The influence of sclerostin-Wnt signaling on bone and mineral metabolism during lactation and weaning

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Directed by Professor Yumie Rhee

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"*Ask, and it shall be given you;*
Seek, and ye shall find;
Knock, and it shall be opened unto you"
(Matthew 7:7)

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TABLE OF CONTENTS

ABSTRACT ····· 1
I. INTRODUCTION ····· 4
II. MATERIALS AND METHODS ····· 6
1. Whole exome sequencing ····· 6
2. Animals ····· 7
3. Dual-energy X-ray absorptiometry (DXA) ····· 8
4. Microcomputed tomography ····· 8
5. Serum biochemical parameters ····· 9
6. Statistical analysis ····· 9
III. RESULTS ····· 10
1.Whole exome sequencing data of subjects in pregnancy and lacation-associated osteoporosis (PLO)
1-1. Clinical characteristics of 12 women ····· 10
1-2. Candidate PLO genes ····· 10
1-3. Candidate PLO-associated pathways ····· 14
2. Effect of lactation
2-1. Bone density and microarchitectures ····· 17
2-2. Serum sclerostin level ····· 20
2-3. Bone turnover markers ····· 20
2-4. Correlation between parameters during lactation ····· 21
3. Effect of duration of lactation
3-1. Bone density and microarchitectures ····· 23
3-2. Serum sclerostin level ····· 25
3-3. Bone turnover markers ····· 25

4. Effect of duration of lactation

4-1. Bone density and microarchitectures ····· 26

4-2. Serum sclerostin level ····· 28

4-3. Bone turnover markers ····· 31

5. Effect of genotype

5-1. Bone density and microarchitectures ····· 31

5-2. Serum sclerostin level ····· 33

5-3. Bone turnover markers ····· 33

IV. DISCUSSION ····· 37

V. CONCLUSION ····· 42

REFERENCES ····· 43

ABSTRACT (IN KOREAN) ····· 47

LIST OF TABLES

Table 1. Clinical characteristics of 12 patients ·····11
Table 2. 23 known “bone”,“bone mineral density”, “osteopo-” gene mutations ·····12
Table 3. Differences in femoral μCT phenotypes by lactation at 1-week ·····18
Table 4. Differences in femoral μCT phenotypes by lactation at 3-week ·····19
Table 5. Correlation between sclerostin and bone turnover markers and measures of bone mass and structures in lactating mice ·····22
Table 6. Differences in femoral μCT phenotypes by duration of lactation in wild type and DMP1-Sost mice ·····24
Table 7. Differences in femoral μCT phenotypes between 3-week lactation and weaning ·····29
Table 8. Microstructures in μCT at 2-week time after weaning compared to age-matched non-lactating mice in both genotype ·····30
Table 9. Microstructures in femoral μCT phenotype by genotype ·····32
Table 10. Serum sclerostin level at each time ·····33
Table 11. Serum bone turnover markers at each time ·····34

LIST OF FIGURES

Fig. 1. Whole exome sequencing methods ·····6
Fig. 2. Study design ·····7
Fig. 3. Pathway approach for candidate PLO gene ·····15
Fig. 4. Common, low, and rare variants in LRP5/6 and SOSTDC1 gene in our patients ·····16
Fig. 5. Effect of lactation at 1 and 3 week on BMD ·····17
Fig. 6. Effect of lactation on serum sclerostin ·····20
Fig. 7. Effect of lactation on bone turnover markers ·····21
Fig. 8. Effect of duration of lactation on BMD ·····23
Fig. 9. Effect of duration of lactation on serum sclerostin ·····25
Fig. 10. Effect of duration of lactation on bone turnover markers ··26
Fig. 11. Effect of weaning (3 week lactation vs. weaning) ·····26
Fig. 12. Effect of weaning (age-matched non-lactating vs. weaning) ·····27
Fig. 13. Effect of weaning on serum sclerostin ·····28
Fig. 14. Effect of weaning on bone turnover markers ·····31
Fig. 15. Effect of genotype on BMD and BMD ·····31
Fig. 16. Comparisons in spine and femur BMD during lactation and weaning period ·····35
Fig. 17. 3D reconstruction of distal and midshaft femur during lactation and weaning period ·····36

ABSTRACT

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Lactation is a state of rapid bone loss for milk production; however, the maternal bone undergoes a rapid remineralization after weaning. Sclerostin, encoded by the gene SOST, is exclusively secreted from osteocytes and has important regulator of bone remodeling by inhibiting Wingless and Int-1 (Wnt) signaling by binding to extracellular domain of low-density lipoprotein receptor-related protein 5/6 (LRP5/6). However, the role of sclerostin-Wnt signaling for controlling bone remodeling during lactation and weaning has not been studied. In this study, we hypothesized that sclerostin related with Wnt signaling would affect bone metabolism and microstructures during lactation and weaning, and pregnancy and lactation associated osteoporosis (PLO) would

be related with mutation of Wnt signaling pathway. We used whole-exome sequencing (WES) to reveal associations between genetic variants and PLO patients with multiple vertebral fractures. We also used dual energy X-ray absorptiometry (DXA), micro-computed tomography (μCT) to evaluate and compared the effect of sclerostin on bone mass and microarchitectures of wild type and DMP1-Sost transgenic mice, in which sclerostin is overexpressed by osteocyte. As a result, WES showed 8 out of 12 patients showed *LRP5/6* mutation encoding the extra-cellular signaling domain of LRP5/6, which sclerostin or Wnt signaling agonists bind to. In animal study, lactation significantly lead to decreased spine and femur bone mineral density (BMD) at 1-week and 3-week of lactation; especially cortical microstructure (cross-sectional thickness and cross-sectional area) at mid-shaft femur were significantly deteriorated. At 2-week after weaning, incomplete recovery of femur BMD and cortical microstructure at mid-shaft femur in both wild and DMP1-Sost mice; however, spine BMD and trabecular microstructures at distal femur were recovered only in wild type mice, suggesting that sclerostin-overexpression would affect bone formation, especially in trabecular dominant bone compartment during weaning period. In conclusion, *LRP5/6* mutations related with Wnt pathway, would be one of the plausible risk factors of failure of maternal bone mineral adaptation to severe bone loss during lactation and weaning contributing to PLO. To activate Wnt signaling pathway during lactation and after weaning for compensation of bone loss, control of

sclerostin level by osteocytes would be important to maintain bone mineral metabolism.

