DNA Sequence analysis of the PCR Products of the MCT118 locus in Japanese DNA Samples

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INTRODUCTION

MCT118 (D1S80) locus is widely used for forensic DNA typing. Many population studies have been reported by using allelic ladder marker. However, the whole DNA sequences of each allele of MCT118 locus have not been reported. In this report the whole DNA sequence of MCT118 locus including both flanking regions and repeat regions has been revealed and the difference between different alleles has been analyzed.

METHOD

DNA extraction:

Genomic DNA was extracted from the human blood or bloodstain by phenol-chloroform method as described by Sakai (1991).

PCR amplification and purification:

PCR amplification of MCT118 locus was performed as follows:
10ng of genomic DNA was in 40ul of PCR buffer (66.7mM Tris-Cl (pH 8.3), 1.7mM $(NH_4)_2SO_4$, 1mM each dNTP, 2 mM each primer for MCT118 primer (Kasai 1990), 3mM $MgCl_2$, 0.17mg/ml BSA, 10mM 2-mercapto-ethanol, 10% DMSO, and 2U of AmpliTaq polymerase), and amplified as described by Kasai 1990.

PCR products were separated by agarose gel electrophoresis. Bands of PCR products were visualized with ethidium bromide under UV transilluminator, and then excised and purified as described by Heery 1990. Purified PCR products were cloned into the pCRII™ vector (*invitrogen*) followed with the protocol of TA cloning kit. Each cloned allele was confirmed with PCR amplification and electrophoresis, and then performed further sequencing analysis.

DNA Typing:

Amplified PCR products were separated with 5% native polyacrylamide gel electrophoresis along with allelic ladder markers precipitated with ethanol before loading (Sekiguchi 1994). Then, the gel visualized with ethidium bromide under UV transilluminator was photographed and analyzed by FragmeNT Software (Molecular Dynamics).

DNA Sequencing:

Cloned plasmids were sequenced with the reagents from the Taq DyeDeoxy Terminator Cycle Sequencing Kit and Taq Dye-primer Cycle Sequencing Kit (ABI), and analyzed with ABI 373A DNA sequencer. To confirm the sequence of each

allele, another sequencing reaction was performed with SequiTherm Long Read Kit with labeled (-29) forward primer and RV primers as the sequencing primers, and analyzed with LiCOR dNA sequencer.

RESULTS

Eleven alleles of MCT118 locus were succeeded in cloning and sequencing. PCR fragments of the cloned alleles were typed as the same repeat number with the threshold value of ±0.3 repeat, and they were appeared to be the same size.

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allele
   14 : ABCDDE--GHI-----------------I-IILG    A: TCAGC CCAA GGAAG
   16 : ABCDDE-CGHI-----------------IHIILG    B: ACAGA CCACA GGCAAG
   18 : ABCDDE-CGHI----------------IHIKIILG    C: GAGGA CCACC GGAAAG
   18 : ABCDDE-CHHI----------------IHIKIILG    D: GAAGA CCACC GGAAAG
   23 : ABCD-E-CGHI---JJIIH-------IIHIKIILG    E: GAAGA CCACA GGCAAG
   24 : ABCDDE-CGHI---JJIIH-------IIHIKIILG    F: GAGGA CCACA GGCAAG
   25 : ABCDDE-CGHI---JJIIH------IIIHIKIILG    G: GAAGA CCACC GGCAAG
   27 : ABCD-E-CGHI---JJIIHIIH---IIIHIKIILG    H: GAGGA CCACC GGCAAG
   28 : ABCDDE-CGHI---JJIIJIIH---IIIHIKIILG    I: GAGGA CCACC AGGAAG
   28 : ABCDDE-CGHI---HHIIHIIH---IIIHIKIILG    J: GAAGA CCACC GGCAAG
   28 : ABCDDE-CGHI---JJIIJIIJ---IIIJIKIILG    K: GAGAA CCACC AGGAAG
   28 : ABCDDE-CGHI---JJIIHIIHIK---IHIKIILG    L: GAGGA CCACT GGAAAG
   31 : ABCDDEFCGHI--IHJIJHJIJIJIIH--IKIILG
   32 : ABCDDE-CGHI-H-HIIHIJJIIHIIHIIIKIILG
   35 : ABCDDEFCGHIIHIHJIJIJIKIIKIIIHIKIILG
 5' flanking region sequence (116 bp)
     GAAAC TGGCC TCCAA ACACT GCCCG CCGTC CACGG CCGGC CGGTC CTGCG
     TGTGA ATGAC TCAGG AGCGT ATTCC CCACG CGCCA GCACT GCATT CAGAT
     AAGCG CTGGC TCAGT G
 3' flanking region sequence (32 bp)
 CCTGC AAGGG GCACG TGCAT CTCCA ACAAG AC
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Fig. 1 The composition of the sequence of repeat units in the MCT118 locus. The repeat units are shown with the proposed nomenclature shown above.

