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Forensic Science International: Genetics Supplement Series 1 (2008) 39–40
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Research article

Comparative study of D1S80 typing by capillary electrophoresis and sequencing

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Received 17 August 2007; accepted 9 October 2007

Abstract

The D1S80 locus is very useful for personal identification in Japan. To obtain a correct allele over 45, we examined PCR amplification product of the allele over 45 both by direct sequencing and fragment analysis using capillary electrophoresis. Direct sequencing finally determined the allele as being 57. However, it was calculated to be an allele of 56 by comparison with size markers for capillary electrophoresis. The difference could be attributed to the electrophoretic size markers. This finding indicates that the direct sequencing may be useful to determine the allele over 45 in the D1S80 locus.

Keywords: D1S80 locus; DNA typing; Sequence; Fragment analysis; Personal identification

1. Introduction

D1S80 (GenBank accession number D28507) is a variable number of tandem repeat locus located on chromosome 1p35–36 with a repeat unit of 16 bp. With alleles defined by the number of repeat units, the D1S80 locus is highly polymorphic in Japan [1]. At present, D1S80 typing method has been employed by fragment analysis using capillary electrophoresis. However, alleles over the region of the allelic ladder marker are not correctly typed using this method. The allele is estimated to be an allele over 45 similar to those identified previously by polyacrylamide gel electrophoresis. However, the allele over 45 is frequently observed [2,3]. This present study investigated the repeat structure of 16 bases from an allele over 45 in D1S80 locus. PCR amplification product of the allele over 45 was examined both by direct sequencing and by fragment analysis using capillary electrophoresis.

2. Materials and methods

Samples of DNA extracted from blood using by QIAamp® DNA Mini Kits (QIAGEN) were amplified by PCR as described by Fujii et al. (1996) [4]. The products were analyzed using the ABI Prism® 3130 Genetic Analyzer and Gene Mapper Software (Applied Biosystems). The nucleotide sequence of the PCR product was used for direct sequencing. Sequencing reaction was performed using the Big Dye Terminator v1.1 Cycle Sequencing Ready Reaction Mix (Applied Biosystems Japan.) with the purified alleles as templates, and primer MCT118N-2 (5′-TCA GCC CAA GGA AGA CAG ACC-3) or MCT118(814–21) as the primer for sequencing the forward or reverse strand, respectively.

3. Results and discussion

Fig. 1 summarizes the relationship between the heterozygote peak height ratio (HPR) and the difference in heterozygote alleles (DHA) for D1S80 typed by fragment analysis. The average of HPR was 1.05 (ranging from 0.5 to 2.72). High value such as 2.7, 1.74 and 1.92 HPR are obtained from 18–57, 33–57 and 29–48 alleles respectively. Thus HPR values may serve as an indicator in the typing of an allele over 45. The mean reported HPR generated from 2 ng of amplified DNA is 0.755 [4]. PCR amplification product of an allele over 45 was examined both by direct sequencing and fragment analysis using capillary electrophoresis. We found that it included a noteworthy region of the 16 base repeated sequencing. Based

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doi:10.1016/j.fsigss.2007.10.130

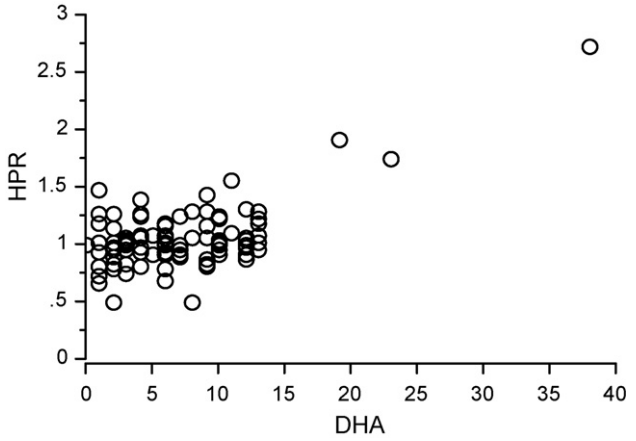

Fig. 1. The relation of HPR and DHA for D1S80 typing by fragment analysis.

on this region, it could finally be determined as an allele 57 by direct sequencing. However, it was calculated to be an allele 56 using the size marker of capillary electrophoresis as shown in Table 1. The difference may be attributed to the size marker of capillary electrophoresis. This finding indicates that the direct sequencing method may be useful to determine an allele over 45 in the D1S80 locus.

In addition, PCR amplification products at the D1S80 locus of original primer pairs [1] contained the undesirable bands during D1S80 typing [5].

Table 1
Estimation of alleles over 45 using the size marker of capillary electrophoresis.

D1S80 type	Allele	Peak size	Calculated allele
33-OL*	33	544.18	
	Over 45	907.04	56 (33 + 22.68)
18-OL*	18	303.83	
	Over 45	907.64	56 (18 + 37.74)

OL*: Off ladder.

Conflict of interest

None.

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