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Ethnic Differences in Allelic Frequencies of Two $(CA)_n$ Microsatellite Markers Located on Chromosome 5q

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The characteristics of allelic polymorphisms of the two (CA)n microsatellite (p599 and λ599) markers spanning the long arm of chromosome 5 were studied in 52 DNA samples from unrelated inhabitants of Seoul (Korea) by using the polymerase chain reaction (PCR) to investigate differences in allele frequencies between Korean and Caucasian populations. The 6 alleles were observed for p599 (CA)n with a polymorphism informative content (PIC) value of 0.71 and 9 alleles for λ599 (CA)n with a PIC value of 0.82. The observed heterozygote frequencies of the loci were estimated to 0.730 and 0.846, respectively. Several allele frequencies of two loci showed significant differences between Korean and Caucasian populations. Genotype data from the two loci were consistent with the Hardy-Weinberg equilibrium by χ^2 test. Linkage disequilibrium between p599 (CA)n and λ599 (CA)n loci was observed in χ^2 test between the observed and expected frequency of allelic association. The probability of matching calculated at each locus was 0.104 for p599 (CA)n and 0.043 for λ599 (CA)n, respectively. These results demonstrate the need to determine population-specific allele frequency distributions for polymorphic markers when performing genetic linkage studies in racially defined several populations.

The genes for spinal muscular atrophy (SMA) and schizophrenia disorder 1 (SCZD1) have been mapped to chromosome 5q11.2-q13.3 (Brzustowicz et al., 1990). The recessive acute and chronic chiidhood-onset SMAs are the types which map to a single locus on chromosome 5 showing strong linkage to DNA loci D5S6 and D5S39 (Brzustowicz et al., 1990; Sherrington et al., 1991). Genetic linkage analysis aimed at identifying disease susceptibility genes may indicate why some diseases are more common in certain ethnic groups. However, there is some evidence for non-allelic heterogeneity (Gilliam et al., 1990). The linkage analysis is also complicated in that SMA and SCZD1 have a variable age of onset and can be confused with other clinically overlapping neurological disorders.

DNA polymorphisms are an extremely effective tool in many fields of molecular genetics. Recently a new class of polymorphic markers broadly refered as microsatellite have been applied to the studies in human genetics (Litt and Luty, 1989; Weber and May, 1989). Length polymorphisms associated with tandem repeat variation of microsatellite sequences consisting of 2-5 nucleotides for each repeat have been used extensively in studies of human evolution (Bowcock et al., 1991; Di Rienzo et al., 1994; Deka et al., 1995; Jorde et al., 1995). Since the discovery of these sequences in human genome, thousands of such loci have been characterized. They were primarily developed and used as gene-mapping markers because they are highly polymorphic, uniformly dispersed throughout the human genome, and have relatively easy genotypes (Dietrich et al., 1994; Gyapay et al., 1994). These studies have demonstrated that a great majority of the loci that have been well characterized have heterozygosity levels in Caucasians > 70%. However, few studies, so far, have attempted to characterize the population-genetic properties of this class of polymorphic $(CA)_n$ (Bowcock et al., 1991; Di Rienzo et al., 1994).

Microsatellite DNA polymorphisms provide also a rich source of markers in the human genome for linkage mapping (Weber and May 1989). Several CA dinucleotide repeat sequences have been located to the SMA and SCZD1 regions on chromosome 5q (Brzustowicz et al., 1990; Gilliam et al., 1990; Sherrington et al., 1991). Because several studies of population-specific allele frequency distributions have demonstrated extensive interpopulation variation at

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microsatellite loci (Deka et al., 1991, 1992; Edwards et al., 1991, 1992), the frequency of each marker allele in the targeted populations should be defined when using linkage analysis for locating disease-susceptibility genes. From this viewpoint, the general application of these markers in mapping genetic disease by linkage disequilibrium test is possible. For their importance in genetic mapping, there is a rapidly expanding database about the distribution of these repeats and about the number and frequency of alleles in several populations in current. Therefore, microsatellites have features of polymorphic markers useful for the physical and genetic mapping of the human genome, disease diagnosis, and personal identification in the medical, populational, and forensic studies. However, genetic polymorphisms in the SMA and SCZD1 region on chromosome 5 have not been well-characterized in world populations.

