ORIGINAL RESEARCH

SIRTUIN 1 Gene Polymorphisms are Associated With Cholesterol Metabolism and Coronary Artery Calcification in Japanese Hemodialysis Patients

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Objectives: *Sirtuin 1* (*SIRT1*), a longevity gene, protects cells against oxidative and genotoxic stress. This study aimed to investigate the association of *SIRT 1* gene single-nucleotide polymorphisms, namely, rs7895833, rs7069102, and rs2273773 with lipid profiles and coronary artery calcification score in 219 Japanese hemodialysis (HD) patients.

Methods: Genotyping of these polymorphisms was performed using polymerase chain reaction with confronting two-pair primers assay.

Results: The A allele frequency of rs7895833 and G allele frequency of rs7069102 were significantly lower in HD patients (0.228 and 0.131, respectively) than those in 803 control subjects (general population) (0.289 and 0.181, respectively) ($P = .010$ and $P = .012$, respectively). However, the allele frequency of rs2273773 was not significantly different from that in the control subjects. Multivariate analysis adjusted for age and duration on HD demonstrated that the serum levels of total and low-density lipoprotein cholesterol were significantly high in G allele carriers of rs7069102 compared with CC genotype in male HD patients. Coronary artery calcification score was significantly high in C allele carriers of rs2273773 in all and male HD patients.

Conclusions: *SIRT 1* polymorphisms, rs7069102 and rs2273773, are associated with abnormal cholesterol metabolism and coronary artery calcification, respectively, in Japanese HD patients, especially in males.

SILENT INFORMATION REGULATOR 2 (Sir2) proteins mediate the health-promoting effects of caloric restriction, which includes the retardation of aging in lower organisms such as yeast, flies, and worms.[1] At least seven Sir2 homologs, sirtuins (SIRT) 1 to 7, have been identified in human beings.[2]

SIRT1, the most extensively studied family member, couples protein deacetylation with NAD(+) hydrolysis and links cellular energy and redox state to multiple signaling and survival pathways. Cell-type and context-specific activation of sirtuins increases resistance to metabolic, oxidative, and hypoxic stress in different tissues. In particular, SIRT1 plays a central role in mediating the beneficial effects of caloric restriction, and its activation associates with longevity and the attenuation of metabolic disorders. Thus, *SIRT1* is a longevity gene, and it protects cells against

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oxidative and genotoxic stress by deacetylating a large number of substrates such as p53 and forkhead transcription factors.[3,4] SIRT1 deacetylates and activates forkhead transcription factor 1 that increases the expression of adiponectin, a key adipose-derived hormone in the regulation of insulin resistance and vascular dysfunction. SIRT1 also has an important function in endocrine signaling, specifically in glucose and fat metabolism.[5,6] SIRT1 activates liver X receptor (LXR) proteins α and β that play an important role in the regulation of lipid metabolism.[7] In adipose tissue, SIRT1 interacts with peroxisome proliferator–activated receptor γ to repress its transcriptional activity, leading to inhibition of adipogenesis.[5] Thus, SIRT1 is associated with lipid metabolism, and *SIR1* polymorphism might affect lipid profiles. However, there is no report on *SIRT1* polymorphisms in hemodialysis (HD) patients.

This study aimed to investigate the association of *SIRT1* gene single-nucleotide polymorphisms (SNPs), namely, rs7895833, rs7069102, and rs2273773 with lipid profiles and coronary artery calcification score (CACS) in Japanese HD patients. Three SNPs (rs7895833, rs7069102, and rs2273773) were selected because they covered almost all common variations of the *SIRT1* gene in Japanese.[8]

Methods

Study Subjects

This study included 219 Japanese subjects taking HD at Meiyo Clinic, Aichi, Japan.[9] The patients included 119 men and 100 women. Mean age was 60.4 ± 13.3 (standard deviation [SD]) years, and the average of dialysis duration was 9.1 ± 8.3 (SD) years. The allele frequencies of rs7895833, rs7069102, and rs2273773 were compared with their respective values for general population (803 control subjects) (280 men and 523 women; mean age, 61.3 ± 10.3 [SD] years) undergoing health checkup at Yakumo Town, Hokkaido. This study ensures compliance of human studies with the Helsinki Declaration of 1975 as revised in 1996 and was approved by the Ethics Committee of Nagoya University Graduate School of Medicine in 2004.

