequency >5%) (81% in Africans), and due to high 'linkage disequilibrium' between SNPs, 'only' 300,000 tag SNPs are needed to cover the whole genome in European-origin populations.

This freely available public resource provides information about SNP validation, frequency and assay conditions, and correlation structure of alleles across the human genome. The vision of the HapMap project predicts that it should be a powerful resource for genetic association studies, and for studying the genes contributing to variation in response to environmental factors, in susceptibility to infection, and in the effectiveness of and adverse responses to drugs and vaccines.

New paradigms – large structural genetic variations

Large genomic rearrangements in the hepatocyte nuclear factor-1 β (TCF2) gene are the most frequent cause of maturity-onset diabetes of the young type 5

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Background: Maturity-onset diabetes of the young (MODY)5 is caused by mutations in the transcription factor hepatocyte nuclear factor-1 β gene (TCF2). However, in 60% of the patients with a phenotype suggesting MODY5, no point mutation is detected in TCF2. The authors have hypothesized that large genomic rearrangements of TCF2 that are missed by conventional screening methods may also cause MODY5.

Methods: In 40 unrelated patients presenting with MODY5 phenotype, TCF2 was screened for mutations by sequencing. Nearly half ($n = 18$) of the patients had a TCF2 point mutation detected. The 22 patients without point mutations were then screened for TCF2 rearrangements by the quantitative multiplex PCR of short fluorescent fragments (QMPSF).

Results: Among the 40 patients, the overall detection rate was 70%: 18 had point mutations, 9 had whole-gene deletions, and 1 had a deletion of a single exon. Similar phenotypes were observed in patients with mutations and in subjects with large deletions.

Conclusions: One-third of MODY5 cases may result from large deletions of TCF2, which are missed on gene sequencing methods. Because QMPSF is more rapid and cost-effective than sequencing, the authors propose that patients with phenotypes consistent with MODY5 should be screened first using QMPSF. In addition, other MODY genes should be screened for large genomic rearrangements. MODY5 and other types of MODY may be more prevalent than previously reported.

MODY5 is suggested by a phenotype of autosomal dominant hyperglycemia associated with renal cysts or impaired renal function, and sometimes also Müllerian aplasia. In a large series of patients with phenotypes suggestive of MODY5, the authors show that large deletions of TCF2 accounted for 50% of the patients who had been classified as MODY-X because conventional screening had failed to detect a TCF2 mutation. While MODY5 is a rare condition, this study is a good illustration that while the 'in vogue' single nucleotide polymorphism (SNP) may be much more amenable to large studies and high-throughput assay methods, large genomic rearrangements may also contribute a large proportion of the inheritance of both rare and common diseases. These include deletions, duplications, large-scale copy-number variants (CNVs), insertions, inversions and translocations, and may occur at the microscopic or submicroscopic level. In a recent review, Feuk et al. [2] describe that, in the past year alone, hundreds of submicroscopic CNVs and inversions have been identified. Around 1 in 20 human proteins are likely to contain tandem repeat copy-number polymorphisms within coding

regions. Such variants can encompass millions of DNA base pairs, containing entire genes and their regulatory regions, and are likely to contribute greatly to human diversity and disease susceptibility.

New genetic associations: with childhood obesity

Variants of ENPP1 are associated with childhood and adult obesity and increase the risk of glucose intolerance and type 2 diabetes

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Background: The authors previously identified a locus on chromosome 6q16.3-q24.2 associated with childhood obesity, including a 2.4-Mb region that is common to eight genome scans for type 2 diabetes (T2D) or obesity. A regional candidate gene is ectonucleotide pyrophosphatase phosphodiesterase, ENPP1 (also called PC-1), as ENPP1 directly inhibits insulin-induced conformational changes and activation of the insulin receptor.

Methods: Variants in this gene were genotyped in 6,147 subjects.

Results: A three-allele risk haplotype (K121Q, IVS20delT-11 and A→G+1044TGA; QdelTG) was associated with childhood obesity (OR = 1.69, $p = 0.0006$), morbid or moderate obesity in adults (OR = 1.50, $p = 0.006$ and OR = 1.37, $p = 0.02$ respectively) and T2D (OR = 1.56, $p = 0.00002$). This obesity-associated ENPP1 risk haplotype contributed to the observed chromosome 6q linkage with childhood obesity, as suggested by the genotype IBD sharing test. The haplotype was associated with an increased risk of glucose intolerance and T2D in obese children and their parents, and was related to higher serum levels of soluble ENPP1 protein in children. A long ENPP1 mRNA isoform, which includes the obesity-associated A→G+1044TGA SNP, was expressed in pancreatic islet β cells, adipocytes and liver only.