In attempt to provide markers to help these problems, we analyzed the genetic variations of the two $(CA)_n$ microsatellite (p599 and λ599) loci located on human chromosome 5q in the Korean population and compared them with those reported for the Caucasian population. Furthermore, we have studied the extent of allele frequency variations at two loci and examined the conformity of genotype frequencies to their Hardy-Weinberg prediction and the extent of genotypic association between the two loci.

Materials and Methods

Subjects

Blood samples were obtained from 52 healthy unrelated inhabitants of Seoul, Korea.

Genomic DNA

Genomic DNA was extracted from buffy coats using the method, with some modification, of John et al. (1991). Buffy coats were obtained from whole blood by centifugation at 1000×g for 5 min at 4℃. To each buffy coat, equal volume of WCLB [white cell lysis buffer; 10 mM Tris-HCl (pH 7.6); 10 mM EDTA (pH 8.0); 50 mM NaCl] was added and treated with 20mg/ml of proteinase K at 65℃ for 1 h. Proteins and cell debris were removed by sequential phenol/chloroform extraction. When the supernatant became clean, the DNA was precipitated with 0.6 volume of isopropanol. After washing with 80% ethanol for twice, the pelleted DNA was dried under vacuum and dissolved in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0).

Radioactive PCR assay

The primers for the two microsatellite markers were synthesized by a DNA synthesizer (Applied Biosystem 381 A) and purified through oligonucleotide-purifying cartriges. Sequences of oligonucleotides for two microsatellites (P1, P2 for p599 and L1, L2 for λ 599) are as follows: P1: 5'-ATTCAGTACTGCTGAAG-G-3', P2: 5'-CCATTCCTACTTTGCCTT-3'; L1: 5'-CAG-TCCTCGTGGAATCATGC-3', L2: 5'-TATTTGCACTTA-TTTACTGCTCC-3'. The PCR was performed essentially according to the method of Saiki et al. (1988). Approximately 20 ng of genomic DNA was amplified in a total volume of 20 μl containing 20 pmole of each primer; 200 μM each of dGTP, dATP, and dTTP; 25 μM dCTP; 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.01% gelatin, 5 μCi ^{32}P-dCTP, and 1 unit of *Taq* DNA polymerase. A total of 25 cycles amplification were performed. Each cycle consisted of denaturation at 93℃ for 30 sec; annealing at 48℃ with P1/P2 primers and at 57℃ with L1/L2 primers for 30 sec; and extention at 72℃ for 1 min.

Amplified products were denatured by addition of 10 μl 95% formamide, 20 mM EDTA and heating to 95℃ for 2 min. The two microliter were mixed with an equal volume of sequencing loading buffer and the alleles were separated by electrophoresis in 6% denaturing polyacrylamide DNA sequencing gel [acrylamide:bis-acrylamide (39: 1), 7 M urea containing 90 mM Tris-borate buffer pH 8.3 and 2 mM EDTA] for 3-4 h at 55 W. The gel was fixed, dried and exposed to high sensitivity X-ray film (Kodak) for 20 h at -70℃. The PCR allele sizes were determined by running M13 mp18 DNA sequencing ladder in each gel for single base pair resolution and individuals were genotyped by comparing with known sequences of M13 mp18 DNA.

Statistics

Allele frequencies of the two CA repeat markers in the Korean population were calculated directly by dividing the number of positive for allele by the number of chromosomes tested (n=104). To evaluate the precision of the estimates, 95% confidence intervals were also calculated, taking into consideration that allelic frequencies of a locus are multinomially distributed.

Unbiased estimates of expected heterozygote frequencies (heterozygosity or *H*) were computed as

$$H = n[1 - \sum_{j=1}^{K}(n_j/n)^2] \cdot (n - 1)$$

where n_1, n_2, n_3, $\cdots$ n_k are the allele count of K alleles at each locus. In a randomly mating population following HWE, heterozygosity is equivalent to the frequency of observed heterozygotes. The heterozygosity was used to calculate the expected total number of heterozygotes at a locus. The total number of distinct homozygous and heterozygous genotypes were computed by previously described theory

(Chakraborty et al., 1988).

