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# Analysis of Risk Factors Affecting Incidence of Osteoporosis and Fragility Fractures in Liver Transplant Recipients

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Study Design A  
Data Collection B  
Statistical Analysis C  
Data Interpretation D  
Manuscript Preparation E  
Literature Search F  
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**Background:** Fragility fractures caused by osteoporosis are common complications seen in recipients of organ transplantation who survive long term. Although many risk factors have been identified for osteoporosis after organ transplantation, none of them have been recognized as the main cause of development of the condition. Several studies have examined vitamin D receptor (*VDR*) gene single-nucleotide polymorphisms (SNPs) for their influence on bone mineral density (BMD) and fracture risk, but with variable results. We aimed to elucidate the risk factors that affect incidence of osteoporosis and fragility fractures in liver transplant recipients.

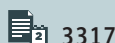
**Material/Methods:** In this study, we monitored incidence of fragility fracture and osteoporosis in 45 patients who had been evaluated with dual-energy X-ray absorptiometry (DXA) after liver transplantation. We also analyzed the association between *VDR* SNPs such as BsmI, Apal, FokI, and TaqI with osteoporosis and fracture incidence in 27 patients in our cohort in whom SNPs were evaluated and DXA performed after liver transplantation.

**Results:** Osteoporosis was diagnosed in 17 of 45 patients in whom BMD was measured after liver transplantation. Of the patients with osteoporosis, 15 (88.2%) subsequently had fragility fractures. The incidence of postoperative osteoporosis was significantly higher in the recipients who had alcoholic liver cirrhosis as their primary disease. Interestingly, there were significantly more patients with a homozygous BsmI GG genotype in the group diagnosed with osteoporosis.

**Conclusions:** Our study suggests that patients who undergo liver transplantation and have alcoholic liver cirrhosis or the BsmI GG genotype may be at increased risk for osteoporosis. Further research is necessary to confirm these findings.

**Keywords:** Liver Transplantation • Osteoporosis • Polymorphism, Single Nucleotide • Receptors, Calcitriol

**Full-text PDF:** <https://www.annalsoftransplantation.com/abstract/index/idArt/925475>



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## Background

Fragility fractures caused by osteoporosis are common complications seen in long-term survivors of organ transplantation. These fractures lead to a decrease in activities of daily living and often affect the prognosis of a transplant recipient [1]. A rapid decrease in bone mineral density (BMD) frequently occurs soon after transplantation and is accompanied by a marked increase in fracture probability. Various factors, including long-term use of steroids, aging, undernutrition, and hypogonadism, can predispose an individual to osteoporosis [2,3]. Although many risk factors for osteoporosis after organ transplantation have been identified, a single causative factor has not been identified in development of the condition.

In patients with end-stage cirrhosis who are awaiting liver transplantation, bone metabolism abnormalities are usually advanced. Liver dysfunction, which plays an important role in bone metabolism, promotes osteoporosis after liver transplantation. Therefore, it would be very useful if development of postoperative osteoporosis could be predicted before liver transplantation in patients with end-stage cirrhosis. It has been reported that BMD before transplantation does not predict fracture incidence after liver transplantation [4]. Studies of candidate genes and genome-wide association have identified single-nucleotide polymorphisms (SNPs) associated with osteoporosis incidence. SNPs in several genes have been shown to affect BMD [5]. Among the candidate genes, the vitamin D receptor gene (*VDR*), which encodes a nuclear hormone receptor, was the first to be proposed as the major locus for genetic control of BMD. *VDR* plays an important role in regulating calcium (Ca) homeostasis by binding the ligand 1,25-dihydroxycholecalciferol (1,25(OH)<sub>2</sub>D<sub>3</sub>), which increases Ca absorption [6]. Several SNPs within *VDR* have been associated with variations in BMD and fracture risk; therefore, *VDR* is considered a candidate for genetic regulation of bone strength and metabolism [7]. Several studies have examined *VDR* gene SNPs, such as BsmI (alleles B/b, SNP G>A, rs1544410), Apal (alleles A/a, SNP C>A, rs17879735), FokI (alleles F/f, SNP T>C, rs2228570), and TaqI (alleles T/t, SNP T>C, rs731236), for their influence on BMD and fracture risk [8,9]. Many studies have reported the effects of *VDR* SNPs on osteoporosis, but the results have varied, and no unified view about the roles of these SNPs has emerged so far [10,11]. Possible reasons for the variability in associations between *VDR* gene polymorphisms and osteoporosis include the ethnicity of the subjects, environment, primary disease, and lack of standardization and different size of the samples. In this study, we analyzed predictors of fragility fracture and osteoporosis in liver transplant patients whose BMD was measured and fracture incidence monitored after transplantation. We also assessed the relationship between fragility fractures and osteoporosis diagnosed by using T score and young adult mean (YAM), and clinical risk factors