Conclusion: Several variants of ENPP1 seem to have a primary role in mediating insulin resistance and in obesity and T2D development. This suggests a common molecular mechanism to both conditions.

The K121Q polymorphism of the ENPP1/PC-1 gene is associated with insulin resistance/atherogenic phenotypes, including earlier onset of type 2 diabetes and myocardial infarction

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Background: Insulin resistance (IR) is a key feature of type 2 diabetes (T2D) and coronary artery disease (CAD). The K121Q polymorphism of the ENPP1/PC-1 gene has been associated with IR. The aim of study is to evaluate the role of the 121Q variant on T2D and CAD risks.

Methods: Three population groups from Italy and USA were studied: 638 non-diabetic controls, 535 type 2 diabetic patients without CAD and 434 patients with CAD.

Results: The 121Q variant carrier frequency increased across the three groups (27.4, 28.8 and 33.2%, respectively; $p = 0.027$). Among diabetic patients, risk of developing T2D before age 65 and of having myocardial infarction (MI) by age 50 were higher in 121Q carriers than in non-carriers (OR = 2.26, 95% CI 1.26–4.03, $p = 0.006$ and OR = 3.17, 1.46–6.88, $p = 0.007$; respectively). The 121Q variant was also associated with increased risk for CAD in diabetic patients who did not smoke (1.47, 1.01–2.18; $p = 0.049$).

Conclusion: The ENPP1/PC-1 121Q polymorphism increased the risk of diseases related to the IR-atherogenic phenotype. Moreover, among diabetic patients, this variant was also associated with earlier onset of type 2 diabetes and myocardial infarction.

These papers bring a new insight into the molecular mechanisms of common obesity, and obesity-related pathologies such as type 2 diabetes and coronary artery disease. In a previous paper, Meyre et al. showed linkage to chromosome 6q in families with early-onset obesity. ENPP1 (also known as alkaline phosphodiesterase-1) was firstly a candidate for type 2 diabetes since it directly inhibits insulin receptor signaling. In this study, analysis of the DNA variations of ENPP1 showed an association between a combination of variants and childhood obesity, morbid or moderate obesity in adults and also with type 2 diabetes. These data support the idea that primary insulin resistance may lead to childhood obesity; other studies have reported that insulin resistance-induced fasting hyperinsulinemia predicts subsequent obesity in children of various ethnic groups.

Previous studies have suggested a common genetic predisposition to obesity and diabetes, and ENPP1 constitutes the first example of a genetic determinant of both obesity and type 2 diabetes. The second paper also suggests that the mutation Q121 in ENPP1 may predispose to obesity with a high adverse metabolic impact; diabetic patients carrying this mutation had a higher risk of early myocardial infarction. Patients with such forms of obesity should require careful attention to complications of insulin resistance.

Interestingly, the protein product of ENPP-1 is also a bone and cartilage enzyme with both 5'-nucleotide phosphodiesterase I and nucleotide pyrophosphohydrolase activity that modulates physiologic mineralization and pathologic chondrocalcinosis by generating inorganic pyrophosphate. It was hypothesized for many years that, as for liver-bone-kidney alkaline phosphatase, expression of a gene for an enzyme with these properties might be shared by cells from bone, cartilage, and liver.

ACDC/Adiponectin polymorphisms are associated with severe childhood and adult obesity

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Background: Common single nucleotide polymorphisms (SNPs) in the ACDC adiponectin encoding gene have been associated with insulin resistance and type 2 diabetes in several populations.

Methods: Four SNPs (-11,377C>G, -11,391G>A, +45T>G and 276 T>G) were investigated in 2,579 French Caucasians (1,229 morbidly obese and 1,330 controls).

Results: Unlike previous studies showing that alleles -11,377G and +276G increased risk of type 2 diabetes, the opposite alleles -11,377C (odds ratio 1.23, $p = 0.001$) and +276T (1.19, $p = 0.006$) were associated with morbid obesity. In 634 obesity trios there was a trend to overtransmission (56.7%) of a risk haplotype including -11,377C and +276T ($p = 0.097$). In normal 400 trios, the obesity haplotype was associated with higher adiponectin levels, suggesting a role of hyperadiponectinemia in weight gain. A promoter region SNP -11,391G>A was associated with higher adiponectin levels in obese children ($p = 0.005$) and in normal children ($p = 0.00007$). In vitro transcriptional assays showed a positive link between -11,391A and ACDC activity.

Conclusion: Variations at the ACDC/adiponectin gene are associated with risk of morbid obesity. However, the mechanisms remain to be elucidated.

