Looking Farther Afield . . .

We would like to introduce a new section in Cancer, Epidemiology, Biomarkers and Prevention (CEBP) called "Looking farther afield . . . ." This editorial feature will involve summarizing one or more recent articles that have been published elsewhere and providing commentary about the potential relevance to the CEBP readership. Initially, topics of interest will be solicited from the CEBP editors, but readers are enthusiastically invited to make suggestions and to contribute. It is anticipated that "Looking farther afield . . . " will become a bimonthly feature in CEBP.

Julie Ross
Senior Editor

Folate, a Turncoat?

There is a growing body of evidence that nutrition during pregnancy may influence the risk of chronic disease (including cancer) in childhood and possibly, adult life. For example, it is well-established that folic acid supplementation during early pregnancy reduces the risk of neural tube defects (R. E. Stevenson *et al.*, Pediatrics, *106:* 677–683, 2000). There are also recent studies to suggest that folic acid supplementation may reduce the risk of childhood cancer (J. R. Thompson *et al.*, Lancet, *358:* 1935–1940, 2001; A. F. Olshan *et al.*, Epidemiol, *13:* 575–580, 2002). Much of this evidence, however, is from observational studies, and the biological mechanism(s) underlying these associations are largely unknown. CpG methylation is one mechanism by which certain genomic regions are silenced. Dietary factors, including methyl donors, are necessary for the synthesis of *S*-adenosylmethionine, which is required for CpG methylation. It has been suggested that early nutrition may be important in providing methyl donors for CpG methylation. Most of the human genome displays little variability with respect to methylation levels, making this an unlikely mechanism for early nutrition to influence later disease. Instead, research has focused on methylation and transposable elements (including DNA transposons and retrotransposons). Transposons are common and potentially mobile sequences of DNA that are scattered throughout the genome. More than 35% of human DNA is estimated to be derived from transposons (J. A. Yoder *et al.*, Trends Genet., *13:* 335–340, 1997). Normally, transposons are highly methylated and thus silenced. Depending on where they are inserted in DNA (which appears to be random for a subset of these elements), transposons can end up silencing neighboring genes.

An intriguing animal study comes from Waterland and Jirtle (Mol. Cell. Biol., *23:* 5293–5300, 2003), who investigated the role of dietary supplementation of pregnant mice and the effect on coat color. The mouse *agouti* gene signals coat phenotype by controlling the color produced by follicular melanocytes; this transcription occurs in a promotor region of exon 2 of the *agouti* (*A*) *allele*. Viable agouti (A^{vy}) mice are yellow, whereas the loss-of-function (silenced) non-agouti (*a*) homozygous allele mice are black. This A^{vy} mutation occurs because of the insertion of a retrotransposon element adjacent to the gene. Expression of this gene (determined through coat phenotype) appears to depend on the level of CpG methylation of the retrotransposon. In this study, it was hypothesized that dietary supplementation of mice during pregnancy could change the coat color of offspring. Prior to mating, a/a (black coat) female mice were randomly assigned to the standard NIH-31 diet or the NIH-31 diet supplemented with methyl donors and cofactors including folic acid, vitamin B12, choline chloride, and betaine. They were mated with A^{vy}/a males and supplementation was provided throughout pregnancy and lactation. A^{vy}/a offspring were examined in both groups. Interestingly, the offspring of the supplemented dams had a coat phenotype that was shifted toward a darker or mottled coat color compared with the lighter coat color in offspring from the unsupplemented dams. The investigators also showed that the methylation status of the promoter region of the *agouti* gene was highly correlated with the methylation status of the adjacent transposon gene. They conclude that there is a localized epigenetic instability of methylation that arises from an interaction between the transposon and its nearby genetic region. Thus, genes that manifest a transposon region adjacent to a promoter region of DNA could be influenced by early nutrition or perhaps other environmental exposures.

Comment. This is a fascinating study of how early environmental factors (in this case, maternal diet) can influence gene expression throughout the life of the offspring. In this study, the authors reported that DNA methylation (as well as coat color) was maintained throughout adulthood; thus, the effects appear permanent. These findings are particularly relevant because of the demonstrated importance of folic acid in humans. Many food stuffs are now fortified with folic acid in the United States, and folic acid is highly recommended as a supplement in pregnancy. Given the results of this study, it will be important to determine whether there may be long-term unappreciated consequences of being exposed to high levels of folic acid *in utero*. For example, could the silencing of transposons that are adjacent to beneficial genes (such as tumor suppressor genes) also occur? Could there be consequences for the immune system? Clearly, additional animal and human studies are needed to both replicate and expand on these results. —Julie A. Ross, University of Minnesota, Minneapolis, MN

Johnny Bee Good!

