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Original article

ApoB C7623T polymorphism predicts risk for steroid-induced osteonecrosis of the femoral head after renal transplantation

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Abstract

Background. Nontraumatic osteonecrosis of the femoral head (ONFH) is caused by disruption of blood flow. This disease often occurs in association with steroid treatment. The pathology of steroid-induced ONFH remains unclear, although abnormalities in lipid metabolism have been reported to be involved. In this study, we examined the differences of gene polymorphism frequencies of apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1), which are important proteins for lipid transport, as well as of lipid parameters, between ONFH cases and referent patients among those who were subjected to renal transplantation.

Methods. Subjects were 158 cases who had undergone renal transplant, including 34 cases that were diagnosed as ONFH after renal transplantation and 124 cases that were not. Four single nucleotide polymorphisms including C7623T and G12619A for the *ApoB* gene and G75A and C83T for the *ApoA1* gene were analyzed using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and Taqman real-time PCR chemistry. Also, serum levels of low-density lipoprotein (LDL), high-density lipoprotein (HDL), ApoB, and ApoA1 were measured. Their relationship to ONFH was statistically evaluated.

Results. A higher frequency of 7623TT or CT of the *ApoB* gene was observed in ONFH cases than in referent patients ($P = 0.033$), resulting in an elevated odds ratio that was statistically significant (adjusted odds ratio = 6.37, 95% CI = 1.53–26.5, $P = 0.011$). No significant relationship was observed between other genes and ONFH. Regarding lipid parameters, a higher value of ApoB/ApoA1 ratio was observed in cases ($P = 0.045$).

Conclusion. For the prediction of ONFH, it is useful to analyze *ApoB* C7623T and plasma ApoB/ApoA1 ratio before the administration of steroids.

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Introduction

Non-traumatic osteonecrosis of femoral head (ONFH) is an intractable disease that is pathophysiologically characterized by ischemic necrosis of the femoral head and deterioration of hip joint function, and these changes significantly affect patient quality of life.[1] Many ONFH cases develop in association with steroid treatment, and alcohol consumption is also a contributing factor.[1] Pathophysiological features of steroid-induced ONFH are (i) it develops at a very early stage during treatment,[2] (ii) the necrotic lesion can be large, therefore making preservation of the femoral head difficult, requiring invasive total hip replacement, and (iii) it is an iatrogenic disease.

We found that single nucleotide polymorphism (SNP) of the ATP-binding cassette *B1* (*ABCB1*) gene[3] is related to ONFH. However, because multiple factors are involved in the risk of ONFH,[1] it is necessary to clarify other related gene polymorphisms to predict the occurrence of necrosis. Determination of individual differences in polymorphisms before steroid administration would enable screening for the risk of ONFH.

Steroid-induced ONFH has been reported to be related to lipid metabolism abnormalities.[4,5] Lipoproteins

play an important role in lipid metabolism and lipid transport in the body. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) are included in lipoproteins, and the proteins apolipoprotein B (ApoB) and apolipoprotein A1 (ApoA1) are involved in the structure of lipoprotein by surrounding triglyceride (TG) and total cholesterol (TC). LDL, with ApoB as the main constituent protein, transports lipids from the liver to the periphery, whereas HDL, with ApoA1 as the main constituent protein, transports lipids from the periphery to the liver. An association of elevated serum LDL and ApoB as well as decreased serum HDL and ApoA1 has been reported in coronary artery disease.[6] Other studies have also shown that serum ApoB/ApoA1 ratio is significantly elevated in steroid-induced, alcohol-induced, and idiopathic ONFH,[7] and femoral necrosis was shown to be significantly correlated with LDL/HDL ratio in a model of femoral necrosis in rabbits treated with steroids.[8] When considering vascular pathology, ApoB and ApoA1 are important factors[7] because elevated blood ApoB and ApoA1 levels reflect the status of enhanced lipid transport to the peripheries, including bone tissue.

