Mitochondrial DNA Copy Number in Peripheral Blood Is Associated with Femoral Neck Bone Mineral Density in Postmenopausal Women

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ABSTRACT. ***Objective.*** It has been suggested that mitochondrial dysfunction is related to aging and metabolic disorders. Yet there are few studies of the relationship between bone mineral density (BMD) and mitochondrial content in humans. We investigated the relationship between BMD and mitochondrial DNA (mtDNA) copy number in peripheral blood of postmenopausal women.

Methods. The study included 146 postmenopausal women. Enrolled subjects were taking no medications and had no disorders that altered bone metabolism. We measured BMD using dual-energy x-ray absorptiometry and leukocyte mtDNA copy number using real-time polymerase chain reaction. Anthropometric evaluations and biochemical tests were performed.

Results. Patients with osteopenia or osteoporosis had lower mtDNA copy numbers than normal subjects ($p < 0.0001$). Femoral neck BMD was negatively correlated with age ($r = –0.01$, $p = 0.04$) and with serum levels of adiponectin ($r = –0.22$, $p = 0.01$) and osteocalcin ($r = –0.31$, $p = 0.0001$). Serum levels of 25-OH vitamin D ($r = 0.32$, $p < 0.0001$) and mtDNA copy number ($r = 0.36$, $p < 0.0001$) were positively correlated with femoral neck BMD. Multiple regression analysis showed that mtDNA copy number ($ß = 0.156$, $p < 0.001$) was an independent factor associated with femoral neck BMD after adjustment for age, body mass index, waist circumference, waist-hip ratio, blood pressure, homeostatic model assessment of insulin resistance, high-sensitivity C-reactive protein, adiponectin, osteocalcin, homocysteine, lipid profiles, 25-OH vitamin D, and regular exercise. mtDNA copy number was not related to lumbar BMD.

Conclusion. Low mtDNA content in peripheral blood is related to decreased femoral neck BMD in postmenopausal women. Our findings suggest that mitochondrial dysfunction may be a potential pathophysiologic mechanism of osteoporosis in postmenopausal women. (First Release May 15 2012; J Rheumatol 2012;39:1465–72; doi:10.3899/jrheum.111444)

Key Indexing Terms:
BONE DENSITY | MITOCHONDRIAL DNA | OSTEOPOROSIS
POSTMENOPAUSAL | LEUKOCYTES

Osteoporosis is a medical problem and a major social concern worldwide because of the increasing elderly population[1]. In particular, osteoporotic fractures increase morbidity and medical expenditures in postmenopausal women[1,2]. Low bone mineral density (BMD) is well established as the best predictor of osteoporotic fracture as well as a major determinant of osteoporosis. However, the core pathophysiologic mechanisms of osteoporosis, a complex process involving numerous cellular components, cytokines, hormones, growth factors, nutrition, and several signaling pathways[1,3], have not been clearly elucidated. It has been reported that low BMD is associated with not only environmental factors such as oxidative stress[4,5] and inflammation[6] but also genetic factors such as family history of osteoporosis[7] and copy number variation (CNV) of the human genome[8]. Specifically, CNV of *UGT2B17* gene was associated with osteoporosis in Chinese subjects[8].

Mitochondrial dysfunction is related to the aging process. Reduced mitochondrial quality or content may be associated with several aging-associated disorders, including cancer[9], cardiovascular disease[10], type 2 diabetes[11], and metabolic syndrome[12]. Mitochondria play a crucial role in energy metabolism through the electron transport chain, a process that inevitably produces reactive oxygen species (ROS) as a byproduct. Mitochondrial DNA (mtDNA) is considered to be vulnerable to free radical attacks. Moreover, a specific mtDNA deletion is associated with oxidative stress in peripheral blood mononuclear cells (PBMC) of male patients with severe osteoporosis[13] and mtDNA variants in PBMC have

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been found to contribute to the development of osteoporosis[14]. These results suggest that osteoporosis may be related to mitochondrial dysfunction.