The etiology of cancer is generally believed to involve an interaction between an environmental exposure and a genetically susceptible individual. Over the last 10 years, many epidemiological studies investigating environmental exposure have been expanded to include an analysis of genetic susceptibility in participants. The design of such studies typically presupposes that the exposure and the genotype are independent. However, an increasing body of work suggests an important influence of genotype on some aspects of behavior and, therefore, potentially a lack of independence of exposure on genotype.

A recent paper [C. W. Whitfield, Science (Wash. DC), *302:* 296–298, 2003] regarding bee behavior has demonstrated a relationship between gene expression and behavior in an animal setting. In this study, the researchers investigated gene expression profiles in bees that performed different tasks in the

hive. Typically, in the first few weeks of adult life, bees participate in brood care (nursing), and in the remaining 5–7 weeks, they shift to gathering nectar and pollen for the hive (foraging). Individually, however, bees perform tasks based on what the colony needs at the moment. Moreover, like humans, they perform at different speeds; some bees are simply harder workers than other bees. Initially, the investigator examined gene expression in the brains of bee nurses and foragers. In a panel, they represented about 5500 genes and found that gene expression differed by 39% in the brains between nursing and foraging bees. Visual inspection of gene expression allowed the investigators to predict the behavior type correctly in 57 of the 60 bees examined. Fifty cDNAs were singled out as most predictive of behavior as either a forager or a nurse; several of these appear to be involved in intercellular signaling.

This fascinating study turns our thoughts to the relationship between genotype and behavior in humans. Twin studies have suggested an important genetic effect on some behaviors. For example, a study of 3997 twin pairs illustrated the substantial genetic component associated with starting to smoke and continuing to smoke with 50% of a variance in risk of smoking initiation and 70% of the risk of persistent smoking being attributed to genetics (W. R. True, Addiction, *92:* 1277–1287, 1997). Examples of studies of polymorphism in specific genes abound and include studies of antisocial alcoholism and serotonin-related polymorphism (E. M. Hill, Psychiatric Genet., *3:* 143–153, 2003) and the relationship between smoking behavior and dopamine receptor genes [J. Yoshimasuk, Epidemiology, *4:* 183–192, 2003; R. F. Tyndalers *et al.*, Ann. Med., *2:* 94–124, 2003; and E. F. McKinney, Pharmacogenetics, *10:* 483–491, 2000, among others). This last paper examined the association between polymorphism and dopamine metabolic enzymes and tobacco consumption in smokers. Activation of central dopaminargic pathways is associated with psychological gratification ("reward") and has been associated with dependence; these pathways are activated by nicotine. In this study, 225 smokers were typed to assess whether the genotype in the genes dopamine β-hydroxylase (DBH), monoamineoxidase (MAO), and catechol *O*-methyl transferase (COMT), were related to smoking behavior. In this study, smokers with a *DBH 1368 GG* genotype smoked fewer cigarettes than those with other genotypes with the effect being statically significant only in women. Smokers with a *MAO 1460 TT/TO* genotype smoked more cigarettes than those with other genotypes. More heavy smokers (>20 a day) had the *DBH 1368 A* allele when compared with light smokers (<10 a day). The authors suggest that these enzymes helped determine a smoker's requirement for nicotine and may explain why some people are predisposed to tobacco addiction and some find it very difficult to stop smoking.

Genotype may influence other aspects of behavior. A study reported in 1999, suggested that cytochrome P-450 polymorphism might predict the use of hormone replacement therapy (HRT). In this study of 749 postmenopausal women the investigators (H. S. Feigelson *et al.,* Cancer Res., *59:* 3908–3910, 1999) showed that women who carried the *CYP17 17 A2/A2* genotype were about one-half as likely as women with an *A1/A1* genotype to be current HRT users (odds ratio, 0.52; 95% confidence interval, 0.31–0.86). The reason for this association was unclear, and past HRT use was not influenced by genotype. A possible explanation for the influence of genotype on behavior might be that the response to HRT is different and less favorable in persons of a certain genotype. However, this remains highly speculative and this work needs to be validated and repeated by others.

Taken together, these data indicate that the association between genotype and behavior may be complex and a lack of independence of an exposure and a gene of interest should be considered as a confounder in gene-environment studies. In addition, the impact of genotype may be seen only after an exposure occurs, *e.g.*, flushing in response to alcohol in persons of Asian descent (*e.g.*, S. E. Luczak *et al.*, J. Stud. Alcohol, *63:* 74–82, 2002; F. Sun *et al.*, Behav. Genet., *32:* 229–236, 2002). Investigation of the association between genotype and what are perceived as antisocial behaviors becomes particularly difficult with the risk of stigmatization of individuals who may be "programmed to behave badly." The interrelationship of genotype and exposure remains a challenge for future investigations. —Stella M. Davies, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio and Julie A. Ross, University of Minnesota, Minneapolis, MN