This Month in the *Journal*

This month in the *Journal,* Martin Richards and Vincent Macaulay have contributed an editorial, to accompany a paper by Finnilä et al., that reports the largest set of human mtDNA sequences to date. The editorial describes the use of mtDNA in studies of human evolution, from its beginnings in the late 1970s to the present. The complete mtDNA sequences that are coming out now give the highest level of phylogenetic resolution possible, and they suggest that the basic structure of earlier phylogenetic networks was generally accurate.

Craniometaphyseal Dysplasia, *by Reichenberger et al.* (*p. 1321*)

The locus for autosomal dominant craniometaphyseal dysplasia, on chromosome 5p, was limited to 2–3 Mb by Reichenberger et al., and it was found to contain only two fully described genes and several expressed sequence tags. In this skeletal disorder, there is progressive thickening and increased mineral density of the craniofacial bones. The *TRIO* gene in the critical region was excluded as a candidate by direct sequencing of DNA from affected individuals. The *ANK* gene, on the other hand, was mutated in affected individuals from five different families. *ANK* is predicted to encode a multipass transmembrane protein. Two in-frame deletions and an in-frame insertion were all found in a putative cytosolic domain of ANK, between transmembrane domains 8 and 9, and these mutations appear to create a dominant-negative protein. Functional studies suggest that ANK is involved in the transport of intracellular pyrophosphate into the extracellular bone matrix. These pyrophosphate levels are critical for the regulation of bone mineralization. Therefore, the authors propose that mutations in *ANK* lead to decreased pyrophosphate levels in the bone matrix and, subsequently, to the phenotype of increased mineral density and progressive thickening of cranial bones.

Mechanism of N-Terminal PAH Missense Mutations, *by Gjetting et al.* (*p. 1353*)

More than 400 different mutations in the *PAH* gene, encoding phenylalanine hydroxylase (PAH), have been associated with hyperphenylalaninemia. PAH converts L-phenylalanine to L-tyrosine, and reductions in this activity cause phenylalanine to build up in the blood. Almost all *PAH* mutations lie outside the region coding for the active site of the enzyme, and the missense mutations in other areas of the gene are thought to be located at key structural residues and at residues at domain interfaces. Gjetting et al. find evidence for another category of missense mutation in *PAH*—that is, mutations that reduce binding of phenylalanine. Two motifs in the regulatory domain of PAH are conserved in all eukaryotic organisms examined, and there appears to be an overrepresentation of mutations in the residues forming these motifs. Regulatory domains of PAH containing naturally occurring mutations in the conserved motifs were expressed as fusion proteins with maltose-binding protein. The fusion proteins showed some increased aggregation but, in general, appeared structurally sound on reducing and native gels. However, the mutations reduced or eliminated phenylalanine binding to the protein. The authors suggest that the activity of some forms of mutated PAH may depend on the concentration of phenylalanine in the liver, thereby providing a possible explanation for the discordant genotype-phenotype associations that are seen with mutations in this domain.

NPC Variant Detection and Mutation Analysis, *by Sun et al.* (*p. 1361*)*; and* **NPC1** ***Mutations, Protein, and Phenotypes,*** *by Millat et al.* (*p. 1373*)

Niemann-Pick type C disease (NPC) is a lysosomal storage disorder characterized by the accumulation of unesterified cholesterol in the lysosomes and late endosomes. Clinical manifestations of NPC are varied, with a range of ages at onset, affected organs, and degrees of neurologic impairment. In addition, there are two biochemical variants of the disease, termed "classic" and "variant," that are based on the degree of impairment in cellular cholesterol trafficking. Sun et al. have defined a new diagnostic method for variant NPC, which uses BODIPY-lactosylceramide (BODIPY-LacCer). Normal targeting of this fluorescent sphingolipid analog was seen when it was incubated with skin fibroblasts of the variant phenotype. In contrast, cells with the classical NPC phenotype showed transport to endosomes/lysosomes rather than to the Golgi. If the variant cells were preincubated with LDL, localization of BODIPY-LacCer to the endocytic system increased. Sun et al. tested this fluorescence system as a diagnostic method and found that they could identify variant cells as NPC in 22 of 23 trials in this system. Mutations in *NPC1* correspond to one complementation group of NPC. To study the different clinical and biochemical manifestations of NPC, both Sun et al. and Millat et al. looked for *NPC1* mutations in a series of affected individuals. They identified several novel mutations, the majority of which were missense