for osteoporosis. Finally, we aimed to elucidate risk factors that affect the incidence of osteoporosis and fragility fractures in liver transplant recipients.

## Material and Methods

### Study Patients

The cohort for the present study consisted of 253 consecutive patients who underwent transplantation with a primary living or deceased donor liver at the Hiroshima University Hospital from June 1991 to December 2017. Of these patients, 45 recipients who had been randomly evaluated with dual-energy X-ray absorptiometry (DXA; Hologic QDR-1000) after their liver transplant were enrolled. In all cases, more than 1 year had passed since the liver transplantation. In detail, BMD measurement was performed in 21 patients within 1 to 2 years after transplantation; in 9, it was done 2 to 3 years later; and in 15 patients, BMD was measured 3 years later. In patients with and without osteoporosis or fragility fractures, age, sex, percentage of postmenopausal women, serum levels of bone metabolism markers before transplantation (Ca, phosphorus [P], intact parathyroid hormone [PTH]), body mass index (BMI) at the time of DXA, duration of steroid therapy after transplantation, percentage of individuals with cirrhosis due to hepatitis C virus (HCV), long-term use of diuretics (>1 month) after transplantation, and presence or absence of Type 2 diabetes mellitus (T2DM) were compared. Using peripheral blood-derived DNA collected before liver transplantation, we analyzed *VDR* SNPs in 141 recipients who gave consent for this genomic study. None of the patients in this cohort had undergone retransplant during the follow-up period. This study was approved by the Institutional Review Board of Hiroshima University (No. Hi-77) and the study protocol conformed to the ethics guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all subjects.

### DNA Extraction and *VDR* Genotyping

Genomic DNA was extracted from the recipients' peripheral blood mononuclear cells using the Wizard SV Genomic DNA Purification System (Promega Corporation, Madison, Wisconsin, United States) according to the manufacturer's protocol. DNA quality was determined by 1% agarose gel electrophoresis followed by staining with ethidium bromide. Purity of DNA was determined by measuring the optical density of the samples at 260 nm and 280 nm using a Nanodrop Analyzer spectrophotometer. Polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis was used to identify the *VDR* polymorphisms FokI (T/Crs2228570), BsmI (G/A rs1544410), Apal (G/T rs7975232), and TaqI (T/C rs731236), as previously described [12]. The primers and restriction enzymes used

**Table 1.** Primers and restriction enzymes used in the study.

Locus	Primers	
Fok1	Forward primer	5'-AGCTGG CCC TGG CAC TGA CTA TGC TCT-3'
	Reverse primer	5'-ATG GAA ACA CCT TGC TTC TTC TCC CTC-3'
Bsm1	Forward primer	5'AGTGTGCAGGCGATTCTAG3'
	Reverse primer	5'ATAGGCAGAACCATCTCTCAG3'
Apa1	Forward primer	5'-CAG AGC ATG GAC AGG GAG CAA G-3'
	Reverse primer	5'-GCA ACT CCT CAT GGC TGA GGT CTC A-3'
Taq1	Forward primer	5'CAA CCA AGA CTA CAA GTA CCG CGT CAG TGA3'
	Reverse primer	5'CAC TTC GAG CAC AAG GGG CGT TAG C3'

in this study are listed in **Table 1**. PCR was performed as follows: An initial denaturation cycle at 95°C for 3 minutes was followed by annealing for 30 cycles at 95°C for 30 seconds, incubation at primer-specific temperatures for 30 seconds and 72°C for 30 seconds, and the final 1-step extension at 72°C for 5 minutes. The restriction endonucleases FokI, BsmI, ApaI, and TaqI were used to digest polymorphic sites of the *VDR* gene. Enzyme-specific restriction reactions were performed for 5 minutes at 37°C for the BsmI, FokI, and ApaI enzymes and at 65°C for the TaqI enzyme.

