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Distribution of Two Deoxyribonuclease I Gene Polymorphisms

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Deoxyribonuclease I (DNase I) polymorphism n exon 8 (A2317G) designated as *DNASE1* is considered to be one of the susceptibility genes for gastric and colorectal carcinoma, and myocardial infarction. Moreover, the presence of a variable number of tandem repeat (VNTR) polymorphism, designated as *HumDN1*, in intron 4, was found. In this study, the simultaneous genotyping of *DNASE1* and *HumDN1* polymorphisms within the DNase I gene was performed in Ovambo, Turk, Mongolian, Korean and Japanese populations. Genotype distributions of *DNASE1* and *HumDN1* loci differed among these populations. While there was a general uniformity for the two polymorphisms in the three Asian populations, a significant difference in genotype distribution were found between the Ovambo and Turk populations. The *DNASE1*1* and *HumDN1*3* alleles were found to be the most predominant in the Ovambos, with the Turks showing the highest allele frequency for *DNASE1*2, HumDN1*4* and *HumDN1*5*. From this study, there was the existence of a certain genetic heterogeneity in the distribution of these two DNase I polymorphisms. Furthermore, linkage disequilibrium between these two polymorphisms was revealed in all of the populations except the Ovambos. The combination of the two polymorphisms within a DNase I gene may be potentially useful for clinical purposes and in population genetic studies.

Key words: DNase I, *DNASE1*, *HumDN1*, SNP, VNTR polymorphism

INTRODUCTION

Deoxyribonuclease I (DNase I, EC 3.1.21.1) polymorphism was the first effective biochemical marker for forensic individualization from small aged urine stains and used socks, when useful PCR-based DNA typing was not yet available (1,2). At least six codominant alleles have been demonstrated by isoelectric focusing (3-5), and of these six alleles, *DNASE1*1* (A2317) and *DNASE1*2* (G2317) based on single nucleotide polymorphism (SNP) (A2317G) have been shown to be common alleles. *DNASE1* polymorphism so far has been detected in various body fluids (1, 6-10), and in addition to forensic purposes, research on DNase I polymorphism has expanded into clinical applications. *DNASE1*2* has been suggested as a risk factor of liver disease, colorectal carcinoma, and gastric carcinoma in the Japanese (11-13). Moreover, recent studies have reported that the prevalence of 1-2 and 2 phenotypes is significantly more frequent in patients with myocardial infarction than in patients with other heart disease such as angina pectoris, heart failure and valvular disease (14).

Yasuda *et al*. (15) have confirmed a novel 56-bp variable number of tandem repeat (VNTR) polymorphism, designated as *HumDN1* in intron 4 of the human DNase I gene. In that study, *HumDN1* VNTR polymorphism was shown to differ between Japanese and German populations. However, the nature of *HumDN1* VNTR polymorphism in other populations is still unclear. Therefore, in the previous study (16), we developed a method for the simultaneous genotyping of SNP (A2317G) and 56-bp VNTR polymorphism within DNase I gene, and using this method, we determined the allele frequencies of both polymorphisms in Ovambo, Turk, Mongolian, Korean and Japanese populations and revealed wide differences between these ethnic groups. Furthermore, in the present study, we also evaluated the linkage disequilibrium between SNP (A2317G) and 56-bp VNTR polymorphisms.

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MATERIALS AND METHODS

Samples and DNA extraction

Blood or bloodstain samples were collected from Ovambos (Bantus) from Namibia, Turks in the Adana area (Southern Turkey), Mongolians from Ulaanbaatar, Mongolia, Koreans from Busan, Korea, and blood samples were collected from 374 healthy unrelated Japanese donors living in four prefectures (Tottori [Yonago district], Shimane [Izumo d.] (17) Ehime [Matsuyama d.], and Fukuoka [Kurume d.]) and after obtaining written informed consent from each participant. Genomic DNA was prepared from the buffy coat or cotton bloodstain using a QIAamp DNA mini kit (QIAGEN Inc., Chatsworth, CA).

Genotyping

A genotyping assay for the simultaneous detection of SNP and VNTR polymorphisms within DNase I gene was performed. Two PCR primer sets were used for simultaneous genotyping. One was for SNP analysis with a mismatched PCR method (18): 5'-ATCGTGGTTGC AGGGATGCTGCCTC-3' (sense) and 5'-AGTTCAAC AGGTGTGGGGAG-3' (antisense); the other was for VNTR polymorphism analysis: 5'-GAGCGCTACCTG TTCGTGTACAG-3' (sense) and 5'-CACCGCAGACA CCTGGTCAGGC-3' (antisense), both as previously reported (15, 18). Co-amplification in a single test tube was performed in a 25-μl reaction mixture using approximately 5 ng of DNA. The reaction mixture contained 1x buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl), 1.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM dNTPs and 1.25 U of *Taq* polymerase (AmpliTaq Gold; Applied Biosystems, Foster City, CA.). PCR was performed with a protocol consisting of an initial denaturation at 94 ℃ for 7 min, followed by 30 cycles with denaturation at 94 ℃ for 10 s, annealing at 60 ℃ for 30 s and extension at 72℃ for 1 min; followed by a final extension at 72℃ for 7 min. Following amplification with PCR, the PCR product was digested with *Xho* I to detect SNP (A2317G) by a mismatched PCR-RFLP method (15). The digests were separated in 8 % polyacrylamide gel, and the patterns on the gels were visualized by silver staining as described previously (19). Genotyping was performed by side-to-side comparisons of constructed allelic ladders.

