

Letter to the editor

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Dear Sir,

In "Association Between DNA Variant Sites in the Apolipoprotein A5 Gene and Coronary Heart Disease in Chinese" (*Metabolism*. 2005;54:568-572), Liu et al report an intriguing finding, that the apoA5 S19W polymorphism genotype frequencies differ significantly between patients with heart disease and control subjects. However, the degree of the difference is extreme, with 44 of 483 patients with coronary heart disease (CHD) but 0 of 502 controls having W-containing genotypes, resulting in $P = .0000001$ and an infinite odds ratio for the association with CHD. The extreme nature of that difference, combined with the absence or near absence of the W allele in other reports in Chinese (0.1% in 2711 Singapore Chinese [1] and 0.0% in our unpublished study of 214 Hong Kong Chinese) and Japanese (0.6% in 154 Japanese Americans [2]), suggests that the results might be because of experimental error.

Incomplete digestion with the restriction enzyme in one batch of samples may be the likeliest possibility. The W allele was detected as a polymerase chain reaction (PCR) product that remained undigested after incubation with the *TaqI* restriction enzyme at 65°C. If patient and control samples were processed separately, and if some samples in one batch of patient with CHD PCR products partly evaporated during the high-temperature incubation (which I have seen happen sometimes, due possibly to pipetting error or loose tube lids or plate covers), the resulting change in concentrations may inhibit the enzyme. Thus, some PCR products would be only partly digested, artifactually producing SW genotypes. The authors may easily check this possibility by regotyping a few patient and control samples, including several of the SW samples. The authors may have already performed this confirmation test, but their article did not mention doing so. In general, one may prevent the above potential problem by processing patient and control samples together on the same PCR plates or in adjacent tubes rather than in separate batches.

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References

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- [2] Austin MA, Talmud PJ, Farin FM, et al. Association of apolipoprotein A5 variants with LDL particle size and triglyceride in Japanese Americans. *Biochim Biophys Acta* 2004;1689:1-9.

Reply

Dear Sir,

Thank you very much for your letter and transferred letter from Dr Larry Baum. The question raised by Dr Larry Baum is quite understandable. Indeed, the polymerase chain reaction product may remain incompletely digested in certain conditions, as mentioned by Dr Larry Baum, and lead to decrease of the SS genotype. However, in our experiments, (1) we have confirmed the SW genotype by repeating the polymerase chain reaction–restriction fragment length polymorphism analysis; (2) we used the overdigestion with overdosed restriction enzyme to avoid the possible incomplete digestion; (3) we usually sequenced part of the samples taken randomly with different genotypes to make sure of the results; (4) and, in our case, we had not found any case of loose tube lids or plate covers during incubation. Thus, evaporation leading to errors, as mentioned in the letter, was unlikely in our case.

Still, the technical pitfalls mentioned by Dr Larry Baum are worthy of the attention of all researchers.

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