### Diagnostic Criteria for Osteoporosis and Fragility Fracture

Osteoporosis was diagnosed by calculating the T score and measuring the YAM of BMD in the lumbar vertebrae of patients using DXA. Following the Japan Osteoporosis Society guidelines for the prevention and treatment of osteoporosis (2015 edition) [13], osteoporosis was defined in this study if a patient met 1 of the following 3 criteria: T score  $\leq$ -2.5 standard deviations (SD), YAM <70%, or YAM <80% with bone fracture. A fragility fracture was defined as a non-traumatic fracture caused by a slight external force, with the target fracture sites including the thoracic spine, proximal femur, rib, pelvis, proximal humerus, distal rib, and lower femur.

### Statistical Analyses

Data were reported as means $\pm$ SD or means $\pm$ standard error of mean for continuous variables and as frequencies (%) for categorical variables. The incidence of fragility fractures, diagnostic markers for BMD (T score and YAM), and clinical risk factors were compared in individuals with and without osteoporosis, using the *t* test for continuous variables and Pearson's chi-squared test for categorical variables. Relationships between each *VDR* SNP and osteoporosis incidence were analyzed using the Pearson's chi-square test whenever appropriate. Statistical analyses were performed with JMP Genomics statistical software, version 8 (SAS Institute, Cary, North Carolina, United States). All

**Table 2.** Characteristics of the patients in whom bone mineral density was assessed (n=45).

Mean age (year)		56.4
Sex	Male	23
	Female	22
Primary disease	HCC	19
	HCV	13
	HBV	6
	Alcohol	5
	PBC	3
	PSC	2
	AIH	2
	NASH	2
	Wilson	2
Others	6	

HCC – hepatocellular carcinoma; HCV – hepatitis C virus; HBV – hepatitis B virus; PBC – primary biliary cirrhosis; PSC – primary sclerosing cholangitis; AIH – autoimmune hepatitis; NASH – non-alcoholic steatohepatitis. \* Including multiple diseases.

*P* values presented are 2-sided. *P*<0.05 was considered statistically significant. A sample size of 20 evaluable patients was calculated, assuming an alpha level of 0.05 and a power of 80%.

## Results

### Relationship Between Osteoporosis and Fragility Fracture Incidence in Liver Transplant Recipients

**Table 2** lists the clinical characteristics of 45 participants. The average age was 56.4 years, with 23 men and 22 women. There

**Table 3.** Relationship between bone mineral density and fragility fracture. There was a significant difference in frontal or lateral T-scores and young adult mean between the patients with and without fragility fractures.

	With fracture n=19	Without fracture n=26	p Value
Lumbar Frontal T score	-2.56±1.02	-1.38±1.48	<0.01
Lumbar Frontal YAM	70.67±13.09	86.24±16.10	<0.01
Lumbar Lateral T score	-4.00±1.31	2.48±1.09	<0.01
Lumbar Lateral YAM	61.93±12.17	78.55±10.71	<0.01

**Table 4.** There were no significant differences between the groups with and without osteoporosis in bone metabolism marker abnormalities, sex, presence or absence of menopause, body mass index, hepatitis C virus-related disease, or duration of administration of steroids or diuretics. A difference was found in the incidence of fragility fracture due to osteoporosis only in the patients with alcoholic liver cirrhosis.

	With osteoporosis n=17	Without osteoporosis n=28	p Value
Incidence of fragility fracture (%)	88.2	14.3	<0.001
Age	56.6±9.2	56.2±11.6	0.919
Postmenopausal women (%)	29.4	50.0	0.175
Preoperative Ca (mg/dl)	9.5±1.0	9.4±0.6	0.609
Preoperative P (mg/dl)	3.6±0.8	3.2±0.7	0.178
Preoperative iPTH (pg/ml)	74.2±60.5	54.7±49.3	0.563
BMI (kg/m <sup>2</sup> )	23.7±3.7	22.5±3.2	0.28
Duration of steroid therapy after transplantation (month)	19.9±29.2	18.6±13.8	0.843
Liver cirrhosis(HCV) (%)	35.3	25.0	0.46
Liver cirrhosis (alcohol) (%)	23.5	3.8	0.039
Diuretics (more than 1 month postoperation) (%)	58.8	46.4	0.42
DM (%)	17.6	3.8	0.108

