Shimane J. Med. Sci., Vol.35 pp.37-42, 2019

Lack of Association of a Single Nucleotide Polymorphism (rs1053874) in the DNase I Gene With Plural Tissue Weight

Yoshikazu TAKINAMI[1)], Junko FUJIHARA[2)], Kaori KIMURA-KATAOKA[2)], Ken INOUE[3)], Yoshihiko KOMINATO[4)], Kazuo YAMADA[2)], Haruo TAKESHITA[2)]

[1)]*Department of Emergency and Critical Care Medicine, Shimane University Faculty of Medicine, Izumo, 693-8501, Japan*

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

[3)]*Health Service Center, Kochi University and Kochi Medical School, Kochi, 780-8520, Japan*

[4)]*Department of Legal Medicine, Gunma University Graduate School of Medicine, Maebashi, 371-8511, Japan*

(Received September 11, 2018; Accepted October 31, 2018)

Financial support: This work was supported by JSPS KAKENHI Grant Number 18H03065 (Grants-in-Aid for Scientific Research) to H.T.

Conflict of interest: The authors declare no competing financial interests.

Background: We have found the association of single nucleotide polymorphism (SNP) rs6180 in the growth hormone receptor (GHR) gene with plural tissue weight. On the other hand, the DNase I gene (DNASE1) is considered to be one of the susceptibility genes for liver disease, gastric and colorectal carcinoma, and myocardial infarction, based on the polymorphic study of rs1053874. Therefore, we investigated the association of rs1053874 in DNASE1 with plural tissue weight in the present study.

Materials and methods: Blood samples (n = 198; 99 female and 99 male) were collected from Japanese subjects autopsied in Shimane Prefecture, and genomic DNA was extracted. SNP (rs1053874, A > G substitution) was analyzed using a polymerase chain reaction, followed by a mismatched-restriction fragment length polymorphism analysis.

Results: Except for the pancreatic weight and appendix, all parameters were significantly related to gender. All parameters were significantly related to age except for the left and right renal weight. In the present study, we focused on analyzing the potential effect of a variation in DNASE1 and investigated the effect of SNP rs1053874 on human organ weight using autopsied samples.

Conclusions: In the present study, the rs1053874 polymorphism does not contribute to organ weight, cardiac hypertrophy index, or body surface areas, and these associations were not observed in Japanese subjects. This study is the first to investigate the association of SNP rs1053874 (A > G substitution) in DNASE1 and data routinely measured at autopsy, such as organ weight.

Key words: deoxyribonuclease I, tissue weight, single nucleotide polymorphism, rs1053874

INTRODUCTION

Deoxyribonuclease I (DNase I, EC 3.1.21.1) is an endonuclease that preferentially attacks double-stranded DNA in a Ca^{2+}-dependent manner to produce oligonucleotides with 50-phospho and 30-hydroxy termini [1].

DNase I has been postulated to be one of the candidate nucleases responsible for internucleosomal DNA degradation during apoptosis [2]. Previously, we demonstrated that human DNASE1 is genetically polymorphic and controlled by six codominant alleles at chromosome 16p13.3 [3]. Three common phenotypes, 1 and 2 (homozygotes) and 1-2 (heterozygote), determined by two common alleles, *DN-*

Corresponding author: H. Takeshita, MD, Ph.D
Department of Legal Medicine, Shimane University Faculty of Medicine, 89-1 Enya-cho, zumo, Shimane 693-8501, Japan
Tel: +81-853-20-2156
Fax: +81-853-20-2155
E-mail: htakeshi@med.shimane-u.ac.jp

*ASE1*1* and **2*, have been found in Japanese and Caucasian populations, and the molecular difference between *DNASE1*1* and **2* is due to an A-G transition occurring at position 2317 in exon 8, regarded as a single nucleotide polymorphism (SNP) of A2317G, resulting a Gln-Arg substitution at amino acid position 222 of the mature enzyme (Gln222Arg polymorphism) [4]. It is shown as rs1053874 in the NCBI data base. Thus, human DNase I exhibits polymorphism at both the protein and DNA levels. Moreover, 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 [5].