Major SNPs of the *ApoB* gene include C7623T in exon 26 and G12619A in exon 29, which are associated with coronary artery disease[9–11] and serum levels of LDL and ApoB.[12,13] Major SNPs of the *ApoA1* gene include G-75A in the promoter region and C83T in intron 1, which are also associated with coronary artery disease[14] and serum levels of HDL and ApoA1.[6,14] However, the relationship between these gene polymorphisms and ONFH has not yet been elucidated.

We hypothesized that the gene polymorphisms and blood levels of ApoB and ApoA1, whose relationships with coronary artery disease have been reported, also have some effects on ONFH. Therefore, in an effort to clarify the risk factors for steroid-induced ONFH, we examined the relationships between steroid-induced ONFH and *ApoB* and *ApoA1* polymorphisms as well as lipid parameters.

Patients and methods

Study design

Steroid-induced osteonecrosis develops after renal transplantation.[15] The current study examined 34 patients with ONFH (case) and 124 patients who did not develop ONFH (reference group) following renal transplantation in our university from 1983 to 2004. ONFH was diagnosed according to previously published criteria.[16] Because ONFH does not develop in renal transplant recipients later than 12 months after the initiation of steroid administration,[2] patients in whom no necrosis was found in the femoral head, nor in the knee, shoulder, and ankle joints at 1 year after renal transplantation, were regarded as reference patients. Patients with any of the following conditions were excluded from this study: (i) those requiring dialysis as a result of loss of kidney graft function; (ii) those who had been diagnosed with ONFH before transplantation; (iii) those whose magnetic resonance (MR) findings did not satisfy the diagnosis criteria (e.g., bandlike low signals in the femoral head in T_1-weighted images); (iv) hip joint disease, such as acetabular dysplasia and osteoarthritis before transplantation; (v) those who received renal transplantation before the introduction of cyclosporine in 1982; and (vi) those who did not agree to participate in this study.

This study was approved by the ethical review board on human genome/gene analysis research of our university, and a written informed consent was obtained from each participating patient.

Clinical information

For all 158 subjects, we retrospectively examined sex, age at transplantation, type of transplanted kidney (living or cadaveric), status of acute rejection, type of immunosuppressant used after transplantation (cyclosporine or tacrolimus), and steroid administration protocol.

All patients received intravenous injection of methylprednisolone 500 mg during surgery and intravenous injection of prednisolone (PSL) 50 mg on the day of the surgery. After the day following surgery, the patients received oral administration of PSL 50 mg/day for 3 or 7 days, then PSL 40 mg/day for 4 or 7 days. The dose was reduced to 30, 25, 20, and 17.5 mg/day every 7 days to 10 mg/day by 6 months later. Total oral dosage given to each patient by the fourth postoperative week was a mean 924.6 mg.

Analysis of SNPs in ApoB and ApoA1 genes

A number of SNPs are recognized for *ApoB*. Among them, C7623T and G12619A are the most frequently reported in the context of blood levels and their relationship with ischemic diseases[9–11]; they are also reported as targets of meta-analysis.[17] For *ApoA1*, G-75A and T83C are the most frequently reported SNPs for their relationship with blood lipid levels and ischemic heart diseases.[6,14]

Among the 158 patients, *ApoB* C7623T and G12619A could be analyzed in 155 and 157 patients, respectively, and *ApoA1* G-75A and T83C in 153 patients each. Genomic DNA was obtained from peripheral blood by using a DNeasy Tissue Kit (Qiagen, Hilden, Germany). *ApoB* C7623T and *ApoA* G-75A and C83T were analyzed by polymerase chain reaction restriction-fragment