Adiponectin is the most profusely secreted protein from adipose tissue. It has marked insulin sensitizing effects, and could play a positive role in the relationship between obesity and insulin resistance. In this regard, this study presents some intriguing results: the two alleles -11,377C and +276T were associated with childhood and morbid adult obesity and with higher adiponectin levels, whereas in previous studies the alternative alleles -11,377G and +276G were associated with increased risk of diabetes. The authors suggest that adiponectin could promote weight gain by its insulin-sensitizing action in adipose tissue. Just as PPAR- γ agonists improve insulin sensitivity but stimulate body fat gain, adiponectin gene and protein levels may therefore distinguish which obese children will become insulin-resistant. Another issue not raised by this paper concerns the failure of current assays to differentiate between types of circulating adiponectin, as not all circulating forms may be functional. This could explain some discrepancies in some studies showing association with disease but not with adiponectin levels, or even apparent contradiction in the associations, as in this paper. Further investigations are required to accurately measure the active circulating form(s) of adiponectin.

A further complexity is the presence of two adiponectin receptors, ADIPOR1 and ADIPOR2, which serve as receptors for globular and full-length adiponectin and mediate increased AMP-activated kinase and PPAR- α ligand activities, as well as fatty acid oxidation and glucose uptake by adiponectin.

A POMC variant implicates β -melanocyte-stimulating hormone in the control of human energy balance

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Background: The melanocortin system plays a critical role in energy homeostasis. The melanocortin-4 receptor (MC4R) has two pro-opiomelanocortin (POMC)-derived ligands: α - and β -MSH. Since β -MSH is absent in rodents, attention has been principally focused on α -MSH.

Methods: The POMC gene was screened in 538 patients with severe, early-onset obesity.

Results: Five unrelated probands were heterozygous for a rare missense variant in the POMC region encoding β -MSH (Tyr221Cys). The percentage of carriers among cases was higher than in a UK Caucasian population ($p < 0.001$). Moreover, the variant cosegregated with obesity/overweight in affected family members. The variant peptide showed reduced ability to bind to, and activate MC4R signaling compared to wild-type β -MSH. Obese children carrying the Tyr221Cys variant were hyperphagic and presented an accelerated linear growth, and in that respect closely resembled MC4R-deficient children.

Discussion: These results suggest that β -MSH regulates human energy homeostasis.

The identification of rare mendelian monogenic human obesity syndromes has been instrumental in defining the role of several key molecules in appetite control, such as leptin and the leptin receptor. Such studies have also shown a major role of melanocortins and their central receptor MC4R. Melanocortins are derived from the larger precursor molecule pro-opiomelanocortin (POMC). The main peptide previously investigated is α -melanocyte-stimulating hormone (MSH), as in rodents the N-terminal cleavage site necessary for the generation of β -MSH is absent. However β -MSH is present in the human hypothalamus. This study shows that a rare missense mutation in the region encoding β -MSH is overrepresented among patients with early-onset obesity. As suggested by their studies of the peptide structure, this variant could predispose to obesity by reduced ability to bind and activate MC4R. Indeed, the similarities in clinical phenotype between obese children carrying the β -MSH mutation and those with MC4R mutations support a common physiological pathway. These findings illustrate that human genetics can provide insights into the important physiological roles of species-specific peptides that are not possible through the study of rodent models.

Co-occurrence of two partially inactivating polymorphisms of MC3R is associated with pediatric-onset obesity

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Background: The MC3R gene may play an important role in energy homeostasis, as suggested by both human linkage studies and knockout mouse models.

Methods: A pair of missense variants (Thr6Lys and Val81Ile) in MC3R were analyzed in 355 overweight and non-overweight children.

Results: Both variants were more frequent in African-American than in Caucasian children. Compared with wild-type or heterozygous children, the 8.2% of children homozygous for both variants had higher BMI and BMI SD scores ($p < 0.0001$), more body fat ($p < 0.001$), greater plasma leptin ($p < 0.0001$) and insulin concentrations ($p < 0.001$), and were more insulin-resistant ($p < 0.008$). In vitro studies showed that the double mutant MC3R was partially inactive; receptor-binding sites were fewer and signal transduction and protein expression were diminished.

Conclusion: By impairing MC3R expression, this double MC3R variant may predispose to excessive body weight in children.

Association of melanin-concentrating hormone receptor 1 5' polymorphism with early-onset extreme obesity

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Background: Most of the discoveries of monogenic forms of human obesity have been aided by murine models. Such models also show that melanin-concentrating hormone receptor 1 (MCHR1) plays an important role in downstream orexigenic activity of the leptin-melanocortin pathway.

