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Characterization of microsatellite markers covering chromosome 1 in the Korean and Japanese populations

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Microsatellite markers are considered to be very promising genetic markers for genetic linkage analysis. The majority of the markers are as informative as in Caucasians but there are significant ethnic differences in the genetic variations. In order to investigate the genetic variations in the Korean and Japanese populations and their ethnic differences, 51 microsatellite marker loci spanning the whole human chromosome 1 were arranged from a commercially available set (ABI PRISM Linkage Mapping Set-HD5, Applied Biosystems, Foster City, CA, USA), and then determined the allelic frequencies and heterozygosities for these marker loci in the 96 unrelated Korean subjects and 96 unrelated Japanese subjects. Of all 51 markers tested, significant differences were observed when microsatellite allele frequency pattern of Korean was compared with those of Caucasian, while this pattern was highly similar between Korean and Japanese populations. Our data indicate that an extensive verification of public microsatellite markers in a particular population study should be undertaken prior to their linkage studies. Moreover, this information should facilitate genetic linkage studies of various hereditary diseases, especially in the Koreans and Japanese.

Key words : Korean, Japanese, Microsatellite markers, Chromosome 1

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Recent advances in genetic techniques have facilitated the molecular dissection of the human genome and provided an insight into its morbid anatomy. These genomic analyses, including the construction of extensive framework linkage maps[1-3] have been greatly aided by the discovery of simple tandem repeat polymorphisms[4,5] (STRPs; also called microsatellite). Microsatellites, an essential tool for genetic linkage analyses, are selected in genetic studies on the basis of both informativeness and their positions with

respect to one another on the genetic map. Genetic mapping locates one gene locus relative to another gene locus that is known by the frequency of recombination for Mendelian traits, or the concordance or discordance of affected sib pair for polygenic traits. 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.

Chromosome 1 is the largest of the chromosomes and occupies 9% of total. It has 252 Mbp and approximately 900 genes have been detected in it. It has been reported that chromosome 1 has candidate regions related to high blood pressure,[6-8] diabetes,[9] rheumatoid arthritis,[10] localized aggressive periodontitis[11] and mandibular prognathism.[12] Mandibular prognathism (MP) (Mc Kusick No.176700) is the best-known facial phenotype with a varying prevalence relative to populations.[13] It was reported that the prevalence of MP is the highest in Asian populations (approximately 15%) and the lowest in Caucasian populations (1%).[14,15] A genome-wide linkage analysis to identify loci susceptible to MP was conducted using Korean and Japanese sib-pairs by Yamaguchi et al.[12] They reported that 2 non-parametric linkage analyses, GENEHUNTER -PLUS and SIBPAL, were applied and nominal statistical significance of linkage to MP was detected at chromosome 1p36, 6q25, and 19p13.2 by using 10 cM microsatellite markers. The chromosome 1p36 represented the highest significance among them. Therefore chromosome 1 was selected as a target and 5 cM microsatellite markers for further studies related to MP were used to reveal the similarity between Korean and Japanese populations.

Several screening sets of microsatellite markers have been described in the literature,[16-19] and such marker sets designed for genome-wide screening have recently become commercially available. Most of them have been prepared on the basis of information on Caucasians, as their allelic frequencies are more readily available. Differences in both the allelic frequency and the heterozygosity of many microsatellite markers between Caucasians and Japanese have been found.[20] But, these have not been well characterized in Koreans and there was no comparison of the allelic frequency and the heterozygosity of many microsatellite markers between Koreans and any other populations.

To verify ethnic differences and informativeness of microsatellite markers of chromosome 1 between Koreans and Japanese for genetic linkage analysis, we analyzed the differences in the allelic frequencies and the heterozygosities between Koreans and Japanese of the 51 microsatellite marker loci spanning the whole human chromosome 1.

SUBJECTS AND METHODS

Genomic DNA was extracted from 96 unrelated Korean subjects and 96 unrelated Japanese subjects. Buccal cells were collected from Korean subjects and DNA was isolated from buccal cells using a BuccalAmp DNA Extraction Kit (Epicentre Technologies, WI, USA). Blood samples were collected from Japanese subjects and DNA was isolated from whole blood cells using a QIAamp DNA Blood Kit (QIAGEN GmbH, Hilden, Germany).

An ABI PRISM Linkage Mapping Set-HD5 (Applied Biosystems, Foster City, CA, USA), which contains fluorescence-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 μl, containing 5 ng of genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.6 μl 10 × PCR buffer, 2 pmol of each primer, and 0.15 Unit AmpliTaq Gold (Applied Biosystems). After a pre-PCR heating step for 12 min at 95℃, 35 cycles of amplification (15 sec at 94℃ for denaturing, 15 sec at 55℃ for annealing, and 30 sec at 72℃ for extension) were performed in GeneAmp 9700 thermal cycler (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 (Appiled Biosystems).