Lipid and CACS Measurement

Blood samples were obtained after 12 hours of fasting. The following biochemical parameters were determined by standard laboratory methods based on Japan Society of Clinical Chemistry: total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein (LDL) cholesterol. CACS was measured by using 16-row multidetector computed tomography (Aquilion 16, Toshiba Medical Systems Corporation, Tokyo, Japan).

Genotyping of *SIRT1* Gene SNPs

Three tagging SNPs, namely, rs7895833, rs7069102, and rs2273773 were selected from the HapMap database that, together with constructed haplotypes, covered 100% of the common variations of the *SIRT1* gene in Japanese. The genotyping of rs7895833 in the promoter region, rs7069102 in intron 4, and rs2273773 in exon 5 was performed using polymerase chain reaction with confronting two-pair primers assay.[10]

Confronting pairs of primers (four primers in all) are as follows:

(1) rs7895833
Forward primer 1: CCCAGGGTTCAA CAAATCTATGTTG
Forward primer 2: GGTGGTAAAAG GCCTACAGGAAA
Reverse primer 1: GCTTCCTAATCTCC ATTACGTTGAC
Reverse primer 2: CCTCCCAGTCAAC GACTTTATC

The region containing this polymorphism was amplified by PCR with these primers with the initial denature at 95°C for 10 minutes, followed by 30 cycles at 95°C for 1 minute, at 64°C for 1 minute, at 72°C for 1 minute, and additionally at 72°C for 5 minutes. PCR products were visualized on a 2% agarose gel with ethidium bromide staining. Genotyping was performed as follows: 320 and 241 bp for AA genotype; 320, 241, and 136 bp for AG genotype; and 320 and 136 bp for GG genotype.

(2) rs7069102
Forward primer 1: GTAGCAGGAACTAC AGGCCTG
Forward primer 2: GAGAAGAAAGAA AGGCATAATCTCTGC
Reverse primer 1: CTATCTGCAGAAATA ATGGCTTTTCTC
Reverse primer 2: GATCGAGACCAT CCTGGCTAAG

PCR with these primers was performed under the same conditions as that for rs7895833. Genotyping was performed as follows: 391 and 277 bp for CC genotype; 391, 277, and 167 bp for CT genotype; and 391 and 167 bp for TT genotype.

(3) rs2273773
Forward primer 1: GTGTGTCGCATCC ATCTAGATAC
Forward primer 2: CTCTCTGTCACAAA TTCATAGCCT
Reverse primer 1: GTAGTTTTCCTTCC TTATCTGACAG
Reverse primer 2: CTGAAGTTTACTAA CCATGACACTG

The region containing this polymorphism was amplified by PCR with these primers with the initial denature at 95°C for 10 minutes, followed by 30 cycles at 95°C for 1 minute, at 63°C for 1 minute, at 72°C for 1 minute, and additionally at 72°C for 5 minutes. PCR products were visualized on a 2% agarose gel with ethidium bromide staining. Genotyping was performed as follows: 314 and 228 bp for CC genotype; 314, 228, and 135 bp for CT genotype; and 314 and 135 bp for TT genotype.

Statistical Analysis

All results are expressed as mean ± SD. Significance was defined as a P value of $<.05$. The analysis was done by using PASW statistics 18 (SPSS Japan Inc., Tokyo, Japan). Hardy–Weinberg equilibrium testing was performed by using the χ^2 test. Student t test and multivariate analysis adjusted for age and duration on HD were performed in the comparison of the mean values between the different genotype groups.

Results

Frequency of *SIRT1* Gene SNPs

Table 1 shows general characteristics of control subjects and HD patients. There is no significant difference in age between control subjects and HD patients ($P = .369$).