Methods: In 180 morbidly obese adults and 87 morbidly obese children, the human MCHR1 gene was sequenced over 3.5 kb of coding, untranslated and intronic regions plus 2 kb of putative promoter region, leading to a total of >2.4 Mb of sequencing. Then, the most common SNPs (minor allele frequency >5%) were genotyped in 557 morbidly obese adults, 552 obese children, and 1,195 non-obese non-diabetic controls, in order to investigate the possible polygenic role of MCHR1.

Results: Among the 39 found, 7 SNPs encode an amino acid change. One mutation (R248Q) cosegregated with the obesity phenotype in one pedigree, but was also found to be a rare polymorphism in control subjects. One plausible promoter SNP, the rs133068, was associated with a lower risk of obesity in children only (allele frequency $p = 0.006$; genotype frequency $p = 0.004$). Results were more significant under a dominant model ($p = 0.001$, odds ratio 0.695 [95% CI 0.560–0.863]). Similar results were found when analyzing adults and children together ($p = 0.006$, 0.783 [0.658–0.930]).

Conclusion: Those results suggest that SNPs in MCHR1 could be involved in severe early-onset forms of obesity.

These papers highlight two lesser known molecules in leptin's central downstream pathway. In the hypothalamus, leptin upregulates anorexigenic neuropeptides, such as α -MSH, which binds and activates both MC4R (melanocortin-4 receptor) and MC3R, and downregulates orexigenic neuropeptides such as MCH. The role of MC4R is better known, but MC3R may also be important in childhood obesity. MC3R knockout mice have an increased fat mass, and three human coding sequence variants have been reported: a heterozygous missense sequence variant Ile183Asn that inactivates MC3R function has been identified in 2 obese individuals to date [3]. The two other variants reported by Feng et al. had individually not been shown to affect MC3R function or obesity risk, but this study now shows that the double mutant combination may very significantly disrupt receptor function and increase childhood-onset obesity.

As in a previous study [4], Bell et al. sequenced the MCH receptor 1 (MCHR1) in severely obese individuals, and found one rare coding mutation R248Q which cosegregated with obesity in one family, but was not associated with obesity in the larger case-control study. However, they also found a significant childhood obesity risk association with the more common promoter SNP rs133068. While childhood cases were compared to adult controls, the findings suggest that common MCHR1 variants could contribute to childhood obesity. MCHR1 is a potential drug target for obesity [5] and some MCH analogs have already been developed, which activate MCHR1 as effectively as MCH in mice. If related drugs are developed, common MCHR1 variants could potentially modify their pharmacological effects on weight gain.

New genetic associations: with sex hormone genes

The valine allele of the V89L polymorphism in the 5 α -reductase gene confers a reduced risk for hypospadias

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Background: Hypospadias is one of the most common malformations in man, with an incidence of 1:300 in newborn boys, however no gene has been identified to cause isolated hypospadias. Steroid 5 α -reductase A (SRD5A2) is a key enzyme for androgenic function. The V89L polymorphism in the SRD5A2 gene

influences enzyme function: compared to the valine wild-type, the leucine variant confers 30% lower enzyme activity.

Methods: 158 hypospadias cases and 96 unaffected controls were genotyped for this V89L polymorphism.

Results: The wild-type V89 allele was significantly protective for hypospadias (odds ratio for homozygous individuals, 0.24; 95% confidence interval, 0.14–0.41). This association was seen regardless of heredity, ethnicity, and severity of phenotype. The authors also sequenced a selected material of 37 sporadic cases of more severe hypospadias for mutations in the androgen receptor AR, SRD5A2, and 17 β -hydroxysteroid dehydrogenase HSD17B3 genes and found only two previously described mutations, one in the AR and one in the SRD5A2 gene.

Conclusion: Functional polymorphisms in androgen metabolism may contribute to hypospadias risk. The etiology is likely to be complex, including multiple genes as well as environmental factors.

Does variant luteinizing hormone (V-LH) predispose to improper testicular position in late pregnancy?

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Background: Undescended testes affect 2–9% of newborn boys. The etiology is probably heterogeneous, but an insufficient androgen effect is a recognized cause. A common genetic variant (V) form of LH occurs in apparently healthy individuals. Compared with wild-type (WT) LH, the V-LH has increased bioactivity in vitro but shorter half-life in vivo.

Methods: 93 cryptorchid (59 uni- and 34 bilateral) and 211 healthy boys were screened for the presence of V-LH. Two immunofluorometric assays, one detecting WT-LH and the other detecting both WT- and V-LH, were used to measure LH concentrations. The ratio of two LH measurements was used to assess the V-LH status.