Linkage analysis for the two $(CA)_n$ microsatellite (p599 and λ599) was performed by computer Pascal language program and were calculated for each marker, with the significance of association evaluated by χ^2 test, corrected for multiple test by multiplying the corresponding p value by the number of alleles observed in the two microsatellite markers.

To demonstrate that the two loci analyzed can also be used for forensic individualization, the probability that two randomly selected unrelated individuals have identical genotypes (probability of matching or nondiscrimination) was calculated (p^2).

Results

Two $(CA)_n$ microsatellite loci, p599 and λ599, were amplified with oligonucleotides flanking the CA repeats on chromosome 5q. These polymorphic regions have been individually subcloned and sequenced from the phage clones p105-599 (D5S76) and lambda 599.1 (D5S36) (Mankoo et al., 1991; Sherrington et al., 1991). Genomic DNA from 52 unrelated Korean individuals were amplified by PCR and typed for both of the microsatellites (Fig. 1). Genotype data for these two $(CA)_n$ microsatellite p599 and λ599 loci were determined in 6% denaturing polyacrylamide DNA sequencing gel (Fig. 1) Both DNA strands of the amplified products were radiolabeled and the alleles have distinct appearances based on the sequence marker (M13 mp18) of the DNA strand. Amplified DNAs from the p599 $(CA)_n$ and λ599 $(CA)_n$ are shown in figure 1A and B, respectively, and ranged in size from 92 to 108 bp, enabling precise determination of allele lengths (Table 1).

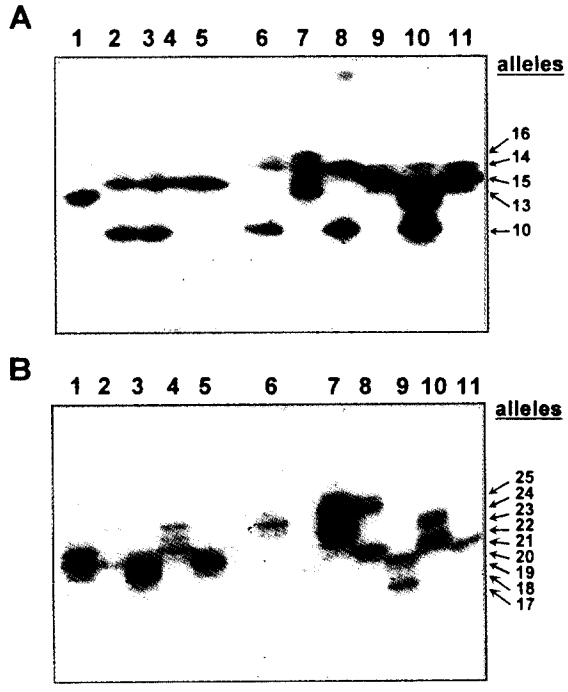

Fig. 1. Radioactive PCR assay for two $(CA)_n$ microsatellite polymorphism. **A.** Autoradiograph of DNA sequencing gel illustrating discrete allele for p599 $(CA)_{10-16}$ (*lane 1*, 13/13; *lane 2*, 10/14; *lane 3*, 10/14; *lane 4*, 14/14; *lane 5*, 14/14; *lane 6*, 10/15; *lane 7*, 13/16; *lane 8*, 10/15; *lane 9*, 14/14; *lane 10*, 10/13; *lane 11*, 14/15). **B.** Autoradiograph of DNA sequencing gel illustrating discrete allele for λ 599 $(CA)_{17-25}$ (*lane 1*, 19/20; *lane 2*, 19/23; *lane 3*, 18/19; *lane 4*, 20/23; *lane 5*, 19/19; *lane 6*, 23/23; *lane 7*, 22/25; *lane 8*, 20/25; *lane 9*, 17/19; *lane 10*, 21/24; *lane 11*, 19/19). Numbers of repeats (alleles) are designated in the right side of each figure.

