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Original Article

Polymorphism of LPL Locus in Japanese and Comparison of PCR Amplification Efficiency from Degraded DNA between LPL Locus and the D21S11

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Abstract

The short tandem repeat (STR) polymorphism of the lipoprotein lipase (LPL) locus was amplified by PCR and analyzed using denaturing polyacrylamide gel electrophoresis followed by silver staining. Among 158 DNA samples from the Japanese population, six alleles were observed. When the sequences of the allelic products were compared, each allelic segment contained 7 and 9–13 TTTA tetranucleotide repeat motifs. Genotypic distribution met Hardy-Weinberg expectations, and included heterozygosity was 48.8%. Most of the Japanese genotypes allele 10. When PCR amplification efficiency for the LPL locus from degraded DNA was compared with that for the D21S11 locus in terms of amplification size, increase in amplification size showed a considerable influence on amplification efficiency, producing inaccurate amplification, such as unbalanced amplification, or amplification of non-target PCR products. These results suggest that reduction in amplification size increases the accuracy and efficiency of PCR amplification from highly degraded DNA.

Key words: Short tandem repeat (STR)—LPL—Japanese population—D21S11—Degraded DNA

Introduction

Short tandem repeat (STR) polymorphisms are widely used for forensic purposes such as in personal identification and paternity testing because of their high level of individual variation. There are many reports on the frequency of the STR polymorphisms located at intron 6 of the lipoprotein lipase (LPL) locus^{1–11,14–18}. Because the allelic sizes of this locus are comparatively small (111–

135 bp), it is considered to be suitable for the detection of polymorphisms from highly degraded DNA.

In this study, we examined allelic frequencies at the LPL locus in the Japanese population, and applied this polymorphism to material examination from degraded DNA, comparing the amplification attitude at this locus with that at the D21S11 locus¹³ which has larger PCR product sizes (approximately 100 bp) than the LPL locus, and is one of the

most widely used STR loci in the forensic field.

Materials and Methods

1. Samples

Genomic DNA was extracted from blood samples from 158 healthy unrelated Japanese individuals. Informed consent was obtained from the blood donors, and this study was approved by the ethical committee of Tokyo Dental College. Leukocyte preparations from the blood were suspended in saline-EDTA (75 mM NaCl, 24 mM EDTA) containing 1% sodium dodecyl sulfate (SDS) and digested with 0.1 mg/ml of proteinase K at 55°C overnight, followed by treatment with RNase at 55°C for 2 hrs. DNA was extracted with phenol/chloroform, precipitated with ethanol, and re-suspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA at pH 7.6).

Samples of 47 pairs of teeth (tooth sample) and cotton used for hemostasis after extraction of teeth (blood stain sample) were obtained from patients at a dental clinic. Informed consent was also obtained from the patients. To isolate DNA from the teeth, the enamel layer of each tooth was scraped with a dental instrument and the remaining material was crushed into a powder using a "diamond mortar", a special device for crushing hard tissues, followed by decalcification in 3 ml of 0.5 M EDTA for three days. The tooth powder was centrifuged, washed in TE twice, and re-suspended in TE, followed by proteinase K digestion. DNA was extracted twice with phenol/chloroform, precipitated with ethanol, and re-suspended in TE. These tooth samples included highly decayed teeth, remaining roots, teeth with infected root canals, root-canal-treated teeth, and teeth broken in the course of tooth extraction. Samples of cotton used for hemostasis after extraction of teeth were washed in sterile physiological saline solution twice, soaked in TE containing 1% sodium dodecyl sulfate (SDS), and digested with 0.1 mg/ml of proteinase K at 55°C overnight. The supernatant solution was

transferred to another tube, and DNA was extracted as described above.

2. PCR amplification

Segments of LPL were enzymatically amplified from genomic DNA using the following oligonucleotides as primers: primer 1: 5'-ATCTGACCAAGGATACTGGGATATA-3', primer 2: 5'-CCTGGGTAAGTCTGAGCGAGACTGTGTC-3'¹⁹⁾. Each amplification of LPL was performed in a 40 µl mixture containing 10 ng of genomic DNA, 10 mM Tris-HCl at pH 8.3, 50 mM KCl, 1.5–2.5 mM MgCl₂, 0.01% gelatin, 200 µM dNTP, 0.5 µM of each primer and 1.5 U of AmpliTaq DNA polymerase or 1.25 U of AmpliTaq Gold (Applied Biosystems). The following PCR conditions were used for amplification using AmpliTaq DNA polymerase: 94°C for 5 min, followed by 28 cycles of denaturation at 94°C for 30 sec, annealing at 68°C for 30 sec and extension at 72°C for 60 sec. After the 28th cycle, an extra step was performed at 72°C for 10 min to extend the template completely. The PCR conditions used for amplification using AmpliTaq Gold were 94°C for 9 min, followed by 30 cycles of denaturation at 94°C for 30 sec and annealing/extension at 60°C for 60 sec. After the 30th cycle, an extra step was performed at 60°C for 10 min to extend the template completely. When performing re-amplification of the first PCR (dual-PCR), 1 µl of the 1st PCR solution was used under the same conditions as those for the initial PCR reaction.