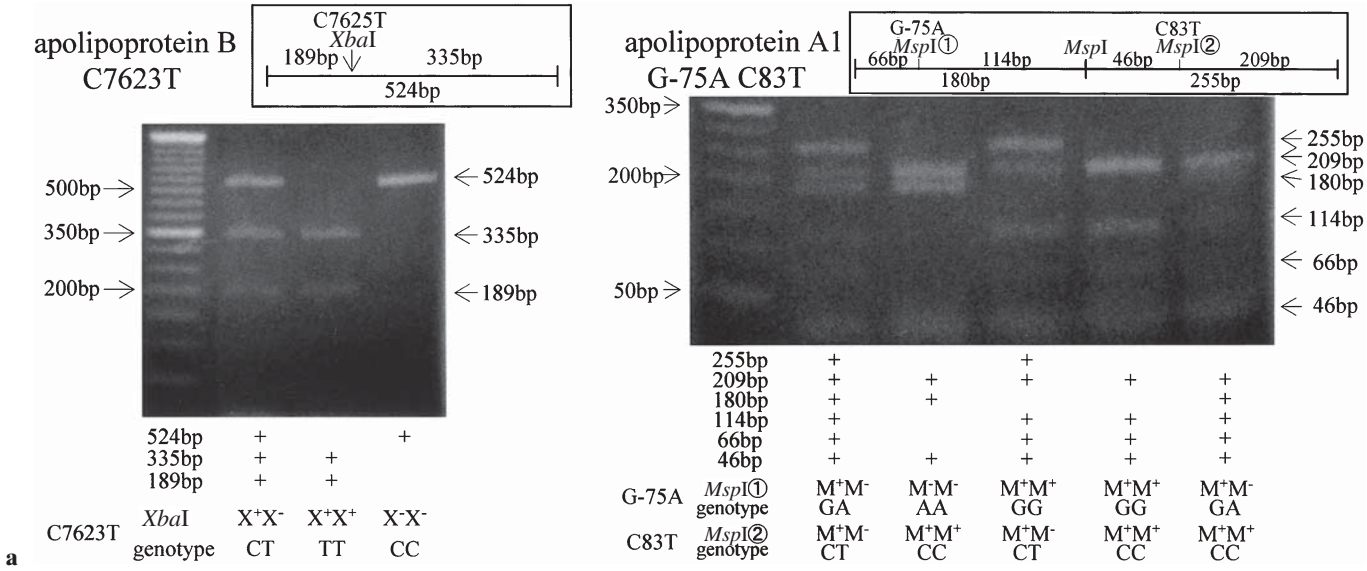

Fig. 1. Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) of apolipoproteinB C7623T (**a**) and apolipoprotein A1 G-75A and C83T polymorphisms (**b**). X^+, X^-, *apoB* allele with or without, respectively, the *Xba*I restriction site; M^+, M^-, *apoA1* allele with or without, respectively, the *Msp*I restriction site

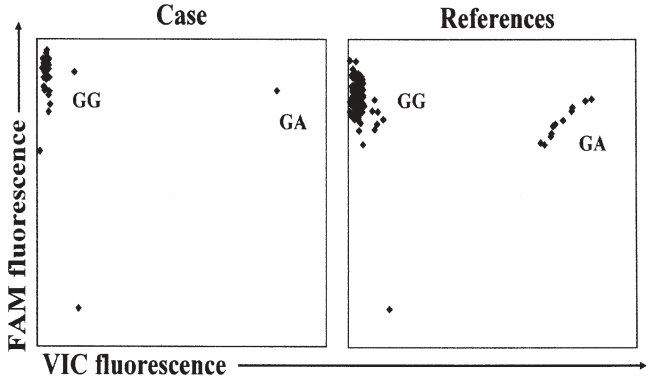

Fig. 2. Genotyping of apolipoprotein B G12619A polymorphism using real-time PCR (Taqman) genotyping assay

length polymorphism (PCR-RFLP), and *ApoB* G12619A was analyzed by real-time PCR (Taqman) genotyping assay. For the analysis of *ApoB* C7623T, 5′-GAAATTCAGGCTCTGGAACTAC-3′ (f), and 5′-GGAATCCTCAAATCTGTTAGGGG-3′ (r) were used as the primers, and *Xba*I was used as the restriction enzyme.[18] Genotypes were classified based on the presence or absence of three respective bands at 524, 335, and 189 bp (Fig. 1a). For *ApoA1* G-75A and C83T, 5′-AGGGACAGAGCTGATCCTTGAACTCTTA AG-3′ (f) and 5′-TTAGGGGACACCTAGCCCTCA GGAAGAGCA-3′ (r) were used as the primers and *Msp*I was used as the restriction enzyme.[6] Genotypes of G-75A and C83T were classified based on the presence or absence of six respective bands at 255, 209, 180, 114, 66, and 46 bp (Fig. 1b). For *ApoB* G12619A, 5′-GGATAACGTGTTTGATGGCTTGGTA-3′(f) and 5′-ATCAATGAGTGAGTCAATCAGATGCTT-3′ (r) were used as the primers, and TTACTC AAAAATTCC and TTACTCAAGAATTCC were used as probe sequences for each allele. To produce fluorescence at separate wavelengths, each of the probes was labeled with different fluorophores (VIC or FAM).[19] Genotypes were classified by analyzing the intensities of the distributions of these two fluorescences (Fig. 2). The differences of these gene polymorphism frequencies between cases and references were examined statistically.