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

Frequency (%)	Heterozygosity		
	Koreans	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

Genotype data derived from Caucasians for each marker was obtained from the Genome Database (GDB, http://www.gdb.org). The microsatellite marker positions were obtained online (http://research. marshfieldclinic. org/genetics/).

The IRB (internal review board) of Busan National University Hospital and Showa University approved this study and all subjects gave 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 among Koreans, Japanese and Caucasians (Table 1). Heterozygosity values higher than 0.6 were 92.1% in Koreans, 94.1% in Japanese, and 99.5% in Caucasians. There were also ethnic differences of heterozygosities of microsatellite markers among populations (Fig 1). The differences of heterozygosities between Koreans and Japanese from 0.00 to 0.05 is 39.2, from 0.05 to 0.10 is 29.4, from 0.01 to 0.15 is 15.7, from 0.15 to 0.20 is 13.7, and above 0.02 is 2.0. The differences of heterozygosities between Koreans and Caucasians from 0.00 to 0.05 is 25.5, from 0.05 to 0.10 is 19.6, from 0.01 to 0.15 is 31.4, from 0.15 to 0.20 is 13.7, and above 0.02 is 9.8. Comparing the differences between Korean-Japanese and Korean-Caucasian, we found that for values of 0.00-0.10 the Korean-Japanese difference is larger, for

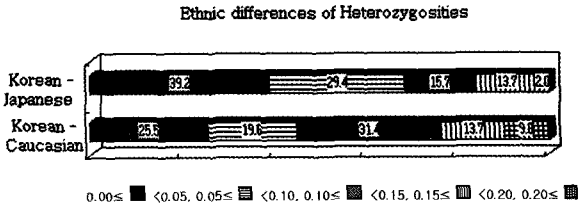

Fig 1. Ethnic differences of heterozygosities of microsatellite markers among the populations.

0.10-0.15 the Korean-Caucasian difference is larger, for 0.15-0.20 there is no difference, and for values above 0.20 the Korean-Caucasian difference is larger. The Korean DNA samples all had lower heterozygosity values than those of Caucasians. Heterozygosity values below 0.20 occurred in 98% of the samples between Korean and Japanese populations.

The mean heterozygosity of the markers was 0.67 (Korean: range 0.23-0.91, Japanese: range 0.29-0.92), and 4 Korean markers (7.9%, D1S2660, D1S2713, D1S2833, D1S423) and 3 Japanese markers (5.9%, D1S2660, D1S2713, D1S423) were found to show heterozygosities lower than 0.6 (Table 2). Of the markers examined, 5.9% in Koreans and 3.9% in Japanese showed heterozygosities below 0.5, insufficient for linkage analysis, while such low informativeness was not found in any of the markers in Caucasians.

DISCUSSION

Genetic variations have been used for the identification of disease related genes. Among those genetic variations, not only single nucleotide polymorphisms (SNPs) but also microsatellites are the most common genetic variations between individuals and registered at a frequency of approximately 3000 or more in the human genome. Therefore, microsatellites can be used to facilitate genetic mapping studies that may lead to a better understanding of the genetic basis for complex diseases. They are considered as very promising genetic markers for genetic linkage analysis.

The newly introduced ABI PRISM Linkage Mapping Set-HD5 (LMS-HD5)[21] is a set of optimized microsatellite markers originally identified in the

Table 2. Heterozygosities of tested markers

Locus	cM	Heterozygosity		
		Koreans	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

* : the microsatellite markers of below 0.60 heterozygosities

Genethon Human Linkage Map[22] that provides an average 5 cM genome-wide coverage. LMS-HD5 utilizes the dye set 6-FAM, HEX, NED, and ROX, and the reverse primers are tailed on the 5′-end to minimize plus A inconsistencies. The set incorporates the 400 markers originally in the Linkage Mapping Set-MD10 (previously LMS V2) and 411 newly designed markers, for a total of 811 markers for whole chromosomes. For this study, 51 microsatellite markers were used for human chromosome 1 of the Korean and Japanese populations and the allelic frequencies and heterozygocities of these markers were investigated.