Key Words: Wnt signaling pathway, DMP1-hSOST transgenic mice, sclerostin, LRP5/6, whole exome sequencing, pregnancy lactation associated osteoporosis

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I. INTRODUCTION

Pregnancy and lactation are associated with a change of maternal bone and mineral metabolism[1,2]. To meet fetal and neonatal demands during pregnancy and lactation without requiring increased intakes of calcium, calcium inevitably comes from the maternal skeleton[3]. This results to deterioration of bone mass in the mother. Normally, after weaning, maternal skeleton undergoes to regain mineralization[1] and lactational loss of bone density is completely reversed within one year in most women[4-6]. Rarely, a failure of maternal adaptations in mineral and skeletal homeostasis in a period of pregnancy, lactation, and weaning, could induce pregnancy- and lactation-associated osteoporosis (PLO), presented as vertebral compression fractures[7-11]. Since PLO is a rare condition, and there is no rationale to screen bone mineral density of young pregnant women during a peripartum period without previous fracture, to evaluate and find the etiology of PLO is very difficult[12].

Lactation is a state of rapid bone loss for milk production. Bone turnover is markedly increased during lactation and causes net resorption[13]. To maintain the

bone homeostasis, bone resorption occurs by osteoclast-mediated bone resorption and osteocytic osteolysis[14-17]. Osteoclast-mediated bone resorption was mainly known as a mechanism by which bone is resorbed to release calcium during lactation; however, osteocytic osteolysis has been recently revealed as potential mechanism by which the mineral is mobilized from cortical bone, the potential for mineral bone suggesting important role of osteocyte in pregnancy. Osteocytes have been also revealed to have important role of actively removing mineralized tissue during and replacing it during the recovery period after weaning[18-20].

Sclerostin, encoded by the gene SOST, is secreted in bone by mature osteocytes. Sclerostin binds to low-density lipoprotein receptor-related protein 5/6 (LRP5/6), inhibiting osteoblastic bone formation by canonical Wnt pathway [21]. While there is no question that sclerostin has a central role in bone biology, the impact of sclerostin on bone metabolism during lactation and weaning has not been well studied yet. So, we hypothesized that sclerostin related to Wnt signaling is essential for maintaining maternal bone mass and microarchitectures in lactation and weaning [21].

To demonstrate the relation of to Wnt signaling and sclerostin in pregnancy- and lactation – associated osteoporosis (PLO), we evaluated 1) the gene mutations related to Wnt signaling pathway in our PLO women by whole-exome sequencing; 2) the impact of sclerostin on bone density and microarchitecture during lactation and weaning periods by using DMP1-Sost transgenic mice, in which sclerostin is overexpressed by osteocytes.

II. MATERIALS AND METHODS

1. Whole exome sequencing

We enrolled 12 women who had vertebral fractures with pregnancy and lactation osteoporosis and analyzed whole exome sequencing (WES). WES was done by SureSelect v4+UTR (71Mbp region). Sequencing data was processed, ingle nucleotide polymorphism, indel were found by HaplotypeCaller. After quality control and filtering, variants on candidate PLO genes were screened. After processing the sequencing data, single nucleotide variations (SNV), short insertion or deletions (Indels) were discovered. Silent variants and coding non-silent variants were removed after quality control. We used candidate gene analysis. First, “Bone”, “bone mineral density” and “osteopo-” related gene-mutations based on human gene-mutation database (HGMD)[22] were matched to our samples. Second, Osteoporosis, Wnt pathway, pregnancy related genes were chosen (Fig. 1).

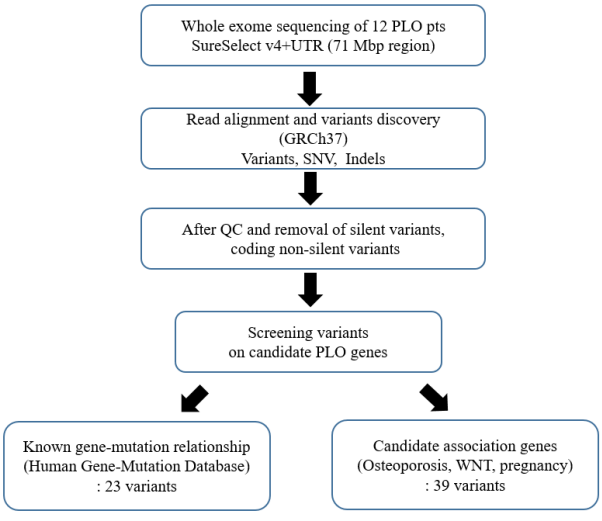


Figure 1. Whole-exome sequencing methods

2. Animals

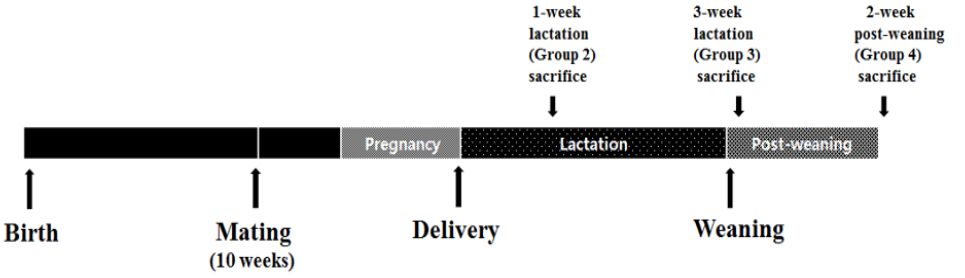


Figure 2. Study design

DMP1-hSOST transgenic mice were generated by inserting the human Sost cDNA (I.M.A.G.E. clone ID 40009482, American Tissue Culture Collection, Manassas, VA, USA) downstream from a 12-kb DNA fragment containing 8 kb of the 50-flanking region, the first exon, the first intron, and 17 bp of exon 2 of the murine DMP1 gene and upstream from a 140-bp fragment containing the rabbit beta-globin polyadenylation sequence.[23] Transgenic mice were produced by microinjection of purified DNA into pronuclei of C57BL/6 mice at the transgenic mouse core facility of the University of Arkansas for Medical Sciences. DMP1-Sost mice were born at the expected Mendelian frequency, were fertile, and exhibited normal size and weight [24].