Measurement of lipid parameters

Serum levels of LDL, HDL, ApoB, and ApoA1 were measured in 112 patients (20 cases and 92 reference patients) from whom blood could be collected again after the DNA sampling. The blood samples were collected before breakfast at 13–158 months after renal transplantation (mean, 106 months; steady state).

Also, ApoB/ApoA1 and LDL/HDL ratios were calculated. The relationships between these lipid parameters and ONFH were examined statistically. These lipid parameters were also compared with each SNP.

Statistical methods

To estimate the minimum sample size required, genotype CT or TT for *ApoB* C7623T was assumed as the primary exposure. An odds ratio of genotype CT or TT to genotype CC of 5.0, a frequency of genotype CT or

TT in the reference group of 5%, $\alpha = 0.05$, and $\beta = 0.2$ were set. Assuming that three times as many as many reference subjects as the case subjects would be enrolled, the minimum subject number required to detect the aforementioned odds ratio with significance was estimated to be 160 in total, including 40 cases and 120 references.

Statistical significance was assessed using the chi-square test, Fisher's exact test, Wilcoxon's rank sum test, or Student's *t* test. The crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated by a logistic regression model. These analyses were all conducted using the Statistical Analysis System (SAS, version 9.0).

Results

Relationship between patient characteristics and ONFH

ONFH was not related to sex, transplanted kidney type, presence or absence of acute rejection, or corticosteroid dose (Table 1). However, it was significantly related to the administration of immunosuppressants after renal transplantation ($P = 0.027$). In a logistic regression model, a significant relationship was found between ONFH and the administration of immunosuppressants by univariate and multivariate analyses (crude OR = 0.35; 95% CI = 0.14–0.91; $P = 0.031$, adjusted OR = 0.27; 95% CI = 0.08–0.92; $P = 0.037$). A significant relationship was also found between ONFH and age at surgery by multivariate analysis (adjusted OR = 1.05; 95% CI = 1.01–1.09; $P = 0.017$).

The influence of gene polymorphisms on ONFH

Genotype frequency of *ApoB* C7623T polymorphism was CC (91.7%), CT (7.7%), and TT (0.6%), that of G12619A was GG (92.3%), GA (7.7%), and AA (0%), that of *ApoA1* G-75A was GG (70.6%), GA (26.8%), and AA (2.6%), and that of C83T was CC (88.2%), CT (11.8%), and TT (0%), which was consistent with other previous reports that examined the frequency of these polymorphisms in Japanese subjects (Table 2).

Comparisons of polymorphisms and ONFH are shown in Table 3. Regarding *ApoB* C7623T, 6 patients (18.2%) of the 33 ONFH patients and 7 patients (5.7%) of the 122 referent patients are involved in the CT or TT genotype. The ratio of patients with CT or TT genotype was significantly higher in ONFH patients ($P = 0.033$, Fisher's exact test). The CT and TT genotypes had a significantly higher risk of ONFH than the CC genotype in both univariate and multivariate logistic regression analyses that included sex, age, immunosuppressant, kidney, acute rejection, steroid administration, and polymorphisms (crude OR, 3.65; 95% CI, 1.14–11.74, $P = 0.030$; adjusted OR, 6.37; 95% CI, 1.53–

