This Month in Genetics

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Prenatal Diagnosis from Maternal Blood

Although risks associated with amniocentesis and chorionic villus sampling have decreased over the years, these invasive procedures are still associated with a risk of miscarriage somewhere in the neighborhood of 1 in 350 to 1 in 400. The risks are higher if the obstetrician has less practice with the techniques or does them infrequently. These risks, not to mention the fact that women have to agree to having big needles or catheters put where they'd rather not, can make prenatal diagnosis scary. Because women have to provide blood samples for other tests during pregnancy, wouldn't it be great if they could simply provide an extra tube of blood for use in prenatal diagnosis of aneuploidy? Fan et al. have brought us one step closer to making this a reality. They were able to detect the most common trisomies, for chromosomes 13, 18, and 21, through Solexa/Illumina high-throughput sequencing of cell-free DNA from maternal plasma. Focusing on the appropriate chromosomes, they looked for overrepresentation of sequence tags. In all cases, they were able to pick out the trisomic pregnancies as outliers from the distribution of values from disomic pregnancies. Added benefits of the technique are that it is polymorphism independent, it theoretically could be expanded to look for other forms of aneuploidy—such as unbalanced translocations—and the procedure can be run for about $700. The number of pregnancies they looked at was small, but this work demonstrates that their technique is promising.

H.C. Fan et al. (2008). Proc. Natl. Acad. Sci. USA. Published online October 6, 2008. 10.1073/pnas.0808319105.

Long-Range Control of Sonic Hedgehog

Holoprosencephaly (HPE) encompasses a range of anomalies that are a result of improper separation of the hemispheres and ventricles in the developing forebrain. One of the key genes that has been implicated in autosomal-dominant nonsyndromic HPE is sonic hedgehog (*SHH*). Although we know that perturbations of SHH level can have dire consequences, we know little about how it is regulated. Jeong et al. went mutation hunting in patients with HPE in the hopes of further defining regulatory sequences for *SHH*. In one affected individual, they identified a C-to-T change in an enhancer 460 kb upstream of the *SHH* coding sequence. This putative mutation is located in a conserved 10 bp sequence within SBE2, a highly conserved enhancer known to regulate *SHH* expression in the brain. In an interaction that is perturbed by the C-to-T change, Jeong et al. reveal that the transcription factor Six3 binds to this sequence and activates *SHH* transcription. Mutations in *SIX3* also cause holoprosencephaly, and HPE-associated missense changes in the homeodomain or the Six domain also reduce or prevent the Six3-SBE2 association. This work provides a mechanistic link between two HPE-associated genes through the demonstration that *SIX3* directly regulates *SHH* expression in the developing forebrain.

Y. Jeong et al. (2008). Nat. Genet. Published online October 5, 2008. 10.1038/ng.230.

Autophagy Defects in Crohn's Disease

We all know that recycling is a good thing to do. But did you know that ignoring that recycling could lead to Crohn's disease? One of the genes that has recently been implicated in the development of Crohn's disease is *ATG16L1*, which encodes a protein predicted to be involved in autophagy, nature's recycling system. Both Saitoh et al. and Cadwell et al. developed mouse models deficient for *Atg16L1* and found that, indeed, autophagy is disrupted. The two groups follow different avenues to explore what this means for Crohn's disease. In the work by Saitoh et al., disruption of autophagy enhances production of the inflammatory cytokines IL-1β and IL-18 by macrophages. The relevance of this finding is demonstrated in an induced model of colitis. Mice lacking Atg16L1 in hematopoietic cells are exquisitely sensitive to this colitis, a reaction that can be alleviated by sopping up the excess cytokines with antibodies to IL-1β and IL-18. While Cadwell et al. were studying their *Atg16L1* hypomorphic mice, they discovered defects in granule exocytosis in Paneth cells, a specialized component of the innate immune system found in the intestinal epithelium. Similar Paneth cell disruptions are observed in intestinal specimens from Crohn's disease patients homozygous for the *ATG16L1* risk allele. These changes are accompanied by increased expression of genes involved in the intestinal injury response. Apparently, not keeping up with your cellular recycling throws the innate immune response in the intestine out of whack. The mechanistic link between

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the two systems will be the next part of this story to uncover.

K. Cadwell et al. (2008). Nature. Published online October 5, 2008. 10.1038/nature07416; T. Saitoh et al. (2008). Nature. Published online October 5, 2008. 10.1038/nature07383.

A Double-Stranded RNA Trigger of Age-Related Macular Dystrophy?

We Americans tend to think of virally caused blindness as a concern that is reserved for other parts of the world. With the study by Yang et al., perhaps this attitude has been naive. Their work implicates double-stranded RNA (dsRNA) in the development of the most common cause of visual impairment in the developed world, age-related macular degeneration (AMD). Because inflammatory cascades are believed to be perturbed in AMD, the authors looked for association of AMD phenotypes with markers in the toll-like receptors *TLR3* and *TLR4*. In three separate case-control samples, a SNP in *TLR3* was associated with geographic atrophy, a component of the dry form of AMD. To understand this association, the authors looked at TLR3 activation in a cell-culture model. TLR3 activation by a prototypical dsRNA induces cell death. This holds true in retinal pigment epithelial cells homozygous for the risk allele in *TLR3*, but in cells heterozygous for the protective *TLR3* allele, cell death was reduced by half. Moving this to an in vivo model, injecting a dsRNA into mouse eyes induces a geographic-atrophy-like phenotype, an effect that is not observed in mice lacking *Tlr3*. In their experimental models, dsRNA clearly can play a role in the death of retinal cells; proof that there is a natural viral source of this dsRNA and that activation of TLR3 plays a major role in AMD is still required.

Z. Yang et al. (2008). N. Engl. J. Med. 359, 1456–1463. 10.1056/NEJMoa0802437.

The DNA-Vinci Code

Triplet codons. TATA boxes. Polyadenylation signals. You'd think that by now, we would understand all of the cues within the DNA sequence. It seems that there are still some mysteries hidden therein. Take transcription-factor binding sites. Although their function is conserved, the precise binding sites of highly conserved transcription factors vary between species. When human and mouse are compared, less than one-third of binding sites align, even when you look at the same protein binding the promoters of orthologous genes (Odom et al. 2007, Nat. Genet. 39, 730–732). To figure out whether the DNA sequence or the nuclear environment plays a bigger role in these differences in transcriptional regulation, Wilson et al. studied transcription in a mouse strain that carries human chromosome 21. They reasoned that transcription from this human chromosome would be more similar to other human chromosomes 21 if genetic sequence is the main driver of transcriptional regulation, and it would be more similar to the mouse chromosome if nuclear environment is more important. Through chromatin-immunoprecipitation experiments, they demonstrate that transcription-factor binding and the positions of histone modifications are largely driven by the underlying genetic sequence rather than by the local environment. Gene expression from the human chromosome is also much more highly correlated with expression from wild-type human cells than it is with orthologous mouse genes. It's sort of like the Da Vinci Code—we don't recognize all of the DNA sequence information that governs transcription-factor binding and gene expression, but the instructions must be in there somewhere. We just have to look hard enough.

M.D. Wilson et al. (2008). Science 322, 434–438. 10.1126/science.1160930.