Statistics

The 2-way R x C contingency test was used to detect population differences in *HumDN1* VNTR genotype frequency. χ^2 -analysis was performed to evaluate the Hardy-Weinberg equilibrium. Principal component analysis (PCA) based on *HumDN1* VNTR genotypes was performed to compare the five populations. A p value of less than 0.01 was considered to indicate statistical significance. These statistical analyses were executed by the program STATCEL (OMS Publishing Inc.). Linkage disequilibrium was estimated on Ehwin freely available on the website (ftp://linkage.rockefeller.edu/software/eh).

RESULTS AND DISCUSSION

Simultaneous genotyping of *DNASE1* and *HumDN1* polymorphisms was performed for five populations. Electrophoresis revealed the presence of 26 different patterns with sharp bands and without extra bands. Genotype distribution of *DNASE1* in the five populations are shown in Table 1. The gene frequencies of

Table 1. Distribution of A2317G (*DNASE1*) in human DNase I gene of five ethnic populations

Population	Group size	Number by DNase I genotype		
		1 (A/A)	1-2 (A/G)	2 (G/G)
Ovambo	104	86	17	1
Turk	108	6	33	69
Mongolian	220	60	130	30
Korean	379	124	202	53
Japanese	114	40	51	23

the *DNASE1* alleles were estimated for these five populations, and genotype distributions were found to be in Hardy-Weinberg equilibrium except for the Mongolian population. We previously have investigated genetic polymorphism of DNase I in Ovambo and Turk populations, demonstrating the existence of genetic heterogeneity in the worldwide distribution of DNase I polymorphism (20). All of the Ovambo and Turk samples in the present study are included in the previous study. Among the five populations, Ovambos showed a significantly higher frequency of the *DNASE1*1* allele ($P < 0.001$), with the Turks showing the highest allele frequency for *DNASE1*2*. Among the three Asian populations, the distribution of *DNASE1* was similar among Mongolian, Korean, and Japanese populations, with the frequency of *DNASE1*1* the highest in Korean, followed by Japanese and Mongolian populations. Yasuda *et al.* (5) previously proposed that the *DNASE1*2* allele must have been produced by point mutation in exon 8 from ancestral *DNASE1*1* allele and showed that the *DNASE1*1* allele is conserved in certain non-human (21). It is feasible that the *DNASE1*1* allele is an ancestral monomorphic gene. Interestingly, most of the Ovambos in the study population had the *DNASE1*1* allele (Table 1).

Genotype distribution of *HumDN1* in the five populations are shown in Table 2. Eleven genotypes were identified in these populations. Allele frequencies of the *HumDN1* locus have been shown to differ between populations. The Ovambo population, had the highest frequency for the *HumDN1*3* allele among the five populations. The predominant alleles in the Mongolian, Korean, and Japanese populations were first *HumDN1*3*, followed by *HumDN1*4* and then *HumDN1*5*. On the other hand, in the Turks, *HumDN1*4* and *HumDN1*5* were the most predominant alleles, and the *HumDN1*2* allele was not found. Allele 6 was found only in the Korean, possibly due to the large sample size. A previous study showed these five alleles in both Japanese and German, while the *HumDN1*2* allele was not found in German subjects, though the other four alleles were detected. There was significant difference between the Japanese and German populations (15). We found no other rare type allele in the present study, and the genotype distributions were found to be in Hardy-Weinberg equilibrium except for Turk and Korean populations. Between these three Asian populations, no significant differences were found by the R x C contingency test, indicating that there is a general uniformity for the *HumDN1* VNTR polymorphism in these Asian populations. However, significant differences in genotype distribution were revealed among the Asian, Ovambo and Turk populations (data not shown).

These findings lead us to conclude that the genetic structure of the DNase I gene differs among these five ethnic groups. Inter-ethnic differences in genetic structure were previously shown in the Ovambo and Turk populations: Takeshita *et al.* (22) revealed the existence of a specific variant in Ovambo and Turk populations of *CYP2A6* polymorphism.