Table 1. Clinical information

	ONFH (+) Cases ($N = 34$)	ONFH (–) References ($N = 124$)	*P* value	Univariate Crude OR (95% CI)	*P* value	Multivariate Adjusted[a] OR (95% CI)	*P* value
Sex							
Male	22 (19.5)	91 (80.5)	0.320	0.67 (0.30–1.49)	0.322	0.86 (0.34–2.17)	0.748
Female	12 (26.7)	33 (73.3)		1		1	
Age							
Median (Range)	40.8 (20–64)	36.1 (9–63)	0.074##	1.03 (0.99–1.06)	0.054	1.05 (1.01–1.09)	0.017
Mean	39.5	35.0					
Immunosuppressant							
Tacrolimus	6 (11.3)	47 (88.7)	0.027*	0.35 (0.14–0.91)	0.031	0.27 (0.08–0.92)	0.037
Cyclosporine	28 (26.7)	77 (73.3)		1		1	
Kidney							
Living	27 (19.7)	110 (80.3)	0.163**	0.49 (0.18–1.34)	0.163	0.93 (0.28–3.12)	0.912
Cadaveric	7 (33.3)	14 (66.7)		1			
Acute rejection							
Present	5 (19.2)	21 (80.8)	0.163**	0.85 (0.30–2.44)	0.756	1.25 (0.35–4.53)	0.733
Absent	29 (22.0)	103 (78.0)		1		1	
Steroid dose							
Large	19 (30.2)	44 (69.8)	0.070###	2.02 (0.52–7.84)	0.312	1.87 (0.33–10.7)	0.484
Middle	12 (15.4)	66 (84.6)		0.85 (0.21–3.41)	0.817	1.14 (0.20–6.41)	0.886
Small	3 (17.7)	14 (82.4)		1 (Trend: $P = 0.072$)		1 (Trend: $P = 0.324$)	

The distribution of subjects is expressed as number and percentage in parentheses

Statistical analyses were performed using the following tests: * chi-square test; ** Fisher's exact test; ## Wilcoxon's rank sum test; ### Mantel extension method

[a] This model includes sex, age, immunosuppressant, kidney, acute rejection, steroid administration, and polymorphisms

Table 2. Frequencies of four polymorphisms

		This study		Reports[21–23]		
		n	Frequency (%)	*n*	Frequency (%)	*P* value
C7623T	CC	142	91.7	92	92.0	1.000**
	CT	12	7.7	8	8.0	
	TT	1	0.6	0	0.0	
	Total	155		100		
G12619A	GG	144	92.3	118	86.8	0.120*
	GA	12	7.7	18	13.2	
	AA	0	0.0	0	0.0	
	Total	156		136		
G-75A	GG	108	70.6	157	74.4	0.584*
	GA	41	26.8	51	24.2	
	AA	4	2.6	3	1.4	
	Total	153		211		
C83T	CC	135	88.2	193	91.5	0.308*
	CT	18	11.8	18	8.5	
	TT	0	0.0	0	0.0	
	Total	153		211		

Statistical analyses were done using *chi-square test; **Fisher's exact

Table 3. Comparisons of polymorphisms and osteonecrosis of the femoral head (ONFH)

	ONFH (+) Cases *n* (%)	ONFH (–) References *n* (%)	*P* value	Univariate		Multivariate[a]	
				Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
ApoB C7623T	(*n* = 33)	(*n* = 122)					
CT TT	6 (18.2)	7 (5.7)	0.033**	3.65 (1.14–11.7)	0.030	6.37 (1.53–26.5)	0.011
CC	27 (81.8)	115 (94.3)		1		1	
ApoB G12619A	(*n* = 34)	(*n* = 123)					
GA AA	1 (2.9)	11 (8.9)	0.465**	0.31 (0.04–2.48)	0.269	0.55 (0.06–5.11)	0.602
GG	33 (97.1)	112 (91.1)		1		1	
ApoA1 G-75A	(*n* = 33)	(*n* = 120)					
GA AA	11 (33.3)	34 (28.3)	0.577*	1.27 (0.55–2.89)	0.577	1.41 (0.57–3.50)	0.459
GG	22 (66.7)	86 (71.7)		1		1	
ApoA1 C83T	(*n* = 33)	(*n* = 120)					
CT TT	4 (12.1)	14 (11.7)	1.000*	1.05 (0.32–3.42)	0.942	1.39 (0.39–5.05)	0.615
CC	29 (87.9)	106 (88.3)		1		1	

CI, confidence interval
Statistical analyses were done using *chi-square test, **Fisher's exact test
[a]This model includes sex, age, immunosuppressant, kidney, acute rejection, steroid administration, and polymorphisms

26.5, $P = 0.011$). These data showed that risk of ONFH was significantly higher in the patients with the T allele. No significant relationship was observed between ONFH and the *ApoB* G12619A, *ApoA1* G-75A, and *ApoA1* C83T genotypes.