Ca – calcium; P – Phosphorus; iPTH – intact parathyroid hormone; BMI – body mass index; HCV – hepatitis C virus; DM – diabetes mellitus.

was no bias in the primary diseases. **Table 3** shows the relationship between diagnostic indicators of BMD (T score and YAM) and fragility fracture incidence. In both posterior-anterior and lateral views of the lumbar vertebrae, all of the indicators were significantly correlated with fracture incidence ( $P<0.01$ ). Fragility fractures were observed in 32 (12.6%) of the 253 liver transplant recipients in our hospital. Of the 45 patients who underwent DXA scan after liver transplantation, 17 were diagnosed with osteoporosis. Of them, 15 patients (88.2%) subsequently developed fragility fractures. In contrast, only 4 of 28 patients (14.3%) who did not meet the criteria for osteoporosis had fragility fractures, indicating that a diagnosis of osteoporosis positively correlated with subsequent fragility fracture incidence in liver transplant recipients (**Table 4**).

#### Clinical Risk Factors for Osteoporosis

We assessed the effects of several common clinical risk factors [14,15] on osteoporosis incidence in the 45 recipients examined with DXA after liver transplantation. There were no significant differences in age, percentage of postmenopausal women, bone metabolism markers (Ca, P, PTH), BMI, steroid exposure period, percentage of individuals with cirrhosis due to HCV, long-term use of diuretics, or T2DM between the patients with and without osteoporosis. However, incidence of postoperative osteoporosis was significantly higher in the transplant recipients who had alcoholic liver cirrhosis as their primary disease than in the other patients (23.5% vs 3.8%,  $P=0.039$ ) (**Table 4**).

**Table 5.** There were no significant differences between the groups with and without fragility fractures in bone metabolism marker abnormalities, sex, presence or absence of menopause, body mass index, hepatitis C virus-related disease, alcohol-related disease, or duration of administration of steroids or diuretics.

	With fracture n=19	Without fracture n=26	p Value
Incidence of osteoporosis (%)	78.9	7.7	<0.001
Age	55.8±10.2	56.7±11.2	0.771
Postmenopausal women (%)	26.3	53.8	0.065
Preoperative Ca (mg/dl)	9.4±0.9	9.4±0.6	0.95
Preoperative P (mg/dl)	3.2±0.9	3.4±0.5	0.274
Preoperative iPTH (pg/ml)	73.7±64.1	43.8±17.7	0.337
BMI (kg/m <sup>2</sup> )	23.3±3.9	22.7±3.1	0.591
Duration of steroid therapy after transplantation (month)	21.6±25.4	17.1±21.1	0.519
Liver cirrhosis(HCV) (%)	26.3	30.8	0.745
Liver cirrhosis (alcohol) (%)	21.1	3.8	0.07
Diuretics (more than 1 month postoperation) (%)	73.7	53.8	0.68
DM (%)	10.5	7.7	0.741

Ca – calcium; P – Phosphorus; iPTH – intact parathyroid hormone; BMI – body mass index; HCV – hepatitis C virus; DM – diabetes mellitus.

### Clinical Risk Factors for Fragility Fractures

We also assessed the effects of several common clinical risk factors on fragility fractures in the 45 recipients examined with DXA after liver transplantation. However, there were no significant differences in age, percentage of postmenopausal women, serum bone metabolism markers (Ca, P, PTH), BMI, steroid exposure period, percentage of individuals with cirrhosis due to HCV, long-term use of diuretics, or T2DM between the patients with and without osteoporosis (Table 5).