Segments of D21S11 were enzymatically amplified using the following oligonucleotides as primers: primer 1: 5'-ATATGTGAGTCAAT-TCCCAAG-3', primer 2: 5'-CTGTATTAGT-CAATGTTCTCCAG-3'¹³⁾. Each amplification of D21S11 was performed as described above, but with 2.5–3 mM MgCl₂ and 1.5 U of AmpliTaq Gold. PCR conditions were 94°C for 9 min, followed by 33 cycles of denaturation at 94°C for 30 sec and annealing/extension at 53°C for 60 sec. After the 33rd cycle, an extra step was performed at 53°C for 10 min to extend the template completely. When performing dual-PCR, 3 µl of the 1st PCR solution was used under the same conditions

as those for the initial PCR reaction.

The PCR products were electrophoresed in 6% denaturing polyacrylamide gels and detected by silver staining.

3. Sequencing analysis

Each amplified DNA fragment was eluted from silver stained gels using the "crush and soak" method¹²⁾, re-amplified by PCR, and directly sequenced using the Dye Terminator Cycle Sequencing Kit (Applied Biosystems). DNA sequencing was performed on the 373A DNA Sequencer (Applied Biosystems).

Results

1. LPL typing

Six kinds of allelic products of different sizes were identified at the LPL locus among 158 Japanese individuals (Fig. 1). Each allele represented a 4bp difference, and each allelic segment contained 7 and 9–13 TTTA tetranucleotide repeats in the sequences of the 111 bp and 119–135 bp fragments met. The allele frequencies obtained are shown in Table 1. Genotypic distribution meets Hardy-Weinberg expectations ($\chi^2=5.10$, $df=1$, $0.2 < p < 0.5$) (Table 2) and heterozygosity was 48.8%. The allelic frequency distribution was not significantly different from that reported in other Japanese populations (Table 1).

2. Detection of LPL locus from degraded DNA

The LPL locus was amplified from DNA obtained from the teeth and blood stain samples obtained from each of the 47 donors. When either on amplification product was obtained, or only a faint band was obtained, re-amplification from the first PCR mixture (dual PCR) was carried out. We found that the genotypes of 44 sample, pairs of teeth and blood (93.6%) matched each other; however, in three pairs of samples, they did not (Fig. 2A). In one pair of samples, two allelic products with a similar intensity were amplified in the blood stain sample, while the tooth sample showed a ladder pattern. In another pair

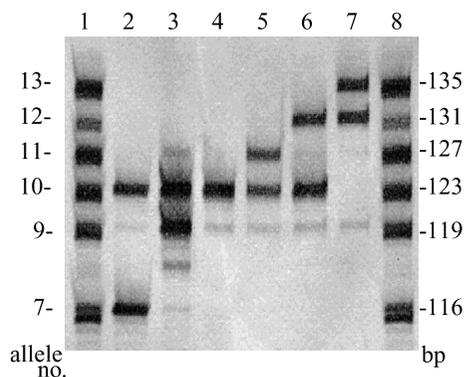


Fig. 1 Electrophoresis of LPL in 6% denaturing gel followed by silver staining

Sizes of fragments are indicated on right, and allele designations are indicated on left.

Lane 1: ladder marker, lane 2: genotype 7/10, lane 3: genotype 9/10, lane 4: genotype 10/10, lane 5: genotype 10/11, lane 6: genotype 10/12, lane 7: genotype 12/13, lane 8: ladder marker

of samples, two allelic products with similar a intensity were amplified in the tooth sample, while the blood stain sample showed unbalanced amplification of the alleles and an additional faint band with different sizes from those of the target bands. In another pair of samples, two allelic products with a similar intensity were amplified in the blood stain sample, while the tooth sample showed well-amplified different bands in addition to the target bands (Fig. 2A).

3. Detection of D21S11 locus from degraded DNA

We amplified the D21S11 locus from 44 DNA samples in which the same genotypes of the LPL locus were obtained from both the blood stain and the tooth samples. The PCR product sizes of the D21S11 locus using the present primers ranged from 213–239 bp. When no amplification product was obtained, or only a faint band was obtained, dual PCR was carried out. In the blood stain samples, the genotypes of only 29 samples (65.9%) could be determined, while those of 15 samples (34.1%) could not. Among the latter, three samples showed additional bands and 12 samples were not amplified clearly (Fig.

