WEB WATCH

Human Ageing Genomics Resources

• http://genomics.senescence.info/index.html

If you are working on or are interested in human ageing, you should know about the Human Ageing Genomics Resources (HAGR): a web site that brings together databases and computational tools to help understand the biology of ageing.

The resource started in 2002 as a collaborative project at the University of Namur, Belgium. A searchable and browsable database of genes that are related to human ageing, GenAge, forms the core of the resource. Each gene page contains nomenclature, cytogenetic and protein information. Among other useful features are links to relevant publications and a list of orthologues with links to NCBI Entrez. There are also further external links to OMIM, Swiss-Prot and GeneCard, to name but a few.

Another database, AnAge, caters for those who work on ageing in other species. It has information on ageing in *Drosophila melanogaster*, *Caenorhabditis elegans*, red and purple sea urchin, and 2,469 chordate species!

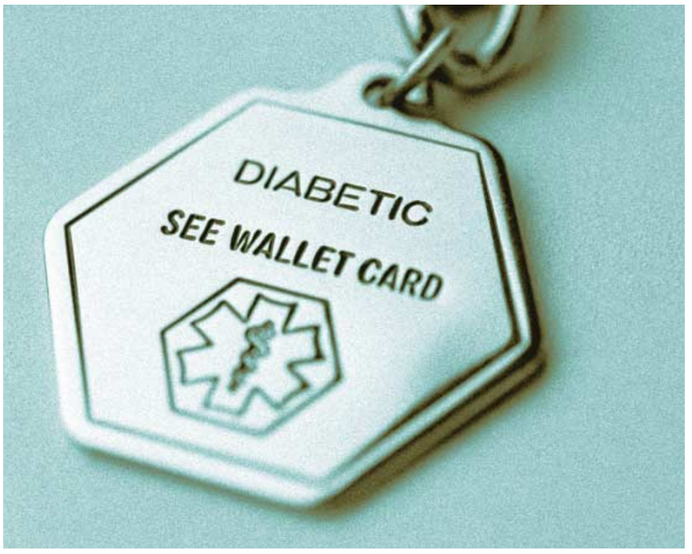
But databases are not all the HAGR has to offer: there is also the Ageing Research Computational Tool — ARCT. This Perl-based toolkit allows you to generate phylogenetic profiles locally or through NCBI's BLAST, to data-mine multiple sequences to find regulatory or functionally important regions, to display protein–protein interactions and phylogenetic trees, and to access other data-analysis programs such as ClustalW and Gibbs.

The project team is busy making continuous improvements to HAGR. For example, the inclusion of gene-expression information is on the cards. The team plan to include genes that are differently expressed between young and old tissues, focusing mainly on microarray data.

Magdalena Skipper

HUMAN GENETICS

Dissecting diabetes



Two new association studies have revived hopes that research into unusual monogenic forms of diabetes can provide pointers to the genetic basis of susceptibility to the common multifactorial form of the disease.

Maturity-onset diabetes of the young (MODY) is an autosomal dominant form of the disease that usually develops before an individual reaches 25 years. The hepatocyte nuclear factor-4α gene (*HNF4α*) that underlies one form of MODY encodes a β-cell transcription factor that is involved in insulin secretion. The hope has been that variation in this gene might also help to explain susceptibility to type 2 diabetes (T2D) — the much more common late-onset form.

Despite linkage evidence for a susceptibility locus in the *HNF4α* region, previous attempts to identify *HNF4α* variants associated with T2D, which focussed on the coding sequence of this gene, had failed. Now, however, Latisha Love-Gregory and Kaisa Silander, with their respective colleagues, have independently gathered convincing evidence for a link between T2D and variation in the region of an alternative promoter for *HNF4α* (P2) that drives transcription of what is probably the gene's dominant splice variant in pancreatic β-cells.

Love-Gregory *et al.* used linkage disequilibrium (LD) to map a 78-kb candidate region that included *HNF4α* and P2. They identified haplotype tagging SNPs (htSNPS) that would represent the most common haplotypes across this region in Ashkenazi Jews. The authors then compared frequencies of the nine

DEVELOPMENT

A new twist to bone formation

The identification of a novel anti-osteogenic domain by Gerard Karsenty and colleagues, reported in *Developmental Cell*, has revealed that the differentiation of osteoblasts — the cells that are responsible for bone formation — is more complex than was previously suspected.

Building on work that identified *Runx2* as the master gene for osteoblast differentiation, the authors wanted to find out why it is expressed four days before osteoblasts appear. Taking this delay as evidence for the involvement of other regulatory proteins, and considering that some developmental bone diseases result from increased (presumably premature) bone formation, they wondered if *Runx2*, which encodes a transcription factor, might be negatively regulated.

Increased bone formation in cranial sutures is seen in *Twist1*$^{+/-}$ mice and also in Saethre–Chotzen patients who are heterozygous for *TWIST1* inactivation. So, Karsenty and co-workers hypothesized that Twist1 (and perhaps its relative, Twist2) negatively regulates Runx2 — and therefore osteoblast differentiation.

***In situ* hybridization in embryonic day (E)12–15 mice confirmed that osteoblast differentiation is seen only after the decrease of *Twist* gene expression. In addition, they found that when the *Twist* genes are not expressed, osteoblasts differentiate prematurely. Moreover, the authors showed that inactivation of the *Twist* genes can rescue a *Runx2* haploinsufficiency phenotype. So, it seems that *Runx2* interacts with both *Twist1* and *Twist2*.**

Twist1 was found to inhibit osteoblast differentiation without affecting *Runx2* expression, so how exactly do the Twist proteins exert their effect? DNA co-transfection experiments revealed that Twist1 and Twist2 specifically inhibit the transactivation function of Runx2. Using Twist1 deletion mutants in these experiments, Karsenty and colleagues identified the domain responsible for the inhibition — the Twist box. It turns out that this novel anti-osteogenic domain of 20 amino acids, which is only present in Twist1 and Twist2, binds directly to the Runt DNA-binding domain of Runx2, and

htSNPs in individually genotyped cases and controls of Ashkenazi-Jewish descent. The pay-off for this well-designed candidate-gene-association study was the identification of a htSNP in the P2 region that was associated with T2D.