Principal component analysis (PCA) was conducted using the *HumDN1* data from the five populations, as well as that from a prior study of a German (15) population (Fig. 1). The PCA plotted the six populations into four general groupings. The Turks and Germans were relatively closely positioned, as were

Table 2. Distribution of 56-bp VNTR (*HumDN1*) polymorphism in human DNase I gene of five ethnic populations

Population	Group size	Number by DNase I VNTR genotype										
		2/2	2/3	2/4	2/5	3/3	3/4	3/5	4/4	4/5	5/5	5/6
Ovambo	104	5	6	2	0	70	18	0	2	1	0	0
Turk	108	0	0	0	0	12	10	17	17	35	17	0
Mongolian	220	0	3	0	1	58	53	36	24	35	10	0
Korean	379	1	5	2	0	118	99	83	21	34	15	1
Japanese	114	3	0	0	0	46	22	19	8	10	6	0

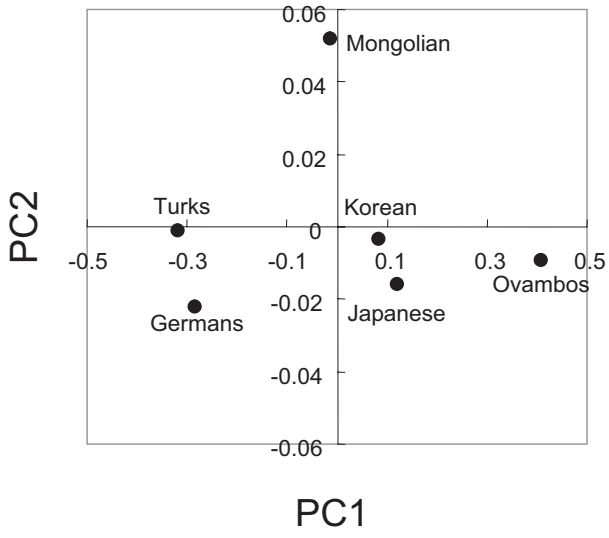

Fig. 1. Two-dimensional principal component analysis based on *HumDN1* VNTR genotypes in Ovambo, Turk, Mongolian, Korean, Japanese, and German [15] populations. Distribution of populations was plotted against the PC1 and PC2.

the Koreans and Japaneses. Surprisingly, the Mongolians fell into a somewhat isolated position relative to among the Asian populations. Mongolia is located in central Asia and consists of more than 20 tribes and ethnic groups, some of which are related to neighboring Turk populations. However, Mongolians have shown to be distant from Turks insofar as HLA-typing (23). We have previously shown that the mutant frequency of the *CYP1A2*-163A* allele in Mongolians is the lowest among 14 populations (24). These latter two studies, together with our present data, lead us to surmise that Mongolians may have a very specific genetic structure.

The association between the *DNASE1* and *HumDN1* polymorphism genotype was investigated.· χ^2- analysis revealed strong linkage disequilibrium ($P < 0.001$) between these polymorphisms in all the populations except the Ovambos ($P > 0.1$). The χ^2 values were 7.86, 44.5, 66.0, 150, and 76.2, in the Ovambos, Turks, Mongolians, Koreans and Japanese, respectively. Table 3 shows the association between *DNASE 1* and *HumDN1* polymorphisms of all subjects (n = 925) in the five populations. The *HumDN1*3* allele was significantly associated with the *DNASE1*1* allele, and both *HumDN1*4* and *HumDN1*5* were associated with *DNASE1*2* in all five populations.

DNASE1 and *HumDN1* polymorphisms distribution has up to now been surveyed in only two populations; this study is the first to demonstrate the existence of genetic heterogeneity in a wide distribution of two DNase I polymorphisms using the simultaneous genotyping method. In Japanese populations, it has been reported that *DNASE1*2* is related to liver disease, gastric carcinoma, colorectal carcinoma and acute myocardial infarction (12-14, 25). Clinically, DNase I activity in serum can be used as a novel diagnostic marker for the early detection of acute myocardial infarction and transient myocardial ischemia (26). Furthermore, recent studies have demonstrated that *DNASE1*2* was more comment in systemic lupus erythematosus patients when compared to control groups (27, 28).

In conclusion, this study has developed the simultaneous genotyping of *DNASE1* and *HumDN1* and established a population database from five ethnic groups for these two loci. This database may serve to elucidate the involvement of *DNASE1* and *HumDN1* polymorphisms in diseases.

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Table 3. Association between A2317G (*DNASE1*) and 56-bp VNTR (*HumDN1*) polymorphisms in all examined populations

		HumDN1										
		2/2	2/3	2/4	2/5	3/3	3/4	3/5	4/4	4/5	5/5	5/6
DNASE1												
1 (A/A)	(n = 316)	9 (0.029)	11(0.035)	2 (0.006)	0 (0)	236 (0.747)	34 (0.108)	7 (0.022)	10 (0.032)	4 (0.012)	2 (0.006)	1 (0.003)
1-2 (A/G)	(n = 433)	0 (0)	2 (0.005)	2 (0.005)	1 (0.002)	56 (0.129)	160 (0.370)	134 (0.309)	28 (0.065)	40 (0.092)	10 (0.023)	0 (0)
2 (G/G)	(n = 176)	0 (0)	1 (0.006)	0 (0)	0 (0)	12 (0.068)	8 (0.045)	14 (0.080)	34 (0.193)	71 (0.403)	36 (0.205)	0 (0)

Numbers shown in parentheses are frequency.

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