The frequencies of genotypes in *SIRT1* gene in control subjects and HD patients are shown in Table 2. As for HD patients, the allele frequencies of the rs7895833 polymorphism of the *SIRT1* gene were 0.772 for the G allele and 0.228 for the A allele, those for the rs7069102 polymorphism were 0.869 for the C allele and 0.131 for the G allele, and those for the rs2273773 polymorphism were 0.662 for the T allele and 0.338 for the C allele. The genotype distributions for the SNPs (rs7895833, rs7069102, and rs2273773) were in Hardy–Weinberg equilibrium ($P = .87$, $P = .67$, and $P = .37$, respectively).

In control subjects, the allele frequency for the A allele in rs7895833 was 0.289; the allele

Table 1. General Characteristics of the Subjects

	Total	Male	Female
HD patients			
n	219	119	100
Age (year)	60.4 ± 13.3	58.7 ± 13.3	62.5 ± 13.1
HD duration (year)	9.09 ± 8.26	8.75 ± 8.53	9.50 ± 7.94
Creatinine (mg/dL)	12.72 ± 2.89	13.75 ± 3.00	11.50 ± 2.20
Total cholesterol (mg/dL)	168.7 ± 35.9	162.4 ± 35.1	176.8 ± 35.9
HDL cholesterol (mg/dL)	44.0 ± 12.8	40.7 ± 11.0	47.5 ± 13.6
LDL cholesterol (mg/dL)	98.6 ± 29.3	94.1 ± 26.4	104.6 ± 31.7
Triglyceride (mg/dL)	135.3 ± 104.4	147.6 ± 132.1	123.6 ± 57.8
CACS	1,323 ± 2,110	1,432 ± 2,061	1,086 ± 1,693
Control subjects			
n	803	280	523
Age (year)	61.3 ± 10.3	63.1 ± 10.6	60.3 ± 10.0
Creatinine (mg/dL)	0.69 ± 0.17	0.82 ± 0.16	0.61 ± 0.11
Total cholesterol (mg/dL)	214.0 ± 32.5	207.1 ± 30.6	217.7 ± 33.0
HDL cholesterol (mg/dL)	59.0 ± 13.8	53.7 ± 12.5	61.9 ± 13.7
LDL cholesterol (mg/dL)	134.9 ± 30.4	130.7 ± 29.0	137.2 ± 30.9
Triglyceride (mg/dL)	100.2 ± 60.6	113.4 ± 75.0	93.2 ± 49.9

HD, hemodialysis; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data presented as mean ± standard deviation (SD).

Table 2. Comparison of Allele Frequencies Between Control Subjects and HD Patients in Genotypes of SIRT1 Gene

	Control Subjects	HD Patients	*P* Value
rs7895833			
GG	408	130	
GA	316	78	
AA	74	11	
A allele frequency	0.289	0.228	.010
rs7069102			
CC	544	164	
CG	223	51	
GG	34	3	
G allele frequency	0.181	0.131	.012
rs2273773			
TT	387	99	
TC	308	92	
CC	103	28	
C allele frequency	0.322	0.338	ns

ns, not significant.

frequency for the G allele in rs7069102 was 0.181; and the allele frequency for the C allele in rs2273773 was 0.322. In HD patients, the allele frequencies of the A allele in rs7895833 and of the G allele in rs7069102 were significantly lower than those in healthy subjects ($P = .010$ and $P = .012$, respectively). However, the allele frequency of rs2273773 was not significantly different from that in healthy subjects.

Association of *SIRT1* Gene SNPs With Lipids and CACS

Table 3 shows association of the *SIRT1* gene SNPs with lipids and CACS in HD patients. The serum levels of total cholesterol and LDL cholesterol were significantly high in G allele carriers of rs7069102 compared with CC genotype in male HD patients. CACS was significantly high in C carriers of rs2273773 compared with TT genotype in all and male HD patients. However, there was no significant association of rs7895833 with lipids or CACS.

Table 4 shows multivariate analysis adjusted for age and duration on HD. The serum levels of total cholesterol and LDL cholesterol were significantly high in G allele carriers of rs7069102 compared with CC genotype in male HD patients. CACS was significantly high in C allele carriers of rs2273773 in all and male HD patients.