Table 1 Allele frequency distribution of LPL system in various populations

	Total No.	Alleles								Heterozygosity (%)	Reference No.
		7 (111)	8 (115)	9 (119)	10 (123)	11 (127)	12 (131)	13 (135)	14 (139)		
Japanese (Present study)	158	0.3	0	0.9	68	8.9	20.3	1.6	0	48.8	
Japanese	224	0.7	0.2	0.9	73	9.6	14.7	0	0	43.6	16
Japanese (Fukui)	84	0	0	0.6	70.8	10.1	17.9	0.6	0	45.6	17
Japanese (Shiga)	119	0	0	0	70.2	7.6	21.8	0.4	0	45.4	17
Japanese (Gunma)	134	0	0.4	1.9	65.3	11.6	20.8	0	0	51.6	17
Chinese (Taiwan)	100	0	0	0	85.5	0.2	12.5	0	0	25.3	8
Chinese (Chaoxian)	50	0	0	27	50	13	9	1	0	65.2	9
Chinese (Han)	50	0	0	13	55	14	17	1	0	63.2	9
Chinese (Tujia)	50	0	0	14	62	9	13	2	0	57.1	9
Chinese (Miao)	50	0	0	54	27	16	3	0	0	60.9	9
Chinese (Bai)	50	0	0	48	24	24	4	0	0	65.3	9
Western Romanian	108	0	0	32.4	33.8	26.4	32.4	3.2	0.9	60.5	2
Silesian	147	0	0	5.8	40.5	25.2	24.5	4.1	0	70.7	3
Caucasian Hungarian	223	0	0	4	40.8	26.7	24.9	2.9	0.7	70.0	4
Caucasian Austrian	550	0.3	0	5.3	38.5	27.8	25.1	2.6	0.4	70.8	5
African-American	210	0	0.2	2.9	50.2	24.4	20.7	3.3	0.2	64.4	10
Caucasian-American	204	0	0.2	4.7	41.2	28.7	20.3	4.9	0	70.0	10
Hispanic-American	219	0	0.2	14.6	37	15.1	27.2	5.9	0	74.2	10
Caucasian	88	0	0	2	33	25	32	8	0	71.9	1
Galician	113	0	0	8.8	37.2	26.1	19	8	0.9	74.3	11
Serbia and Montenegro	323	0.2	0	0.7	40.6	25.4	24.1	2.9	0	71.2	6
South-east Poland	714	0.1	0.3	4.7	44.3	23.1	25.2	2.1	0.1	68.5	7
Saudi Arabian	127	1.2	0	8.7	22.4	48.4	16.1	3.2	0	68.1	15
Brazil (Rio de Janeiro)	30	0	1.7	8.6	31	36.2	20.7	3.4	0	72.1	14

Allele numbers correspond to number of repeat units present in amplified fragments deduced from size.

Table 2 Distribution of LPL genotype frequencies

Genotype	Observed	Expected	χ^2 values
10/10	66	73.06	0.68
10/11	22	19.12	0.43
10/12	53	43.62	2.02
11/12	6	5.71	0.14
Others	11	16.49	1.83
Total	158	158.00	5.10
	df=1	0.2<p<0.5	

All classes with less than five events were pooled.

2B). In the tooth samples, the genotypes of 35 samples (79.5%) could be determined, while those of nine samples (20.5%) could not. Among the latter, six samples showed

additional bands, unbalanced amplification of the alleles or a ladder pattern (Fig. 2B). No amplification products were obtained from the remaining three samples.

Discussion

The recent increase in volume of DNA polymorphic data available has meant that they are becoming more useful not only in personal identification, but also in the determination of geographic origin in forensic science. At present, mitochondrial and Y-chromosomal DNA polymorphisms are more informative in determining geographic origin than autosomal polymorphisms. The application of autosomal DNA polymorphisms for