By contrast, Silander *et al.* were building on previous mapping work on non-insulin-dependent diabetes mellitus (NIDDM) by the Finland–United States investigation of NIDDM Genetics (FUSION) study on affected sibling-pair families from Finland, which indicated that there might be a T2D-susceptibility locus in the 20q13.12–20q13.13 region. Their approach was to compare the frequencies of 291 SNPs in this region between pooled DNA from FUSION cases and controls. A different strategy in a different population but an equally successful result: another SNP in the P2 region associated with T2D. More intensive follow-up SNP genotyping around *HNF4α* identified another T2D-associated SNP in the same region, in near perfect linkage disequilibrium with the first.

Commendably, the two groups collaborated to follow up each other's work, and found that the SNPs that they originally identified were in near perfect linkage disequilibrium with each other. Overall, they identified four SNPs in the P2 region that are associated with T2D in both populations and, in doing so, immeasurably strengthened the overall findings.

So, for diabetes at least, those who study rare Mendelian versions of complex diseases in the hope that it will tell us something about the multifactorial disorder seem to be on the right track. However, these results also beg the question: how many candidate-susceptibility genes have been wrongly discarded because only coding SNPs have been examined in association or linkage studies?

Nick Campbell

References and links

ORIGINAL RESEARCH PAPERS
Love-Gregory, L. D. *et al.* A common polymorphism in the upstream promoter region of the hepatocyte nuclear factor-4α gene on chromosome 20q is associated with type 2 diabetes and appears to contribute to the evidence for linkage in an Ashenazi Jewish population. *Diabetes* **53**, 1134–1140 (2004) | Silander, K. *et al.* Genetic variation near the hepatocyte nuclear factor-4α gene predicts susceptibility to type 2 diabetes. *Diabetes* **53**, 1141–1149 (2004)
WEB SITES
Alan Permutt's laboratory:
http://www.genetics.wustl.edu/molgen/permutt.html
Francis Collins' laboratory:
http://www.genome.gov/10000351

therefore decreases the ability of Runx2 to bind to DNA. Finally, the authors showed that *in vivo* mutagenesis of the Twist box also leads to premature osteoblast differentiation.

So, this work shows that a decrease in the expression of the *Twist* genes relieves the Twist-box-inhibition of Runx2 and so triggers osteoblast differentiation. It also reveals that Saethre–Chotzen syndrome results from premature RUNX2 activity. Not only that — it also identifies the Twist box as the earliest regulator of osteblast differentiation.

Natalie Wilson

References and links

ORIGINAL RESEARCH PAPER Bialek, P. *et al.* A Twist code determines the onset of osteoblast differentiation. *Dev. Cell* **6**, 423–435 (2004)
WEB SITE
Gerard Karsenty's laboratory:
http://imgen.bcm.tmc.edu/molgen/facultyaz/karsenty.html

IN BRIEF

GENOME EVOLUTION

The evolutionary gain of spliceosomal introns: sequence and phase preferences.

Qiu, W.-G. *et al. Mol. Biol. Evol.* 10 Mar 2003 (doi:10.1093/molbev/msh120)

Whether introns were introduced into the eukaryotic genome early or late in evolution has been a matter of hot debate for some time. Qiu and colleagues have systematically analysed the distribution and reading-frame phase of introns in ten gene families to infer their evolution. They show that the introns were probably not present in the common ancestors of these families and that the bias towards phase-0 introns is probably the result of repeated and recent phase-biased intron gain rather than the retention of an ancestral bias.

HUMAN GENETICS

The effects of human population structure on large genetic association studies.

Marchini, J. *et al. Nature Genet.* 28 Mar 2004 (doi:10.1038/ng1337)

Assessing the impact of population stratification on genetic association studies.

Freedman, M. L. & Reich, D. *et al. Nature Genet.* 28 Mar 2004 (doi:10.1038/ng1333)

Undetected population structure can lead to false positives in large-scale association studies. Freedman *et al.* show that, contrary to what has been proposed, stratification can be difficult to detect with only a few unlinked markers. So, some population stratification might be present even in well-designed studies. Marchini *et al.* assess the effects of stratification in three example populations and show how even modest stratification has effects that increase with sample size. Their results could inform large-scale study designs.

TECHNOLOGY

A resource for large-scale RNA-interference-based screens in mammals.

Paddison, P. J., Silva, J. M., Conklin, D. S. *et al. Nature* **428**, 427–431 (2004)

A large-scale RNAi screen in human cells identifies new components of the p53 pathway.

Bers, K. & Hijmans, E. M. *et al. Nature* **428**, 431–437 (2004)

Two groups have created a tremendous resource for carrying out large-scale genetic screens in mammalian cells. Using similar strategies, Hannon and colleagues and Bernards and colleagues constructed short hairpin (sh) RNA-expression libraries, each of which includes ~25,000 shRNA expression cassettes that target between 8,000 and 10,000 human genes (the first library also targets ~5,500 mouse genes). shRNA-carrying cassettes can be packaged in retroviruses for transfection and tracked in mixed populations thanks to the use of DNA barcodes. While testing their libraries, Bernards' group identified modulators of p53-dependent proliferation arrest, whereas Hannon's group used it to screen for defects in human proteasome function.