However, there have been few studies on the relationship between BMD and mitochondrial function in humans. It has been suggested that mtDNA content, measured by copy number, reveals mitochondrial gene stability and biogenesis and reflects mitochondrial function[15]. We investigated the relationship between BMD and mitochondrial function as assessed by mtDNA content in peripheral blood of postmenopausal women.

MATERIALS AND METHODS

Study subjects. Our observational cross-sectional study included 146 postmenopausal women aged 51–72 years who had not experienced vaginal bleeding for more than 1 year after their last menstruation and had not received hormone therapy. All participants were patients who visited the primary healthcare clinic of Chung-Ang University Hospital in Seoul for osteoporosis screening between October 2009 and May 2010. Subjects were excluded if they were taking any medications that alter bone metabolism, such as bisphosphonates, selective estrogen receptor modulators, tibolone, corticosteroids, antiretroviral agent, or estrogens, and those with histories of prior osteoporotic fractures, calcium or vitamin D supplementation, thyroid disorders, chronic renal disease, or cancer. All subjects completed a lifestyle questionnaire regarding alcohol consumption, smoking status, physical exercise, and age at menopause. Alcohol consumption was defined as consumption of 72 g or more of alcohol per week. Subjects who reported that they were smoking at the time of the study were considered to have a smoking habit. Regular exercise was defined as physical exercise performed for at least 30 min each session, more than twice a week, for more than 6 months. Data regarding past and current medical diseases and medications were collected from medical records. The Institutional Review Board of Chung-Ang University Hospital approved our study, and all subjects provided written informed consent.

Mitochondrial DNA copy number in peripheral blood. DNA from peripheral leukocytes was extracted from 1 ml whole blood using a commercial kit (Qiagen, Valencia, CA, USA). Relative mtDNA copy number was measured using real-time polymerase chain reaction (PCR) with the Light Cycler-Fast Start DNA Master SYBR Green I kit from Roche Molecular Biochemicals (Pleasanton, CA, USA). mtDNA quantity was normalized by simultaneous measurement of the nuclear gene ß-globin[16]. Forward and reverse primers for ß-globin were 5'-GAA GAG CCA AGG ACA GGT AC-3' and 5'-CAA CTT CAT CCA CGT TCA CC-3', respectively, and forward and reverse primers for the mitochondrial ND1 gene were 5'-AAC ATA CCC ATG GCC AAC CT-3' and 5'-AGC GAA GGG TTG TAG TAG CCC-3', respectively. After denaturation at 95°C for 300 s, DNA samples were subjected to 40 cycles of incubation at 95°C for 0.1 s, 58°C for 6 s, and 72°C for 18 s. The number of PCR cycles necessary to produce 20 ng of DNA product was defined as the threshold cycle number (Ct), and the mtDNA copy number was calculated using the following equation: relative copy number = $2^{\Delta Ct}$ ($\Delta Ct = Ct_{ß\text{-}globin} - Ct_{ND1}$).

Lumbar and femoral neck BMD. BMD of the lumbar spine (L1 to L4) and right femoral neck were measured by dual-energy x-ray absorptiometry (DEXA) using DEXXUM-T (Osteosys, Ltd., Seoul, Korea) and was expressed as grams of mineral per area (g/cm^2). DEXA quality assurance procedures were conducted every third day to ensure scanner reliability according to the guidelines of the manufacturer and maintenance checks were carried out by the manufacturer every quarter.

Classification of osteoporosis and osteopenia. T score at the femoral neck and lumbar spine was calculated by comparing the measured BMD with the mean peak BMD of a normal adult population of the same age[17]. Osteoporosis and osteopenia were defined as a BMD T score ≤ –2.5 or between –1.0 and –2.5, respectively, in either femoral neck or lumbar spine, according to the World Health Organization classification[18]. Lumbar spine T score was determined from the total spine BMD, calculated as the L1-L4 bone mineral content divided by the L1-L4 area.