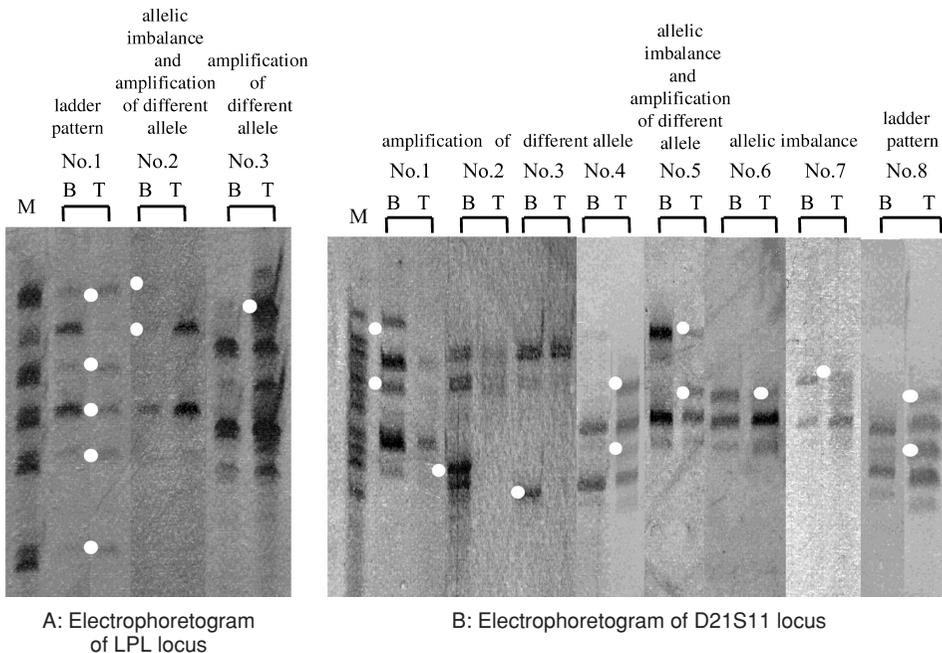


Fig. 2 Electrophoretogram of LPL and D21S11 loci obtained from tooth and blood stain samples showing misleading amplification

Source of samples and phenomena observed (indicated by white dots) are shown at top. Allelic imbalance means unbalanced amplification of alleles. T shows tooth sample, B shows blood sample, and M shows allelic ladder.

such purposes we found that except at the HLA locus, remains to be systematically studied. In this study, the variation of the LPL locus was not so high in the Japanese population. The expected heterozygosity at the locus was 48.8%, and was not significantly different from that in other Japanese populations (43.6–51.6%)^{16,17}. In contrast, higher values of heterozygosity (60.5–74.3%) have been observed in most populations in Europe, America, and the Middle East, apart from in African-Americans (Table 1)^{1–11,14,15}. The most striking difference in allelic distribution between the Japanese and those other populations was the frequency of allele 10, which showed a distribution of 65.3–73% (Table 1) for the former and 22.4–44.3% for the latter. When the expected frequency of genotypes not containing allele 10 was calculated, the corresponding value was 0.07–0.12 for the Japanese and 0.35–0.60 for the other populations. Loci, such as the LPL locus, which are not polymorphic in certain populations but

are polymorphic in other populations may be referred to the estimation whether a certain individual belongs to in determining or the latter population.

The success rate of PCR amplification of the LPL locus using degraded DNA obtained from blood stain and tooth samples was considerably high (94%). Dual PCR of the samples with faint or no amplification products at initial amplification resulted in visualization of PCR products. In many samples, genotypes from the teeth and blood stains matched each other, which suggests that correct types were obtained in these samples. However, we could not exclude the possibility of coincidence here, as amplification may have been carried out on contaminated DNA. One of the main reasons for unsuccessful amplification from forensic materials is the presence of inhibitors in those samples. As dual PCR decreases the influence of inhibitors in PCR reaction, it is worth using dual PCR for amplification from degraded DNA.

Using the 44 DNA samples in which the same genotypes were obtained at the LPL locus, we amplified the D21S11 locus at larger amplification sizes of 90–110 bp. This increase in amplification size decreased the success rate of PCR amplification to 60–88%. Increase in amplification size in the present samples considerably affected amplification efficiency. This may be partly attributable to the sizes of the amplifiable template DNA. In addition, the present results also demonstrated that increase in amplification size not only affected amplification efficiency, but also increased unbalanced amplification and amplification of non-target PCR products. If size or number of copies of amplifiable template DNA decreases, unbalanced amplification may occur, because of insufficient copies of template DNA corresponding to one of the alleles. Amplification of non-target bands may be partly caused through contamination by foreign DNA. However, amplification of non-target bands may result mainly from incomplete PCR products from degraded template DNA increasing mispairing of repeat regions during PCR amplification, as non-target bands appeared at the positions corresponding to the PCR products with constant repeat sizes. These results suggest that one of the most important premises for successful PCR amplification and increase in accuracy may be reduction of amplification size according to degree of template DNA degradation¹⁸⁾. In this study, we did not investigate the influence of PCR inhibitors in isolated DNA. Because inhibitors in isolated DNA differ depending on the source of the forensic material, they greatly influence the success or failure of PCR amplification. Therefore, we have to find for a more efficient method of DNA isolation which removes inhibitors from forensic materials.

In conclusion, we demonstrated that 1) reduction in PCR amplification size increased the success rate of PCR amplification from degraded template DNA, 2) that increase in amplification size of STR in such samples increased the chance of false amplification such as unbalanced amplification of alleles

and amplification of additional bands to the target bands. Although the LPL locus is not so highly polymorphic in the Japanese population, it may be useful in determination of geographic origin of individuals as it is more polymorphic in many other populations.

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