Although significant differences in microsatellite allelic frequency were detected among different ethnic groups, the comparison of allelic frequencies between Koreans and Japanese showed a high similarity of microsatellite allelic frequency patterns. Genetically, Koreans are most analogous to the Mongolian and are related to the Japanese. This lends genetic evidence to the ethnohistoric account of the origin of Koreans from Central Asia.[23] Most authors generally agree that Koreans are considered as a North Asian group.[24] Korean and Japanese populations may share some common genetic structure that could reflect recent gene flow and some amount of admixture of Y chromosomes between these 2 populations.[25] These results may reflect the 'out of Northeast-Asia hypothesis' of the origin of the Japanese population.[26] Recently, it has been reported that the Asian population had the smallest number of distinct SNP haplotypes. Furthermore, allele frequency patterns between Koreans and Japanese were reported to be compa-rable with previous data within the Japanese population,[27] suggesting a common origin of ancestry, as expected from the close geographical location of the 2 countries.[28]

Ikari[29] reported that the microsatellite markers (ABI PRISM Linkage Mapping Set-MD10 established among approximately 10 cM were tested in 64 unrelated Japanese. These included 30 markers tested in our investigation. His findings for the heterozygosities testedin the same markers were very similar to ours. Our marker set was established on average every 5 cM and these gave a more informative framework for genome-wide screening

for the disease susceptibility loci for chromosome 1. Further study needs to substitute 4 markers for Koreans and 3 markers for Japanese having heterozygosities lower than 0.6 for other informative markers in the corresponding loci. This would give more valuable information for genetic linkage studies for Koreans and Japanese.

Chromosome 1 includes positional candidate genes of interest such as alkaline phosphatase, heparan sulfate proteoglycan 2 and matrilin-1. These materials revealed their acts for bone formation and growth, cell growth and differentiation and tissue organization. Therefore, we supposed that chromosome 1 would play an important role in growth of the craniofacial region and would be related to hereditary malformations such as MP and cleft lip and palate. Yamaguchi et al.[12] reported that chromosome 1, 6 and 19 included some susceptible loci for MP according to a genome-wide linkage analysis using Korean and Japanese sib-pairs. This led us to choose chromosome 1 as our experimental subjective.

Our results suggest that public microsatellite markers should be verified to whether they are optimal for genetic linkage analyses of specific populations. Especially, highly similar patterns of allelic frequencies in Koreans and Japanese are suggestive of a common origin of ancestry. Therefore, the genetic information obtained here should facilitate genetic linkage studies of various hereditary diseases related to chromosome 1 in Korean and Japanese families.

CONCLUSIONS

Microsatellites are considered to be very promising genetic markers for genomic analysis. However, it has been demonstrated that there are significant ethnic differences in the genetic variations.

In the present study, we compared the allelic frequencies and heterozygosities in Korean and Japanese populations of all of the 51 microsatellite marker loci spanning the whole human chromosome 1 which is one of the susceptible loci for mandibular prognathism. Significant differences have been observed when heterozygosities and allelic frequencies of Koreans were compared with those of Caucasians, whereas these results were highly similar between Korean and Japanese populations.

Public microsatellite markers should be verified whether they are optimal for genetic linkage analyses of hereditary diseases in specific populations before being used.

Our results dictate that the same microsatellite markers could be used for genetic linkage studies of various hereditary diseases related to chromosome 1 in Korean and Japanese families.

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국문초록

한국인과 일본인에서 1번 염색체에 부착되는 microsatellite marker의 특징

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Microsatellite marker는 유전연관분석을 위한 매우 유용한 유전표지이다. 그러나 대부분의 marker들은 서양인의 정보를 이용하고 있으므로 다른 종족에서 사용할 때는 종족간에 존재할 수 있는 유전 변이의 현저한 차이를 검증해야 한다. 한국인과 일본인 집단에서 종족간 유전 변이를 조사하기 위하여, 각각 96명의 비 혈연관계의 한국인과 일본인 개체들에서 DNA를 채취하였다. 그리고 microsatellite set(ABI PRISM Linkage Mapping Set-HD5, Applied Biosystems, Foster City, CA, USA)을 이용하여 1번 인간 염색체 전 부위에 걸쳐 51개의 microsatellite marker들을 배열하고 부착된 marker들의 위치를 분석하여 대립유전자 빈도와 이형질성을 결정하였다. 그 결과, 한국인과 서양인 집단 사이에는 현저한 차이를 보였으나 한국인과 일본인 집단 사이에서는 매우 유사하였다.

본 연구의 결과는 유전 연관 연구에 앞서 일반적으로 상용되는 microsatellite marker에 관한 광범위한 검증을 반드시 시행하여야 한다는 것을 나타낸다. 또한 한국인과 일본인 집단 사이에서 유사하게 나타난 대립유전자 빈도와 이형질성은 두 민족간의 동질성이 높다는 것을 의미하므로 두 민족을 대상으로 한 1번 인간염색체와 관련된 유전 질환의 유전 연관 연구를 시행할 때 동일한 microsatellite marker의 이용 가능성을 제시하였다.

주요 단어 : 한국인, 일본인, 1번 염색체, Microsatellite marker

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