Original

Characterization of Microsatellite Markers Covering Chromosome 1 in the Korean and Japanese Population

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Abstract : Microsatellite markers are useful markers for genetic linkage analysis. However, it has been demonstrated that ethnicity plays a significant role in genetic variation. To investigate genetic variation between the Korean and Japanese populations, 51 microsatellite marker loci spanning the entire human chromosome 1 were acquired from a commercially available set (ABI PRISM Linkage Mapping Set-HD5). The allelic frequencies and heterozygosities for these marker loci in 96 unrelated Korean subjects and 96 unrelated Japanese subjects were determined. Of the 51 markers tested, significant differences were observed when the microsatellite allele frequency pattern of Koreans was compared with those of the Caucasian model, whereas the pattern was highly similar between the Korean and Japanese populations. Our data indicate that an extensive verification of public microsatellite markers in a particular population, especially in Koreans, should be undertaken prior to linkage studies. Moreover, our findings should facilitate genetic linkage studies of various hereditary diseases.

Key words : allele frequency, heterozygosity, microsatellite markers, chromosome 1

Introduction

Microsatellites are an essential tool for genetic linkage analyses. They are selected in genetic studies on the basis of both their value in terms of information and their positions with respect to one another on the genetic map. Genetic mapping locates one gene locus relative to another, the position of which is known by the frequency of recombination for Mendelian traits, or the concordance or discordance of affected sib pairs for polygenic traits. Microsatellites markers are identified using a suitable computer program. These markers then have to be tested for their uniqueness and suitability in linkage analyses studies. They are assessed according to the level of heterozygote frequency of the microsatellite alleles as well as the relative allele frequency. Because microsatellites are distributed prolifically throughout the genome and are highly variable in repeat length and in polymorphisms, they have become powerful tools for genetic mapping of disease susceptibility loci.

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Several screening sets of microsatellite markers have been described in the literature¹⁻⁴⁾, and such marker sets designed for genome-wide screening have recently become available commercially. Most of these marker sets have been prepared according to information obtained from the Caucasian population, as their allelic frequencies are more readily available. Yet significant differences were observed when the SNP allele frequency pattern of Koreans was compared with that of Caucasians and Africans⁵⁾. Differences in both the allelic frequency and the heterozygosity of many microsatellite markers between Caucasians and Japanese have also been found⁶⁾.

In the present study, we compared the allelic frequencies and heterozygosities between Korean and Japanese populations for all of the 51 microsatellite markers spanning human chromosome 1, which contains over 3000 genes and over 240 million base pairs, of which ~90% have been determined.

Subjects and Methods

Genomic DNA was isolated from 96 unrelated Korean subjects and 96 unrelated Japanese subjects, from both whole blood using a QIAamp DNA Blood Kit (QIAGEN GmbH, Hilden, Germany) and from buccal cells using a BuccalAmp DNA Extraction Kit (Epicentre Technologies, madison, WI, USA). An ABI PRISM Linkage Mapping Set-HD5 (Applied Biosystems, Foster City, CA, USA) containing fluorescent labeled primer pairs for 51 microsatellite markers was used for genotyping. Polymerase chain reaction (PCR) amplification of each DNA segment of interest was performed in 96-well plates in a volume of 6 μ l, containing 5 ng of genomic DNA, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.6 μ l 10 $\times$ PCR buffer, 2 pmol of each primer, and 0.15 U AmpliTaq Gold (Applied Biosystems). After a pre-PCR heating step for 12 min at 95 $^{\circ}$ C, 35 cycles of amplification (15 s at 94 $^{\circ}$ C for denaturing, 15 s at 55 $^{\circ}$ C for annealing, and 30 s at 72 $^{\circ}$ C for extension) were performed in GeneAmp 9700 thermal cyclers (Applied Biosystems). The PCR products were combined into pools, analyzed on ABI 377 DNA sequencers, and genotyped using GeneScan (version 3.1) and Genotyper (version 2.5) software (Applied Biosystems). Caucasian-derived genotypic data (heterozygosity) for each marker was obtained from the Genome Database (GDB; <http://www.gdb.org>) and from an online source (<http://research.marshfieldclinic.org/genetics/>). The Ethical Committee of Pusan National University and Showa University approved this study, and all subjects gave their written informed consent.

Results

Among the 51 microsatellite markers in the original set (ABI PRISM Linkage Mapping Set HD-5), heterozygosity was different in a significant fraction of the markers between Koreans, Japanese and Caucasians (Table 1). Differences in heterozygosity of over 0.2 between Koreans and Caucasians were found in 5 markers (9.8%), and between Korean and Japanese in only 1 marker (2.0%) (Fig. 1). The mean heterozygosity of the markers was 0.67 (Korean: range 0.23–0.91, Japanese: range 0.29–0.92). In addition, four Korean markers (7.8%) and three Japanese markers (5.9%) were found to show heterozygosities lower than 0.6 (Table 2). Of the markers examined, 5.9% of the Korean group and 3.9% in the Japanese population showed heterozygosities below 0.5, which is insufficient for linkage analysis. This low level of usefulness was not found for any of the Caucasian markers.