Anthropometric and biochemical evaluation. One trained examiner conducted all anthropometric measurements. Body weight was measured to the nearest 0.1 kg with the subjects wearing light clothing and no shoes using an electronic scale. Height was measured to the nearest 0.1 cm using a stadiometer. Waist circumference was measured on standing subjects midway between the lowest rib and the iliac crest, and hip circumference was measured at the maximal protrusion of the greater trochanter. Body mass index (BMI) and waist-hip ratio (WHR) were calculated. Blood pressure was measured in the sitting position after a 10-min rest period.

Biochemical tests were performed on blood samples collected after overnight fasting (> 12 h). Serum levels of fasting glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-C), triglyceride, and high-sensitivity C-reactive protein (hs-CRP) were measured using an Advia 1650 Chemistry system (Siemens, Tarrytown, NY, USA). Low-density lipoprotein cholesterol (LDL-C) was calculated using Friedewald's formula [LDL-C = total cholesterol – HDL-C – (triglyceride/5)] if serum triglyceride level was < 400 mg/dl. Fasting insulin levels were measured by electrochemiluminescence immunoassay (Roche, Indianapolis, IN, USA) and insulin resistance was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR) index [(insulin (μlU/ml) × fasting blood glucose (mg/dl)/18)/22.5]. Serum osteocalcin and 25-OH vitamin D were measured using an electrochemiluminescence immunoassay (Roche). Plasma adiponectin levels were measured using an enzyme immunoassay kit (AdipoGen, Seoul, Korea) with inter- and intraassay variability of 4.63% ± 0.82% and 2.72% ± 0.52%, respectively.

Statistical analysis. Data are presented as mean ± SD or number (%). Variables such as triglycerides, adiponectin, and mtDNA copy number were log-transformed to approximate a normal distribution. Subjects were classified into normal, osteopenia, and osteoporosis groups according to BMD T score. Clinical characteristics were compared among the 3 groups with ANOVA for continuous variables and chi-square test or Fisher's exact test for categorical variables. Pearson's correlation coefficients were calculated to evaluate the relationships between lumbar or femoral neck BMD and clinical variables. Significance was defined at the 0.05 level. To confirm an independent association between mtDNA copy number in peripheral blood and BMD, a stepwise multiple linear regression analysis was performed to exclude the influence of potential confounding variables such as age, BMI, WHR, 25-OH vitamin D, osteocalcin, adiponectin, homocysteine, and hs-CRP. We defined potential confounding variables as those that showed associations with mtDNA copy number by Pearson's correlation analysis in this study or those shown to have relationships with mitochondrial function in past studies. Significance for entry into the model used the 0.15 level automatically determined in stepwise regression. All calculations were performed using the SAS 9.1 package (SAS Institute, Cary, NC, USA).

RESULTS

The mean age of the subjects was 57.40 ± 6.07 years, and the mean femoral neck and lumbar BMD were 0.918 ± 0.098 and 0.958 ± 0.174 g/cm^2, respectively. Among the 146 postmenopausal women, 54 (36.99%) and 36 (24.66%) were included in the osteopenia and osteoporosis groups, respectively. Table 1 shows the clinical characteristics among the 3 groups. There were significant differences among the groups in femoral neck BMD ($p < 0.0001$), 25-OH vitamin D ($p = 0.001$), and osteocalcin ($p = 0.001$), as well as mtDNA copy number ($p < 0.0001$). The numbers of current smokers (0.68%), alcohol drinkers (3.42%), and patients using antidia-

Table 1. Clinical characteristics of study subjects.

