This Month in Genetics

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Fix-It Man

Although many aneuploidies arise from maternal nondisjunction, Turner syndrome is an exception. In the majority of cases of monosomy X, the missing sex chromosome is paternal. Lange et al. have evidence that, in some cases, this may result from the loss of a structurally defective Y chromosome that is an unintended byproduct of a mechanism designed to maintain Y chromosome integrity. Because the majority of sequence on the Y does not have homologous sequence with which to recombine, it is predicted to accumulate deleterious mutations. Counteracting this problem, the Y chromosome has a set of large palindromic sequences that can serve a similar function. The palindromes are maintained by gene conversion in an intrachromosomal mechanism that allows a mutant sequence within the palindrome to be corrected with its sister sequence. Lange et al. define a likely mechanism by which this occurs and the consequences when this mechanism falters. The authors propose a model in which double-strand breaks are resolved with palindromic sequences within a chromatid or between sister chromatids in a mechanism that does not involve crossover. An alternative resolution to the double-strand breaks is an isodicentric Y chromosome, which would arise via interchromatid crossover. Indeed, the authors find physical evidence of this alternative resolution with the identification of 51 individuals who possessed an isodicentric Y formed via this mechanism. The researchers weren't just lucky to find these rare alternative products; this study was made possible by their collection of >2000 individuals likely to have a sex-chromosome defect, which was collected over 25 years. The inherent instability associated with a Y chromosome with two centromeres means it is likely to be lost during mitosis. In fact, the researchers found individuals who were mosaic for an isodicentric Yp chromosome and a 45,XO constitution. These individuals were phenotypically female, despite the fact that they had two copies of the sex-determining gene *SRY*, and some of them had phenotypic features of Turner syndrome. The likelihood of sex reversal increased with the intercentromeric distance on the isodicentric chromosome, suggesting that this increases competition between the two centromeres, which leads to additional chromosome instability. If an isodicentric Y were lost early in development, full-blown Turner syndrome without detectable mosaicism for the isodicentric Y would be the result, providing a plausible explanation for the largely paternal origin of this aneuploidy.

Lange et al. (2009). Isodicentric Y chromosomes and sex disorders as byproducts of homologous recombination that maintains palindromes. Cell 438, 855–869.

Histone Demethylation Reveals Loci for DNA Methylation Imprints in Oocytes

How does a germ cell know which loci should be genetically imprinted? DNA methylation via DNMT3A is central to the parent-of-origin-specific gene expression in imprinted regions, but we haven't clearly understood how this methyltransferase knows which genetic regions to modify. Ciccone et al. now provide evidence that this is at least partly determined through the activity of the KDM1B. This protein is a histone H3 lysine 4 (H3K4) demethylase whose activity is required to remove a histone modification that is protective against DNA methylation during oogenesis. In mice, a female deficient for KDM1B produces oocytes that have excess H3K4 methylation. The resulting embryos lack appropriate DNA methylation imprints at multiple genes that are normally maternally methylated. This failure to establish the appropriate epigenetic marks leads to a loss of monoallelic expression of these genes and the embryos do not survive. Thus, histone methylation protects some loci from DNA methylation by DNMT3A, and KDM1B can expose some of these loci to the activity of the methyltransferase. However, this does not clear up the original question of DNMT3A specificity. At least in a bulk assay on chromatin, KDM1B's demethylase activity is wide ranging and does not appear to have the specificity that is needed to determine which loci are imprinted. The authors propose that the timing of KDM1B expression in oocytes might govern which imprinted loci it controls and that DNMT3A together with its cofactor potentially has additional specificity that allows it to select between the unprotected loci. It remains to be seen whether there are other histone demethylases that are needed for establishment of imprinting at loci that are not affected by KDM1B.

Ciccone et al. (2009). KDM1B is a histone H3K4 demethylase required to establish maternal genomic imprints." Nature, in press. Published online September 2, 2009. 10.1038/nature08315.