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mutations. The mutations were scattered throughout the protein but were somewhat concentrated in the luminal cysteine-rich loop of NPC1. In all but two of the variant cell lines for which *NPC1* mutations were identified, at least one of the mutations was in the cysteine-rich loop. Surprisingly, no *NPC1* mutations were found in 5 of the 12 variant cell lines discussed in the article by Sun et al. Each study identified mutations that were associated with the variant biochemical phenotype; however, the P1007A mutation that, in the article by Millat et al., was identified as associated with only the variant phenotype was found, by Sun et al., in a cell line with the classical phenotype. Furthermore, although the classical phenotype is ascribed to the I1061T mutation, Millat et al. find that, in combination with the P1007A mutation, this mutation gives a variant phenotype. They suggest that one copy of a variant-associated mutation is sufficient for expression of the variant phenotype, even if the second mutation has been associated with the classical phenotype. As more *NPC1* mutations are identified, these genotype-phenotype correlations should certainly become better defined.

New, Prevalent Mutation That Results in MCAD Deficiency, *by Andresen et al.* (*p. 1408*)

In populations of European descent, there is a high carrier frequency of a mutation, 985A→G, in the gene for medium-chain acyl-CoA dehydrogenase (MCAD). This high frequency, along with the fact that the resulting disorder, MCAD deficiency, is treatable with a simple dietary regimen, makes this disease a good candidate for a newborn-screening program, and these screens, which look for abnormal acylcarnitine levels in blood spots, are now used worldwide. The goal of this study by Andresen et al. was to evaluate the tandem mass-spectrometry method used in the newborn-screening programs. They gathered a sample of >900,000 blood spots from the United States, on the basis of which they identified 62 newborns with abnormal acylcarnitine levels. Many (63%) of the newborns identified in the screen were homozygous for the 985A→G mutation, and another 34% were heterozygous for the mutation. Seven of these heterozygotes also had a 199T→C mutation, a mutation that had not been previously identified. A discrepancy between the frequency of 985A→G alleles identified through screening of newborns and the frequency of this allele in people identified clinically has been thought to result from false positives in the screen. Instead, this discrepancy could be explained by the identification of 199T→C because it is as a relatively common mutation, it is associated with abnormal acylcarnitine levels, and it has not been found in clinically affected patients.

LD and Genotyping Errors, *by Akey et al.* (*p. 1447*)

Although linkage disequilibrium (LD) has been used extensively in gene mapping and evolutionary genetics studies, the effects that genotyping errors have on general measurements of LD have not been systematically characterized. Akey et al. have more precisely calculated these effects and have discovered that even a small genotyping error rate for single-nucleotide polymorphisms can have significant effects on measurements of LD, particularly if the frequency of the minor allele is low. These studies were performed with both a stochastic-error model, in which there is an equal probability that the alleles at a locus will be genotyped incorrectly, and a directed-error model, in which there is a systematic genotyping error for a particular allele at a locus. The differences between these models tend to diverge as the minor-allele frequency increases. Akey et al. also compared the effects that genotyping errors have on four different measures of LD: D', r, Q, and d. When error rates are low, these measures do not differ substantially; however, as the error rate increases, the differences become more apparent. The measures Q and d were, in general, more robust to error than were D' and r, but, overall, the choice of LD measure was also affected by the allele and haplotype frequencies and by the error model. Thus, no one LD measure was found to be superior, in every situation, to the others.

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