Characteristics	Normal†, N = 56	Osteopenia†, N = 54	Osteoporosis†, N = 36	p††
Age, yrs	56.63 ± 6.25	57.48 ± 5.55	58.67 ± 6.40	0.29
Body mass index, kg/m^2	24.87 ± 2.66	24.16 ± 2.19	24.15 ± 2.15	0.35
Waist circumference, cm	82.34 ± 6.38	81.93 ± 6.75	82.97 ± 7.89	0.78
Waist-hip ratio	0.85 ± 0.05	0.85 ± 0.05	0.86 ± 0.05	0.35
Blood pressure, mm Hg				
Systolic	123.70 ± 16.04	121.24 ± 12.13	125.11 ± 13.28	0.41
Diastolic	72.57 ± 10.10	71.81 ± 8.00	72.92 ± 8.80	0.83
Glucose tolerance index				
Fasting glucose, mg/dl	89.93 ± 21.73	88.83 ± 14.41	86.27 ± 19.94	0.66
Fasting insulin, μIU/ml	5.80 ± 4.94	5.93 ± 2.97	6.51 ± 6.47	0.77
HOMA-IR	1.45 ± 2.12	1.32 ± 0.76	1.46 ± 1.71	0.90
Lipid profile				
Total cholesterol, mg/dl	203.86 ± 32.52	206.51 ± 31.31	213.63 ± 39.32	0.40
Triglyceride*, mg/dl	108.48 ± 51.58	122.94 ± 83.41	159.67 ± 40.22	0.04
HDL-cholesterol, mg/dl	56.77 ± 12.26	55.02 ± 11.80	54.98 ± 10.94	0.72
LDL-cholesterol, mg/dl	125.39 ± 30.63	124.73 ± 31.51	126.64 ± 31.56	0.93
Inflammatory index				
hsCRP, mg/ml	0.15 ± 0.25	0.18 ± 0.37	0.22 ± 0.62	0.64
Adiponectin*, μg/ml	6.68 ± 5.30	5.74 ± 2.95	7.23 ± 4.05	0.24
Homocysteine*, μmol/l	12.82 ± 14.29	11.45 ± 2.72	11.44 ± 4.93	0.99
Bone metabolism index				
Femoral neck BMD, mg/cm^2	0.997 ± 0.062	0.912 ± 0.059	0.804 ± 0.071	< 0.0001
Lumbar BMD, mg/cm^2	0.962 ± 0.155	0.953 ± 0.171	0.961 ± 0.207	0.91
25-OH vitamin D, ng/ml	19.46 ± 9.76	12.91 ± 4.54	14.64 ± 9.10	0.001
Osteocalcin, ng/ml	10.85 ± 3.50	12.60 ± 4.00	14.04 ± 4.93	0.001
Mitochondrial DNA copy number*	1.24 ± 0.20	1.11 ± 0.18	1.09 ± 0.24	< 0.0001
Regular exercise**	20 (35.71)	20 (37.04)	11 (30.56)	0.43

Data are mean ± standard deviation or number (%). * Log-transformation to improve distribution. ** Physical exercise performed at least 30 min more than twice a week, more than 6 months. † Normal: T score ≥ –1.0; osteopenia: –2.5 < T score < –1.0; osteoporosis: T score ≤ –2.5. †† ANOVA or chi-square test. HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein.

betes (1.37%) or antihypertensive agents (10.27%) were extremely low in all subjects and not significantly different among the 3 groups.

Table 2 shows the associations between femoral neck BMD or lumbar BMD and measured variables. Femoral neck BMD was negatively correlated with age (r = –0.01, p = 0.04) and with serum levels of adiponectin (r = –0.22, p = 0.01) and osteocalcin (r = –0.31, p = 0.0001), and positively correlated with serum levels of 25-OH vitamin D (r = 0.32, p < 0.0001). The relationship between mtDNA copy number and femoral neck BMD (r = 0.36, p < 0.0001) and lumbar BMD (r = –0.07, p = 0.42) is shown in Figure 1.