Furthermore, *DNASE1* is considered to be one of the susceptibility genes for liver disease [6], gastric and colorectal carcinoma [7], and myocardial infarction [5] from the polymorphic study in the rs1053874. Over the last decade, since the discovery of the utility of its genetic polymorphism for forensic purposes, research on DNase I has expanded into clinical applications [8]. However, the essence of DNase I relevance to disease has not been clarified.

On the other hand, information on externally visible traits such as gender, eye and hair color, and height provided by DNA-based investigations would be valuable for forensic personal identification [9]. We have investigated the association of various SNPs with human height [10, 11]. The specimens used in our previous study were obtained at autopsy, and data other than height, such as organ weight, can be applied for association study. In the previous study, we also reported on our investigation into the association of various SNPs with human organ weight, and we have found an association of SNP rs6180 in the growth hormone receptor (GHR) gene with plural tissue weight [12]. In the present study, we evaluated the rs1053874 polymorphism of human *DNASE1* with the correlation of each phenotype and organ weight in our autopsy samples to investigate the essence of *DNASE1* as a disease-susceptive gene.

.

MATERIALS AND METHODS

Study population

Blood samples (n = 198; 99 female and 99 male) were collected from Japanese subjects autopsied in Shimane Prefecture. The height, weight, tissue weight, and cardiac parameters in the autopsy characteristics from the study are shown according to gender in Table 1. As shown in Table 1, gender differences were observed in most parameters; independent statistical analysis was performed in females and males. Genomic DNA was extracted from blood samples collected at autopsy using a QIAamp DNA Blood Mini Kit (Qiagen N.V., Venlo, Netherlands). The study, including the use of genomic DNA derived from autopsy cases, was reviewed and approved by the Human Ethics Committee of Shimane University School of Medicine (No. 1678).

SNP typing

In this study, one SNP (rs105374, A > G substitution) was analyzed by polymerase chain reaction (PCR), followed by restriction fragment length polymorphism (RFLP) analysis with a slight modification of Yasuda *et al.* [4]. Since the substitution sites corresponding to this SNP neither suppressed nor created any known restriction enzyme recognition sites, we used a mismatched PCR-amplification method for genotyping. The incorporation of a deliberate mismatch near the 3'-terminus of a PCR primer allowed the creation of each enzyme recognition site. Primers for the specific amplification of the DNA fragments encompassing a substitution site corresponding to the SNPs were newly designed on the basis of the nucleotide sequences of rs1053874-F (5'- GGCAGGTCCCA GGGCTCTTAGTTT-3') and rs1053874-R (5'- GAGTCGGGAACAACG-GCGACT-3')". The underlined residue indicates the mismatched nucleotide that creates a *Hinf* cutting site when the substitution site is G. Amplification was performed in a 25-μl reaction mixture using approximately two ng of DNA. The reaction mixture contained a buffer (15 mM Tris-HCl, pH 8.0, 50 mM KCl), 1.5 mM MgCl2, 0.5 μM of each primer, 200 μM of the dNTPs, and 1.25 U of Taq polymerase (AmpliTaq Gold; Applied Biosystems, Foster City, CA, USA). PCR was performed with a protocol consisting of initial denaturation at 94ºC for three min followed by 30 cycles with denaturation at 96ºC for one min, annealing at 50ºC for