Discussion

HD patients showed significantly low frequencies of the A allele of rs7895833 and the G allele of rs7069102 compared with age-matched general population. Multivariate analysis adjusted for age and duration on HD demonstrated that the serum levels of total cholesterol and LDL cholesterol were significantly higher in G allele carriers of rs7069102 compared with CC genotype in male HD patients. CACS was significantly higher in C allele carriers of rs2273773 in all and male HD patients. Thus, the present study first demonstrates that *SIRT1* polymorphisms are associated with cholesterol metabolism and coronary artery calcification in Japanese HD patients.

Only a few human genetic association studies on SIRT1 have been published so far. Variation in the *SIRT1* gene was not associated with longevity in a case–control study comparing long-lived individuals with younger subjects,[11] and all-cause mortality was not influenced by *SIRT1* genetic variation in the Leiden 85-plus study.[12]

SIRT1 genetic variation is related to obesity.[13,14] Zillikens et al.[13] reported that the A allele carriers of rs7895833 showed an increase in body mass

Table 3. Variables According to Polymorphisms in SIRT1 Gene

	rs7069102			rs2273773		
	CC	CG + GG	*P* Value	TT	TC + CC	*P* Value
All						
n				99	120	
CACS				901 ± 1625	1582 ± 2065	.007
Male						
n	90	29		59	60	
Total cholesterol (mg/dL)	158.7 ± 35.3	174.0 ± 32.3	.040			
LDL cholesterol (mg/dL)	91.1 ± 26.1	104.1 ± 25.3	.024			
CACS				943 ± 1,800	1,913 ± 2,199	.010

Table 4. Multivariate Analysis Adjusted for Age and HD Duration

	Adjusted *P* Value	
	rs7069102	rs2273773
All		
CACS	ns	.013
Male	.036	ns
LDL cholesterol	.030	ns
CACS	ns	.014

ns, not significant; CACS, coronary artery calcification score.

index. Armand et al.[14] reported that there was a significant difference in the G allele frequency of rs7069102 between obese subjects and controls (0.70 and 0.67, respectively), and thus, G allele carriers of rs7069102 are at a higher risk of obesity than noncarriers. Based on these reports, A allele carriers in rs7895833 and G allele carriers in rs7069102 tend to be obese, and thereby at a high risk for cardiovascular disease. Obesity induces high mortality in general population.

The present study demonstrates that HD patients showed significantly low frequencies of the A allele of rs7895833 and the G allele of rs7069102 compared with healthy subjects. We hypothesize that chronic kidney disease patients with A allele carriers of rs7895833 and the G allele carriers of rs7069102 might tend to die because of obesity-induced cardiovascular disease, before they enter into end-stage renal disease requiring HD treatment, and consequently, the HD patients might have lower frequencies of A allele in rs7895833 and of G allele in rs7069102.

The serum levels of total cholesterol and LDL cholesterol were significantly higher in G allele carriers of rs7069102 compared with CC genotype. These results indicate that G allele carriers of rs7069102 are at high risk for hypercholesterolemia. CACS was significantly higher in C carriers of rs2273773 compared with TT genotype in all and male HD patients. Therefore, the C allele carriers of rs2273773 may be at a high risk for coronary artery disease. CACS is regarded as an index of the severity of atherosclerotic vascular disease and may predict future adverse cardiovascular events, especially in HD patients.[15] Thus, *SIRT1* polymorphism may be associated with development of cardiovascular disease in HD patients.

SIRT1 activates LXR that operates as cholesterol sensor to protect the organism from cholesterol overload, and reduces cholesterol loading in macrophages, consequently protecting against atherosclerosis.[16] Thus, G allele carriers of rs7069102 and C allele carriers of rs2273773 might have reduced activities of SIRT1 and LXR, thereby leading to hypercholesterolemia and atherosclerosis.

The different allele frequencies in rs7895833 and rs7069102 between HD patients and control subjects might have an impact on survival. Further follow-up study of the HD patients will be required to determine if the *SIRT1* polymorphisms might affect their survival.

Practical Application

It might be useful to examine SIRT1 polymorphism for management of cholesterol and coronary artery calcification in Japanese HD patients.

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