Comparison of lipid parameters and ONFH

Lipid parameters were measured to evaluate their association with ONFH (Table 4). A significant difference was found in the ApoB/ApoA1 ratio, i.e., the mean, median, and range of the ApoB/ApoA1 ratio were 0.65, 0.62, and 0.38–0.93 in the ONFH group, whereas they were 0.58, 0.54, and 0.19–1.19 in the reference group ($P = 0.045$, Wilcoxon rank sum test). No association was observed between ONFH and serum levels of HDL, LDL, ApoB, and ApoA1, as well as the LDL/HDL ratio.

Comparison of lipid parameters and SNPs

The lipid parameters were compared with four gene polymorphisms: *ApoB* C7623T and G12619A, *ApoA1* G-75A, and C83T. Lipid parameters were not related to *ApoB* C7623T and G12619A or *ApoA1* G-75A (data not shown). Significant relationships were found

Table 4. Comparisons of lipid parameters and ONFH

		ONFH (+) Cases ($n = 20$)	ONFH (−) References ($n = 93$)	P value
HDL (mg/dl)	Median	66.0	70.0	0.161**
	(Range)	40.0–119.0	36.0–147.0	
	Mean	66.5	72.6	
LDL (mg/dl)	Median	119.5	112.0	0.470**
	(Range)	69–180	48–289	
	Mean	120.5	116.9	
LDL/HDL ratios	Median	1.91	1.60	0.227**
	(Range)	0.98–3.23	0.33–3.95	
	Mean	1.93	1.76	
ApoA1 (mg/dl)	Median	151	160	0.257*
	(Range)	110.0–221.0	94.0–276.0	
	Mean	152.3	161.3	
ApoB (mg/dl)	Median	99.5	84.0	0.094**
	(Range)	60.0–136.0	50.0–205.0	
	Mean	96.5	88.7	
ApoB/ApoA1 ratio	Median	0.62	0.54	0.045**
	(Range)	0.38–0.93	0.19–1.19	
	Mean	0.65	0.58	

Statistical analyses were done using * Student's t test and ** Wilcoxon rank sum test

between *ApoA1* C83T and serum ApoB level, LDL/HDL ratio, and ApoB/ApoA1 ratio ($P = 0.044$, $P = 0.050$, and $P = 0.020$, respectively).

Discussion

Lipid metabolism abnormality,[4,5] hypercoagulability,[20] and vascular endothelial damage[21] have all been reported as causes of ONFH. Regarding lipid metabolism abnormality, it is considered that the transport of lipids from central to peripheral tissues is enhanced by elevated serum LDL/HDL ratio, and resultant fat embolism[4] leads to the inhibition of circulation within the bone marrow. Some studies have associated ONFH with serum ApoB/ApoA1 ratio and LDL/HDL ratio.[7,8] It is suggested that there are individual differences in the process of steroid-induced ONFH because an identical dose of steroid leads to ONFH in some patients and not in others. In this study, we investigated the relationship between lipid metabolism abnormality and ONFH using SNP analysis as a way to examine individual differences.

