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Parentally imprinted allele typing at a short tandem repeat locus in intron 1a of imprinted gene *KCNQ1*

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Abstract

A short tandem repeat (STR) in the intron 1a of paternally imprinted gene, *KCNQ1*, is evaluated as a new probe for use in parentally imprinting allele (PIA) typing. This typing can determine the inheritance of one allele from father by the methylation difference. Allelic and genotypic frequencies of the STR were determined using samples from 175 unrelated Japanese and 170 unrelated Germans. The polymorphism information contents were 0.652 and 0.634 for the Japanese and the Germans, respectively, indicating usefulness in individual identification. This method was applied to five Japanese families consisting of 19 individuals. Genomic DNA was digested by methylation-sensitive restriction endonucleases, *Hha*I and *Hap*II, followed by PCR amplification using two-step sandwich primer sets and the products were analyzed on polyacrylamide gel electrophoresis. For all of the families, each child's paternal allele given by PIA typing corresponded to one of the two alleles from father, not the two from mother, that were determined by the STR genotyping. The results demonstrate that this STR probe is feasible for use in PIA typing and that its typing method can contribute to paternity testing.

Keywords: Human *KCNQ1* gene; Paternally imprinted allele typing; DNA methylation; STR; Paternity testing

1. Introduction

Since forensic DNA evidence was first presented in the US court system in 1986 [1], a large number of STRs, VNTRs and SNPs have been found and some of them are useful as genetic markers for forensic investigation. However, universally locating on autosomes, most of these markers are hard to apply in the cases of discriminating the parental-derived alleles using conventional DNA typing methods. Recently, we developed a new method called parentally imprinted allele (PIA) typing for paternity testing and identity determination [2,3]. This PIA typing is based on the differential methylation of parental alleles at a VNTR locus upstream of the H19 gene and the combination of a methylation-sensitive *Hha*I digestion with a polymerase chain reaction (PCR) method detects only the paternal allele. More markers for PIA typing will provide a more reliable examination in forensic DNA testing. Therefore, we chose an STR in intron 1a of the imprinted gene, *KCNQ1* [4], by searching a DNA database as a marker of that type. The *KCNQ1* gene, encoding a protein for a kind of voltage-gated potassium channel and required for the repolarization phase of the cardiac action potential and potassium homeostasis in the inner ear [5,6], is located on chromosome 11p15.5 and is neighbor to a large number of imprinted genes [4,7,8]. This paper examines the feasibility of this new STR by performing a genotype survey of Japanese and Germans and family examinations. Here, we describe the utility of the STR for use in PIA typing and its applicability to paternity testing.

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2. Materials and methods

2.1. Samples

Blood samples were collected from 175 unrelated Japanese and 170 unrelated Germans for population studies, and from five Japanese families consisting of 19 individuals for PIA typing. Genomic DNA was isolated using proteinase K digestion and extracted from blood samples using phenol/chloroform. All individuals gave informed consent prior to inclusion in this study.

2.2. Primers

Two primer pairs were designed, based on the GenBank sequence data (accession number: AJ006345). One pair consisted of primers A (forward) 5′-AGTAAAGGCCTTC GGACAGTG-3′ and B (reverse) 5′-GTAGTATCATTTA GCGCGTTC-3′, while the other was composed of primer A and C (reverse) 5′-GAATCCCTGCCTGATTTCACG-3′. For amplification of the STR fragment, primers A and B were used. Primers A and C were included in the PCR reactions for amplification of the 1.2 kb fragment containing the STR, and the three *Hha*I and three *Hap*II restriction sites.

2.3. Identification of STR polymorphism in intron 1a of the KCNQ1 gene

The STR fragment was amplified by PCR in a 15 μl of reaction mixture comprising 10 ng template DNA, 30 pmol of each primer (A and B) and 0.75 U of FastStart Taq DNA Polymerase (Roche, Germany), as recommended by the manufacturer. The PCR was performed according to the following conditions: initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 72 °C for 1 min, with a final extension at 72 °C for 7 min. The amplified products were separated by 8% polyacrylamide gel electrophoresis and visualized using ethidium bromide or silver staining. Each allele sequence of the amplified STR was confirmed by sequence analysis with an ABI Prism310 Genetic Analyzer (Applied Biosystems, Foster, CA). The alleles were designated corresponding to the number of repeat units.

Table 1
Frequency distribution (a) and forensic efficiency values (b) of an STR polymorphism in intron 1a of imprinted gene *KCNQ1* from 175 Japanese and 170 unrelated German samples

(a) Frequency distribution

Allele	Japanese, $n=175$	German, $n=170$
16	–	1 (0.003)
15	8 (0.023)	16 (0.047)
14	60 (0.171)	158 (0.465)
13	119 (0.340)	103 (0.303)
12	8 (0.023)	4 (0.012)
11	3 (0.009)	–
10	14 (0.040)	2 (0.006)
9	2 (0.006)	–
8	–	–
7	1 (0.003)	–
6	135 (0.386)	56 (0.165)
Total	350 (1.000)	340 (1.000)

(b) Forensic efficiency values

	Japanese	German
Alleles identified	9	7
Heterozygosity	0.691	0.629
PIC (polymorphism information content)	0.652	0.634
Genotype identified	19	14
Discrimination power	0.861	0.837
χ^2	21.727 ($P>0.100$)	8.323 ($P>0.100$)

2.4. PIA typing

Approximately 500 ng of genomic DNA was digested with 10 U each of *Hha*I and *Hap*II (TaKaRa, Japan) at 37 °C for 4 h in a 20 μl digestion system with 2 μl of 0.1% BSA. The digested DNA was diluted to concentrations of 2.000, 0.400, 0.080 and 0.016 ng/μl with sterilized water. For the amplification of the 1.2 kb fragment, PCR was performed in a total volume of 10 μl, using 1 μl of each dilution as a template, 20 pmol of each primer (A and C), and 0.5 U of FastStart Taq DNA Polymerase. The PCR conditions were as follows: initial denaturation at 94 °C for 4 min, 30 cycles at 94 °C for 30 s, 57 °C for 1 min, 72 °C for 1 min and extension at 72 °C for 7 min. One microliter of the PCR product was subjected to the STR assay described above.

3. Results

3.1. The STR polymorphism and its population study

The STR polymorphism was examined in the Japanese and German populations. Sequence analysis of the discrete alleles showed it as a TGGA repeat locus, ranging from 6 (167 bp) to 16 repeats (207 bp). The allele frequencies and the forensic efficiency values of the STR are summarized in Table 1a and b, respectively. In the Japanese samples, Allele 6 and Allele 13 of nine alleles identified were frequent (0.386 and 0.340, respectively). The heterozygosity and polymorphism information content (PIC) were 0.691 and 0.652, respectively. On the other hand, seven alleles were identified in the German samples, with the frequency of Allele 14 being the highest (0.465). The heterozygosity and PIC were 0.629 and 0.634, respectively. Allele 16 was detected in the German samples, but not in the Japanese samples. Conversely, Alleles 7, 9 and 11 were found in the Japanese samples, but not in the German samples thus far examined. Thus, the frequency distribution of this locus differs significantly in the two populations ($P<0.01$).