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DNase II Polymorphisms (2630T>C (Ser145Ser) and 2901G>A (Val206Ile)) in Japanese From Shimane Prefecture, Koreans, and Three Africans

Kaori KIMURA-KATAOKA[1], Junko FUJIHARA[1], Toshihiro YASUDA[2], Hisakazu TAKATSUKA[1], Koji TAKAYAMA[1], Tomonori MURO[3] and Haruo TAKESHITA[1]

[1]*Department of Legal Medicine, Shimane University Faculty of Medicine, Izumo 693-8501, Japan*

[2]*Division of Medical Genetics and Biochemistry, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan*

[3]*Criminal Investigation Laboratory, Shimane Prefectural Police Headquarters, Matsue 690-8510, Japan*

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Recently, SNPs and haplotypes in the DNase II gene were shown to be associated with renal disorder in SLE patients. In this study, *DNASE2*Ser145Ser* and *DNASE2*Val206Ile* polymorphisms were determined by using the PCR-RFLP technique in healthy subjects of Japanese, Koreans, Ovambos, Ghanaians, and Xhosans (n=871). For *DNASE2*Ser145Ser*, the Japanese and Korean populations exhibited higher 2630T frequencies than the Ovambos, Ghananians, and Xhosans, whose allele frequencies were higher for 2630C. Population data from the HapMap Project showed similar allele frequencies. For *DNASE2* Val206Ile*, Koreans were the only population with the *DNASE2*Val206Ile* polymorphism. A previous study revealed an association with the risk of renal disorders in Korean SLE patients and the *DNASE2* Ser145Ser* polymorphism. In this study, we verified significant ethnic diversity in the *DNASE2*Ser145Ser* polymorphisms. It is questionable whether a correlation between the risk of renal disorder in SLE patients and the *DNASE2*Ser145Ser* polymorphism exists in populations other than the Korean population.

Key words: African, DNase II, Korea, polymorphism, Shimane, SLE

INTRODUCTION

Deoxyribonuclease II (DNase II, EC 3.1.22.1) is one of two distinct types of DNase present in mammalian tissues and body fluids (1-3). It hydrolyzes double-stranded DNA to 3'-phosphoryl oligonucleotides under acidic conditions in the absence of metal ions. It has been suggested that DNase II may be responsible for the internucleosomal DNA degradation that is characteristic of apoptosis (4). Shin et al. hypothesized that the insufficient DNA degradation caused by the disruption of DNase II expression may lead to some form of autoimmune dysfunction, particularly systemic lupus erythematosus (SLE) (5). SLE is characterized by the defection of phagocytosis, and clearing of immune complexes and of apoptotic cells. Moreover, Shin et al. demonstrated that SNPs (-1066G>C, +2630T>C (Ser145Ser), and +6235G>C) and haplotypes in the DNase II gene are associated with renal disorders in Korean patients with SLE (5). They first reported associations between the DNase II polymorphisms and disease. Principally, the non-synonymous substitution, which results in an amino acid replacement, is more likely to produce functional alterations of the protein than the synonymous substitution. However, in the study by Shin et al., the synonymous substitution without the amino acid replacement (2630T>C (Ser145Ser)) exhibits an association with renal disorders with SLE, while the non-synonymous substitution (2901G>A (Val206Ile)) does not. In order to clarify this discrepancy, we investigated 2630T>C (Ser145Ser) and 2901G>A (Val206Ile) as a preliminary step. Since Shin et al. surveyed only Koreans, we analyzed Japanese subjects from Shimane Prefecture, Koreans, and three Africans and compared our results with those from previous studies.

Correspondence: H. Takeshita, Department of Legal Medicine, Shimane University Faculty of Medicine, Izumo, 693-8501, Japan
Tel: +81-853-20-2156
Fax: +81-853-20-2155
E-mail: htakeshi@med.shimane-u.ac.jp

MATERIALS AND METHODS

Biological samples

Blood or bloodstain samples were collected from 89 Japanese (Shimane Prefecture), 408 Koreans (Busan of South Korea), 91 Ghanaians (Ghana), 188 Ovambos (Bantusin Namibia), and 95 Xhosas (Cape Town of South Africa). Informed consent was obtained from each participant. Genomic DNA was prepared from the buffy coat or cotton bloodstain using a QIAamp DNA mini kit (QIAGEN Inc., Chatsworth, CA).

