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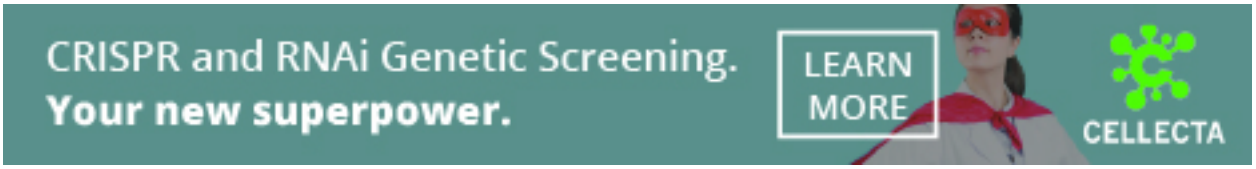

Mistyping ACE Heterozygotes

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Human angiotensin I-converting enzyme (ACE) insertion/deletion (I/D) polymorphism has been proposed to be a significant predictor of risk for myocardial infarction (MI) among individuals otherwise at low risk for heart attack.[1] The insertion sequence (288 bp) located within intron 16 of the human dipeptidyl carboxypeptidase 1 (DCP1) gene is a member of the *Alu* family.[2] Recently, Rigat et al.[3] developed a rapid PCR-based assay for identifying the ACE genotypes. The procedure involves PCR amplification of genomic DNA utilizing a set of primers flanking the insertion sequence, which allows discrimination among the three ACE genotypes: II, DD, and ID.

In genotyping a large pedigree in which one of the parents was an II homozygote, we were disturbed to encounter several DD genotypes among the offspring (Fig. 1, left). This was not a case of mistaken paternity as judged by several other criteria (not presented here). Furthermore, on repeating the PCR amplification under a slightly different condition, all of the DD genotypes amplified as ID (Fig. 1, right). However, on several other occasions, we observed that ID genotypes amplify as DDs. This led us to conclude that amplification of the I allele is sometimes suppressed in an ID heterozygote so that the latter can be mistyped as DD. This phenomenon was also observed by Perna et al.[4] during amplification of an *Alu* I/D polymorphism within the human TPA gene. Although the mechanism for this suppression is not currently understood, the mistyping is of utmost concern because it is the ACE/DD genotype that has been shown to be significantly at a higher risk for MI when compared with the ID or the II genotype.[1]

To resolve the mistyping of ID to DD, we examined several experimental parameters and found that inclusion of 5% dimethylsulfoxide (DMSO) in the reaction mixture greatly improved amplification of the I allele in an ID heterozygote (Fig. 2, left). In several trials with DMSO the ID heterozygotes amplified faithfully. However, to safeguard against any ID to DD mistyping we devised an additional PCR amplification protocol. This included utilization of a new sense primer (see legend to Fig. 2) from the 5′ end of the insertion sequence, along with the standard antisense primer,[3] re-