Ten-week-old DMP1-hSOST transgenic (TG) or wild type (WT) female mice were mated with wild C57BL6 (WT) male, and after female mice became pregnant, males were removed. After birth, lactating females were randomly assigned to three groups in both genotypes according to the time of

lactation or weaning; lactation day 7, day 21, and day 14 after weaning. After finishing lactation or weaning, mice were sacrificed (Fig 2). Age matched non-lactating control mice, of which pups were removed right after birth, were also maintained until each time point in both wild type and DMP1-hSOST transgenic mice; no-lactation until day 7, day 21, and day 35. All age-matched animals were ± 3 days of lactating animals. Mice were fed a diet containing 0.81% calcium and 0.64% phosphorus (LabDiet, St. Louis, MO, USA) and water and were maintained on a 12-hour light/dark cycle.

3. Dual-energy X-ray absorptiometry (DXA)

Bone mineral density (BMD) was measured by DXA using an InAlyzer densitometer (Medikors, Korea). Mice were sacrificed and T12 to L3 vertebra and left femur were dissected, cleaned of soft tissue, fixed at 10 % formalin for 3 weeks, and stored in 70% ethanol. The region of interest (ROI) for lumbar spine was L1, L2, and L3. Whole femur was measured for total femoral areal BMD.

4. Microcomputed tomography (μCT)

The microarchitecture of the left femur were analyzed by scanning at 1.5mm below the growth plate for trabecular bone, and at 4.5mm below the growth plate for cortical bone by μCT (Skyscan 1076, Kontich, Belgium).

5. Serum biochemical parameters

Blood sampled were collected at the facial vein, and centrifuged at 3000 relative centrifugal force (RCF). Serum sclerostin (R&D Systems, Minneapolis, MN, USA), the amino-terminal propeptide of type 1 procollagen (P1NP)(IDS, Boldon, U.K), cross-linked C-telopeptide (CTX) (IDS, Boldon, U.K) were measured with using enzyme-linked immunosorbent assays.

6. Statistical analysis

Statistical analyses were performed using SPSS 22.0 (PASW Statistic, SPSS, IBM, Chicago, IL, USA) and GraphPad Prism version 6.0 for Windows (GraphPad Software, La Jolla, CA, USA). Differences between two groups were tested by t-test were used. Spearman correlation test were used to analysis correlation tendency. Data are presented as mean ± SD or n (%). A *P*-value <0.05 was considered to be statistically significant

III. RESULTS

1. Whole-exome sequencing data of subjects in pregnancy and lactation osteoporosis (PLO)

1-1. Clinical characteristics of 12 women

All women had more than one vertebral compression fractures. Ten women were primiparas. The mean age and body mass index (BMI) of women were 31.2 ± 2.7 years and 20.3 ± 2.1 kg/m^2, respectively (Table 1). No subjects had a history of fractures before pregnancy, and 2 women had a family history of osteoporosis or vertebral fractures. The duration of lactation was 3 [2-3] months, documentation of fractures of all women by any imaging examination was within 3 months. Subjects had lower lumbar spine bone mineral density (BMD) (0.620 ± 0.181g/cm^2, Z-score = -3.0 ± 0.7).

1-2. Candidate PLO genes

All variants type were all disease-associated polymorphism (DP) or disease-associated polymorphism with additional supporting functional evidence (DFP). Ten of twelve women have heterozygous or homozygous mutations of gene mutations enc oding receptor activator of nuclear factor κ B (RANK) (*TNFRSF11A*) [25], osteoprotegerin (*TNFRSF11B*)[26], a decoy receptor for RANK, calcitonin receptors (*CALCR*)[27], PR domain zinc finger protein 2 (*PRDM2*)[28,29], interacting with estrogen receptor alpha suggesting that bone resorption and mineral me tabolism are also importantly involved in PLO women (Table 2).

Table 1. Clinical characteristics of 12 patients

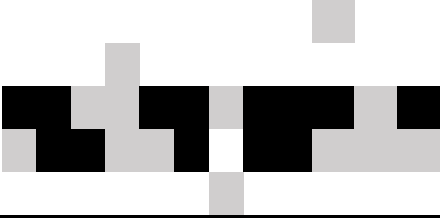
ID	Age	Family or past history	height (cm)	weight (kg)	BMI	parity	duration of lactation (month)	Fx_site	Vertebral Fx	LS aBMD (g/cm^2)	LS Z-score	FN aBMD (g/cm^2)	FN Z-score	TH aBMD (g/cm^2)	TH Z-score
1	30	.	161	44.5	18.3	2	15	T8,10,L2,L3	4	0.704	-2.9	0.734	-0.2	0.784	-0.6
2	29	.	168	54	18.6	1	8	T9,11,12,L1	4	0.545	-4	0.488	-2.4	0.6	-2.2
3	30	.	159.3	42.7	17.2	1	3	L1,3,4,5	4	0.743	-2.8	0.517	-2.2	0.574	-2.3
4	32	.	165	60	22.4	1	3.3	T8,9,11	3	0.733	-2.5	0.62	-1.2	0.706	-1.3
5	32	Hypothyroidism (patient herself)	159	63	23.7	1	3	T12	1	0.66	-2.5	0.711	-0.4	0.799	-0.5
6	30	Vertebral Fx (grand mother and mother)	164	53	20.4	1	2	T6,8,12,L1,3	5	0.641	-2.9	0.578	-1.5	0.712	-1.2
7	32	.	156	50	18.5	1	1	T12,L2	2	0.725	-2.4	0.538	-1.9	0.597	-2.2
8	31	Osteoporosis (mother)	158	53	21.2	1	0	T12,L2,4	3	0.576	-3.4	0.478	-2.5	0.564	-2.5
9	33	.	160	46	19.5	1	2	T5,T12	2	0.47	-4.6	0.491	-2.3	0.596	-2.2
10	30	.	156	50	20.8	1	3.3	T11,12,L1,2,5	5	0.849	-2.7	0.88	-1	0.839	-1.2
11	27	.	160	59	23.1	2	3	T2,T9,T11,L1,L2-5	8	0.175	-2.5	0.603	-1.3	0.797	-0.5
12	38	.	162	58	22.4	1	2.3	T8,9,10,11,12,L1~5	10	78.5 (vBMD by QCT)		0.53	-1.65	0.637	-1.46