DNase II genotyping method

*DNASE2*Ser145Ser* and *DNASE2*Val206Ile* polymorphisms were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The primer sets for the detection of *DNASE2*Ser145Ser* and *DNASE2*Val206Ile* based upon the nucleotide sequence of the human DNase II gene are shown in Table 1. Genomic DNA samples (1μL) were added to PCR mixtures (9 μL) consisting of nuclease-free water, 5μM of each primer, and GoTaq® Green Master Mix (Promega Corp., Madison, WI). After initial denaturation at 94 ℃ for 5 min, amplification was performed by denaturation at 94 ℃ for 1 min, annealing at 61 ℃ for *DNASE2*Ser145Ser* and 56 ℃ for *DNASE2*Val206Ile* (Table 1) for 1 min, and extension at 72 ℃ for 1 min for 30 cycles, followed by a final extension at 72 ℃ for 5 min. After amplification with PCR, 10 μL PCR products were digested with restriction enzymes (Table 1) in a 20 μL reaction volume at 37 ℃ for 5 hours. The digests (10 μL) were separated in an 8% polyacrylamide gel in a TBE buffer at a constant voltage (300 V). The patterns on the gels were visualized by silver staining as described previously (6). Genotyping was performed by side-to-side comparisons of constructed allelic ladders.

RESULTS AND DISCUSSION

In the present study, we analyzed two DNase II polymorphisms from synonymous and non-synonymous substitutions in Japanese (Shimane Prefecture), Korean, and three African populations based upon the previous study by Shin et al. (5). The digestion products for *DNASE2*Ser145Ser* and *DNASE2*Val206Ile* revealed the presence of three different patterns illustrated in Table 1.

The previous study (5) revealing weak associations between *DNASE2*Ser145Ser* and the risk of renal disorder in SLE patients was the first study reporting any relationship between DNase II polymorphisms and disease. The result of *DNASE2*Ser145Ser* analysis in our study of Japanese, Korean, Ovambo, Ghanaian, and Xhosa DNAs is shown in Table 2. The Japanese and Korean populations exhibited higher 2630T frequencies than the Ovambo, Ghananian, and Xhosa populations, whose allele frequencies were higher for 2630C. Population data from the HapMap Project showed similar allele frequencies. The allele frequency of the Japanese from Shimane Prefecture (0.72) was consistent with those from Tokyo Prefecture (0.74). The data from our present study and from Shin et al. for Koreans (2630T frequency of 0.65 and 0.64, respectively) resembled the HapMap Project data for the Chinese (2630T frequency of 0.66). The HapMap Project showed similar allele frequencies between Africans and Europeans. Geographically, Nigeria and Ghana are neighboring countries in central Africa, while Namibia and South Africa are located in south Africa. Although the Nigerian allele frequency was expected to be similar to that of the Ghanaian and the Ovambo (Namibia) allele frequency was expected to resemble that of the Xhosa (South Africa) according to the HapMap Project, the Nigerian 2630C allele frequency of 0.74 corresponded only to that of the

Table 1. Primer sequence, annealing temperatures, and restriction enzymes for PCR-based genotyping

Gene	Primer	Sense/ Antisense	Sequence	Temp (℃)	Restriction enzyme	Product size (bp)
2630 (T to C)	2630-F	Sense	5'-CAAGGGGAGGAGGAAATGCAA-3'	61	*Pvu II*	TT: 213
	2630-R	Antisense	3'-GCTGGTCCACAGTGTACCTAA-5'			TC: 213, 159, 54
						CC: 159, 54
2901 (G to A)	2901-F	Sense	5'-GACTTGGAGAATGTGGTCAACGGC-3'	56	*HpyCH4IV*	AA: 123
	2901-R	Antisense	3'-TTCAAGTCGTTAAACCTCTA-5'			AG: 123, 94, 29
						GG: 94, 29

Table 2. Genotype distributions of 2 SNPs (2630T>C(Ser145Ser)and 2901G>A(Val206Ile)) of the *DNASE2* gene