Genotypes of the p599 $(CA)_n$ and λ599 $(CA)_n$ alleles were shown in Fig. 1A and B, and their allele frequencies in Korean population are summarized in Table 1. From the survey of 52 unrelated Koreans, 6 alleles were observed for p599 $(CA)_n$ ranging in size from $(CA)_{10}$ to $(CA)_{16}$ (Table 1). A total of 9 alleles from the same individuals were observed for λ599 $(CA)_n$ ranging in size from $(CA)_{17}$ to $(CA)_{25}$ (Table 1). Both of the two $(CA)_n$ microsatellite loci exhibited polymorpism in the sizes of the amplified fragements. Alleles always differed in sizes by multiples of 2 bp (from CA strand to CA strand bands), a result consistent with the concept that the number of tandem dinucleotide repeat is the variable factor. One p599 allele (CA_{10}) and two λ599 alleles (CA_{17} and CA_{19}) were included, which were not reported in the study on Caucasians (Sherrington et al., 1991). The frequency and actual base pair size of the alleles at the two microsatellites are given in Table 1. Korean population has 31.7% of CA_{14} and 28.9% of CA_{20}, repectively, as the most common alleles for p599 $(CA)_n$, and λ599 $(CA)_n$.

To determine whether the population is in HWE, exact test of homogeneity was carried out. Because the loci analyzed have a large number of alleles, expected and observed frequencies of homozygotes

Table 1. Allele frequency and PCR product size of the two $(CA)_n$ microsatellite loci

Repeat type	Size	PCR product size (bp)	Frequency (%)	Confidence interval (95%)
p599 $(CA)_n$				
1	CA_{16}	107	8.65	(0.015 0.158)
2	CA_{15}	105	6.73	(0.004 0.131)
3	CA_{14}	103	31.73	(0.200 0.435)
4	CA_{13}	101	23.08	(0.124 0.337)
5	CA_{11}	97	0.96	(0.000 0.034)
6	CA_{10}	95	28.85	(0.174 0.403)
λ599 $(CA)_n$				
1	CA_{25}	108	4.81	(0.000 0.097)
2	CA_{24}	106	15.38	(0.066 0.243)
3	CA_{23}	104	11.54	(0.032 0.186)
4	CA_{22}	102	6.73	(0.004 0.124)
5	CA_{21}	100	14.42	(0.059 0.232)
6	CA_{20}	98	28.85	(0.179 0.403)
7	CA_{19}	96	7.69	(0.009 0.137)
8	CA_{18}	94	8.65	(0.014 0.149)
9	CA_{17}	92	1.93	(0.000 0.051)

Table 2. Chi-square test for homozygotes and heterozygotes of the two $(CA)_n$ microsatellite loci observed in this study

Microsatellite locus	No. of alleles	No. of individuals	Homozygotes		Heterozygotes		χ^2
			Obs	Exp	Obs	Exp	
p599	6	52	14	12.9	38	39.1	0.125
λ599	9	52	8	8.3	44	43.7	0.013

Obs, observed numbers; Exp, expected numbers

and heterozygotes were compared. The distribution of the genotypes at the two microstellite loci is in HWE (χ^2=0.125 for p599; χ^2=0.013 for λ599). The Hardy-Weinberg formulation was calculated by comparing observed and expected genotypes. A test of χ^2 based on the total number of distinct homozygous and heterozygous genotypes of the two $(CA)_n$ microsatellites observed in Korean population is presented in Table 2. Comparison between the expected and observed frequencies of homozygotes and heterozygotes by χ^2 are also summarized in Table 2. The observed heterozygosities at the two microsatellite loci were 0.73 and 0.85, respectively. Using these allele frequencies, also, we calculated a PIC of 0.71 for p599 $(CA)_n$ and 0.82 for λ599 $(CA)_n$, respectively (Table 3).

These two $(CA)_n$ microsatellites are assumed to be within 7.7 kb of each other (Sherrington et al., 1991). We did not perform a haplotype analysis with two $(CA)_n$ microsatellite loci because no families were used in this study. The resulting genotypes were analyzed by the computer program Pascal language. Linkage disequilibrium between p599 $(CA)_n$ and λ599 $(CA)_n$ loci was apparently observed in χ^2 test between the observed and expected frequency of allelic association except the $(CA)_{22\text{-}23}$ alleles for λ599 $(CA)_n$ locus (data not shown).