### Relationship Between VDR SNPs and Osteoporosis

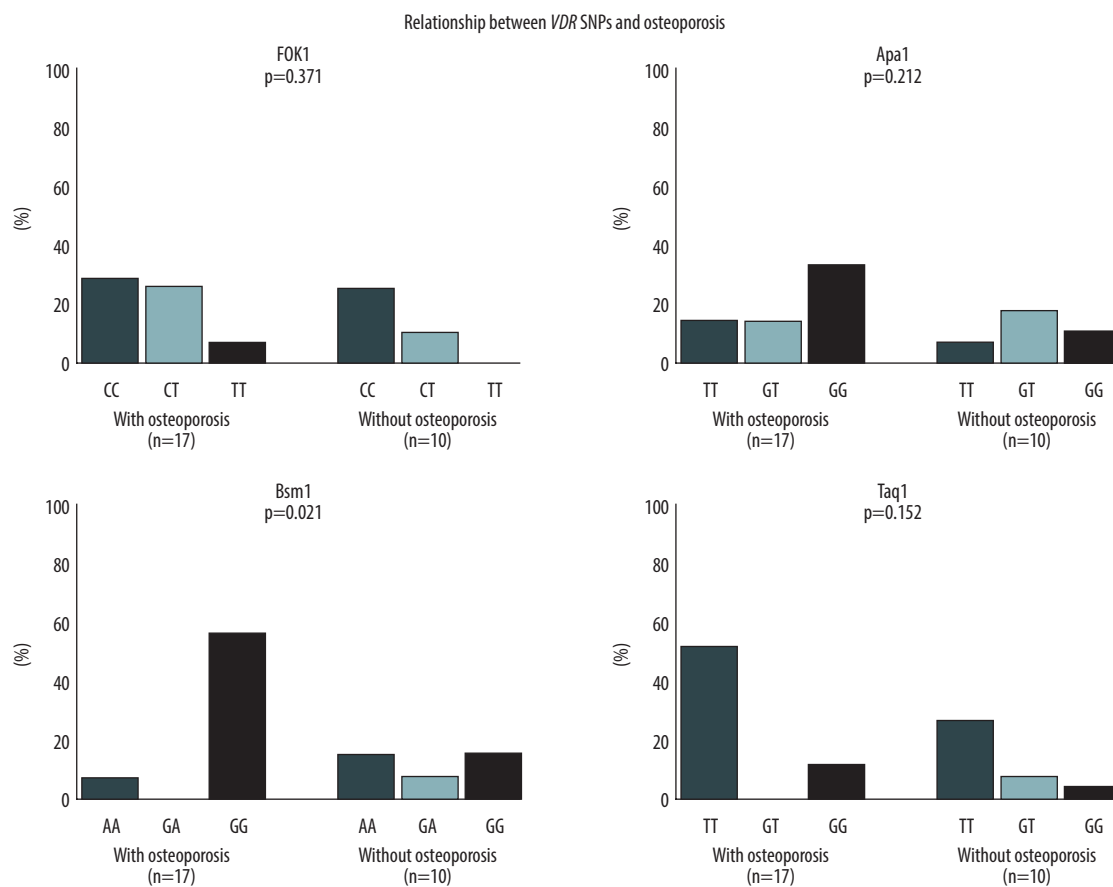
In 27 of 45 patients who underwent DXA after liver transplantation, 4 VDR SNPs (FokI, BsmI, ApaI, and TaqI) were studied. Seventeen of the 27 patients were diagnosed with osteoporosis. There were no significant allele differences in FokI, ApaI, or TaqI polymorphisms between the patients with and without osteoporosis (Figure 1). However, significantly more patients diagnosed with osteoporosis had the homozygous BsmI GG genotype, whereas no significant differences in BsmI alleles were observed in the non-osteoporosis group, indicating that liver transplant candidates with the BsmI GG genotype had an increased risk of osteoporosis ( $P=0.021$ , Figure 1).

### Relationship Between VDR SNPs and Fragility Fracture Incidence

The relationship between each VDR SNP and fragility fracture incidence is shown in Figure 2. In the 27 patients who underwent DXA after liver transplantation and who had fragility fractures after liver transplantation, each VDR SNP was analyzed. There were no significant differences in SNP polymorphism genotypes between patients with and without fragility fractures. In particular, the BsmI GG genotype, which was significantly associated with osteoporosis, did not correlate with fragility fracture incidence.

### Discussion

We first analyzed the relationship between osteoporosis and fragility fracture incidence in liver transplant recipients. The diagnosis of osteoporosis in the present study was positively correlated with subsequent fragility fracture incidence. We also found that having alcoholic liver cirrhosis as the primary disease was a clinical risk factor for osteoporosis after liver transplantation. Furthermore, VDR SNP analyses indicated that a BsmI GG genotype was associated with an increased risk of osteoporosis.