Table 1. Comparison of data among different genotypes of rs1053874 in autopsied subjects

				multiple regression		
	AA (n = 81)	AG (n = 91)	GG (n = 26)	gender	age	SNP
Height (cm)	163(131-181)	158 (133-175)	162 (147-174)	$p < 0.01$	$p < 0.01$	0.111
Weight (kg)	52 (26-95)	54 (27-100)	58 (40-76)	$p < 0.01$	$p < 0.01$	0.592
Cardiac weight (g)	350 (150-715)	355 (210-850)	372 (270-350)	$p < 0.01$	$p < 0.01$	0.348
Left ventricular weight (g)	177 (66-350)	165 (89-414)	182 (273-112)	$p < 0.01$	$p < 0.01$	0.509
Cardiac hypertrophy index	2.18 (0.95-3.97)	2.31 (1.34-4.86)	2.25 (1.67-3.40)	$p < 0.05$	$p < 0.01$	0.177
BSA (m^2)	1.53 (1.05-2.07)	1.55 (1.09-2.11)	1.59 (1.38-1.83)	$p < 0.01$	$p < 0.01$	0.259
Left ventricular mass index	110 (49-175)	107 (62-201)	111(73-158)	$p < 0.01$	$p < 0.01$	0.259
Left pulmonary weight (g)	430 (185-815)	404 (145-862)	428 (275-685)	$p < 0.01$	$p < 0.01$	0.307
Right pulmonary weight (g)	505 (220-930)	495 (178-905)	492 (290-905)	$p < 0.01$	$p < 0.01$	0.638
Hepatic weight (g)	1205 (510-2600)	1192 (210-2405)	1443 (935-3070)	$p < 0.01$	$p < 0.01$	0.670
Pancreatic weight (g)	72 (15-265)	70 (10-395)	84 (20-265)	0.102	$p < 0.01$	0.998
Left renal weight (g)	140 (65-390)	140(68-680)	158 (105-215)	$p < 0.01$	0.7988	0.998
Right renal weight (g)	130 (25-258)	126 (60-310)	143 (90-240)	$p < 0.01$	0.3895	0.650
Cerebral weight (g)	1300 (1070-1700)	1305 (215-1680)	1358 (1075-1700)	$p < 0.01$	$p < 0.01$	0.739
Appendix (cm)	7.8 (2-16)	8.6 (4-13.5)	9 (6-11.5)	0.6994	$p < 0.01$	0.197

The values were presented as median. (minimum - maximam)
P values different genotypes by univariate regression analysis.

Table 2. Allele frequencies of rs1053874 polymorphism in healthy global populations

population	*n*	A	G	References
Japanese	198	0.639	0.361	This study
Asian (HCB)	90	0.433	0.567	HapMap database
Asian (JPT)	90	0.567	0.433	HapMap database
European	120	0.333	0.667	HapMap database
Sub-Saharan African	120	0.850	0.150	HapMap database

one min, and extension at 72ºC for two min, followed by a final extension at 72ºC for five min. To determine the genotype of each SNP, two μl of the PCR product obtained using each pair of primers was digested with the *Hinf* enzyme（New England Biolabs, Ipswich, MA, USA）at 37ºC for two h in a final reaction mixture volume of 15 μl in accordance with the manufacturer's instructions.

The amplified product from the A allele is resistant to *Hinf* digestion, whereas the G allele was completely digested by *Hinf* to yield 125-bp and 29-bp fragments. The digests were separated in 8% polyacrylamide gel. Nucleotide sequences of the representative subjects were confirmed by direct sequencing of the PCR products in which a substitution site corresponding to each SNP was included; the dideoxy chain-termination method with the BigDye® Terminator Cycle Sequencing Kit was

employed using the 310 Genetic Analyzer (Applied Biosystems) in accordance with the manufacturer's instructions.

Statistical analysis

Multiple regression analysis was used to compare adult height, weight, organ weight, and cardiac parameters among different genotypes. These statistics were collected using the STATCEL2 program (OMS Publishing, Inc., Tokyo, Japan). Differences of $P < 0.05$ were considered to be statistically significant.

RESULTS

The height, weight, organ weight, and cardiac parameters in the autopsy characteristics from the study are shown in Table 1. In the allele and genotype distributions, the frequencies of the C and A alleles of the rs1053874 polymorphism were 63.9% and 36.1%, respectively. Genotype frequencies of the two polymorphisms were in good agreement with the Hardy–Weinberg equilibrium at the loci. Allele frequencies from the present study are similar to those from the NCBI database (Table 2).

