SEQUENCING AND SIZE DETERMINATION OF THE D1S80 INTERALLELE

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As a useful VNTR locus, the D1S80 system has been applied in forensic practice by laboratories worldwide . The D1S80 allele variants are valuable for paternity testing and individual identification. However, there are few reports concerning these variants[1] and their sequencing. In this paper, we report on the variant of allele 27 and its sequencing, based on our previous investigation of the D1S80 polymorphism in the Japanese population.

Materials and Methods

DNA Extraction

Genomic DNA from unrelated Japanese individuals was isolated from blood using proteinase K/SDS lysis and phenol extraction.

PCR Amplification

PCR was performed according to our previous method[2], with the exception that one of the primers was labeled with a fluorescent dye HEX or FAM. For fragment size determination, the amplified samples were run on a 6% denaturing polyacrylamide gel and automatically analyzed by an ABI 373A sequencer using Genescan software.

DNA Extraction from PAG

Amplified products were electrophoresed on a 6% polyacrylamide gel. After electrophoresis, the target alleles which were stained with ethidium bromide were excised from the gel and extracted through GENE CAPSULE. Some extracted DNA was diluted with water and used as template for the next PCR.

Cloning

DNA fragments were isolated from PAG and cloned in pBluescript. Recombinant clones were randomly selected.

Sequence Analysis

Sequencing was performed with a 6% denaturing polyacrylamide gel on an ABI 372A sequencer using the dideoxy chain termination method.

Results and Discussion

On a 6% polyacrylamide nondenaturing gel, the variant showed a different migration rate compared with allele 27 (Fig. 1).

Allele 27 has a higher frequency in Japanese and other Oriental populations compared with Caucasians, Hispanics and African Americans (Tab. 1). In our 121 samples of Japanese individuals, sixteen had allele 27, among which 4 alleles were found to be the variants.

On a denaturing sequencing gel, allele 27 and its variant showed the same migration rate. Fig. 2 shows a Genescan electropherogram, in which the peaks of allele 27 and its variant match closely. This suggests that allele 27 and its variant have the same fragment size.

After treatment with Msp I, allele 27 and its variant showed different electrophoretic band patterns (Fig. 3). Changes in the Msp I cleavage sites were found in the sequence of the repeating units of the variant. As a result, allele 27 had 8 Msp I cleavage sites in the repeating units, while the variant had 9 cleavage sites.

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              195                                   226
Allele 27 :  GAAGACCACAGGCAAG GAGGACCACCGGAAAG
Variant :  - - G - - - - -C- -A- - -  - -A - - - - - - - C- - -
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As shown above, the region of repeating units of the variant from position 195 (counting from the upstream primer combination site) to position 226 showed five base changes. Altogether 18 such base changes were found in the sequence of the repeating units of the variant. The sequences of the upstream flanking regions of allele 27 and its variant were compared and no changes were found. The sequence changes in the repeating units may lead to a conformational change of the variant, which would account for its faster migration rate.

Reference

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Table 1 Distribution of allele 27 in different populations

Population	frequency (%)
Japanese[2]	6.6
	5.0(allele 27)
	1.6 (variant)
Chinese[3]	4.4
Malays[3]	5.5
Indians[3]	1.2
Caucasian[4]	0.7
Hispanic[4]	1.6
African American[4]	0.8

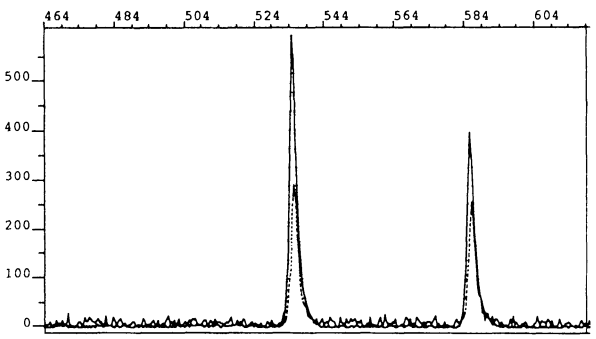

Fig. 2. A Genescan electropherogram. Two samples, 24/27 and 24/variant, were labeled with HEX and FAM respectively and were applied in the same lane. Bold line : 24/27; dotted line : 24/variant.

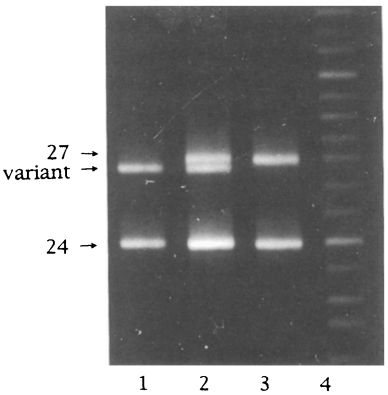

Fig. 1. Electrophoretic band patterns of allele 27 and the variant on a 6% polyacrylamide gel. Lane 1:24 / variant ; lane 2 ; a mixture of the sample 24/27 and the sample 24/variant; lane 3: 24/27; lane 4 : D1S80 allelic ladder.

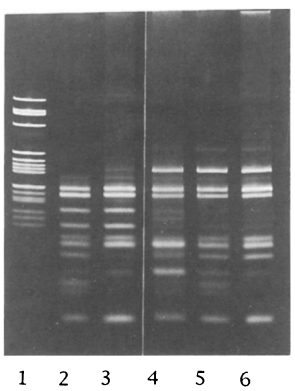

Fig 3. Electrophoretic band patterns of allele 27 and the variant after the treatment with Msp I. Lane 1 : pBR322 - Hae Ⅲ; lane 2-3, variant ; lane 4-5, allele 27; lane 6, RMS