Table 3 shows the independent associations between femoral neck BMD and mtDNA copy number. The multivariate model explained 13% of the variance of femoral neck BMD by mtDNA copy number (ß = 0.156, p < 0.001), 8% by serum adiponectin (ß = –0.047, p < 0.001), 4% by osteocalcin (ß = –0.004, p = 0.01), and 2% by the level of HDL-C (ß = 0.001, p = 0.07) in stepwise multiple regression analysis that included age, BMI, waist circumference, WHR, systolic and diastolic blood pressure, HOMA-IR, hs-CRP, homocysteine, total cholesterol, LDL-C, triglyceride, 25-OH vitamin D, and regular exercise. However, mtDNA copy number was not related to lumbar BMD. Instead, WHR (ß = 0.491, p = 0.07) and osteocalcin (ß = –0.006, p = 0.10) were independent variables associated with lumbar BMD.

DISCUSSION

In this cross-sectional study of postmenopausal women, we demonstrated a significant positive relationship between mtDNA copy number in peripheral blood and femoral neck BMD that was independent of age, obesity, inflammatory markers, bone metabolism-related indices, and exercise.

It has been reported that femoral neck BMD or its changes are related to diverse metabolic indicators such as BMI[19], muscle mass[20], lipid profile[21], insulin resistance[22], metabolic syndrome[23], and circulating adiponectin[24], estrogen[25], or osteocalcin[25,26]. Further, a relationship between low bone mass and cardiometabolic disorders has consistently been proposed. Initially, this was merely regarded as concurrence related to senescence. However, independent of age and traditional risk factors, low BMD was consistently found to be associated with cardiovascular or metabolic disease in a large number of studies[27,28,29]. Despite a relatively clear epidemiologi-

Table 2. Correlation between bone mineral density and other variables.

Variables	Femoral Neck BMD		Lumbar BMD	
	r	p	r	p
Age	–0.01	0.04	–0.11	0.18
Body mass index	0.04	0.67	0.09	0.30
Waist circumference	–0.03	0.70	0.09	0.29
Waist-hip ratio	0.01	0.90	0.14	0.10
Systolic blood pressure	–0.02	0.78	–0.003	0.97
Diastolic blood pressure	–0.04	0.63	0.06	0.50
Fasting glucose	0.14	0.10	0.14	0.08
Fasting insulin	0.01	0.92	–0.03	0.72
HOMA-IR	0.06	0.44	0.01	0.90
Total cholesterol	0.06	0.49	0.03	0.72
Triglyceride*	–0.08	0.36	0.01	0.95
HDL-cholesterol	0.09	0.26	0.07	0.43
LDL-cholesterol	0.07	0.37	0.01	0.95
hsCRP	–0.09	0.26	–0.01	0.86
Adiponectin*	–0.22	0.01	–0.05	0.52
Homocysteine*	0.06	0.46	–0.14	0.10
25-OH vitamin D	0.32	< 0.0001	–0.07	0.41
Osteocalcin	–0.31	0.0001	–0.14	0.10

Coefficients (r) and p values calculated by Pearson correlation method. * Log-transformation to improve distribution. HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein.

cal connection between these factors, the underlying common pathophysiology has not been fully elucidated.

Recently, a large number of studies have shown that low mtDNA copy number in peripheral blood is correlated with mitochondrial-related metabolic disorders or conditions such as insulin resistance[30,31], glucose dysregulation[32], nonalcoholic fatty liver disease[33], elevated homocysteine levels[34], hyperlipidemia[35], and cancers[36,37,38]. Therefore, the mtDNA content of peripheral blood can be used as a surrogate marker of numerous metabolic diseases that are related to mitochondrial dysfunction. Production of ROS and inflammatory cytokines in the PBMC can be elevated in numerous conditions related to oxidative stress, including obesity[39], diabetic nephropathy[40], Parkinson's disease[41], and vitiligo[42]. Mitochondria are a major source of ROS, and ROS-mediated oxidative damage of the mitochondria themselves elevates ROS production. Oxidative injury induces a decrease in mtDNA content in various tissues and oxidative stress levels in folate-deficient liver are inversely correlated with liver mtDNA content[43]. Excessive ROS production induced low mtDNA copy number in arsenic trioxide-treated oocytes[44] and in the muscle of hyperglycemic streptozotocin-treated mice[45]. In addition, oxidative stress can lead to osteoporosis[4,5]. It has been reported that ROS considerably influence the generation and survival of osteoclasts, osteoblasts, and osteocytes through various mechanisms involving FoxOs, Wnt/ß-catenin, and peroxisome proliferator-activated receptor-γ[46]. Therefore, oxidative stress may play an important role in the relationship between low leukocyte mtDNA content and low bone mass.