Multivariate regression analysis including age, gender, post-mortem intervals, and rs1053874 polymorphism was performed. Except for the pancreatic weight and appendix, all parameters were significantly related to gender. All parameters were significantly related to age except for the left and right renal weight. In the present study, we focused on analyzing the potential effect of a variation in the DNase I gene and investigated the effect of SNP rs1053874 on human organ weight using 198 (99 female and 99 male) autopsied samples. As shown in Table 1, rs1053874 was not related to height (cm), weight (kg), cardiac weight (g), left ventricular weight (g), cardiac hypertrophy index, body surface area (m2), left ventricular mass index, left pulmonary weight (g), right pulmonary weight (g), hepatic weight (g), pancreatic weight (g), left renal weight (g), right renal weight (g), cerebral weight (g), or the appendix (cm).

DISCUSSION

The rs1053874 is the only polymorphic SNP in the *DNASE1*with amino acid substitutions and is considered to be related to DNase I function. In rs1053874, the enzyme activity of phenotype AA is significantly higher than that of GG [13], and the Gln222Arg (A2317G) polymorphism in *DNASE1* exhibits ethnic and functional differences [14]. We have already demonstrated a significant association of deoxyribonuclease I polymorphism with liver disease and gastric carcinoma. The rs1053874 polymorphism of the human *DNASE1* is considered to be one of the susceptibility genes for liver disease [6], gastric and colorectal carcinoma [7], and myocardial infarction [5]. For the above reason, we evaluated this polymorphism of the human *DNASE1* with the correlation of each phenotype and organ weight in our autopsy samples to investigate the essence of *DNASE1* as a disease-susceptive gene. Because this rs1053874 polymorphism did not contribute, the correlation between the difference in some kind of disease and phenotype is assumed to be due to other mechanisms. This study is the first to investigate the association of an SNP rs1053874 (A > G substitution) in *DNASE1* and data routinely measured at autopsy, such as organ weight. In the previous study, we also reported on our investigation into the association of various SNPs with human organ weight, and we have found an association of SNP rs6180 in the growth hormone receptor (GHR) gene with plural tissue weight [12]. Moreover, we have shown that an SNP in the *HMGA2* gene is associated with human height [10] and that SNPs in the *LIM homeobox 3-quiescin Q6 sulfhydryl oxidase 2* (*LHX3-QSOX2*) gene and the *IGF1* gene are not related to human height, indicating that the height-related gene differs among populations [11]. Therefore, we have found an association of SNP rs6180 in the GHR gene with cardiac weight, left ventricular weight, cardiac hypertrophy index, BSA, right pulmonary weight, left renal weight, and right renal weight [12]. In the present study, the rs1053874 polymorphism does not contribute in organ weight, cardiac hypertrophy index, and body surface areas, and these associations were not observed in Japanese (Table 1). The difference between no association of *DNASE1 gene* and association of *HMGA2, LHX3-QSOX2* and the *IGF1* genes are is currently under consideration.

On the other hands, there are ethnic differences in this polymorphism, and it would be valid to evaluate this rs1053874 polymorphism in Caucasian and/or African people. On the other hand, there are many other single nucleotide polymorphisms in *DNASE1* [15]. Moreover, there is another genetic variation in a 56-bp variable number of tandem repeat (VNTR) polymorphisms within the human *DNASE1* gene [16]. In the future, we will evaluate above SNPs and/or VNTR polymorphism of the human *DNASE1* with the correlation of each phenotype and organ weight in our autopsy samples. Further accumulative data are needed to clarify the association.

CONCLUSIONS

The rs1053874 polymorphism does not contribute in organ weight, cardiac hypertrophy index, and body surface areas, and these associations were not observed in Japanese.

REFERENCES

1) Yasuda T, Awazu S, Sato W, Iida R, Tanaka Y, Kishi K. Human genetically polymorphic deoxyribonuclease: purification, characterization, and multiplicity of urine deoxyribonuclease I. *J Biochem* (Tokyo) 1990;108:393-8.

