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LETTER TO THE EDITOR

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Sir: We were somewhat surprised to read the article by Nakazawa et al. in the July 2001 issue which reported eight single-nucleotide polymorphisms (SNPs) at the human tissue-type plasminogen activator (t-PA) locus (Nakazawa et al. 2001), which they claim to be novel. The authors even stated that "Until now, polymorphisms in the t-PA gene, except for the Alu I/D polymorphism, have not been investigated." We would therefore like to draw the attention to the paper we published in August 2000 entitled "Identification of eight novel single-nucleotide polymorphisms at human tissue-type plasminogen activator (t-PA) locus: associations with vascular t-PA release in vivo" (Ladenvall et al. 2000). Five of the SNPs that we have already described are identical to those reported by Nakazawa et al. The strong linkage disequilibrium between the Alu I/D polymorphism and the SNPs in exon 6 and intron 10 was already described in our paper. We are also concerned by the fact that Nakazawa et al. used the protocol of Ludwig et al. (1992) without the addition of a second polymerase chain reaction with an insertion-specific primer, because we have shown that this protocol occasionally results in misclassification of the Alu I/D genotype (Jern et al. 1999).

Furthermore, as regards evolutionary aspects, the authors claim that "The Alu insertion is considered to be the ancestral type," thereby overlooking a number of studies that clearly show that the ancestral state is the absence of the Alu element (Batzer et al. 1996; Tishkoff et al. 1996, 2000 and references therein). In those studies, samples from geographically diverse human populations, as well as from four different ape species, were typed for the t-PA Alu I/D polymorphism, and the results show that the insertion event occurred in humans prior to the emigration out of Africa. In the latest extensive work by Tishkoff et al., more than 1000 individuals from 30 populations (including Japanese) were also typed for two dinucleotide short tandem-repeat polymorphisms (STRPs) at the t-PA locus and haplotypes were generated (Tishkoff et al. 2000). From the global pattern of STRP/Alu haplotype variation and linkage disequilibrium, Tishkoff et al. could further elucidate the evolutionary history of this locus.

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Reply to the letter of Ladenvall et al.

The thoughtful letter from Ladenvall et al. illustrates the difficulty in comprehensively describing and comparing gene polymorphisms among human ethnic populations that vary significantly among different ethnic groups in the presence or absence of polymorphisms and frequencies of alleles, especially for single-nucleotide polymorphisms (SNPs). It also illustrates, in the lack of a comprehensive, everyday, updated polymorphism database that covers every ethnicity at present, an honest researcher's struggle to obtain perfect genetic data at an appropriate time during the research course from its start to the final report of the results. We have previously shown that the presence or absence and frequencies of alleles among different ethnicities are markedly different in 30%–40% of human SNPs (Iwasaki et al. 2001; Shinohara et al. 2001). Ladenvall et al. (2000) have presented the results of their polymorphism survey of the t-PA gene in a Swedish population; five polymorphisms coincided with the eight polymorphisms that we described in a Japanese population in a report published this year (Nakazawa et al. 2001). We agree that five of the eight were originally identified in a Swedish population by Ladenvall et al. (2000), which was independently observed in a Japanese population in our study, and the remaining three in our study were novel polymorphisms of the t-PA gene undescribed in any population to the best of our knowledge.

This discussion, in turn, indicates the need to carry out haplotype analysis, linkage disequilibrium analysis, and disease association studies on the basis of the respective ethnic polymorphism information. Results of linkage disequilibrium tests among polymorphisms therefore can vary markedly, depending on the ethnicity of the population that was studied, as has been shown in multiple genes including the angiotensinogen gene. We observed a set of linkage disequilibriums in a Japanese population (Nakazawa et al. 2001), two of which were observed in a Swedish population (Landenvall et al. 2000).

The majority of t-PA researchers, including us, still use traditional methods of genotyping Alu I/D polymorphisms described by Ludwig et al. (1992). As the new improved method described by Jern et al. (1999), we agree it should replace the traditional method in the near future.

Estimation of the evolutionary process that occurred in the human race is an unsolved area of research; several programs for construction of the phylogenic tree of genetic variations have been developed, although no perfect tool has been found to date. Thus, we can only infer the evolutionary process from the ethnicity, sample size, available genealogical data, and characteristics of the program used. We appreciate several inferences about the evolutionary process for t-PA polymorphisms done in different populations (Batzer et al. 1996; Tishkoff et al. 1996, 2000). Some of the conflicting results among these studies and ours reflect structural problems, which need to be solved in the future. We sincerely hope that collaborative efforts to understand the evolutionary process of the t-PA locus among researchers around the world will give further insight into the pathogenesis of human vascular disorders and aid human welfare.

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