However, recent studies demonstrated the redundancy of mtDNA through the stimulation of mitochondrial biogenesis as a compensating mechanism against increased mitochondrial damage induced by oxidative stress[47]. Further, under the same metabolic compromise, tissue-specific differences in mtDNA copy number were also reported[48]. In the latter study, muscle mtDNA copy number in nondiabetic individuals was higher than that in diabetics, whereas leukocyte mtDNA copy number was lower in nondiabetics than in diabetes. These results were explained as being related to characteristics of the

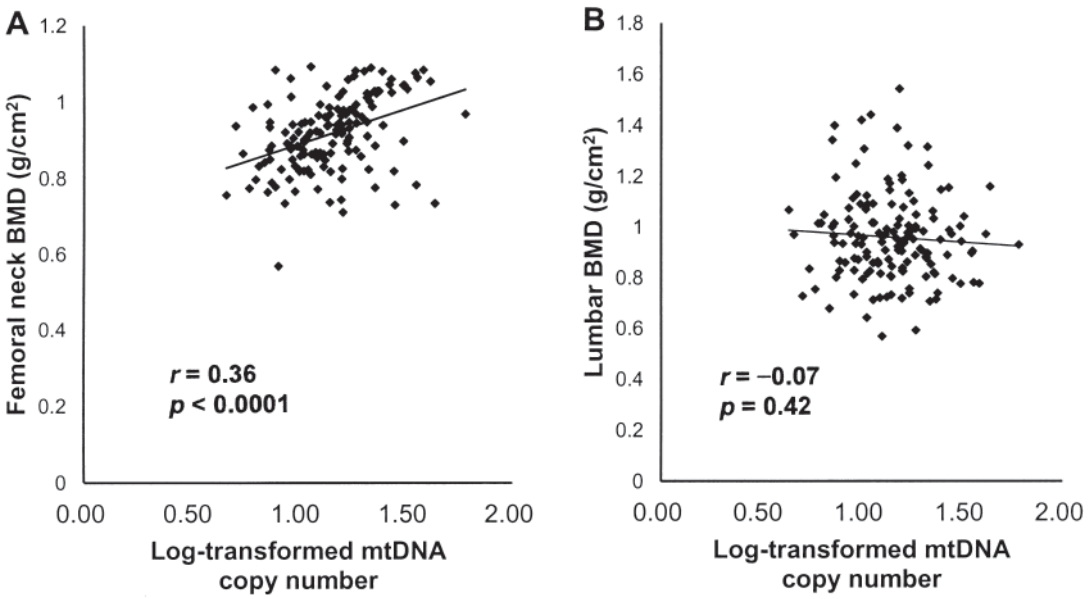

Figure 1. Correlations between log-transformed mitochondrial DNA copy number and femoral neck BMD (A) or lumbar BMD (B). mtDNA: mitochondrial DNA; BMD: bone mineral density.

Table 3. Stepwise multiple linear regression analysis to identify independent clinical variables associated with bone mineral density.

