Original Article

A Promoter Polymorphism of Lamin A/C Gene is an Independent Genetic Predisposition to Arterial Stiffness in a Japanese General Population (The Tanno and Sobetsu Study)

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Aim: We examined the hypothesis that there is a positive, independent association between polymorphisms of lamin A/C gene (*LMNA*) and arterial stiffness in Japanese.

Methods: The subjects were 261 men (mean age, 64.4 ± 0.7 years) selected from inhabitants of the towns of Tanno and Sobetsu in a rural area of Japan who underwent medical check-ups. We conducted clinical examinations, including measurement of bilateral brachial-ankle pulse wave velocity (baPWV) as a marker of arterial stiffness, and genetic analysis. Subjects with atrial fibrillation, subjects with ankle-brachial index < 0.9 , and subjects taking any medication were excluded. We selected two single nucleotide polymorphisms (SNPs) as markers of *LMNA*, 1908C/T in exon 10 and -1030C/T in the promoter region, which we have recently identified. All genotypes were clearly determined by the TaqMan PCR method.

Results: Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT + TT genotype ($1,652 \pm 22.1$ cm/s vs. $1,552 \pm 43.0$ cm/s, $p = 0.039$); however, no significant difference was found for 1908C/T polymorphism. The baPWV was found to be significantly associated with age, body height, systolic blood pressure, and smoking habit; therefore, we next performed multiple regression analysis including these parameters, and found an independent, significant association between baPWV and -1030C/T polymorphism.

Conclusion: Promoter -1030C/T polymorphism of *LMNA* is a possible genetic predisposition to arterial stiffness in the Japanese population.

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Key words; Arterial stiffness, Nuclear lamina, Genetics, Single nucleotide polymorphism (SNP)

Introduction

Hutchinson-Gilford progeria syndrome (HGPS; Online Mendelian Inheritance in Man #176670) is a rare sporadic disorder with premature aging, and

patients with this syndrome are likely to have coronary artery disease, stroke, or other cardiovascular diseases¹⁻³. HGPS induces severe systemic arterial stiffness, which leads to fatal myocardial infarction or stroke before an average age of 13 years. Approximately 80% of HGPS cases are caused by a single base change of C to T in position 1824 on exon 11 of a gene encoding nuclear lamins A and C^{4,5}. Lamins are structural protein components of nuclear lamina, a protein network underlying the inner nuclear membrane that determines nuclear shape and size, and

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constitute a class of intermediate filaments. The gene encoding lamins A and C is named lamin A/C gene (*LMNA*; Gene ID 4000), and it spans approximately 24 kb and contains 12 exons on chromosome 1q21. Alternative splicing within exon 10 of *LMNA* gives rise to 2 different mRNAs that code for prelamin A and lamin C.

HGPS is associated with premature arterial stiffness, and it is therefore thought that *LMNA* is involved in the pathophysiology and genesis of arterial stiffness that occurs concurrently with the accelerated aging process. It has been reported that *LMNA* has several single nucleotide polymorphisms (SNPs). The 1908C/T polymorphism (rs #4641), one of the SNPs on *LMNA*, in exon 10 is associated with a risk for developing metabolic traits, including insulin resistance. A positive association between 1908C/T polymorphism and metabolic abnormalities has been reported in Inuit⁶, Japanese⁷, Pima Indians and Armish⁸.

However, there is no data on the association between *LMNA* and arterial stiffness in a general population. Arterial stiffness is mainly determined by measuring pulse wave velocity (PWV)⁹. PWV reflects systemic arteriosclerosis as well as relating to cardiovascular risk factors¹⁰ and ischemic heart disease in type 2 diabetes mellitus¹¹. PWV is also a predictor of cardiovascular mortality in patients with end-stage renal disease¹² or hypertension¹³ and in elderly individuals¹⁴, independently of age, blood pressure, and cardiac mass.

The purpose of this study was to examine the relationship between polymorphisms of *LMNA* and arterial stiffness in a cross-sectional epidemiological study of a Japanese general population, the Tanno and Sobetsu study.

Materials and Methods

We recruited 586 male inhabitants of Tanno Town and Sobetsu Town who had undergone medical check-ups in 2003. Tanno and Sobetsu are located in Hokkaido, the northernmost island of Japan. The Tanno and Sobetsu study was started in 1977 with a population-based prospective cohort design. Detailed epidemiological findings have already been reported¹⁵⁻¹⁸.