BMI, body mass index; Fx, fracture; LS, lumbar spine; aBMD, areal bone mineral density; TH, total hip

Table 2. 23 known "bone","bone mineral density","osteoporo-" gene mutations in human gene mutation database (HGMD)

Variant id	Gene	HGVS amino acid changes	HGMD accession	Disease	Rs id	Variant Type	P-value	Case MAF	Control MAF	1	2	3	4	5	6	7	8	9	10	11	12
1:12009956:G>A	PLOD1	NP_000293.2: p.A99T	CM068658	Bone mineral density (BMD)	rs7551175	DP	0.589	0.292	0.242												
1:14106394:->CTC	PRDM2	NP_036363.2: p.P703_A704insP	CD043415	BMD	rs2308040	DFP	0.082	0.542	0.364												
3:113187675:C>G	SPICE1	NP_653319.1: p.L275V	CM1312606	BMD	rs16861032	DP	0.085	0.208	0.097												
4:88732692:G>A	IBSP	NP_004958.2: p.G195E	CM093421	Hip BMD	rs1054627	DP	0.687	0.167	0.137												
4:88732874:A>G	IBSP	NP_004958.2: p.T256A	CM1413823	BMD	rs17013182	DP	0.118	0.042	0.161												
4:123814308:G>A	NUDT6	NP_009014.2: p.R209Q	CR110375	BMD	rs1048201	DP	0.431	0.333	0.415												
5:156936364:A>G	ADAM19	NP_150377.1: p.S284G	CM093420	Hip BMD	rs1422795	DP	0.671	0.125	0.158												
6:151936677:G>A	CCDC170	NP_079335.2: p.V604I	CM093417	BMD	rs6929137	DP	0.487	0.25	0.318												
7:93055753:C>T	CALCR	NP_001733.1: p.P447L	CM980299	Lower lumbar BMD	rs1801197	DP	0.725	0.083	0.106												
7:120969769:G>A	WNT16	NP_476509.1: p.G82R	CM127941	BMD	rs2908004	DP	0.161	0.083	0.2												
7:120979089:C>T	WNT16	NP_476509.1: p.T263I	CM127940	Cortical bone thickness/BMD	rs2707466	DP	0.17	0.083	0.197												
8:104427359:A>C	DCAF13	NP_056235.4: p.R47S	CM109930	Total body BMD	rs3134295	DP	0.857	0.333	0.352												
8:104427541:C>T	DCAF13	NP_056235.4: p.P108L	CM109931	Total body BMD	rs3134296	DP	0.719	0.292	0.327												
8:104432545:A>G	DCAF13	NP_056235.4: p.I194V	CM109932	Total body BMD	rs3134253	DP	0.904	0.333	0.346												
8:119964052:C>G	TNFRSF11B	NP_002537.3: p.N3K	CM045665	Osteoporotic fractures	rs2073618	DP	0.387	0.208	0.291												
9:14722477:C>G	CER1	NP_005445.1: p.A65G	CM094855	Low BMD	rs3747532	DP	0.745	0.042	0.058												
11:68115489:A>G	LRP5	NP_002326.2: p.Q89R	CM057461	Lower femoral neck BMD	rs41494349	DP	0.54	0.125	0.088												
12:48272895:T>C	VDR	NP_001017535.1: p.M1T	CM972826	Higher BMD	rs2228570	DFP	0.926	0.417	0.426												

12:56865338:T>C	GLS2	NP_037399.2: p.L581P	CM1312608	BMD	rs2657879	DP	0.482	0.042	0.082
15:74336633:T>C	PML	NP_150241.2: p.F645L	CM1314597	Paget disease of bone	rs5742915	DP	0.015	0.042	0.003
17:42225547:A>C	C17orf53	NP_076937.2: p.T126P	CM093418	Hip BMD	rs227584	DP	0.351	0.167	0.252
18:60027241:T>C	TNFRSF11A	NP_003830.1: p.V192A	CM109815	Paget disease of bone	rs1805034	DFP	0.976	0.333	0.33
20:6750882:T>G	BMP2	NP_001191.1: p.S37A	CM034611	Bone mass	rs2273073	DFP	0.281	0.045	0.122



HGVS, human genome variation society; MAF, minor allele frequencty; DP, disease-associated polymorphism; DFP, disease-associated functional polymorphism, BMD, bone mineral density

1-3. Candidate PLO-associated pathways

We evaluated the genes related with Wnt signaling pathway, osteoporosis, bone, pregnancy, and lactation. *LRP5/6* gene mutations were common parts of Wnt-signaling pathway, osteoporosis and bone (Fig. 3). Figure 4 showed the variants in *LRP5/6* and *SOSTDC1* gene. The arrows show the common, low, rare mutations in *LRP5/6* and *SOSTDC1* gene, all related with Wnt signal pathway important to induce bone formation. Compared to control group, *LRP5* mutations p.Gln89Arg in S05,S09,S10 and p.Ala1330Val in S01,S05,S06,S07,S09, S10 case were found as common variant (minor allele frequency (MAF) > 0.05). Low variants (0.01 < MAF < 0.05), p.Ser127Thr in *LRP6* gene were found in S12. *LRP5* mutations p.Arg701His, p.Tyr1082Cys, *LRP6* mutations p.Ser61Gly, p.Val90Ile as rare variants (MAF < 0.01) were revealed in 3 samples (Fig. 4). Interestingly, *SOSTDC1* mutation p.Leu50Phe was found in S03. *SOSTDC1*, also known as Wise gene, encodes secreted glycoproteins that antagonize WNT signaling by binding to LRP5, LRP6 or both. *LRP5/6* mutations are located in β-propeller extracellular domain of LRP5/6[30], which is crucial to binding to sclerostin, Wnt agonists.