Variables	ß	Standard Error	F Value	p
Femoral neck BMD				
Mitochondrial DNA copy number*	0.156	0.035	21.21	< 0.001
Adiponectin*	–0.047	0.013	13.48	< 0.001
Osteocalcin	–0.004	0.002	6.88	0.01
HDL-cholesterol	0.001	< 0.0001	3.38	0.07
Lumbar BMD				
Waist-hip ratio	0.491	0.27	3.28	0.07
Osteocalcin	–0.006	0.003	2.72	0.10

All variables left in the model are significant at the 0.15 level; no other variable met the 0.15 level for entry into the model. R^2 for femoral neck BMD and lumbar were 0.26 and 0.04, respectively. Mitochondrial DNA copy number was independent variable and regression coefficients (ß) were adjusted for age, BMI, waist circumference, systolic and diastolic blood presure, HOMA-IR, hsCRP, homocysteine, total cholesterol, LDL-cholesterol, triglyceride, 25-OH vitamin D, and regular exercise. BMI: body mass index; BMD: bone mineral density; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; LDL: low-density lipoprotein. * Log-transformation to improve distribution.

cells such as in mitotic (leukocytes) or postmitotic (muscle) cells. However, in endothelial cells, which are mitotic cells, exogenous and endogenous ROS in mitochondria increased mtDNA damage and decreased mitochondrial RNA transcription levels and mitochondrial protein production[49]. In addition, although mtDNA copy number in neurons[50], muscle cells[51], and leukocytes[52] showed a positive correlation with age until middle age, thereafter copy number was negatively correlated with age, as oxidative stress is generally elevated with aging. Further, in hemodialysis patients, increased mtDNA copy number in PBMC was associated with a significant decrease in all-cause mortality[52]. In our study, there was no significant relationship between leukocyte mtDNA copy number and age ($r = –0.21$, $p = 0.17$). Thus, further research is needed to clarify the relationship between oxidative stress and mtDNA biogenesis.

BMD was also highly heritable at different sites in a twin and family study in Koreans[53]. It was recently suggested that CNV of *UGT2B17* may be related with osteoporosis and hip osteoporotic fracture as a genetic factor[8]. CNV indicates a DNA segment of 1 kb or larger that is present in different copy numbers compared to a reference genome, and has been shown to contribute about 18% of the variation in gene expression[8]. It has been reported that CNV reflecting a structural genomic variation are associated with several complex human disorders and conditions[8]. Also, the mtDNA copy number of peripheral blood showed high heritability (65%)[54]. Therefore, the possibility cannot be excluded that quantitative mtDNA variation is genetically involved in mtDNA gene expression, mitochondrial function, and multifactorial human diseases. In particular, the mitochondrial ND1 gene used for primers for genotype mtDNA copy number in our study was also used in the previous study[54] that examined the association between mitochondrial DNA content and heritability. Mitochondrial DNA can be easily damaged by ROS because of the absence of protective histone, reduction of the antioxidative system, and physical proximity to the ROS origin[55]. Mitochondrial DNA copy number can represent both the microenvironmental and genetic factors. Emerging evidence has suggested that development of osteoporosis can be related with mtDNA[13,14,56]. A specific deletion or common variants of mtDNA are associated with severe male osteoporosis[13] or development of osteoporosis[14] in PBMC. Further, BMD, bone mineral content (BMC), and BMC/length in the femur were all decreased in mutator mice of the catalytic subunit of mtDNA polymerase, needed in mtDNA synthesis, at the age of 40 weeks with an increased mtDNA mutation[56]. Although it has been reported that these genetic background factors of mtDNA influence the osteoporosis, a focused molecular mechanism between them has not yet been investigated.

Unexpectedly, mtDNA copy number was not connected with lumbar BMD. We cannot exactly understand the reason for and clinical significance of this finding. However, genetic influences may be lower in lumbar than in femoral neck BMD among females. Whereas Korean males showed the highest heritability (0.76) in lumbar BMD, the heritability of lumbar BMD (0.66) was relatively low compared to pelvic BMD (0.78) in females[53]. In both sexes, lumbar BMD explained only 17% of total variance of BMD in the genetic study[53]. This value was the lowest among variances of other sites examined. And presence of lumbar osteoarthritis can lead to overestimation of BMD measured[57]. Further, it has been reported that there may be differences not only in risk factors but also in potential pathophysiologic factors between low femoral neck and lumbar BMD[24,57]. Further investigation is needed of the underlying mechanisms of the differential findings between mtDNA copy number and femoral neck/lumbar BMD.