2) Rauch F, Polzar B, Stephan H, Zanotti S, Paddenberg R, Mannherz HG. Androgen ablation leads to an upregulation and intranuclear accumulation of deoxyribonuclease I in rat prostate epithelial cells paralleling their apoptic elimination. J *Cell Biol* 1997;137:909-23.

3) Kishi K, Yasuda T, Ikehara Y, Sawazaki K, Sato W, Iida R. Serum deoxyribonuclease I (DNase I) polymorphism: pattern similarities among isozymes from serum, urine, kidney, liver, and pancreas. *Am J Hum Genet* 1990;47:121-6.

4) Yasuda T, Kishi K, Yanagawa Y, Yoshida A. Structure of the human deoxyribonuclease I (DNase I) gene: identification of the nucleotide substitution that generates its classical genetic polymorphism. *Ann HumGenet* 1995;59:1-15.

5) Arakawa K, Kawai Y, Kumamoto T, *et al*. Serum deoxyribonuclease I activity can be used as a sensitive marker for detection of transient myocardial ischaemia induced by percutaneous coronary intervention. *Eur Heart J* 2005;26;2375-80.

6) Yasuda T, Nadano D, Tenjo E, Takeshita H, Nakanaga M, Kishi K. Survey of the association of deoxyribonuclease I polymorphism with disease. *Hum Hered* 1993;43:205-8.

7) Tsutsumi S, Takeshita H, Yasuda T, Kuwano H, Kishi K. Association of DNase I phenotype 2 with colorectal carcinoma risk in Japanese populations. *Cancer Lett* 2000;159:109-12.

8) Yasuda T, Kawai Y, Ueki M, Kishi K. Clinical applications of DNase I, a genetic marker already used for forensic identification. *Leg Med* (Tokyo) 2005;7:274-7.

9) Kayser M, Schneider PM. DNA-based prediction of human externally visible characteristics in forensics:motivations, scientific challenges, and ethical considerations. *Forensic Sci Int Genet* 2009;3:154-61.

10) Takeshita H, Fujihara J, Soejima M, *et al*. Confirmation that SNPs in the high mobility group-A2 gene (HMGA2) are associated with adult height in the Japanese population; wide-ranging population survey of height-related SNPs in HMGA2. *Electrophoresis* 2011;32:1844-51.

11) Fujihara J, Takeshita H, Kimura-Kataoka K, *et al*. Replication study of the association of SNPs in the LHX3-QSOX2 and IGF1 loci with adult height in the Japanese population; wide-ranging comparison of each SNP genotype distribution. *Leg Med* (Tokyo) 2012;14;205-8.

12) Fujihara J, Kimura-Kataoka K, Yasuda T, Sano R, Kominato Y, Takeshita H. Association of a single nucleotide polymorphism (rs6180) in GHR gene with plural tissue weight. *J Genet* 2016;95:189-92.

13) Fujihara J, Takatsuka H, Kataoka K, Xue Y, Takeshita H. Two deoxyribonuclease I gene polymorphisms and correlation between genotype and its activity in Japanese population. *Leg Med* (Tokyo) 2007;9:233-6.

14) Takeshita H, Soejima M, Koda Y, Yasuda T, Takatsuka H, Fujihara J. Gln222Arg (A2317G) polymorphism in the deoxyribonuclease I gene exhibits ethnic and functional differences. *Clin Chem Lab Med* 2009;47:51-5.

15) Kimura-Kataoka K, Ueki M, Takeshita H, *et*

al. Identification of the functional alleles of the nonsynonymous single-nucleotide polymorphisms potentially implicated in systemic lupus erythematosus in the human deoxyribonuclease I gene. *DNA Cell Biol* 2014;33:492-502.

16) Fujihara J, Yasuda T, Iida R, *et al*. Global analysis of genetic variations in a 56-bp variable number of tandem repeat polymorphisms within the human deoxyribonuclease I gene. *Leg Med* (Tokyo) 2015;17:283-6.