Looking Farther Afield

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Synonymous Single Nucleotide Polymorphisms: Now Look Who's Talking

Nonsynonymous single nucleotide polymorphisms (SNP) are considered functional because they result in amino acid changes; few investigators consider synonymous changes when examining potential disease associations. In a recent issue of *Science*, Nackley et al. (1) evaluated phenotypic and enzymatic activity in haplotypes of the human *catechol-O-methyltransferase* (*COMT*) gene. The COMT enzyme degrades catecholamines and seems important in pain sensitivity. COMT is found in two forms: soluble (COMT-S) and membrane bound (COMT-MB). Four SNPs form three major haplotypes: A/G in the COMT-S promoter region, and three in the coding regions of COMT-S and COMT-MB $His^{62}His$ (C/T), $Leu^{136}Leu$ (C/G), and $Val^{158}Met$ (A/G). Several SNP studies, including those of cancer, have focused on $Val^{158}Met$ because of an amino acid change, but results are inconsistent. Using data from White female subjects regarding pain responsiveness (PR), the authors reported the following haplotypes (2): low PR (GCGG), average PR (ATCA), and high PR (ACCG). Notably, the two haplotypes associated with the extremes of PR code for the stable 158 valine variant. Thus, the functional SNP does not explain the variation in phenotype. Moreover, variation in the S-COMT promoter region (A/G) also does not contribute to PR. The authors hypothesized that haplotype structure could be responsible for differences in phenotype. Using the statistical programs, RNA MFOLD and AFOLD, secondary structures were predicted. The structure associated with low PR was short and least stable, whereas that for high PR was long and most stable. Transfection experiments also supported a substantial reduction in enzymatic activity for the high-PR haplotype compared with the low-PR haplotype; average PR was in between the two extremes. These data indicated that reduced activity associated with the high-PR haplotype is likely mediated through local mRNA structure via protein synthesis or degradation. To test this, the authors did site-directed mutagenesis experiments by mutating specific base pairs within the nucleotides; these experiments revealed direct effects on mRNA structure. These results are fascinating as they provide evidence that synonymous nucleotides are critical in maintaining mRNA secondary structure. Further, the results emphasize the importance of considering synonymous SNPs within haplotypes in disease-association studies. In corroboration, another article from *Science* (3) reports that a synonymous SNP in the *multidrug resistance 1* (*MDR1*) gene alters the function of the resultant protein (P-glycoprotein). Importantly, and as expected, the authors found no difference in mRNA levels between the wild-type and the haplotype that includes this SNP. Instead, they found that conformational differences in the protein directly affected substrate specificity. For instance, the IC_{50} for trypsin was ~3.4 times higher for the SNP-containing haplotype than for the wild-type protein. Again, these data argue for consideration of silent mutations in the context of disease association and pharmacogenetic studies. Thus, what does this mean for molecular epidemiology studies? As we move back and forth between candidate gene approaches and whole genome scans, these two studies give one pause: We definitely must not ignore synonymous changes within haplotypes. The findings here also make sense in the context of understanding discrepancies between studies that focus on a single nonsynonymous SNP; it is just not that simple.

Expressions of Interest

Although consideration of synonymous SNPs may require additional head scratching, much of our genotyping approach using nonsynonymous SNPs still seems appropriate. Here, Spielman et al. (4) examined the expression level of genes among racially defined populations. EBV-transformed cell lines from 60 European-derived individuals (CEU), 41 Han Chinese (CHB), and 41 Japanese from Tokyo (JPT) stored as part of the HapMap project were evaluated using Affymetrix Genome Focus Array, which measures expression in ~8,500 genes. When comparing the CEU population with either the CHB or JPT population, 939 and 756 genes differed significantly in expression, respectively. When comparing the CHB with the JPT group, only 27 genes differed significantly in expression. Upon combining the CHB and JPT samples and comparing with the CEU group, 1,097 genes significantly differed in expression. However, most expression differences were not of a great magnitude. Only 35 genes had more than a 2-fold mean expression between the CEU group and the combined CHB/JPT group. Of these genes, *UGT2B17* had the largest difference, with a mean expression 22 times higher than the combined CHB/JPT group. The authors replicated their results using 24 samples from the Han Chinese of Los Angeles; only one gene differed in expression between the Han Chinese of Los Angeles and the CHB/JPT group, but 21 differed significantly from the CEU group. Cluster analyses of all four populations revealed two distinct groups: one consisted of 59 of 60 CEU individuals, whereas the other consisted of all three Asian groups and one CEU individual. To determine whether these differences were driven by genotype, the authors carried out genome-wide analysis for each of the 1,097 phenotypes. For the vast majority of genes, significant expression differences were due to differences in genotype frequency between the populations. For instance, 69% of the CEU group possessed the more highly expressed allele of the *UGT2B17* gene, whereas only 15% of the CHB/JPT group did. This study is reassuring in that the protein product of many genes deemed to have functional significance (e.g., nonsynonymous SNPs) seem to track as expected. Couzin (5), in an editorial in *Science*, remarked specifically about the importance of studying those genes that seem to stand out between groups. Could these genes help explain differences in disease risk? Importantly, the genes that were differentially expressed between the groups are not the usual suspects interrogated to better understand differences in cancer risk between populations. For example, *UGT2B17* is a steroid metabolism gene that may

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also be involved in drug metabolism (5). The authors plan to study other racial and ethnic groups to determine what patterns arise.

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