Postmenopausal status is related to an increased risk of fracture, and most fractures occur in postmenopausal women[58]. Estrogen deficiency is believed to play a role in var-

ious metabolic and endocrinological conditions in postmenopausal women compared with premenopausal women. Indeed, estrogen is known to exert direct and/or indirect effects on mitochondrial function, including stabilization of the mitochondrial membrane, increased mitochondrial and nuclear transcription, and activation of intracellular signaling proteins[59]. Increasing prevalence and risk of fracture could be attributed to decreased mitochondrial function and/or biogenesis due to estrogen deficiency in postmenopausal women compared with premenopausal women, although this remains to be confirmed.

We evaluated diverse variables that might reveal mechanisms underlying the connection between bone and cardiometabolic disorders, including serum levels of osteocalcin, homocysteine, and adiponectin. mtDNA copy number in peripheral blood was independently and positively correlated with femoral neck BMD. Thus, mitochondrial biogenesis may act on common mechanisms underlying the pathophysiology of osteoporosis and cardiometabolic disorders. Unlike other adipocytokines, adiponectin favorably influences energy homeostasis and the chronic inflammatory reaction[60]. Adiponectin also acts positively on bone cells, including stimulation of osteoblast proliferation and inhibition of osteoclastogenesis[61]. However, cross-sectional studies and some large prospective studies have consistently shown that circulating adiponectin levels were inversely associated with BMD[24], consistent with our results. Further, a recent large cohort study reported that serum adiponectin level is associated with increased hip BMD loss in elderly women[24]. Recent results suggest that the maintenance of mitochondrial function is associated with adiponectin[62]. However, mitochondrial biogenesis was not related to serum adiponectin in the liver of adiponectin knockout mice[63]. In our study, we found that plasma adiponectin levels were significantly and inversely correlated with femoral neck BMD and were not associated with mtDNA content in peripheral blood ($r = 0.14$, $p = 0.08$).

Our study has some limitations. We did not examine mitochondrial biogenesis in skeletal muscle, which is generally accepted as the "gold standard" for evaluation of mitochondrial function. However, it has been suggested that the mtDNA content of peripheral blood may reflect that of muscle and liver tissue in rats[64]. Moreover, measurement of mtDNA content is most reliable in the target tissue, as mtDNA content differs between organs. The number of mitochondria, from single to several thousand, in a cell varies widely by organism and tissue type[54]. Each mitochondrion is estimated to contain 2–10 mtDNA copies[54]. It has been reported that mtDNA copy numbers per PBMC, myocyte, neuron, and hepatocyte are 223~854, 1075~2794, 1200~10,800, and up to 25,000, respectively[54]. To elucidate the relationship between mitochondrial dysfunction and osteoporosis, further studies in bone marrow, osteoblasts, osteoclasts, and osteocytes are needed. Next, we did not examine circulating estradiol levels in the subjects. However, because we enrolled women who had been postmenopausal for at least 1 year, serum estradiol levels may not have been that different among subjects. It is also known that there is only a weak association between serum estradiol level and rate of bone turnover in postmenopausal women[17]. In addition, we did not measure the levels of oxidative stress and therefore could not directly investigate the role of oxidative stress as a mediator between mtDNA copy number in peripheral blood and low BMD. As well, it is difficult to identify causation and mechanisms underlying the relationships between mitochondrial biogenesis and low femoral neck BMD with a cross-sectional study design. Finally, the study was not community-based and suffers from potential selection bias.

Our study showed that low mtDNA content in peripheral blood is related to decreased femoral neck BMD in postmenopausal women. Our findings suggest that mitochondrial dysfunction is a potential pathophysiologic mechanism of osteoporosis in postmenopausal women.

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