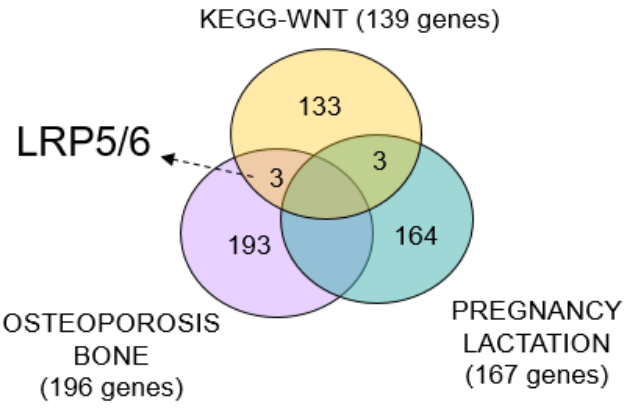


Figure 3. Pathway approach for candidate PLO gene

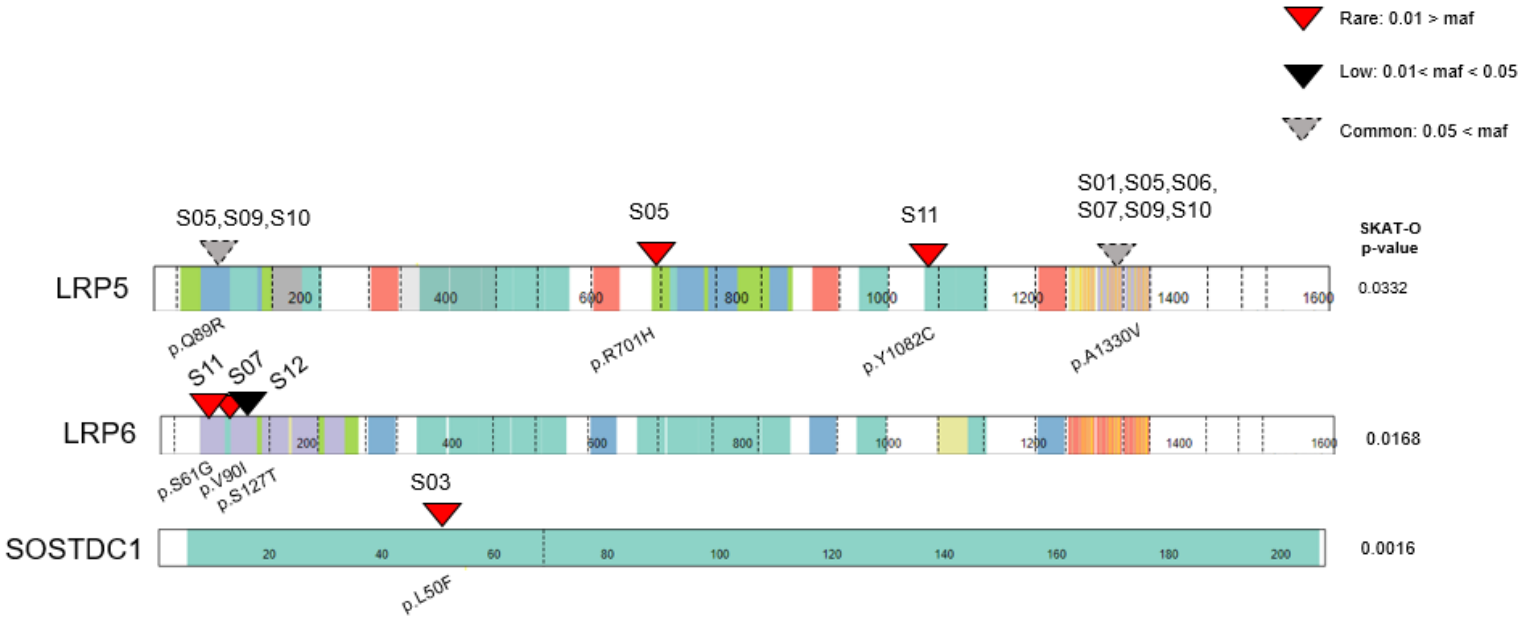


Figure 4. Common, low, and rare variants in LRP5/6 and SOSTDC1 gene in our patients

2. Effect of lactation

2-1. Bone density and microarchitectures

Both early and late lactation lowered the all spine and femur BMD at 1-week or 3-week in lactating mice compared to age-matched non-lactating mice (Fig. 5). Lactation deteriorates trabecular and cortical bone loss at early and late lactation (Table 3-4). Among micro CT parameters, lower cross-sectional thickness, longer endosteal perimeter, lower cross-sectional area showing deterioration of cortical bone.