The subjects completed a standard questionnaire regarding their medical history and their smoking and drinking habits. We measured anthropometric parameters, systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, triglyceride, high-density lipoprotein (HDL) cholesterol, plasma glucose, immunoreactive insulin (IRI), highly sensitive

C-reactive protein (hs-CRP), and adiponectin in all subjects. Brachial-ankle pulse wave velocity (baPWV) and ankle-brachial index (ABI) were measured using Form® PWV/ABI (Omron Colin Co., Ltd., Tokyo, Japan) and the average of right baPWV and left baPWV was adopted¹⁹. Insulin sensitivity was determined by homeostasis model assessment of the insulin resistance (HOMA-IR) index, which was calculated as plasma glucose (mg/dL) × immunoreactive insulin (μU/L)/405. Blood samples were collected in the early morning after fasting for 8–11 hours. Blood pressure was measured twice after 5 minutes of rest, with the subjects seated.

Exclusion criteria were atrial fibrillation, suspected arteriosclerosis obliterans (ASO) defined as ABI on any side lower than 0.9, and taking any medication, in order to rule out drug effects. After excluding 219 of the 586 male subjects according to the above criteria, we conducted genetic analysis. Finally, 261 male subjects were successfully genotyped. All subjects gave written informed consent to participate in the genetic analysis and in all other procedures associated with the study. The Institutional Review Board (IRB) of Osaka University and the IRB of Sapporo Medical University both approved the study protocol.

Genomic DNA was extracted from 200 μL buffy coat using a QIAamp DNA Blood Kit (QIAGEN K. K., Tokyo, Japan). C-to-T transversion at nucleotide position 1908 in exon 10 of the lamin A/C gene (*LMNA* 1908C/T; rs #4641) and C-to-T transversion at nucleotide position -1030 in the promoter region of *LMNA* (*LMNA* -1030C/T; no rs#) were determined by the TaqMan-polymerase chain reaction (PCR) method. The *LMNA* 1908C/T polymorphism was detected using the following primers and probes: forward, 5'-CGA GGA TGA GGA TGG AGA TGA C-3'; reverse, 5'-CCT CAG CGG CGG CTA C-3'; cytosine base (C)-specific probe, 5'-VIC-CAC TCA CGT GGT GGT G-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-CAC TCA CAT GGT GGT G-MGB-3'. The *LMNA* -1030C/T polymorphism was detected using the following primers and probes: forward, 5'-CCA CTA CCT TCT TTC TGG CTG AA-3'; reverse, 5'-ACT AGG TCC CAG ATT TCT GTG GTT-3'; cytosine base (C)-specific probe, 5'-VIC-CAG CCA ATG TTG GGT C-MGB-3'; and thymine base (T)-specific probe, 5'-FAM-ACA GCC AAT ATT GGG TC-MGB-3'. PCR was carried out using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA). PCR conditions were as follows: initial denaturation at 95°C for 10 min, followed by 40 cycles of 92°C for 15 sec and 60°C for 60 sec. The fluorescence level of PCR

Table 1. Baseline characteristics of study subjects ($n=261$)

	Male ($n=261$)
Age (years)	64.6 ± 0.7
BMI (kg/m ²)	23.5 ± 0.2
SBP (mmHg)	133 ± 1.3
DBP (mmHg)	75 ± 0.7
Total cholesterol (mg/dL)	193 ± 2.0
Triglyceride (mg/dL)	112 ± 4.6
HDL cholesterol (mg/dL)	52 ± 0.8
Current smoker (%)	33.0
HOMA-IR	1.0 ± 0.07
hsCRP (mg/dL)	0.107 ± 0.008
Adiponectin (ng/mL)	6.1 ± 0.2
baPWV (cm/s)	1,631 ± 19.7

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

products measured using an ABI PRISM 7900HT Sequence Detector (Applied Biosystems) differentiated the three genotypes of these two polymorphisms.

Associations between the polymorphisms and clinical variables were analyzed using one-way analysis of variance (ANOVA). Differences in genotype or allele distribution were examined by χ^2 analysis. Multiple regression analysis was used to assess the contribution of confounding factors. All numerical values are expressed as the means ± SEM. Significance was defined as $p < 0.05$. All statistical analyses were conducted using JMP software version 5.1.2J for Windows (SAS Institute Inc., Cary, NC, USA).

Results

The 261 male subjects had a mean age of 64.6 ± 0.7 years, mean body mass index (BMI) of 23.5 ± 0.2 kg/m², and mean brachial-ankle pulse wave velocity (baPWV) of 1,631 ± 19.7 cm/sec. **Table 1** shows the baseline characteristics of all study subjects. The genotype frequencies of the two polymorphisms of *LMNA* examined did not significantly differ from the values predicted by the Hardy-Weinberg equilibrium. The frequencies of CC, CT and TT genotypes of exon 10 1908C/T polymorphism were 60%, 32% and 8%, respectively, and the frequencies of CC, CT and TT genotypes of promoter -1030C/T polymorphism were 79%, 16% and 5%, respectively. Since the number of subjects with TT genotype of these two polymorphisms was small, we adopted a recessive model of the

