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Leukotriene C_4 synthase polymorphism and aspirin-induced asthma

To the Editor:

In their interesting study, Van Sambeek et al[1] confirmed the presence of the leukotriene C_4 synthase *(LTC4S)* genetic polymorphism, which was previously described by us.[2] They concluded, however, that there was no association between the C-444 allele and aspirin-induced asthma (AIA), which was observed in the Polish patients in their study. The authors suggested that the confounding effect of population stratification was the most likely explanation for the discrepancy between the 2 sets of results.

Another explanation, which we would like to suggest, is severity of the disease, a factor which was not taken into account either by us in our initial study[2] or by Van Sambeek et al,[1] who did not present any clinical characteristics of their patients.

We have now extended our observations and stratified our AIA patients according to disease severity, on the basis of the requirement for systemic corticotherapy.[3] In a pooled group of 76 patients with AIA, we confirmed the genetic association of the *LTC4S* C-444 allele with the AIA phenotype (relative risk = 2.62, 95% CI 1.38-4.98) when compared with findings in 110 aspirin-tolerant asthma (ATA) patients, although the frequency of the risk allele was lower (0.39) than in the initial report (0.44).[2] Similar comparison of genotypes in subsets of patients with mild asthma (31 AIA vs 33 ATA) who did not require systemic corticotherapy demonstrated virtually identical frequencies; the risk allele prevalences were 0.31 versus 0.33, respectively.

It is likely that in the study of Van Sambeek et al,[1] comprising 61 AIA and 33 ATA patients, such admixture effect could bias results, leading to the negative conclusion. Van Sambeek et al reported on a significant deviation of genotype frequencies from genetic equilibrium within the AIA group (P = .038), consisting of C-444/C-444 homozygote overrepresentation (8 vs 4.6 expected) and heterozygote deficiency (18 vs 24.3 expected). In a recent commentary on association studies for complex respiratory diseases, Silverman and Palmer[4] concluded that "significant deviations from the expected proportions of homozygote and heterozygote classes in a population of case subjects may be caused by association with the disease allele."

The second conclusion of the study by Van Sambeek et al addressed the functionality of the A-444->C polymorphism. In a luciferase reporter system, either allele was expressed in the THP-1 monocytic cell line, and no difference was observed between the constructs. Two methods of transfection, electroporation and diethylaminoethyl-dextran, were used with plasmids having an intact promoter of the *LTC4S* gene. Still, their wild-type construct demonstrated a wide interval of high luciferase activity (between 60-fold and 120-fold), and no positive control was done (eg, luciferase construct with a constitutive promoter).

It is common that transient plasmid reporter systems demonstrate great variability between experiments, partially because of the inherent transfection efficacy. We limited the possibility of the reporter gene activation by other than the mutated DNA motif, using a fragment of the *LTC4S* 5´ region truncated to less than 300 bp. The effect of the A-444->C mutation was moderate and variable but consistently higher (3%-57%) in expression for the C-444 allele–controlled reporter. Moreover, we proposed a molecular mechanism for the unique interaction of the C-444 allele with a transcription factor (H4TF-2).[3]

Functionality of the mutation is regarded as the ultimate criterion for significance of a genetic association. However, in multifactorial diseases, any generalization based on the limited set of experiments should be interpreted with caution.

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Reply

To the Editor:

We appreciate the comments of Drs Szceklik and Sanak on our recent article regarding the 5´ flanking region polymorphism in the