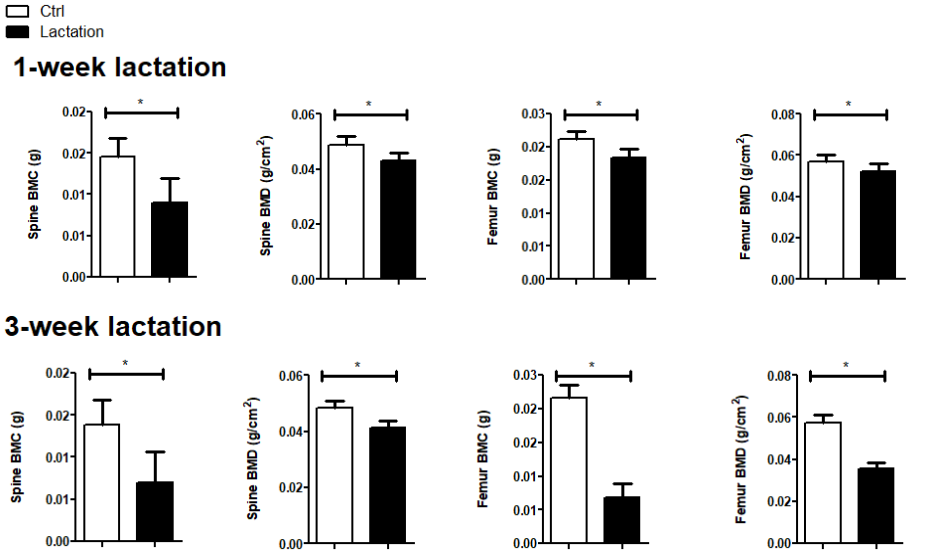


Figure 5. Effect of lactation at 1 and 3 week on BMD

Table 3. Differences in femoral μCT phenotypes by lactation at 1-week

	Wild type							DMP1-Sost						
	non-lactating group (n = 5)			lactating group (n = 5)				non-lactating group (n = 5)			lactating group (n = 5)			
	Mean		SD	Mean		SD	p-value	Mean		SD	Mean		SD	p-value
Distal femur														
TV, mm^3	3.34	±	0.14	2.96	±	0.26	**0.02**	3.22	±	0.30	2.86	±	0.27	0.08
BV, mm^3	0.63	±	0.19	0.60	±	0.17	0.76	0.19	±	0.18	0.13	±	0.15	0.60
BV/TV ,%	18.82	±	5.35	20.04	±	5.18	0.72	5.43	±	4.65	4.22	±	4.56	0.69
Tb.Th, μm	71.69	±	2.13	72.12	±	5.13	0.87	73.31	±	4.96	68.69	±	3.23	0.12
Tb.N,1/mm	2.62	±	0.72	2.76	±	0.59	0.75	0.77	±	0.72	0.62	±	0.68	0.74
Tb.Sp, mm	0.27	±	0.06	0.24	±	0.03	0.42	0.83	±	0.29	0.66	±	0.28	0.39
Conn.D,$1/mm^3$	102.91	±	41.83	111.32	±	50.77	0.18	27.55	±	20.69	15.10	±	19.70	0.36
Midshaft femur														
Cs.Th,mm	0.13	±	0.01	0.17	±	0.02	**<0.01**	0.14	±	0.01	0.15	±	0.01	0.05
periosteal perimeter[mm]	4.73	±	0.12	4.76	±	0.09	0.67	4.80	±	0.20	4.79	±	0.12	0.96
endosteal perimeter[mm]	4.09	±	0.15	3.90	±	0.09	**0.04**	4.15	±	0.19	3.98	±	0.15	0.16
CSA, mm^2	0.60	±	0.02	0.74	±	0.07	**<0.01**	0.63	±	0.07	0.69	±	0.04	0.17
MMI, mm^4	0.27	±	0.02	0.32	±	0.04	**0.02**	0.30	±	0.05	0.31	±	0.03	0.52

Abbreviations: TV, tissue volume; BV, bone volume; BV/TV, percent bone volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D,connectivity density; Cs.Th, crossectional thickness; CSA, crossectional area; MMI, polar moment of inertia. Values are means ± standard deviation (SD).

Table 4. Differences in femoral μCT phenotypes by lactation at 3-week

	Wild type						DMP1-Sost					
	non-lactating group (n = 5)			lactating group (n = 5)			non-lactating group (n = 5)			lactating group (n = 5)		
	Mean	±	SD	Mean	±	SD	p-value	Mean	±	SD	Mean	±	SD	p-value
Distal femur														
TV, mm^3	3.03	±	0.15	3.34	±	0.14	**0.01**	2.50	±	0.22	3.22	±	0.30	**<0.001**
BV, mm^3	0.63	±	0.15	0.63	±	0.19	0.99	0.08	±	0.05	0.19	±	0.18	0.25
BV/TV ,%	20.78	±	4.30	18.82	±	5.35	0.54	3.13	±	1.78	5.43	±	4.65	0.33
Tb.Th, μm	68.13	±	1.95	71.69	±	2.13	**0.03**	66.57	±	4.70	73.31	±	4.96	0.06
Tb.N,1/mm	3.05	±	0.64	2.62	±	0.72	0.35	0.46	±	0.25	0.77	±	0.72	0.40
Tb.Sp, mm	0.26	±	0.12	0.27	±	0.06	0.88	0.68	±	0.17	0.83	±	0.29	0.35
Conn.D,$1/mm^3$	121.78	±	28.36	102.91	±	41.83	0.43	12.12	±	5.35	27.55	±	20.69	0.15
Midshaft femur														
Cs.Th,mm	0.18	±	0.01	0.13	±	0.01	**<0.001**	0.19	±	0.01	0.14	±	0.01	**<0.001**
periosteal perimeter[mm]	4.76	±	0.11	4.73	±	0.12	0.74	4.72	±	0.15	4.80	±	0.20	0.51
endosteal perimeter[mm]	3.80	±	0.09	4.09	±	0.15	**0.01**	3.67	±	0.18	4.15	±	0.19	**<0.01**
CSA, mm^2	0.79	±	0.05	0.60	±	0.03	**<0.001**	0.81	±	0.05	0.63	±	0.07	**<0.01**
MMI, mm^4	0.34	±	0.03	0.27	±	0.02	**0.01**	0.34	±	0.04	0.30	±	0.05	0.16

Abbreviations: TV, tissue volume; BV, bone volume; BV/TV, percent bone volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D,connectivity density; Cs.Th, crossectional thickness; CSA, crossectional area; MMI, polar moment of inertia. Values are means ± standard deviation (SD).

2-2. Serum sclerostin level

Serum sclerostin level was lower in lactating mice compared to non-lactating mice in WT at 1-week lactation and in TG at 3-week lactation (Fig. 6).