Table 2. Comparison of parameters between CC genotype and CT + TT genotype of 1908C/T polymorphism

	CC ($n=157$)	CT + TT ($n=104$)	p
Age (years)	65.3 ± 0.9	63.5 ± 1.2	0.24
BMI (kg/m ²)	23.5 ± 0.3	23.7 ± 0.3	0.58
SBP (mmHg)	133 ± 1.7	136 ± 2.1	0.30
DBP (mmHg)	75 ± 0.9	77 ± 1.1	0.07
Total cholesterol (mg/dL)	194 ± 2.7	190 ± 3.4	0.09
Triglyceride (mg/dL)	112 ± 5.1	113 ± 10.0	0.91
HDL cholesterol (mg/dL)	52 ± 0.9	52 ± 1.7	0.96
Current smoker (%)	32.4	5.2	0.75
HOMA-IR	1.1 ± 0.1	1.0 ± 0.2	0.56
hsCRP (mg/dL)	0.11 ± 0.008	0.082 ± 0.02	0.10
Adiponectin (ng/mL)	6.1 ± 0.2	6.0 ± 0.4	0.80

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

Table 3. Comparison of parameters between CC genotype and CT + TT genotype of -1030C/T polymorphism

	CC ($n=207$)	CT + TT ($n=54$)	p
Age (years)	64.8 ± 0.8	63.9 ± 1.5	0.61
BMI (kg/m ²)	23.6 ± 0.2	23.2 ± 0.4	0.37
SBP (mmHg)	134 ± 1.4	131 ± 2.8	0.27
DBP (mmHg)	76 ± 0.8	74 ± 1.6	0.24
Total cholesterol (mg/dL)	191 ± 2.2	199 ± 4.4	0.38
Triglyceride (mg/dL)	112 ± 5.9	106 ± 7.3	0.56
HDL cholesterol (mg/dL)	53 ± 1.0	52 ± 1.3	0.88
Current smoker (%)	35.4	28.1	0.26
HOMA-IR	1.1 ± 0.1	1.0 ± 0.1	0.27
hsCRP (mg/dL)	0.11 ± 0.01	0.10 ± 0.01	0.43
Adiponectin (ng/mL)	6.0 ± 0.3	6.2 ± 0.3	0.52

Values are expressed as the mean ± SEM or %. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA-IR, homeostasis assessment model of insulin resistance; hsCRP, highly sensitive C-reactive protein; baPWV, brachial-ankle pulse wave velocity

C allele (CC vs. CT + TT) for the two polymorphisms. **Tables 2** and **3** show the clinical parameters of each genotype of the 1908C/T polymorphism and -1030C/T polymorphism of *LMNA*, respectively. Despite previous findings⁶⁻⁸⁾, there was no significant relationship between the T allele of 1908C/T polymorphism and metabolic traits in our study cohort. BaPWV of subjects with CC genotype of -1030C/T polymorphism was significantly greater than that of

Fig. 1. Comparison of brachial-ankle pulse wave velocity (baPWV) according to genotypes of *LMNA* 1908C/T and -1030C/T polymorphisms.

subjects with CT+TT genotype of -1030C/T polymorphism ($1,652 \pm 22.1$ cm/s vs. $1,552 \pm 43.0$ cm/s, $p=0.039$), while there was no significant difference in the genotype of 1908C/T polymorphism (**Fig. 1**). BaPWV showed significant positive correlations with age, systolic blood pressure and smoking habit and a significant negative correlation with body height. We therefore selected these factors as covariates of multiple regression analysis for baPWV and *LMNA* -1030C/T polymorphism (**Table 4**). There was an independent relationship between the genotype of -1030C/T polymorphism and baPWV after adjusting covariates. Mean baPWVs of -1030/CC and -1030/CT+TT genotypes were $1,650 \pm 17.1$ cm/s and $1,571 \pm 32.0$ cm/s, respectively, after adjusting covariates.

Since there was a strong linear correlation between age and baPWV, we analyzed the genotypic difference of -1030C/T in this correlation. The gradient of the regression line of subjects with CC genotype was significantly high compared to that of subjects with CT+TT genotype (data not shown), indicating that subjects with CC genotype of -1030C/T polymorphism might be susceptible to the progression of arterial stiffness by aging.

