FGD4 *Mutations in CMT4H,* by *Delague et al.* (*p. 1*)

***RhoGEF Mutations Cause CMT,* by *Stendel et al.* (*p. 158*)**

Charcot-Marie-Tooth (CMT) diseases are hereditary motor and sensory neuropathies that are categorized in two subsets, demyelinating and axonal. The recessive demylineating form, CMT4, is the most severe type, and six genes at nine CMT4 loci have been identified so far. CMT4H was previously linked to 12p11.21-q13.11; here, Delague et al. screened five genes in the region and identified one missense mutation and one splicing mutation in *FGD4*/FRABIN. *FGD4* is a GDP/GTP nucleotide-exchange factor for a Rho GTPase. To further demonstrate the importance of this gene, the authors overexpressed the wild-type and truncated mutant protein variants in rat primary motoneurons and Schwann cells. Whereas the wild-type protein induced filopodia-like microspikes, the mutant induced fewer spikes that were abnormally shaped. Taking a different approach to identifying new CMT genes, Stendel et al. searched for mutations in GTAPases in various forms of CMT diseases. Their hypothesis was that GTPases controlled the actin and microtubule dynamics that were crucial for the proper maintenance of function of the Schwann cells. By sequencing *FGD4* in 63 patients with CMT, they identified three familial cases and one sporadic case with *FGD4* mutations. The authors' expression studies and work demonstrating the effect of *FGD4* expression on Schwann cells added further support to the role of this gene in CMT.

***Evolution of γ-Crystallin Genes,* by *Plotnikova et al.* (*p. 32*)**

Mutations in the crystallins, a group of major structural components of the eye, are responsible for about half of inherited forms of congenital cataracts. A family with polymorphic congenital cataracts (PCC) was previously used to identify a PCC locus at 2q33-35, which contains the γ-crystallin family. Here, mutation screening of the four functional γ-crystallin genes in the family revealed a P23S mutation in *CRYGD*. Of note, this PCC-causing mutation is the "normal" sequence variant in other species. This led Plotnikova et al. to suspect that the site in the human gene may be an example of a compensated pathogenic deviation (CPD), a situation in which a harmful sequence change has become tolerated because of a compensatory change elsewhere in the molecule. Once such a coincident change had occurred, a reversion of one of the sites to the ancestral sequence would be deleterious. To determine whether CPD is responsible for the new wild-type sequence of the human gene, the authors searched for an interacting site that, when in combination with 23S, did not cause PCC. They report that, in some species, the ancestral serine was tolerated because of a related change at either site 109 or site 136.

***Haplotype Association in Family Studies,* by *Shi et al.* (*p. 53*)**

Various methods exist that are able to use unphased family genotype data to screen for risk-conferring haplotypes. Here, the authors developed max_Z^2, which has some advantages, including the ability to measure parental effects, over existing methods. To equip max_Z^2 with the ability to handle situations in which the causative SNP is not typed or in which a disease haplotype is responsible for disease susceptibility, the authors propose a combination with the fully multivariate Hotelling's T^2 test. When this sum algorithm was compared with existing methods, the power of the new method was consistently strong under various conditions. The method was also applied to previously reported data regarding an association between *IRF6* and orofacial clefts and successfully identified an association with the risk alleles. An additional feature of the method is its ability to nominate alleles that tag for risk haplotypes. This feature will potentially contribute new information to assist with the search for new susceptibility alleles.

RHO *Suppression and Replacement In Vivo,* by *O'Reilly et al.* (*p. 127*)

Over 100 mutations in the gene encoding rhodopsin, *RHO,* have been identified that cause autosomal dominant retinitis pigmentosa (RP). Because of the mutation heterogeneity in this disease, as in many other dominant disorders, developing gene therapy to target specific variants has been deemed inefficient and costly. A more general approach has focused on suppressing both the wild-type allele and the mutant allele while introducing a new functional allele concurrently. Suppression can be obtained using RNA interference (RNAi) to target a site not affected by the mutations, so that all endogenous alleles are rendered inactive, and the replacement gene avoids this suppression by containing sequence changes that destroy the RNAi target site. Although this technique has been successful in vitro, its use had not yet been demonstrated in vivo. To begin, O'Reilly et al. performed analyses of the technique in HeLa cells and retinal explants and report

Am. J. Hum. Genet. 2007;81:i–ii. 0002-9297/2007/8101-0001$15.00
DOI: 10.1086/519851

that both suppression and expression of the replacement gene work well. The authors' rescue of RP phenotypes in transgenic mice is a first step in the potential development of a strategy to treat such diseases.

mtDNA and COX Activity, *by Durham et al.* **(*p. 189*)**

Many individuals with a mutation in their mtDNA are heteroplasmic, with a mix of both the mutant mtDNA and wild-type (WT) mtDNA in their cells. It has been hypothesized that the proportion of mutant-to-WT molecules is indicative of pathogenic effect, but evidence has been lacking for what kind of threshold must be reached for a mutation to inhibit mitochondrial function. It has been difficult to evaluate whether mitochondrial dysfunction is due to a lack of WT mtDNA or a glut of mutant mtDNA, because, even within the same tissue, each cell can contain a different ratio of the two species. By looking at sections of individual muscle fibers, Durham et al. searched for a relationship between this ratio and cytochrome c oxidase (COX) activity. They reported that, for one type of mtDNA mutation, COX-positive cells had a relatively constant amount of WT mtDNA; in contrast, COX-negative cells had a higher total level of mtDNA, and a higher percentage of the molecules were mutant. This supported the prediction that, if WT mtDNA levels are not high enough for activity, a compensatory nonspecific increase of mtDNA can potentially result in high enough levels of WT mtDNA for functionality. But, because the upper limit of total mtDNA is controlled, if the starting proportion of WT mtDNA is too low and this general increase is also increasing the amount of the mutant form, not enough WT mtDNA can be made. In contrast, when a different mutation was examined, the authors observed that, even in the presence of a higher-than-normal level of WT mtDNA, function was still inhibited. It was predicted that this mtDNA mutation somehow interfered with the normal function of the WT mtDNA.

This Month on the Cover

In 1971, Alfred G. Knudson theorized that two mutations were necessary for the development of retinoblastoma, a tumor of the retina (Proc Natl Acad Sci USA 68:820–823). This "two-hit" hypothesis has since been experimentally demonstrated as the etiology behind retinoblastoma and other cancers caused by mutations in tumor suppressors. Retinoblastoma is due to the loss of both functional copies of the tumor-suppressor gene *Rb*. Knudson's hypothesis also helps to explain why inherited forms of retinoblastoma often have an earlier onset than do sporadic cases. People who inherit one mutant allele of *Rb* need to gain only a single induced mutation in the other copy of their gene to develop a tumor. In contrast, people with two wild-type forms of the gene would have to acquire a mutation in each copy, and that would take longer. On the cover is a photograph of a retinoblastoma-affected fundus. Special thanks to Dr. Arun D. Singh, Cole Eye Institute, Cleveland Clinic Foundation, for the image.

Robin E. Williamson
Deputy Editor