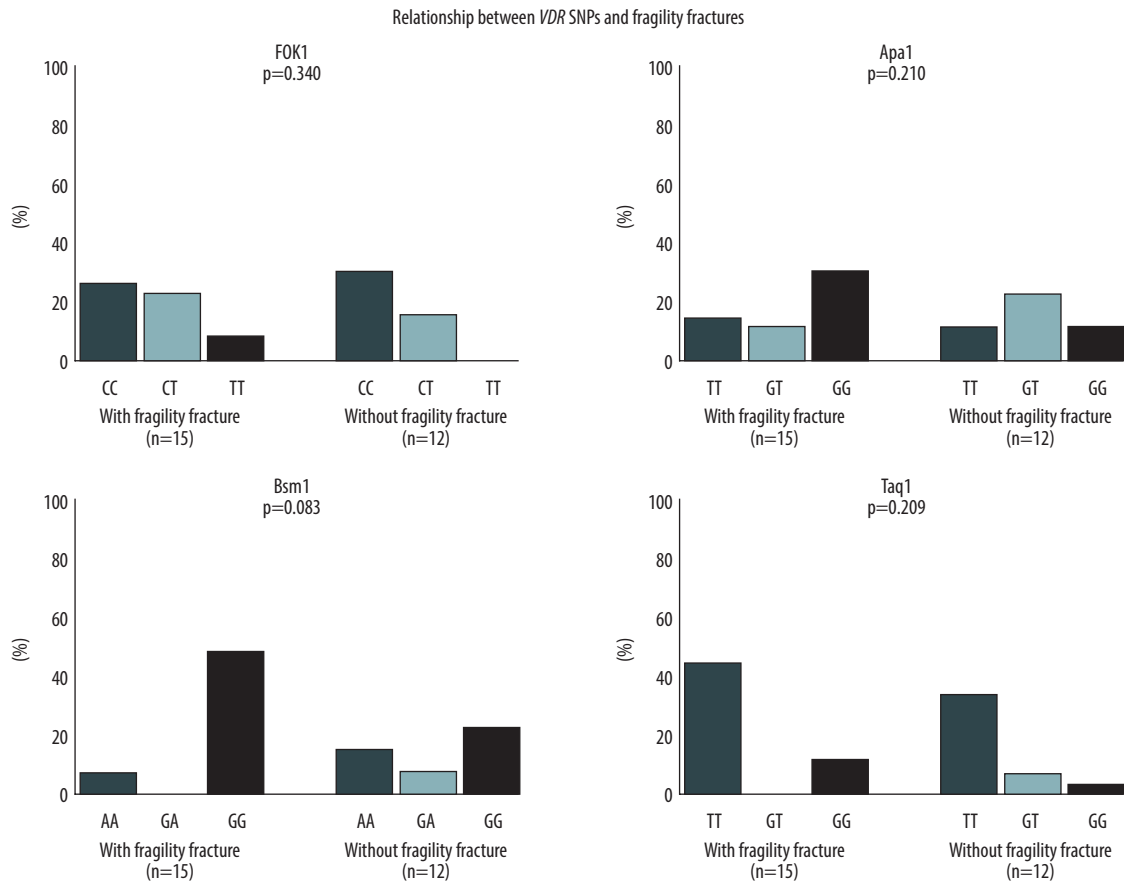
**Figure 1.** Both bone mineral density and single-nucleotide polymorphisms were measured in 27 patients. For Fok1, Apa1, and Taq1, there were no differences related to osteoporosis. However, there was a significant difference in incidence of osteoporosis associated with the VDR (Bsm1) GG group. \*  $P=0.021$ .

Recent medical advances have remarkably improved the survival rate of and prognosis for patients after organ transplantation. It is now necessary to improve management strategies for these individuals to reduce their risk of long-term complications. One such complication is the occurrence of fragility fractures after liver transplantation, which impair activities of daily life and thus may affect the prognosis for recipients who have overcome hepatic failure. Osteoporosis, defined as decreased BMD, is closely correlated with deterioration of bone tissue and disruption of bone microarchitecture; therefore, it could increase risk of fragility fractures [16]. Our data also showed a significant close relationship between osteoporosis and liver transplantation. In patients who have undergone liver transplantation, osteoporotic changes progress rapidly, due to undernutrition, loss of exercise, and/or decrease in physical strength, especially in the first year after surgery [17,18]. The recipients, therefore, remain at risk of subsequent fractures in the earlier postoperative period. Furthermore, similar osteoporotic changes also are seen in many patients with cirrhosis who are awaiting liver transplantation. Several reports