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The Death of Linkage Analysis?

Graduate students, breath a sign of relief; the era of the LOD score may be over. Ng et al. have correctly identified the gene for Freeman-Sheldon syndrome with four unrelated, affected individuals in the absence of prior linkage information or candidate gene analysis. The key to this finding is the ability to detect rare genetic variation, which, beyond causing rare Mendelian disorders, could also play a significant role in complex genetic traits. Sure, large-scale DNA sequencing is continually getting faster and cheaper, but we're not at the point at which it is feasible to perform whole genome sequencing on large study populations in order to find rare variation in that way. Rather than sequencing complete genomes, Ng et al. specifically captured the coding regions on arrays and then sequenced whole "exomes" by using massively parallel sequencing. This vastly decreased the amount of DNA they needed to sequence, and the authors reasoned that it also would allow them to focus on variation that was likely to have a functional impact. Not only did they show that they could sensitively and specifically detect variation in eight exomes of HapMap individuals from three populations, they compared the exome sequences of four individuals with Freeman-Sheldon syndrome with those of the other eight in their study and with the variation in dbSNP and used this information to correctly identify the disease gene. I'm of course being a little snide here; there is still room for linkage analysis in gene hunting, but this work proves that, in some cases, we may be able to skip this step to find coding variation that governs Mendelian traits.

Ng et al. (2009). Targeted capture and massively parallel sequencing of 12 human exomes. Nature 461, 272–276.

Canine Grooming Made Simple

From an Afghan hound's silky fur, which begs to be stroked, to the tight curls of an Airedale terrier, there is a broad range of coats in the domestic dog breeds. You might expect that variation in many genes would govern these differences in coat length and texture, but, remarkably, Elaine Ostrander and her colleagues have identified three mutations that account for the majority of coat phenotypes in the purebred dogs in the United States. For each basic coat trait—hair length, curl, and the presence of the eyebrow and moustache-like tufts called furnishings—the researchers started with a breed that segregated the trait of interest (such as long-haired versus short-haired dachsunds) and performed a genome-wide association study. This approach controlled for other phenotypic features of the breed. They confirmed their strongest associations in a "case-control" sample that included dogs from many breeds and then identified the relevant mutations through DNA sequencing in the smallest shared haplotype between the data sets. For each coat characteristic, a single major mutation was identified, one each in *RSPO2*, *FGF5*, and *KRT71*. These three mutations act in various combinations to produce the vast majority of coat types observed in purebred dogs, which vary from the ancestral, short-haired state that is also found in wolves to the curly coated dogs with furnishings, such as the Bichon-Frisé, that possess all three of the mutations. Perhaps some complex traits are not so genetically complex after all.

Cadieu et al. (2009). Coat variation in the domestic dog is governed by variants in three genes." Science Express, in press. Published online August 27, 2009. 10.1126/science.1177808.

I'd Like to Make a Withdrawal

The completion of a successful genome-wide association study is cause for celebration and constitutes a bridge crossed on the way to dissecting genetic contributions to complex traits. But after the champagne corks have been popped and the dissertations defended, the next huge phase of the project begins in which the reason for the association is explored. How to proceed? For the *IL2RA* association with type 1 diabetes (T1D), Dendrou et al. used a set of individuals without T1D in order to define an endophenotype that could explain the relationship between genetic variation in the region and risk of disease. They were vastly aided by the availability of the Cambridge BioResource, a collection of ~5000 normal volunteers in the Cambridge, UK, area who have donated blood and saliva samples and who can be recalled for further phenotyping on the basis of their genotype. The authors recruited participants who had one of three protective haplotypes in the *IL2RA* region or that were homozygous for the susceptible haplotype at *IL2RA*. Fresh blood samples from these individuals were analyzed by flow cytometry to measure IL2RA (aka CD25) cell-surface expression. Correlations between *IL2RA* haplotype and the level of CD25 expression in specific immune cell subsets were observed. One of the roles of IL-2 signaling is to support the function of T regulatory cells, which are critical for immune tolerance and therefore the prevention of autoimmunity. Beyond highlighting a pathway that could be involved in the development of T1D, the approach taken in the study serves as a model for teasing apart other genetic associations with complex traits and argues for the development of similar Biobanks in other areas of the world.