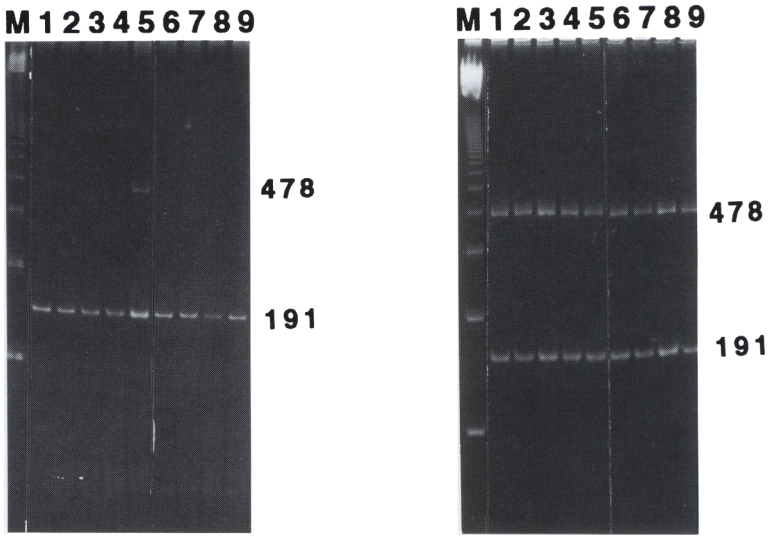

FIGURE 1 Anomalous amplification of ID genotypes as DDs. (*Left*) PTC-100-96V (MJ Research); (*right*) Twin Block System (Ericomp, Inc.). The reaction mixture contained 0.1–0.5 μg of genomic DNA, 4 pmoles of each primer, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 1% Triton X-100, 0.2 mM each dNTP, and 1 unit of *Taq* DNA polymerase (Promega) in a final volume of 20 μl. After an initial denaturation at 93°C for 3 min, the DNA was amplified during 30 cycles of 92°C for 1 min, 57°C for 1 min, and 72°C for 1 min, followed by a final extension at 72°C for 7 min. The samples were analyzed by PAGE (7.5%), stained with ethidium bromide, and photographed. All of the offspring DNA samples, with the exception of sample 5, amplified anomalously as DD on one occasion (*left*), although they amplified correctly as ID on another occasion (*right*). The genotype of the mother was II (Fig. 2, lane *6*).

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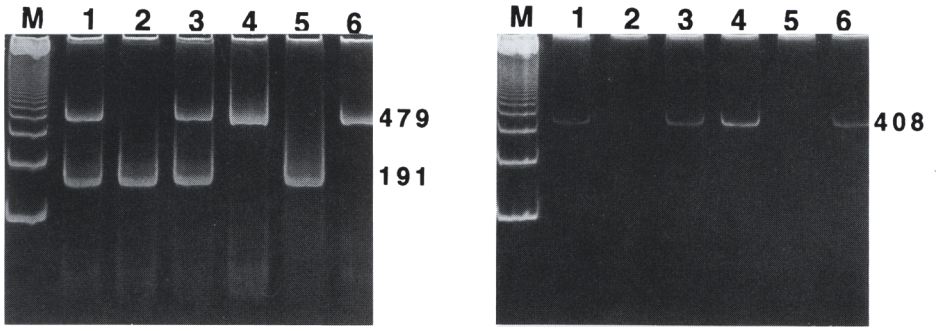

FIGURE 2 Confirmation of the DD genotypes by an insertion-specific second amplification. (*Left*) Standard primers as in Fig. 1; (*right*) the sense primer replaced by an insertion-specific primer: 5′TTTGAGACGGAGTCTCGCTC3′. The reaction and cycling conditions were the same as in Fig. 1, except that 5% DMSO was included in all of the reactions and the annealing temperature was raised to 61°C for the insertion-specific amplification. Samples were analyzed as in Fig. 1. The genotypes of the DNA samples were 1/ID, 2/DD, 3/ID, 4/II (grand child), 5/DD, and 6/II (mother), respectively. In the insertion-specific amplification (*right*), samples 1,3,4, and 6 each showed a band corresponding to 408 bp indicating the presence of at least one I allele, whereas samples 2 and 5 showed no amplification commensurate with their DD genotypes (*left*). Had the latter been truly IDs misamplified as DDs (cf. Fig. 1), each would have shown a 408-bp band in this insertion-specific amplification.

sulting in amplification of a 408-bp fragment from the I allele. Thus, an ID (as well as an II) genotype will show a positive amplification, whereas a DD genotype will show no amplification because of lack of an annealing site for the new sense primer (Fig. 2, right). In practice, only the DNA samples that typed as DD by the standard amplification procedure need to be reamplified in the presence of a positive control (ID/II). This will allow the identification of those ID genotypes that were mistyped as DDs during the first amplification. Utilizing this additional amplification, the typing of the DD and ID polymorphisms can be accomplished with 100% accuracy.

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