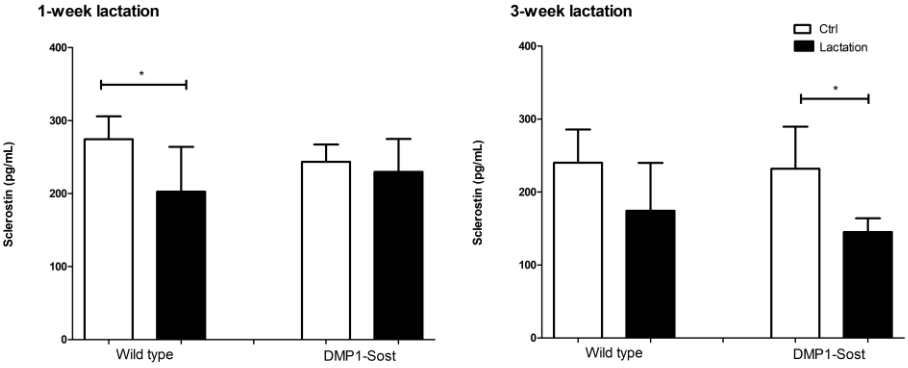


Figure 6. Effect of lactation on serum sclerostin

2-3. Bone turnover markers

According to lactation, P1NP was significantly lower in lactating mice in both WT and DMP1-Sost mice at 1-week lactation. CTX was not significantly different according to lactation at any genotype. At late lactation, P1NP was significantly higher in DMP1-Sost mice. CTX was not significantly different as at 1-week lactation (Fig. 7).

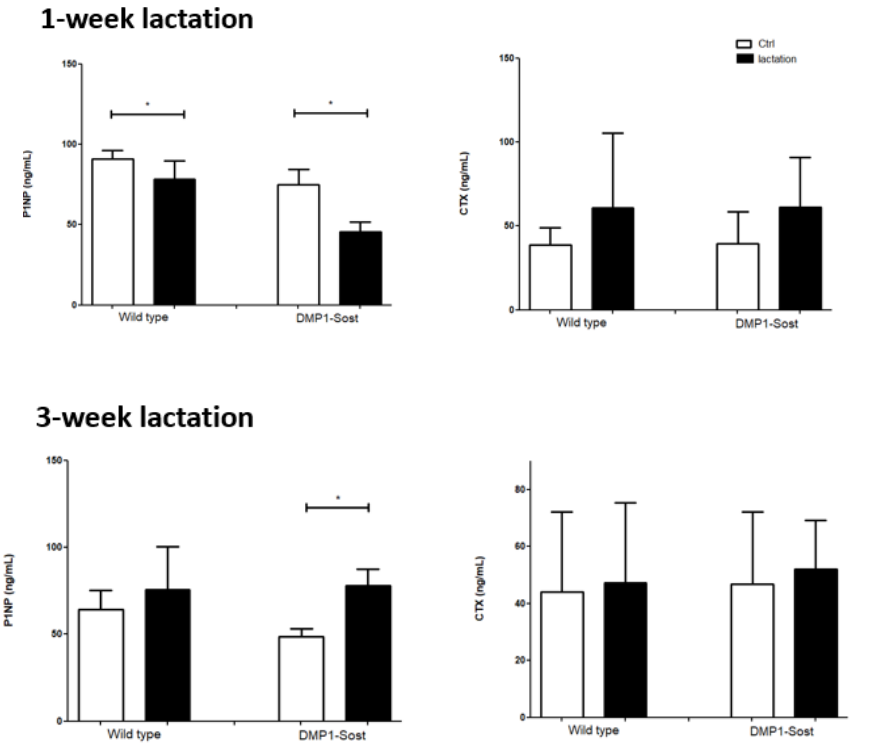


Figure 7. Effect of lactation on bone turnover markers

2-4. Correlation between parameters during lactation

During lactation, the correlation of sclerostin with bone turnover markers and bone density and quality was analyzed (Table 5). There was a significantly negative correlation between P1NP and sclerostin in lactating DMP1-Sost mice, but not in weaned mice.

Table 5. Correlation between sclerostin and bone turnover markers and measures of bone mass and structures in lactating mice

	Sclerostin			
	WT		DMP1-Sost	
	r	p	r	p
Bone turnover marker				
P1NP	-.248	.392	-.591*	.026
CTX-1	0.152	0.605	-.152	.605
Parameters by DXA				
Spine BMD	.638*	.014	.373	.189
Femur BMD	.556*	.039	.697**	.006
Parameters by micro CT				
Trabecular bone parameters				
Tissue volume [mm^3]	.091	.803	.267	.488
Bone volume [mm^3]	.382	.276	.167	.668
Percent bone volume [%]	.394	.260	.117	.765
Bone surface [mm^2]	.418	.229	.317	.406
Bone surface / volume ratio [BS/BV][1/mm]	-.236	.511	.350	.356
Bone surface density [BS/TV][1/mm]	.370	.293	.383	.308
Trabecular thickness,Tb.Th [mm]	.333	.347	-.317	.406
Trabecular number,Tb.N [1/mm]	.370	.293	.317	.406
Trabecular separation,Tb.Sp [mm]	-.224	.533	-.350	.356
Trabecular pattern factor,Tb.Pf [1/mm]	-.309	.385	.483	.187
Structure model index, SMI	-.309	.385	.233	.546
Connectivity	.430	.214	.343	.366
Connectivity density [$1/mm^3$]	.467	.174	.333	.381
Degree of anisotropy,DA	.430	.214	-.083	.831
Cortical bone parameters				
Crossectional thickness,Cs.Th [mm]	.539	.108	.400	.286
periosteal perimeter [mm]	.139	.701	.333	.381
endosteal perimeter [mm]	-.733*	.016	.233	.546
Crossectional area [mm^2]	.527	.117	.450	.224
polar moment of inertia,MMI(polar) [mm^4]	.297	.405	.633	.067
Eccentricity, Ecc	.370	.293	-.494	.177

Spearman`s correlation coefficients presented. Data are shown as r-values.

3. Effect of duration of lactation

3-1. Bone density and microarchitectures

Longer lactation significantly affected more deteriorated femur BMD in both type of mice. Difference of spine BMD according to duration of lactation was not significant (Fig. 8). Cortical bone compartment in mid-femur was significantly lower in both WT and DMP1-Sost mice. In WT, trabecular architectures were not different at between 1-week lactation and 3-week lactation; however, trabecular thickness significantly higher in DMP1-Sost mice at 3-week lactation and other parameters tended to be higher tissue volume, bone volume, percent bone volume, trabecular thickness, connectivity density compared to the parameters at 1-week lactation (Table 6). Significant loss of femoral BMD could be explained by deterioration of cortical parameters (lower Cs.Th and CSA) in both genotype mice. Interestingly, trabecular thickness (Tb.Th) was significantly higher in DMP1-Sost mice.

