This Month in *The Journal*

Robin E. Williamson[1]

Methods of Analyzing Rare Variants

Li and Leal, page 311
The current technologies in use for the identification of genetic variants associated with complex diseases focus on the concept that common variants are responsible for a small but significant risk effect. An alternative, but nonexclusive, hypothesis is that multiple rare variants are responsible for a gene's association with a phenotype. These rare variants are more difficult to identify with methods that are based on linkage disequilibrium and tSNPs because such variants are less likely to be tagged by known SNPs in the region. Also, several different rare variants may be present in a single dataset of affected individuals. To deal with these situations, Li and Leal present techniques for identifying association variants in the presence of high allelic heterogeneity. Their analyses handle sequence data that will become increasingly more available as sequencing technologies improve. The authors compare the single-marker, multi-marker, and collapsing versions of their methods and, on the basis of the results of their investigation, propose a "combined multivariate and collapsing" approach that reaps the benefits of each of the individual methods.

Adaptive Evolution of the *UGT2B17* CNV

Xue et al., page 337
Copy-number variation (CNV) is recognized as a significant source of variation in humans, and differentiation between humans and great apes, as well as among subsets of human populations, suggests that CNV has potentially contributed to evolutionary processes. To assess whether the presence of a region of CNV is due to selection versus genetic drift, Xue et al. decide to evaluate a specific CNV using the techniques already established for detecting signatures of selection at SNPs. The authors choose to study the CNV containing *UGT2B17* because the gene's involvement in steroid and xenobiotic metabolism makes it a strong candidate for evolutionary control of gene dosage. Additionally, the gene resides in a complex region of the genome, and the analysis of such a region might provide an important example for the design of CNV-selection studies. Xue et al. start by re-examining and modifying the reference assembly to obtain an accurate depiction of the structure of the *UGT2B17* alleles. An analysis of the alleles on a panel of samples from around the world reveals that the deletion allele is more frequent in the East-Asian populations than it is in African and European populations. By resequencing the segments flanking the deletion in the four populations studied, the authors perform a number of neutrality tests while taking into account the complexity of the region. The results of these analyses suggest that the region underwent unique selective events in each of the different populations.

Gene Therapy for mtDNA-Related Diseases

Ellouze et al., page 373
Although many advancements have been made in the identification of mitochondrial diseases and in the understanding of their pathogenesis, treatment of such diseases is often quite ineffective. One potential therapeutic method relies on the expression of mitochondrial genes in the nucleus. This technique, termed allotopic expression, has recently been optimized for a few genes, and the successful restoration of mitochondrial function in vitro has been reported. As the next step toward developing a means for treating mitochondrial diseases in humans, Ellouze et al. sought to establish how well allotopic expression works in an in vivo system. In order to do this, the authors first needed to develop a proper animal model in which to test their method. Their previous work has focused on Leber Hereditary Optic Neuropathy (LHON), and they extend that work here. LHON is the most common mitochondrial disease, and most cases are due to mutations in *ND1*, *ND4*, or *ND6*. The authors allotopically express a common mutant variant of *ND4* in adult rat eyes, and evaluation reveals that the treated eyes develop a LHON phenotype. Subsequent treatment with the wild-type gene successfully rescues the disease phenotype and prevents further damage.

DYRK1A Dosage Perturbs REST Levels

Canzonetta et al., page 388
Down syndrome (DS), caused by trisomy of chromosome 21, is characterized by a number of phenotypic features, and a great deal of work has been done to attribute each of the disease features to the increased dosage of specific regions of chromosome 21. Previous work has reported that levels of neuron-restrictive silencer factor,

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encoded by *REST*, are decreased in Down-syndrome brain cells. REST is a key regulator of many neuronal functions and is involved in neuronal differentiation. To further examine this relationship, Canzonetta et al. evaluate the level of *Rest* expression in a transchromosomic mouse model of DS. They begin by looking at pluripotent mouse embryonic stem cells, and they report that the expression of *Rest* is significantly reduced in DS cells in comparison to transcript levels in wild-type cells. By examining a number of mouse DS model cell lines, the authors are able to determine that this effect on *Rest* levels is due to a minimal region that contains just three genes. A concurrent linkage analysis in humans confirms this candidate region. Additional analyses allow the authors to determine that it is the dose of *DYRK1A*, one of the genes in the region, that is responsible for the modulations of *REST* transcript levels. This relationship between DYRK1A and the REST pathway might be a crucial mechanism through which trisomy 21 causes a number of DS neuronal features.

X-linked Dominant Protoporphyria

Whatley et al., page 408

Disruption of one of a number of the genes involved in heme biosynthesis causes porphyria, or the accumulation of porphyrins, which leads to cutaneous or neurological manifestations. Erythropoietic protoporphyria (EPP) is characterized by life-long photosensitivity and is most often due to mutations in *FECH*, the gene encoding the terminal enzyme of heme biosynthesis. However, a significant number of EPP families do not carry mutations in *FECH*. Whately et al. study a subgroup of these *FECH*-mutation-negative families who each have an unusually high level of erythrocyte protoporhyrin in its zinc chelate form. Due to suggestive evidence that this type of EPP is X-linked, the authors choose to examine two X-chromosome genes that are involved in heme formation, *GATA1* and *ALAS2*. One deletion in *ALAS2*, c.1706-1709delAGTG, is identified in six of the families, and a different *ALAS2* deletion, c.1699-1700delAT, is detected in the remaining two families. Of note, the delAGTG mutation is found on five different haplotypes, suggesting that it has independently arisen multiple times. ALAS2 is an erythroid-specific enzyme and has been found to be essential for hemoglobin formation. All previously detected mutations in the gene are loss-of-function alleles and cause X-linked hereditary sideroblastic anemia with iron overload. Here, functional work on the EPP deletion alleles demonstrates that they cause an increase in ALAS2 activity and suggests that a gain-of-function mechanism is responsible for the observed accumulation of protoporphyrin and its zinc chelate in these EPP families.