From a forensic point of view, to give information in regard to the usefulness of a particular locus for forensic individualization, the probability of matching (P^2, the probability that two unrelated individuals will have the same genotype of by chance) was estimated to 0.043 (λ599) and 0.104 (p599), respectively (Table 3). When two loci for the most common genotype were combined, the total matching probability was 3.14×10^{-7}. For application to parentage test, the average power of exclusion was also calculated and shown in Table 3. The power of exclusion refers to the probability of excluding a man who is falsely accused of being the father. Of two loci, λ599 $(CA)_n$ used jointly for parentage testing, the power of exclusion was 0.65.

Discussion

In this study, we compared the allele frequency distributions of two $(CA)_n$ microsatellite (p599 and λ599) markers spanning the long arm of chromosome 5 in the Korean population with those from the Caucasian population. The data presented here, also, should aid genetic linkage studies aimed at finding disease-susceptibility genes in Korean population.

These markers studied here have been previously characterized only in Caucasian population (Sherrington et al., 1991). There were several differences noted between Korean and Caucasian populations in respect to frequency, number of alleles and predominating alleles. Fig. 2A and B show the distributions of allele frequencies at two $(CA)_n$ microsatellite (p599 and λ599) loci in Korean and Caucasian populations. The distributions between these two populations are irregular, showing bimodal or trimodal distributions, and there is considerable variation from p599 $(CA)_n$ locus to λ599 $(CA)_n$ locus in these distributions. The frequency distributions for two $(CA)_n$ loci in the Korean population were bimodal, while the Caucasian population was bimodal for p599 but trimodal for λ599 (Fig. 2). The mechanism by the different modes (i.e., bi- or trimodal) of relative allele frequency distributions in Korean and Caucasian populations is intriguing. Because the two loci are located within D5S36 (non-functional region) on chromosome 5, we think that it is possible that specific allele sizes represent the evolution of allele in different ethnic groups. Bimodal relative allele frequency distributions in Asian populations have also been observed at variable number of tandem repeat (VNTR) loci (Boerwinkle et al., 1989; Hong et al., 1993). It has been suggested that nonidentity of individuals repeat motifs gives rise to these bimodal frequency distributuions via cellular recombination pathway (Boerwinkle et al., 1989). Also, we think that these differences resulted in higher heterozygosity and PIC for these two loci in Koreans than in Caucasians.

The most common allele, also, appears to be different for the two $(CA)_n$ microsatellite loci in Korean and Caucasian populations, and at the two loci, predominant alleles do not coincide between these two populations (Fig. 1A and B) The most frequent genotypes for the two loci have frequencies of 0.317 (p599) and 0.290 (λ599), respectively. The

Table 3. Power of exclusion and matching probability of two $(CA)_n$ microsatellite loci observed in Korean population

Microsatellite locus	No of individuals	No of alleles	Range of alleles (bp)	PIC	Power of exclusion	Matching probability
p599	52	6	95 - 107	0.71	0.52	0.104
λ599	52	9	92 - 108	0.82	0.65	0.043

