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Response to Odawara et al.

The comments of Odawara et al., an example of a case regarding the possible technical pitfall in the methodology of genotyping of insertion/deletion polymorphisms of the ACE gene, raise some interesting and important points. We agree with their opinion that it is important to exclude mistyping in ACE gene polymorphism.

The main finding of our previous study (1) was that ACE gene polymorphism was strongly associated with myocardial infarction in Japanese patients with NIDDM with a higher frequency of the deletion allele (D) than that in control subjects, whereas this locus was not primarily associated with diabetic retinopathy or nephropathy.

Odawara et al. are correct in stating that polymerase chain reaction (PCR) with insertion-specific primers or with dimethylsulfoxide (DMSO) would better lessen the bias originating from the methodology. In fact, we included DMSO in our PCR reaction mixture because we were aware of the possibility of mistyping of the ID genotype as the DD genotype based on a large number of subjects genotyped for the same polymorphism by another group in the same department (2–4). We apologize for not providing the detailed methodology in our report because of the necessity to limit the size of the manuscript. Reactions were performed in a final volume of 50 μl containing 100 ng of genomic DNA, 10 pmol of each primer, 3 mmol/l $MgCl_2$, 50 mmol/l KCl, 10 mmol/l Tris-HCl at pH 8.4, 5% DMSO, and 0.4 U *Taq* polymerase. Primers and PCR conditions were reported elsewhere (3).

Although we did not perform the PCR with an insertion-specific primer, it is unlikely that our results were influenced by the technical pitfall proposed by Odawara et al. for several additional reasons. First, because possible amplification confusion between ID and DD genotypes has been well recognized, repeated tests were carefully performed in samples originally typed as DD. Second, another group in our department has published several reports (2–4), and this method seems to be established. Third, the frequency of the DD genotype in the control subjects was 16.9% (1), which is similar to those (11–17%) reported in Japanese patients (5,6). Fourth, the distribution of the genotypes was in agreement with the Hardy-Weinberg equilibrium both in the subjects as a whole and in each group subdivided by complication status.

Finally, given the likely possibility of mistyping in a few subjects, the similar associations of this genetic marker with ischemic heart disease reported by groups in France (7), Denmark (8), and the U.K. (9), as well as those observed in our study, appear to indicate the importance of the ACE gene in susceptibility to macroangiopathy in NIDDM. Our goals are to broaden the understanding of the contribution of genes to diabetic complications and to provide new methods for prediction, prevention, and cure of the complications.

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Pancreatic Polypeptide Responses to Hypoglycemia in Aging and Diabetes

In younger patients with IDDM, an attenuated pancreatic polypeptide (PP) response to hypoglycemia is felt to be an early marker of autonomic neuropathy because the PP response is mediated by the vagus nerve (1). These studies were conducted to assess PP responses to hypoglycemia in normal elderly and elderly NIDDM patients and to determine if PP responses are a good marker of autonomic dysfunction in these subjects.

Healthy nonobese young (n = 10, age 27 ± 1 years, BMI 22.8 ± 0.6 kg/m^2), healthy nonobese elderly (n = 10, age 74 ± 1 years, BMI 24.5 ± 0.6 kg/m^2), and elderly NIDDM patients (n = 10, age 72 ± 1 years, BMI 25.6 ± 0.9 kg/