Results: The prevalence of V-LH was not different between the control and cryptorchid groups. V-LH prevalence in the total sample was similar to that in the general Finnish population. Among cryptorchid boys, the prevalence of V-LH was dependent on gestational age: 6.7% at GA <37, 20.9% at GA 37–39, and 42.9% at GA of 40–42 weeks. In contrast, the percentage of V-LH status was similar at different gestational ages in all control groups.

Conclusion: The V-LH is not critical for normal testicular descent. However, the increased prevalence of V-LH among cryptorchid boys with GA >40, suggests that the lower hormonal efficacy of V-LH predisposes to improper testicular descent in late pregnancy.

These two studies explore the association between common functional genetic variants in the androgen secretion and metabolism pathway with congenital disorders of the male reproductive tract. The V89L variant in 5 α -reductase type 2 has been consistently reported to associate with lower enzyme activity. The findings by Thai et al. are consistent with the biological rationale of a protective role of androgen, and confirm a previous study in 90 Chinese hypospadias patients [6]. However, the authors admit that the frequency of this variant varies between different populations, and as all the controls were all Swedish Caucasians, perhaps only the results in the 99 cases with similar background are truly valid (OR = 0.46, 0.31–0.69; p = 0.0003).

V-LH arises from two separate amino acid changing point mutations in the LH β -subunit gene; one of these mutations also introduces an extra glycosylation signal. V-LH therefore has different immunologic properties and greater biologic activity as compared to LH, and has been previously linked to menstrual disorders and subfertility. While cryptorchid boys had higher total LH levels than controls, no difference was found in the prevalence of V-LH. The potential effect modification by gestational age is interesting, but may not have been a primary hypothesis, and requires confirmation.

The COMT val158met polymorphism is associated with early pubertal development, height and cortical bone mass in girls

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Pediatr Res 2005;58:71–77

Background: Estrogens regulate bone mass accretion during puberty. Catechol-O-methyltransferase (COMT) converts estrogens to more inactive metabolites. The COMT val158met polymorphism results in a 60–75% reduction in enzyme activity (the met variant confers lower activity = L).

Methods: In a cross-sectional study of 246 healthy pre/early pubertal girls, this polymorphism was investigated for association with skeletal phenotypes measured by dual x-ray absorptiometry or peripheral quantitative computerized tomography.

Results: COMTLL girls were 5.4 cm taller than wild-type homozygotes, and had greater bone mineral content (MC) and larger areas of total body; femur and spine. Cortical BMC measured in the tibia was 9.8% higher in COMTLL due to a larger cortical cross-sectional area, since the cortical volumetric bone mineral density was not associated with COMT genotype. Serum levels of free estradiol and insulin-like growth factor-I were higher in girls with COMTLL. Regression models indicated that the effects of COMT genotype on skeletal growth were exerted via free estradiol levels and current pubertal stage.

Conclusion: The COMTLL genotype is associated with higher free estradiol levels and earlier pubertal development, resulting in an increased skeletal growth. However, the effect of COMT genotype on adult stature and skeletal mass can be determined only after cessation of the growth period.

The heritability for statural growth and pubertal development is estimated to be above 80%, however the specific genes involved are unclear. COMT was initially identified as an essential enzyme in the biosynthesis of catecholamines, including dopamine, epinephrine, and norepinephrine. In addition to that role, COMT also inactivates estrogens. By studying a functional polymorphism in this gene, this paper underlines the role of estrogens in statural growth and puberty timing. COMT genotype was associated with serum levels of free estradiol, height, cortical radial bone growth, earlier puberty in girls and schizophrenia. The findings are further supported by observing a positive correlation between free estradiol and IGF-I levels, since estrogens stimulate growth hormone secretion. One can hope that this paper will provoke further research on the role of this polymorphism on growth from birth to adulthood. The catechol-metabolizing system also has potential pathophysiological significance in estrogen-induced hormonal cancers, degenerative brain disorders, and cardiovascular diseases [7]. The twisted link between that basic pathway and growth and puberty will provide a vast area for future research, and COMT represents a good candidate to investigate links between childhood growth, adult height and adult health.

New genetic associations: with fetal growth

Xenobiotic-metabolizing genes and small-for-gestational-age births: interaction with maternal smoking

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Background: Common variants in xenobiotic-metabolizing genes could modify the effects of exposures such as maternal smoking on the risk of low birth weight for gestational age (SGA).