Characterization of the MCT118 locus by nucleotide sequences revealed a fixed order of 16 bp core repeat units except 1st repeat, which is 14 bp. Figure 1 shows the first 6 repeats units from 5' end and the last 4 units from 3' end are the same stable region among alleles. However, the sequences of the core repeat units between 7th repeat from 5' end and 5th repeat from 3' end seemed to be variable among alleles, and so this region was named variable region (Fig. 1). The 16 bp sequences of core units in variable region consist of 4 kinds of core repeat units (H, I, J, K) and the arrangements of these units were seemed to have some order. Almost every same allele has same mobility in

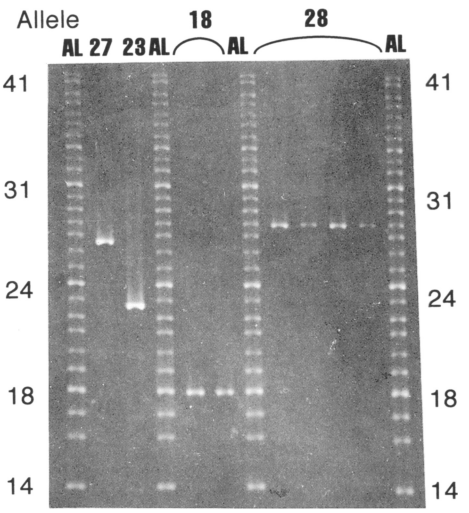

Fig. 2 The electrophoresis of cloned alleles Each 18 and 28 repeats allele is different each other. Their sequences are shown in Fig. 1

native-PAGE regardless of their different sequences in variable region (Fig. 2). However, two alleles, 23 repeat and 27 alleles, have differences in the first 6 repeats, and have slightly fast mobility in native PAGE (Fig. 2). The sequences of the 5' and 3' flanking region of the MCT118 locus were completely same among alleles. Because the ambiguity region was found in 5' flanking region, allele specific PCR was applied and determined the complete sequence. These results showed the correct length of the PCR product was 146 + 16 x N (N: number of repeat units).

DISCUSSION

At the first time, MCT118 typing had been made by calculating the repeat numbers with DNA size marker (e.g. 123 bp marker). Recently, however, allelic ladder marker of MCT118 (D1S80) locus has been available (Baechtel 1993; Perkin Elmer 1993), and it showed fast migration with its calculated molecular weight in native-PAGE (Kasai 1992; Sugiyama 1993). These results indicate the sequence of MCT118 locus may affect its migration in native-PAGE. Twenty-eight and more alleles in MCT118 locus have been already reported (Budowle 1995). Every allele was found almost the same migration with allelic ladder marker, but a few alleles that show slightly fast or slow migration with allelic marker were also found. It has been thought these different migrations might be caused by the sequence deletion within repeat units and/or the unequal sequence composition (Skowasch 1992; Sugiyama 1993; Kloosterman 1993). Several deletions were found within the core repeat units in Col2A1 VNTR (Berg 1993) and ApoB VNTR (Boerwinkle 1989). In MCT118 locus, however, no deletion was found within the core repeat units, and only replacements were found within them. This indicates abnormal migrations in MCT118 locus might be caused not by the deletion of core units but by the substitution of base in the core units. In this study, cloned 23 repeat and 27 repeat alleles have slightly fast mobility from allelic markers. No deletions were found in both alleles but the order of repeat units in stable region was different from other alleles. This implies the possibility that the difference of stable region makes mobility difference. However, some alleles like 28 repeats, which have the same repeat numbers but have different sequences in core repeat units in variable region, had no apparent different mobility in native-PAGE (Fig. 2). So, this implies the order of core units in variable region does not affect the mobility in native-PAGE. Probably, the changes of the order of core units in stable region may affect the mobility in native-PAGE.

Comparing the order of the core repeat units in each allele, the changes of the order have found only in variable region, while there is no change in stable region. The variable region consisted of only 4 kinds of core repeat units in every allele, and the increase or decrease of the repeat numbers in MCT118 locus seemed to happen not by randomly adding or deleting the core units but by concurrently adding or deleting a couple of core units. This implication may cause the discontinuous repeat numbers and the unequal distribution of allele frequency in inter- or intra-population.

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