Dendrou et al. (2009). Cell-specific phenotypes for the autoimmune locus IL2RA using a genotype-selectable human bioresource. Nature Genetics 41, 1011–1015.

This Month in Our Sister Journals

Drosophila Hit the Bottle (or Column, as It Were)

Although in some cases genome-wide association studies have been successful, there are certainly circumstances in which they will not be able to identify the genetic variation associated with a trait of interest. Particularly in cases in which the causative variant is a rare allele or it has only

a modest effect, this type of study is often underpowered. If one can focus an association study to a particular set of candidate genes, you can alleviate the statistical penalty you incur because of multiple testing and you can also tailor the set of markers you choose, on the basis of gene-specific linkage disequilibrium. That's all well and good, but how do you pick the candidate genes? Morozova et al. used a tremendous *Drosophila* resource to study their trait of interest, amount of alcohol consumption, in order to select a candidate gene for study in humans. They have a panel of 40 inbred *Drosophila* lines that originated from a natural population. In a previous paper (Nature Genetics *41*, 299–301), they used this panel to identify over 10,000 genetically variable transcripts that they could group into transcriptional modules. In the current work, they used the transcript data to look for transcript modules that were associated with ethanol sensitivity in the *Drosophila* lines. One of the networks of transcripts includes several metabolic enzymes, including the gene for malic enzyme (*Men*), which is a central connector of different metabolic pathways. There is a human ortholog to *Men* called *ME1*, and the authors selected this gene as the proof of principle that the *Drosophila* network analysis could successfully identify a candidate gene network in humans. Although the effects of variation in *ME1* were small, an association was documented between variation in *ME1* and cocktail consumption in the Framingham Offspring sample. Whereas they found this association by using genotypes at 21 SNPs in a sample of ~1700 individuals, the authors estimate that a genome scan would have to include greater than 7600 individuals in order to find the *ME1* SNP with the largest effect on alcohol consumption because of the lower power associated with this approach. Not only is this an interesting approach to finding candidate genes, but I also found it fun to read about their quantitative method for measuring drunkenness in flies!

Morozova et al. (2009). Alcohol sensitivity in Drosophila: translational potential of systems genetics. Genetics, in press. Published online August 3, 2009. 10.1534/genetics.109.107490.

Are You Positive?

The premise of newborn screening (NBS) is simple: we want to catch as many babies as we can who are affected with certain genetic disorders in order to minimize the effects of these disorders. In practice, though, if you set the cut-off values for a positive screen to maximize the number of affected individuals that you can identify, you will also end up with false-positive screens. Although the false positives can be teased out with further testing, there is evidence to suggest that parents of children who screened falsely positive in NBS will have increased concerned about their child's health and may seek excess healthcare for their child as a result. In the largest study of this issue to date, Lipstein et al. explore this hypothesis in this month's issue of *Genetics in Medicine*. They recruited a set of parents who received false-positive NBS results and matched them with a set of parents who had a child with negative NBS results and then surveyed them in terms of their child's health care utilization over the course of approximately the first 6 to 12 months of the child's life. Although the parents of children with a false-positive NBS reported higher stress levels, they found no evidence that these parents seek more healthcare for their children than do parents of children with normal NBS results. This finding contrasts some previous reports and suggests that excess healthcare utilization does not need to be factored into the costs of newborn screening programs.

Lipstein et al. (2009). Impact of false-positive newborn metabolic screening results on early health care utilization. Genetics in Medicine, in press. Published online September 11, 2009. 10.1097/GIM.0b013e3181b3a61e.