Table 1. Distribution of heterozygosities of microsatellite markers in the original ABI PRISM Linkage Mapping Set HD-5

Heterozygosity	Frequency (%)		
	Korean	Japanese	Caucasians
<0.5	5.9	3.9	0.0
0.5 - 0.6	2.0	2.0	0.5
0.6 - 0.7	52.9	47.1	8.7
0.7 - 0.8	31.4	21.6	38.0
>0.8	7.8	25.4	52.8
>0.6	92.1	94.1	99.5

Fig. 1. Ethnic differences of heterozygosities of microsatellite markers between the different populations

Discussion

Genetic variation has been used for the identification of disease-related genes. Among those genetic variations, SNPs and microsatellites are the most common source of variation between individuals, detected at a frequency of approximately 3000 or more in the human genome. Therefore, microsatellites could be of value for genetic mapping studies, which may lead to a better understanding of the genetic basis for complex diseases. They are therefore considered as promising markers for genetic linkage analysis. This study examined the distribution of 51 microsatellite markers at intervals of approximately 5 cM of allele frequency amongst the Korean and Japanese populations.

Although significant differences in microsatellite allele frequency were detected among the different ethnic groups, the comparison of allele frequencies between Koreans and Japanese showed a high similarity of microsatellite allele frequency patterns. Genetically, Koreans are nearest to Mongolians and are related to the Japanese. This lends genetic evidence to the ethnohistoric account of the origin of Koreans from Central Asia⁷⁾, and most authors agree that Koreans are from a North Asian population group⁸⁾. Japanese and Korean populations may share some common genetic structure that could reflect recent gene flow and some amount of admixture of Y chromosomes between these two populations⁹⁾. These results may also support the "out of northeast-Asia hypothesis" concerning the origin of the

Table 2. Heterozygosities of tested markers

Locus	cM	Heterozygosity		
		Korean	Japanese	Caucasians
D1S468	4.22	0.71	0.69	0.75
D1S2660	10.78	0.34*	0.52*	0.78
D1S214	14.04	0.76	0.72	0.80
D1S450	20.61	0.68	0.80	0.81
D1S2667	24.68	0.71	0.83	0.82
D1S434	29.93	0.61	0.61	0.61
D1S507	33.75	0.65	0.81	0.78
D1S2644	43.72	0.68	0.64	0.81
D1S199	45.33	0.77	0.69	0.84
D1S2864	50.28	0.77	0.83	0.80
D1S234	55.10	0.71	0.77	0.82
D1S2892	70.41	0.91	0.73	0.89
D1S2713	73.81	0.23*	0.37*	0.75
D1S2797	75.66	0.76	0.73	0.73
D1S2652	80.77	0.61	0.67	0.64
D1S2700	87.31	0.79	0.85	0.89
D1S2846	91.89	0.64	0.65	0.56
D1S230	95.31	0.65	0.63	0.79
D1S198	99.30	0.80	0.78	0.80
D1S2841	106.45	0.85	0.77	0.78
D1S500	107.56	0.72	0.62	0.64
D1S207	113.69	0.74	0.79	0.84
D1S2868	126.16	0.64	0.61	0.77
D1S206	134.20	0.68	0.81	0.82
D1S495	136.88	0.61	0.62	0.87
D1S2726	144.38	0.62	0.67	0.75
D1S252	150.27	0.64	0.83	0.82
D1S498	155.89	0.77	0.73	0.82
D1S2635	165.62	0.65	0.72	0.86
D1S484	169.68	0.62	0.68	0.64
D1S2878	177.86	0.76	0.82	0.84
D1S196	181.49	0.61	0.66	0.73
D1S218	191.52	0.73	0.84	0.83
D1S2818	198.30	0.62	0.65	0.70
D1S238	202.73	0.82	0.81	0.86
D1S2877	205.40	0.61	0.63	0.72
D1S413	212.44	0.70	0.64	0.77
D1S249	220.65	0.69	0.64	0.87
D1S245	227.81	0.64	0.62	0.83
D1S425	231.11	0.65	0.62	0.81
D1S227	238.52	0.61	0.72	0.71
D1S213	242.34	0.69	0.81	0.86
D1S2833	245.05	0.59*	0.61	0.83
D1S2709	247.23	0.67	0.89	0.73
D1S2800	252.12	0.73	0.67	0.79
D1S2850	256.26	0.61	0.63	0.65
D1S2785	266.27	0.73	0.92	0.77
D1S304	267.51	0.62	0.61	0.61
D1S2842	273.46	0.64	0.68	0.78
D1S423	277.80	0.44*	0.29*	0.61
D1S2836	285.75	0.61	0.79	0.78

*microsatellite markers with heterozygosities of below 0.60

Japanese population¹⁰⁾. Recently, it was reported that the Asian population has the smallest number of distinct SNP haplotypes. Furthermore, allele frequencies between Korean and Japanese populations are comparable with previous data for the Japanese population only¹¹⁾, suggesting a common origin of ancestry, as expected from the close geographical location of the two countries⁵⁾.

Ikari *et al*¹²⁾ reported that the microsatellite markers (ABI PRISM Linkage Mapping Set-MD10) established among approximately 10 cM were tested in 64 unrelated Japanese individuals. Thirty of these markers were included in our investigation, and our findings on the heterozygosity for these markers supports this previous work.

Our study has one important limitation, in that the ethnic differences in allele frequencies between Koreans and Japanese are based on a relatively small sample size. Since genotype distribution with small sample sizes is sometimes different from the true distribution within a larger population, further studies using a larger sample size need to be conducted. Nevertheless, the genetic information obtained here should facilitate genetic linkage studies of various hereditary diseases, especially in the Korean population.

Abbreviations

Polymerase chain reaction ; PCR Centi-morgans from the telomere of the p arm ; cM

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