have shown, however, that preoperative BMD values in candidates for liver transplantation do not correlate with incidence of postoperative osteoporosis or fragility fracture [19,20]. Rapid postoperative bone mineral loss is an important risk factor for subsequent fragility fracture. Administration soon after liver transplantation of various drugs for osteoporosis, such as risendronate, denosumab, and ibandronate, reportedly is effective in suppressing the decrease in BMD [21-23].

Many studies have shown that pre-transplant bone disease and post-transplant exposure to immunosuppressants, particularly high-dose glucocorticoid therapy, are 2 major risk factors for osteoporosis after organ transplantation [24]. Glucocorticoids likely affect bone and mineral homeostasis indirectly, decreasing calcium absorption from the intestine and increasing renal excretion of calcium. Immunosuppressive drugs could also affect bone remodeling. Lan et al hypothesized about possible pathogenetic mechanisms of post-transplant osteoporosis and fractures caused by immunosuppressive agents such as glucocorticoids, calcineurin inhibitors, and mammalian target of





**Figure 2.** Both bone mineral density and single-nucleotide polymorphisms (SNPs) were measured in 27 patients. There were no differences related to fragility fracture for any of the SNPs.

rapamycin inhibitors [24]. Holm et al reported on a dynamic time trend in primary osteoporosis risk factors over 12 years since 2000. The prevalent risk factors included age, BMI, T2DM, major osteoporotic fracture history, calcium supplementation, and use of thiazide and high-dose prednisone [25]. In addition, many other studies have reported that primary or secondary osteoporosis can be triggered by smoking, alcohol overdose, estrogen deficiency, menopause, malnutrition, use of immunosuppressant therapy, neurodegenerative disease, secondary parathyroid hyperactivity, liver cirrhosis, and hematological disease. In our retrospective study, age, menopause, BMI, long-term glucocorticoid administration, T2DM, cirrhosis due to HCV, bone metabolism markers including PTH level, and long-term use of diuretics were not identified as risk factors. However, alcoholic liver cirrhosis was identified as a risk factor for osteoporosis. Heavy alcohol consumption has been associated with low BMD. González-Reimers et al demonstrated that alcoholism can cause decreased bone synthesis and/or increased bone breakdown [26]. In prospective studies, the risk of bone loss and fracture after liver transplantation was related to BMD and bone turnover was low in many patients with

liver failure. Patients with cirrhosis had a higher prevalence of vitamin D deficiency, and the levels were significantly lower in patients with a class C Child-Pugh score.

Vitamin D is an important factor for BMD metabolism. It potentiates intestinal absorption of Ca, PTH secretion, and bone turnover. These actions are mediated through *VDR*, which specifically binds 1,25-dihydroxyvitamin D<sub>3</sub> and regulates skeletal development, maintenance of skeletal architecture, hormone secretion, and immune function [27]. *VDR* belongs to the nuclear receptor superfamily and comprises 427 amino acids. The *VDR* gene is located on chromosome 12; it has 11 exons and is about 75 kb long. The protein is encoded by exons 2 to 8 [28]. In 1994, an analysis of genetic factors affecting BMD by Morrison et al revealed a *VDR* gene polymorphism that influenced gene cleavage by the restriction enzyme BsmI within the intron region between exons 8 and 9 [29]. The genotype of this *VDR* gene polymorphism significantly affected BMD; BMD was lowest in individuals with the genotype and highest in individuals with BB (uncleaved allele) and bb genotypes (cleaved allele), respectively [29]. Furthermore, Spector et al [30] detected