Discussion

We examined the hypothesis that there is a positive, independent association between polymorphisms

Table 4. Multiple regression analysis for brachial-ankle pulse wave velocity (baPWV)

Term	Estimate	SE	t	p
Age	12.5	1.58	7.9	<0.0001
Body height	0.684	2.55	0.3	0.79
Systolic blood pressure	7.69	1.15	6.7	<0.0001
Smoking habit	-6.34	15.9	-0.40	0.69
-1030C/T	-39.4	17.8	-2.2	0.028
CC vs. CT+TT				

$R^2=0.48$ ($n=261$)

of *LMNA* and arterial stiffness in a cross-sectional study of a Japanese population. Genotype frequencies of the two polymorphisms satisfied the Hardy-Weinberg equilibrium. The mean baPWV of -1030C/T polymorphism was significantly greater in subjects with CC genotype than in subjects with CT+TT genotype; however, no significant difference was found for 1908C/T polymorphism. On the other hand, age, body height, systolic blood pressure, and smoking habit were significantly associated with baPWV. Multiple regression analysis including covariates revealed that subjects with the -1030T allele had a significantly lower level of baPWV.

The 1908C/T polymorphism of *LMNA* has been reported to be related to metabolic abnormalities or insulin resistance in Japanese⁷ and Armish⁸), but a relationship between this polymorphism and arterial stiffness was not found in the present study. In addition, no relationship was found between 1908C/T polymorphism and metabolic traits in our population. This may due to the characteristics of our subjects. Subjects taking any medication were excluded from this study, and the number of subjects with type 2 diabetes mellitus or dyslipidemia was therefore small; however, the promoter -1030C/T polymorphism of *LMNA*, which we have recently identified by direct sequencing in the subjects, was independently associated with baPWV as a marker of arterial stiffness. To our knowledge, this is the first report of a relationship between *LMNA* polymorphism and arterial stiffness.

In addition, we investigated the relation between the prevalence of cardiovascular diseases and SNPs in *LMNA* using a cross-sectional method; however, there was no significant relation between cardiovascular diseases and SNPs. Because the subjects of our study were relatively healthy and had a low prevalence of cardiovascular diseases, our investigation might lack statistical power. A prospective study to elucidate this relation is now ongoing. Because of the shortness of the follow-up period, we do not have valuable results

at present, but we will report the obtained results in the future.

Mutations in *LMNA* have been discovered in a staggering variety of inherited diseases called "laminopathies"²⁰⁾. To date, several laminopathies are more familiar than Hutchinson-Gilford progeria syndrome (HGPS), such as Dunnigan-type familial partial lipodystrophy (FPLD), Emery-Dreifuss muscular dystrophy, Charcot-Marie-Tooth disease, limb-girdle muscular dystrophy, mandibuloacral dysplasia, dilated cardiomyopathy with conduction abnormality and early onset of atrial fibrillation. Laminopathies are caused by a mutation of *LMNA*, and are likely to include cardiovascular diseases^{21, 22)}. The precise mechanisms of this relationship are unknown, but it is speculated that *LMNA* regulates metabolic traits as well as arterial stiffness and thus results in the aging process. HGPS is a laminopathy that is mainly caused by C-to-T mutation in position 1824 of *LMNA* and results in systemic arteriosclerosis. Recent studies have shown that this mutation causes nuclear blebbing induced by anchoring the mutant lamin A (called "progerin", which lacks 50 amino acids near the carboxy terminus) to the inner nuclear membrane, resulting in dysregulated gene transcription, heterochromatin disorganization^{23, 24)}, and increased vulnerability of the nuclear membrane. Numerous abnormalities present in HGPS are common phenomena that occur in cells not only of HGPS patients but also aged individuals in the general population, such as nuclear blebbing, epigenetic changes and increased levels of DNA damage²⁵⁾; therefore, *LMNA* seems to be one of the key genes regulating aging as well as arterial stiffness.

In order to elucidate the function of -1030C/T promoter polymorphism of *LMNA*, we searched for transcription factors likely to bind around -1030C/T, using online databases of TRANSFAC, JASPAR, IMD, and CBIL/GibbsMat (<http://www.cbil.upenn.edu/cgi-bin/tess/tess/>). Motif analysis revealed one transcription factor, CREB-binding protein/CCAAT recognition factor (CBP/CRF), which has homology with the sequence including the -1030T allele. This may result in a difference in *LMNA* expression. Subjects with the -1030T allele are likely to have a strong expression of lamin A and C matrix and might have a stable nuclear membrane against environmental insult, represented by reactive oxygen species.

Our study has several limitations. First, our study was conducted using a small number of subjects in a Japanese population. Second, this study was designed as a cross-sectional method. Although arteriosclerosis occurs mostly in aged individuals, the genotype-phenotype relationship should also be analyzed in a time-

considered, longitudinal study. Third, the mechanisms by which transcriptional activities of *LMNA* are regulated by promoter -1030C/T polymorphism are unclear. Further study is required to clarify the function of promoter -1030C/T polymorphism.

In conclusion, promoter -1030C/T polymorphism of *LMNA* might be associated with arterial stiffness in Japanese independent of metabolic traits. The mechanism of the influence of *LMNA* on arterial stiffness may reveal part of the mechanism of a common condition susceptible to arteriosclerosis, aging.

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