Methods: Two joint studies were conducted: (1) a case-control study of 493 SGA cases (birth weight <10th percentile for sex and gestational age) and 472 controls (at or above the 10th percentile), and (2) a family-based case-control study with approximately 250 case trios (mother, father, and newborn) and around 250 control trios. Logistic regression and a log-linear model were used to analyze the association

between genetic variants such as CYP1A1*2A, CYP1A1*2B, CYP1A1*4, GSTT1, GSTM1, and XRCC3 and SGA. The interaction between genetic variants and maternal smoking was also studied.

Results: Reduced risk of SGA was associated with complete maternal GSTT1 deletion, odds ratio (OR) = 0.63 (95% confidence interval = 0.41–0.97) and also complete newborn GSTM1 deletion OR = 0.74 (0.55–0.98). Increased risk of SGA was associated with partial newborn GSTT1 deletion, OR = 1.40 (1.01–1.95), and newborns homozygous for CYP1A1*2A had an OR of 4.28 (1.02–18.0). The trio-based results were consistent. Significant interactions were observed between maternal smoking in the third trimester and CYP1A1*2A ($p = 0.03$), XRCC3 ($p = 0.03$), and newborn GSTT1 ($p = 0.01$).

Conclusion: Certain genetic variants involved in the metabolism of xenobiotics are directly associated with the risk of SGA, and may also modify the effects of maternal smoking on risk of SGA.

Risk of small-for-gestational age is associated with common anti-inflammatory cytokine polymorphisms

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Background: Anti-inflammatory cytokines play a key role in maintaining pregnancy. Mothers with common variants in anti-inflammatory cytokine gene could have altered risks of spontaneous preterm birth or small-for-gestational age (SGA).

Methods: Polymorphisms in interleukin (IL)-4, IL-5, IL-10, IL-13, and transforming growth factor (TGF- β_1) were genotyped in a nested case-control study drawn from a prospective pregnancy cohort. Women were recruited between 24 and 29 weeks' gestation at obstetric clinics between February 1996 and June 2000. Haplotypes were inferred using the EM algorithm and the bayesian method, PHASE. Semi-bayesian hierarchical logistic regression analyses were implemented to obtain odds ratio (OR) estimates and 95% confidence intervals (CIs) for each polymorphism.

Results: White mothers who carried the 'low-producing' IL-4 CC and IL-10 ATA haplotypes had markedly reduced risk of SGA (for the CC haplotype, 0.2 [0.0–1.2]; for the ATA haplotype, 0.5 [0.3–0.8]). In contrast, in both African-American and white mothers, carriers of the 'high-producing' IL-4(–589)T variant had increased risk of SGA. African-American mothers who carried the IL-4 GCC haplotype had greater risk of spontaneous preterm birth (OR = 2.9; 95% CI = 1.2–7.4).

Conclusion: Variants related to decreased anti-inflammatory cytokine production may lower risk of SGA. Furthermore, the same mechanism that protects against SGA might increase risk of spontaneous preterm birth.

Risk of spontaneous preterm birth is associated with common proinflammatory cytokine polymorphisms

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Background: Preliminary data suggest that common variants in immune response genes may alter the risk of spontaneous preterm birth and small-for-gestational age (SGA). Additional candidate genes were genotyped.

Methods: The authors genotyped polymorphisms in 6 cytokine genes associated with inflammation: interleukin (IL)-1 α , IL-1 β , IL-2, IL-6, tumor necrosis factor (TNF), and lymphotoxin- α (LTA) in a nested case-control study drawn from a prospective pregnancy cohort. Women were recruited between 24 and 29 weeks' gestation at obstetric clinics between February 1996 and June 2000.

Results: Two haplotypes spanning the TNF/LTA genes were associated with increased risk for spontaneous preterm birth in white subjects (for the AGG haplotype, OR = 1.5 [95% CI = 0.8–2.6]; for the GAC haplotype, 1.6 [0.9–2.9]). Additionally, carriers of the GAG haplotype were found to have

decreased risk of spontaneous preterm birth (0.6; 0.3–1.0). The TNF(–488)A and LTA(IVS1-82)C variants, constituents of the AGG and GAC haplotypes respectively, were also strongly associated with increased risk of spontaneous preterm birth.

Conclusion: The results suggest that mothers with common variants in proinflammatory cytokine genes have altered risks for spontaneous preterm birth. Selected TNF/LTA haplotypes were associated with spontaneous preterm birth in both African-American and white subjects. The data do not support an inflammatory etiology for SGA.

These studies show that common genetic variants in the mother that affect maternal metabolism or level of inflammation may alter the risk for preterm delivery and for SGA. Furthermore, the paper by Infante-Rivard reports apparently independent effects of fetal genotypes on risk of SGA, although it is always difficult to completely separate the effects of maternal and fetal alleles.