another polymorphism in exon 9 of *VDR*, identified by the restriction enzyme *TaqI*, and showed that a recognition site for *TaqI* was necessary for cleavage of allele T. BMD was lowest in individuals with the *tt* genotype and highest in individuals with the *TT* genotype, where it is the uncleaved allele. In addition, an association between polymorphisms in the sites cleaved by the restriction enzymes *Apal* [31] and *FokI* [32] and BMD also has been reported. Numerous studies subsequently have been conducted about the association between *VDR* polymorphisms and osteoporosis. However, the above-mentioned correlations were not reliably reproduced in these studies, as they depended on the race and age of the patients. Several recent meta-analyses also have failed to clearly show significant correlations between genotypes, especially those restricted by *BsmI*, and BMD [33-37]. Although many potential factors can account for these discrepancies, a major cause for the variation in these genotype frequencies is attributable to the different populations studied [38]. For example, the frequency of the A allele of the *BsmI* G>A polymorphism varies from 42% in White and 36% in African subjects to 7% in Asian and 1% to 8% in Japanese subjects [39-41]. Most of the previous reports from Japan showed that patients with the *BsmI* A allele type had lower BMD [39,40], which is in contrast to the present study's findings of significantly lower BMD in patients with the *BsmI* GG genotype. Inconsistent results may be due to differences in patient backgrounds. In addition, while our study was performed in liver transplant recipients who were on immunosuppressants, previous studies were conducted in patients who had not undergone transplantation. Dysfunction in intestinal absorption of nutrients necessary for bone metabolism (Ca, vitamin K) is commonly observed in liver transplant recipients, even in the postoperative recovery period. Thus, the differences in the present and previous studies may provide an explanation for the contradictory results. It had been reported that there were no obvious associations between any of the *VDR* genotypes and a decrease in BMD in patients on long-term corticosteroid therapy [41]. Matsuyama et al [42] reported that BMD clearly increased after treatment with 1 alpha-hydroxy vitamin D<sub>3</sub> in individuals with the *BsmI* GG genotype, but not in those with the GA or AA genotype. It has also been reported that patients with the *BsmI* A allele have significantly reduced ability to absorb calcium in their small intestine, and that the effect of *VDR* gene polymorphisms on BMD depends on calcium intake [43,44]. In liver transplant recipients perioperatively, marked reduction in vitamin K intake, reduction in intestinal microflora due to frequent antibiotic administration or transfer to non-vitamin K-producing bacteria, and biliary outflow disorder often are seen. Osteocalcin, a representative marker for osteogenesis, is activated by vitamin K to regulate bone metabolism [45]. Sergeev et al have demonstrated that gamma-carboxylation of *VDR* depends on vitamin K concentration [46]. In other words, the significantly lower BMD in transplant recipients with a *BsmI* GG genotype is

not surprising because of inadequate intake of dietary calcium, decrease in vitamin K intake, and lack of supplementation of vitamin D immediately after liver transplantation. Few reports have shown a relationship between *VDR* SNPs and BMD in the setting of organ transplantation, but Ozel et al reported that first-year BMD analysis after renal transplantation showed significant differences in femur BMD levels: BMD was higher in carriers of *BsmI* G, which is consistent with our results [47]. Thus, the recipients with a *BsmI* GG genotype may be at higher risk of osteoporosis after liver transplantation.

The present study was a retrospective analysis of BMD and *VDR* SNPs randomly measured in liver transplant recipients. It would be desirable to compare bone metabolism markers (Ca, P, PTH) and serum vitamin D and bone turnover marker levels at the time of DXA scanning. However, that was not done because not enough data had been collected at that point. Also, the total steroid dose after liver transplantation is much more important when discussing the relationship with osteoporosis. Because the doses could not be calculated accurately in some cases, the duration of steroid administration was compared. Because of these gaps in the data analyses, the study's conclusions are limited.

A prospective study with a large number of patients should be performed in the future to accurately identify risk factors for osteoporosis and fragility fracture after liver transplantation.

## Conclusions

In conclusion, BMD evaluation using DXA after transplantation may prove useful as a diagnostic tool that could predict risk of subsequent fragility fracture. Our analysis of clinical factors and relevant SNPs showed that postoperative osteoporosis in liver transplant recipients was more common in patients with alcoholic liver cirrhosis and in individuals with the *VDR* *BsmI* GG genotype. The *VDR* SNPs that affected the risks of osteoporosis and fragility fractures were not the same, but it is likely that a complex set of factors are involved in bone metabolism in recipients of liver transplants, such as exposure to immunosuppressants, nutritional status, and bone metabolism in the graft liver. Further investigation is necessary to assess the interplay between these factors.

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## Conflicts of Interest

None.



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