	Population						HapMap			
	Japanese	Korean	Korean*	Ovambos	Ghanaian	Xhosan	Japanese[a]	Chinese[b]	African[c]	European[d]
					+2630T>C (*Ser145Ser*)					
Total populations, *n*	89	408	339	188	91	95	90	90	120	120
Genotype, *n* (%)										
T/T	44(49.5)	169(41.4)	138(40.7)	16 (8.5)	14 (15.4)	9 (9.5)	48(53.4)	44 (48.9)	8 (6.7)	8 (6.7)
T/C	40(45.9)	192(47.1)	161(47.5)	72(38.3)	36 (39.5)	33 (34.7)	38(42.2)	90 (33.3)	46(38.3)	40(33.3)
C/C	5 (5.6)	47(11.5)	40(11.8)	100(53.2)	41 (45.1)	53 (55.8)	4 (4.4)	16 (17.8)	66(55.0)	72(60.0)
Allele frequency										
T	0.72	0.65	0.64	0.28	0.35	0.27	0.74	0.66	0.26	0.23
C	0.28	0.35	0.36	0.72	0.65	0.73	0.26	0.34	0.74	0.77
					+2901G>A (*Val206Ile*)					
Total populations, *n*	89	96	340	188	91	95	-	-	-	-
Genotype, *n* (%)										
A/A	0 (0.0)	0 (0.0)	1 (0.3)	0 (0.0)	0(0.0)	0(0.0)	-	-	-	-
A/G	0 (0.0)	2 (2.1)	12 (3.5)	0 (0.0)	0(0.0)	0(0.0)	-	-	-	-
G/G	89(100.0)	94(97.9)	327(96.2)	188 (0.0)	91(0.0)	95(0.0)	-	-	-	-
Allele frequency										
A	0.00	0.01	0.02	0.00	0.00	0.00	-	-	-	-
G	1.00	0.99	0.98	1.00	1.00	1.00	-	-	-	-

[a-d] Population data from the HapMap Project: Japanese subjects are residents in Tokyo; Chinese subjects are Han Chinese residents in Beijing; African subjects are Yoruba in Ibadan, Nigeria; European subjects are Utah residents with ancestry from northern and western Europe.
* Data obtained by H.D.Shin, et al. (5)

Ovambo (0.72) and Xhosa (0.73). The Ghanaian 2630C frequency was determined to be 0.65, which was inconsistent with the neighboring Nigerian allele frequency. This discrepancy between the expected and the actual allele frequencies of populations in Ghana and other African countries was possibly the result of an insufficient number of DNA samples.

From a previous study (5), the allele frequency of 2901A of *DNASE2*Val206Ile* in Koreans was determined to be 0.02 (Table 2). This allele frequency was significantly lower than the allele frequencies of *DNASE2*Ser145Ser*. To verify this data, we examined the allele frequencies of *DNASE2*Val206Ile* in the same population studied for *DNASE2*Ser145Ser*. The allele frequency of 2901A in our study of the Korean population was determined to be 0.01, which corresponded to that of the previous study (5). Interestingly, other populations we analyzed did not exhibit any polymorphisms, and likewise, any existing *DNASE2*Val206Ile* polymorphisms have not been reported in the HapMap Project. In the present study, we showed that the Koreans were the only population with the *DNASE2*Val206Ile* polymorphism, although other populations that we have not yet analyzed may have it.

Shin et al. revealed an association with the risk of renal disorder in Korean SLE patients and the *DNASE2*Ser145Ser* polymorphism, while no association with *DNASE2*Val206Ile* was detected (5). In this study, we confirmed the presence of the *DNASE2*Ser145Ser* polymorphisms in other populations. Because significant ethnic diversity in the *DNASE2*Ser145Ser* polymorphisms is present, it is questionable whether the correlation between the risk of renal disorder in SLE patients and the *DNASE2*Ser145Ser* polymorphism exists in populations other than the Korean population. Further study is required to examine the association between SLE and *DNASE2*Ser145Ser*, *DNASE2*Val206Ile*, and other SNPs within the DNase II gene.

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