The papers by Engel and colleagues include a total of 22 polymorphisms in 11 genes in 136 spontaneous preterm cases, 180 SGA cases and around 500 controls, and several of the findings are at borderline statistical significance. Larger studies are needed to refine these findings and to include even more candidate genes in the inflammation pathways. Furthermore, one can easily imagine biological interactions between pro- and anti-inflammatory proteins and genetic variants, interactions with maternal infectious status and other environmental factors, and also interactions with fetal genotypes. Very large studies will be needed to provide sufficient power to confidently demonstrate such interactions. Genetic statistical methods will also need to continue to evolve, for example to possibly represent these biological pathways as statistical pathway analyses that may allow different interactions at various stages.

Reviews – the future of genetic epidemiology

INS VNTR class genotype and indexes of body size and obesity: population-based studies of 7,999 middle-aged men and women

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Background: The association between the insulin gene (INS) variable number tandem repeat (VNTR) polymorphism with indexes of body size and obesity in adults is inconclusive. Equivocal and varying reports have been reported.

Methods: These associations were assessed by genotyping a marker for this polymorphism in a series of population-based cohort studies including a total of 7,999 middle-aged UK mainly Caucasian men and women.

Results: There was no convincing evidence that INS VNTR class genotype was associated with indexes of body size and adult obesity.

Conclusion: These data suggest that INS VNTR class is not an important determinant of size and body weight regulation in middle-aged men and women.

In light of this comprehensively negative study in adult men and women, the reported insulin gene VNTR associations with childhood obesity risk could therefore be false positive, limited to the childhood period, or reflect effect modification by early postnatal growth rate. Childhood growth data were unavailable in this study, and contrasting associations with this polymorphism have been reported to depend on early growth patterns [8]. However, such tentative explanations for a conclusively overall negative result will need much confirmation, and the most likely explanation for variable findings in genetic association studies remain quality and size, as discussed in two recent reviews [9, 10] in a recent series on Genetic Epidemiology in the *Lancet*. In 'What makes a good genetic association study?' [9], Hattersley and McCarthy confirm that sample sizes of around 5,000–10,000 may be required to detect genetic associations with effect sizes around 1.2–1.3, and they list a number of sources and ways to minimize errors in such studies. 'Genetic epidemiology and public health: hope, hype, and future prospects'

[10] discusses the hope of future studies using large population-based research biobanks consisting of 100,000–500,000 individuals, which are already recruited or in recruitment.

Food for thought – who else has any data?

Growth hormone (GH) pharmacogenetics: influence of GH receptor exon 3 retention or deletion on first-year growth response and final height in patients with severe GH deficiency

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Background: The presence or absence of exon 3 in the GHR gene has been associated with differences in 1- and 2-year growth responses to human recombinant GH (hGH) therapy in children without GH deficiency (GHD). The association of GHR-exon-3 genotype on the short- and long-term growth responses to hGH therapy in children with GHD was assessed.

Methods: In a university hospital setting, genotype and retrospective data analysis was performed on 75 children with GHD treated with hGH at a mean dose of 33 $\mu\text{g/kg}\cdot\text{day}$. GHR-exon-3 genotype (fl/fl vs. fl/d3 or d3/d3) was assessed by multiplex PCR. Growth velocity was analyzed in 58 children who remained prepubertal during the first year, and adult height in 44 patients after 7.5 ± 3.0 years of treatment.

Results: Clinical and laboratory data at the start of treatment and hGH doses were similar between the different genotype groups. Patients carrying at least one GHRd3 allele had a significantly faster growth velocity in the first year of hGH therapy (12.3 ± 2.6 vs. 10.6 ± 2.3 cm/year; $p < 0.05$) and achieved a taller adult height (SD score: -0.8 ± 1.1 vs. -1.7 ± 1.2 ; $p < 0.05$) compared with patients homozygous for GHRfl alleles.

Conclusion: GHD children who are homozygous for GHR exon 3 fl showed lower short- and long-term responses to hGH therapy. Approximately half of the population is homozygous for GHRfl, and future studies adjusting hGH therapy to genotype may improve outcome.

The d3-growth hormone (GH) receptor polymorphism is associated with increased responsiveness to GH in Turner syndrome and short small-for-gestational-age children

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Background: The deletion polymorphism of exon 3 of the GH receptor gene (d3-GHR) has recently been linked to the growth response to high-dose recombinant human GH (rhGH) therapy in short non-GH-deficient children. The authors tested this association in two distinct groups of non-GH-deficient children: (1) short girls with Turner syndrome and (2) short children born small for gestational age (SGA).

