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Reply to Whitfield

To the Editor:

Dr. John B. Whitfield (Whitfield 2002 [in this issue]) writes to call attention to the variation in alcohol-dependence risk as a function of both the *ADH1B* Arg47His polymorphism *and* specific populations. We do not disagree with his result: our study (Osier et al. 2002) involved, primarily, normal individuals from multiple populations and showed considerable variation in allele frequencies at that site among the populations. In that report, we commented that the different risks of alcoholism associated with alleles at this site could be explained by other relevant variation on the specific haplotype at high frequency in eastern Asia. However, we did not show that haplotyping of the ADH Class I polymorphisms resulted in an unusually high F_{st} but that some individual sites in the gene cluster individually had unusually high F_{st} values. One particular haplotype does have a very large range of variation, but we do not have an appropriate empiric distribution for F_{st} values in multiallelic haplotype systems to show that it is unusually large. We showed that the "protective" allele, *ADH1B*47His,* occurs primarily on a specific haplotype in the Mediterranean and European populations studied but occurs on a different haplotype in eastern Asia. We concluded that the *ADH1B*47His* allele is likely to be old, a conclusion Whitfield reiterates.

We do disagree with some of the conclusions Whitfield reaches. He concludes that the effects of the *ADH1B*47His* allele are not additive. However, because of the strong linkage disequilibrium (LD) across the Class I gene cluster, Whitfield's analysis showing nonadditive allelic effects uses the *ADH1B*47His* allele as a surrogate for the entire haplotype. Those analyses do not provide sufficient evidence to limit the effect to just that allele; some other variant on that haplotype could be relevant. Taken together, the evidence that the risk difference associated with this polymorphism is not the same in Europe as it is in eastern Asia and our demonstration that the haplotype containing the *ADH1B*47His* allele is different in Europe from the one in eastern Asia require us to focus on the entire haplotype and not just on this one site. The nonadditive effects cannot be attributed to the *ADH1B*47His* allele exclusively, as Whitfield himself notes earlier in his introductory paragraph.

Whitfield repeats a common error when he says LD will decrease over time, without noting all of the assumptions involved in that deterministic result. In regions of high disequilibrium caused by very low levels of recombination, the effects of random genetic drift can easily outweigh the deterministic expectation, as we have demonstrated (Calafell et al. 2001). Modern humans have existed outside of Africa for a relatively short time and had small population sizes during much of that time. Thus, drift associated with the expansion out of Africa and diversification around the world can swamp any factors like recombination that tend to reduce LD. Since these ADH cluster genes are involved in alcohol metabolism, we of course have the additional complication of determining to what extent natural selection may have played a role in altering the frequency of particular haplotypes in different geographical regions.

Finally, Whitfield implies it is important to determine whether social factors or LD are responsible for the differences in effects of *ADH1B*47His* in eastern Asia and in Europe. Social factors can indeed be very important in modifying risk associated with different genotypes. For example, it will be difficult to determine the actual risk associated with ADH variation in northern Africa and the Middle East, since most populations in those regions are Muslim and consumption of alcohol is proscribed by their religion. However, we note that the relevant "genetic" component is not strictly differences in LD but differences in what haplotypes are present. A site in the ADH cluster but not in LD with the *ADH1B* Arg47His site could have an epistatic effect such that only those chromosomes with particular alleles in coupling account for the protective effect. Moreover, background genotype clearly differs at the population level between eastern Asian populations and European populations (e.g., Calafell et al. 1998) adding yet another level of confounding on the path to understanding the role of the *ADH1B* Arg47His polymorphism in risk of alcoholism.

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Detecting Polymorphisms and Mutations in Candidate Genes

To the Editor:

Currently, there is no consensus in the literature as to the number and the nature of controls that should be studied to distinguish between polymorphisms and disease-causing mutations (Bridge 1997). The quandary becomes particularly acute when we are trying to determine if a missense alteration in a candidate gene is important (disease associated). How many control samples should be tested, and what other considerations should go into the selection of controls? It is important to consider and report the status, race or ethnic background, and sex (if appropriate) of controls. Furthermore, how many patients should be studied when one is screening a gene for mutations? We have attempted to address these concerns in this letter.

First, one needs to consider if the control subjects could have the same disorder as the case subjects. Where did the control subjects originate, and how were they selected? The use of convenient control subjects (newborn samples, unused diagnostic samples, etc.) may inadvertently include individuals who are carriers or affected. If one uses control subjects selected for a particular study, they may or may not be appropriate for a different study. When studying psychiatric disorders, one needs to ensure that the controls do not have undiagnosed problems. When studying late-onset diseases, one needs to confirm that the control subjects are past the age of onset.

Marchuk (1998) suggested typing controls from similar racial, ethnic, and geographic backgrounds, since allele frequencies can differ between groups. In the past, ignoring this important tenet has caused some mutations to be misclassified. The peripheral myelin protein 22 Thr118Met substitution was believed to be a mutation in Charcot-Marie-Tooth disease, but was found to be a Swedish polymorphism (Nelis et al. 1997). The fibrillin-1 P1148A substitution was initially considered to be a Marfan syndrome mutation in a mixed population of patients, because it had not been found in white or African American control subjects. However, it was later found to be a polymorphism in Asians (Wang et al. 1997). The homeo box A1 A218G polymorphism was reported to increase susceptibility to autism; however, it was found to be more common in African Americans than in whites (Collins et al., in press). Thus, one could misinterpret a negative result if only a single racial or ethnic group is utilized as a control population.

The sex of the control subjects is of obvious importance in testing for polymorphisms in X-linked genes.