There was no significant difference between the allele frequencies of the four genes determined in this study and the genotype frequencies reported in previously published studies conducted in the Japanese population.[22–24] This result indicates that there was no bias in the genetic predisposition of the patient population in this study. In the subjects of this study, genotype frequencies of CT or TT for *ApoB* C7623T and GA or AA for *ApoB* G12619 were low at 8.3% and 7.7%, respectively. As well, the frequency of the CT or TT genotype for *ApoB* C7623T among the reference group and the frequency of the GA or AA genotype for *ApoB* G12619 among the case group were extremely low at 5.7% and 2.9%, respectively. Thus, the positive predictive value by PCR-RFLP or TaqMan-PCR methods is low and the possibility of false-positive results becomes relatively high. However, the magnitude of misclassification depends not so much on the positive predictive value of those test results but on sensitivity and specificity. Sensitivity and specificity are parameters that are less subject to the effect of the frequency of gene polymorphisms. Thus, the magnitude of misclassification, if any, is expected to be at the same level between the case and the reference groups; this is so-called nondifferential misclassification and produces a bias toward an underestimation of the relationship of interest, hence suggesting that the true exposure effect is stronger.

Although *ApoB* C7623T is a non-functional nonsense mutation that does not lead to any amino acid sequence changes, it is believed to be in linkage disequilibrium with other important coding regions.[25] Regarding the relationship to coronary artery disease, it is reported that people with genotype CC of *ApoB* C7623T are more susceptible and those with genotype TT are less susceptible despite their higher serum lipid levels.[9–11] Demant et al. reported that decreased activity of LDL receptor lowers metabolism of LDL, which makes its elimination from serum difficult in patients with *ApoB* C7623T genotype TT.[25] Boekholdt et al. reported that

the T allele of *ApoB* C7623T alters the structure of *ApoB*, increasing the serum level of LDL, which is less likely to cause arteriosclerosis.[17] An important result of this study was that for subjects with genotype TT or CT for the *ApoB* C7623T polymorphism we obtained an adjusted OR of 6.37 for ONFH. This finding suggested that subjects with genotype TT or CT for *ApoB* C7623T are 6.37 times as likely to have ONFH after renal transplantation as those without such a genotype, including accounting for the effects of sex, age, immunosuppressant, kidney, acute rejection, and steroid administration. No significant relationship was observed in this study between ONFH and *ApoB* G12619A or *ApoA1* C83T, both of which are associated with coronary artery disease.[9,14] There may be different pathological mechanisms involved in the process of steroid-induced ONFH compared with those in coronary artery disease.

Regarding the relationship between ONFH and serum apolipoprotein levels, Miyanishi et al. reported the relationship between ONFH and serum ApoB/ApoA1 ratio in a study of Japanese subjects.[7] In the current study, although no relationship was observed between ONFH and serum ApoB level, a significant relationship was also observed between ONFH and serum ApoB/ApoA1 ratio ($P = 0.045$). As a useful serological marker of cholesterol transport, the ApoB/ApoA1 ratio has been emphasized mainly in the field of ischemic heart disease. As it is reported that accumulation of lipids in the bone affects the development of ONFH,[26] the high serum ApoB/ApoA1 ratios observed in this study may have affected ONFH through increased transport of lipids to the periphery.

No significant relationship was observed between *ApoB* C7623T and lipid parameters in this study. No such relationship has been found in other studies that were conducted in Japanese,[27] African,[28] or Finnish[29] subjects, but significant relationships have been observed in European[10] and Brazilian[11] subjects. These results suggest that there may be racial differences in the relationship between *ApoB* gene polymorphisms and serum apolipoprotein or lipoprotein levels.

In this study, among patients with the C7623T polymorphism T allele, some (6 cases) developed ONFH and others (7 cases) did not. Among patients without the T allele, some (27 cases) developed ONFH and others (115 cases) did not. It is considered that multiple genetic and environmental factors, in addition to C7623T polymorphism, contribute to ONFH.[3] Through further investigation to establish the risk of ONFH before steroid administration, it should be possible to prevent steroid-induced ONFH by adjusting steroid doses or providing alternative medication for higher-risk patients.

We investigated the relationship of *ApoB* and *ApoA1* polymorphism, as well as lipid parameters, to steroid-induced ONFH in the Japanese population, and showed that the T allele of *ApoB* C7623T polymorphism and elevated serum ApoB/ApoA1 ratio are risk factors for ONFH. It is useful to analyze these factors before steroid administration to predict the risk of ONFH, and it should be possible to devise so-called tailor-made medicine based on this information for patients who need steroid treatment.

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