Methods: A retrospective study of all rhGH-treated prepubertal children (aged 3.5–14 years) during the last 18 years. Patients with Turner syndrome ($n = 53$) defined by karyotype received a mean rhGH dose of 38 $\mu\text{g/kg}\cdot\text{day}$ (SD ± 8). Short SGA children ($n = 60$), defined as birth length and/or weight < -2.0 SD score plus pretreatment height < -2.0 SD score, received a mean rhGH dose of 56 $\mu\text{g/kg}\cdot\text{day}$ (SD ± 11). Growth prediction for the first year of therapy was calculated on the basis of rhGH dose, age, weight, height, and gender-adjusted midparental height according to the prediction models by Ranke et al. The GHR-exon-3 locus was genotyped using a PCR multiplex assay.

Results: Pre-rhGH therapy there were no significant differences in height, spontaneous height velocity, IGF-I, or IGFBP-3 levels between the three GHR genotype groups. Turner syndrome girls carrying one or two d3-GHR alleles showed a significantly higher 1st year increment in height velocity ($p = 0.019$) and exceeded their 1st year growth prediction ($p = 0.007$), whereas their increments of IGF-I and

IGFBP-3, weight, and height were not significantly different. Short SGA carriers of d3-GHR also grew significantly faster than predicted ($p = 0.023$), but gain of height velocity was not significantly higher ($p = 0.067$). The mean 1st year height gain associated with d3-GHR was around 0.75 cm in SGA and 1.5 cm in Turner syndrome.

Conclusion: The data support an increased responsiveness to high-dose rhGH in association with the d3-GHR genotype. The magnitude of this effect may depend on the cause of the short stature.

Both of these reports positively confirm the study of dos Santos et al. in short non-GH-deficient children [11] reported on in last year's Yearbook, extend those observations to GH-deficient children and Turner syndrome girls, and suggest a nearly 1 SD difference in adult height between genotype groups. The functional effect of this variant is still unclear, but as it is carried by around 50% of all individuals and has an apparently dominant effect, this common marker likely explains a large proportion of the interindividual variation in responses to GH therapy.

Confirmation of the initial association study is a clearly important step in showing a true genetic association, and these two studies, including a total of 188 children, are encouraging. However, publication bias towards the positive findings may distort the real size of the association. At last year's ESPE 2005 meeting, in addition to these papers there were a further 5 studies presented on this association, which have yet to be fully published. Of those studies, only 1 ($n = 15$ children) was also confirmatory, but 4 ($n = 237$ children) found no association. How many of you are also sitting on further, likely negative, unpublished data on this variant? Initiatives such as the Network of Investigator Networks [12], set up to share best practices and methods for genetic association analyses, and in particular to integrate all published and unpublished data, including 'negative' studies, are surely welcome.

Food for thought – what is your biological age?

Obesity, cigarette smoking, and telomere length in women

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Introduction: Obesity and smoking are important risk factors for many age-related diseases. Both are associated with increased oxidative stress, which increases the rate of telomere erosion per replication, and inflammation, which enhances white blood cell turnover. Together, these processes might accelerate the rate of telomere erosion with age.

Methods: In 1,122 white women aged 18–76 years, the association between telomere length in white blood cells and age, body mass index and smoking was assessed.

Results: Telomere length decreased steadily with age at a mean rate of 27 bp per year. Telomeres of obese women were 240 bp shorter than those of lean women ($p = 0.026$). A dose-dependent relation with smoking was recorded ($p = 0.017$), and each pack-year smoked was equivalent to an additional 5 bp of telomere length lost, which is 18% of the yearly rate in the overall cohort.

Conclusions: These results emphasize the pro-ageing effects of obesity and cigarette smoking.

Telomeres cap the ends of chromosomes and protect them from degradation and end-to-end fusion. In cultured somatic cells, telomeres undergo erosion with each cycle of replication. Oxidative stress enhances this erosion. The authors hypothesized that telomere attrition in white blood cells may serve as a marker of the cumulative oxidative stress and inflammation and, consequently, represent a marker for biological ageing. Indeed they found considerable variation in telomere length between individuals, and telomere length decreased progressively and linearly with age.

The data are cross-sectional, so rates of telomere loss can only be estimated. Furthermore, while the population was based on 45 monozygotic and 516 dizygotic twin pairs from an adult twin registry, the authors did not analyze within-pair discordances in BMI or smoking. However, compared to the mean population age of 47.8 years, the authors estimated that the difference in telomere length

between being lean and being obese corresponds to 8.8 years of ageing, and smoking a pack of cigarettes per day for 40 years corresponds to 7.4 years of ageing. Future studies will hopefully also identify those factors that make us younger!

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