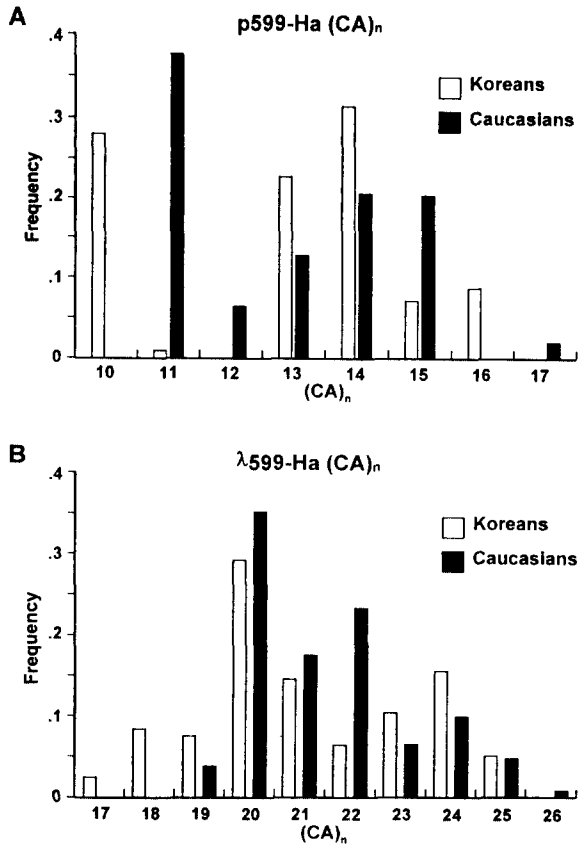

Fig. 2. Distributions of allele frequency of two microsatellite loci in Korean (the present study) and Caucasian (Sherrington et al., 1991) populations. Allele counts were used to calculate and plot the relative allele frequencies of allele at (A) p599 $(CA)_n$ and (B) λ599 $(CA)_n$ loci.

heterozygosities observed for the Koreans were estimated to 0.730 for p599 $(CA)_n$ and 0.846 for λ599 $(CA)_n$, respectively (Table 2). Especially, the greater number of alleles at the λ599 $(CA)_n$ locus results in heterozygosity and PIC values are generally equivalent to or higher than those found in Caucasians. We therefore think that the informativeness of the $(CA)_n$ markers observed in this study was generally superior to that of standard unique sequence probe polymorphisms and was as good as many minisatellite polymorphisms (Nakamura et al., 1987).

For application to parentage test, the average power of exclusion was also calculated and shown in Table 3. The probability of matching (P^2, the probability that two unrelated individuals will have the same genotype by chance) was estimated to 0.104 for p599 and 0.043 for λ599, respectively (Table 3). When two loci for the most common genotype were combined, the total matching probability was 3.14×10^{-7}. However, the result was suggested that the p599 $(CA)_n$ was not apropriate to this calculation because p599 $(CA)_n$ and λ599 $(CA)_n$ are found to be linked in the same chromosome 5. Because linkage disequilibrium between two loci was detected, and alleles from each locus appear to be in HWE, combined heterozygosity for two loci may not be used to calculate accurate genotype frequencies for personal identification. The power of exclusion refers to the probability of excluding a man who is falsely accused of being the father. Of two loci, λ599 $(CA)_n$, which was used jointly for parentage testing, the power of exclusion was 0.65. Therefore, the $(CA)_n$ microsatellite (λ599) marker potentially useful as a marker for individual identification.

These two $(CA)_n$ microsatellites reported by Sherrington et al. (1991) were in the CEPH (Centre d'Etude du Polymorphisme Humain) families. By testing the microsatellite, p599 $(CA)_n$ and λ599 $(CA)_n$ in the same population, they were able to estimate haplotype frequencies. They suggest that there is a strong linkage disequilibrium between p599 $(CA)_n$ and λ599 $(CA)_n$. But we did not perform a haplotype analysis with two $(CA)_n$ microsatellite loci because no families were used in this study. Using each genotype from two microsatellites, however, we found that there are linkage disequilibria between the two loci but not complete one(data not shown). Through linkage disequilibrium between a marker and disease gene, many candidate genes were searched and located in the near vicinity of the markers (Dean et al., 1990). In this sense, two $(CA)_n$ microsatellites located on the same chromosome 5, which shows obvious nonrandom association, should increase the usefulness of mapping, because relatively few individuals are required to establish disequilibrium. These will increase the power of association tests for genetic disease where family data may be defective or a complex mode of inheritance is hypothesized. Also, the result suggest that characters of the markers used to build linkage maps are informative as measured by heterozygosity or PIC and as measured by the average and maximum separations between adjacent markers in cM and can be improved. Thus, we think that these two $(CA)_n$ microsatellites are moderately to highly informative in human genetic studies.

In conclusion, the choice of genetic markers for human linkage analysis is based on the location of the marker with respect to a putative disease gene and degree of polymorphism of the marker. Polymorphisms of two $(CA)_n$ microsatellite studied here is highly informative and will be useful in analyses of other conditions which localize to 5q11.2-q13.3, including one of SMA and SCZD 1 (Brzustowicz et al., 1990). As the Korean and Caucasian populations show, allele frequencies vary significantly from population to population. These results indicate the need to accurately determine population-specific allelic distributions when performing linkage studies in a racially or geographycally defined group.

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