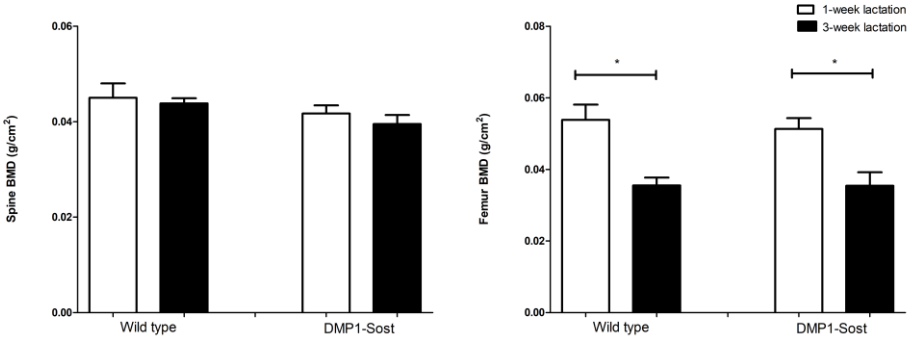


Figure 8. Effect of duration of lactation on BMD

Table 6. Differences in femoral μCT phenotypes by duration of lactation in wild type and DMP1-Sost mice

	Wild type						DMP1-Sost mice					
	1-week lactation (n = 5)			3-week lactation (n = 5)			1-week lactation (n = 5)			3-week lactation (n = 5)		
	Mean		SD	Mean		SD	p-value	Mean	SD	Mean	SD	p-value
Distal femur												
TV, mm^3	3.31	±	0.12	3.34	±	0.14	0.69	3.07 ± 0.17		3.22 ± 0.30		0.37
BV, mm^3	0.79	±	0.07	0.63	±	0.19	0.12	0.09 ± 0.04		0.19 ± 0.18		0.31
BV/TV ,%	23.82	±	2.26	18.82	±	5.35	0.09	3.05 ± 0.97		5.43 ± 4.65		0.30
Tb.Th, μm	72.78	±	4.30	71.69	±	2.13	0.63	60.15 ± 6.40		73.31 ± 4.96		0.01
Tb.N,1/mm	3.28	±	0.30	2.62	±	0.72	0.10	0.51 ± 0.15		0.77 ± 0.72		0.45
Tb.Sp, mm	0.23	±	0.02	0.27	±	0.06	0.19	0.72 ± 0.14		0.83 ± 0.29		0.48
Conn.D,$1/mm^3$	134.73	±	24.29	102.91	±	41.83	0.18	25.50 ± 5.82		27.55 ± 20.69		0.84
Midshaft femur												
Cs.Th,mm	0.15	±	0.01	0.13	±	0.01	<0.01	0.16 ± 0.01		0.14 ± 0.01		0.01
periosteal perimeter[mm]	4.84	±	0.12	4.73	±	0.12	0.18	4.90 ± 0.23		4.80 ± 0.20		0.48
endosteal perimeter[mm]	4.04	±	0.14	4.09	±	0.15	0.53	4.01 ± 0.21		4.15 ± 0.19		0.31
CSA, mm^2	0.70	±	0.04	0.60	±	0.02	<0.01	0.74 ± 0.07		0.63 ± 0.07		0.04
MMI, mm^4	0.32	±	0.03	0.27	±	0.02	0.02	0.35 ± 0.06		0.30 ± 0.05		0.17

Abbreviations: TV, tissue volume; BV, bone volume; BV/TV, percent bone volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D,connectivity density; Cs.Th, crossectional thickness; CSA, crossectional area; MMI, polar moment of inertia. Values are means ± standard deviation (SD).

3-2. Serum sclerostin level

Serum sclerostin level was significantly lower at 3-week of lactation in DMP1-Sost mice compared to that at 1-week lactation (Fig. 9).

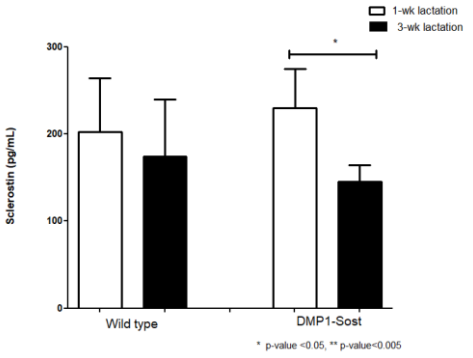


Figure 9. Effect of duration of lactation on serum sclerostin

3-3. Bone turnover markers

P1NP level was not different according the length of lactation in wild type; however, the average of P1NP level of DMP1-Sost mice at 3 week lactation period was significantly higher compared to level of mice at 1-week of lactation. This result could be explained by the significant lower level of sclerostin at 3 week in lactating DMP1-Sost than the level at 1week of lactation. CTX level was not significantly different between two times in both WT and DMP1-Sost mice (Fig. 10).

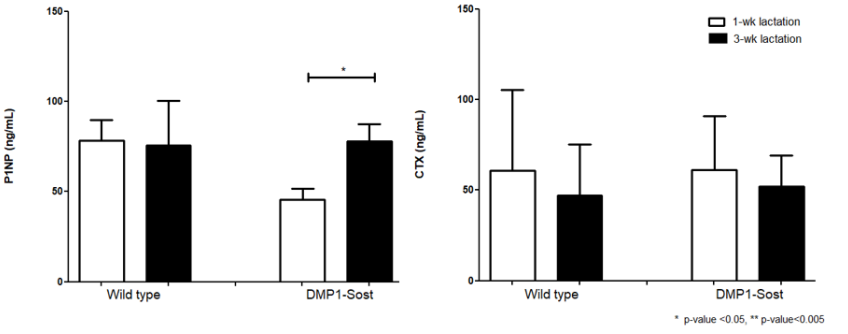


Figure 10. Effect of duration of lactation on bone turnover markers

4. Effect of weaning

4-1. Bone density and microarchitectures

After stopping lactation, femur BMD in wild type and DMP1-Sost mice were significantly higher at 2 weeks after weaning in wild type compared to 3-week lactation; however, femur BMD in both types of mice did reach to the level of age-matched non-lactating control mice (Fig. 11).

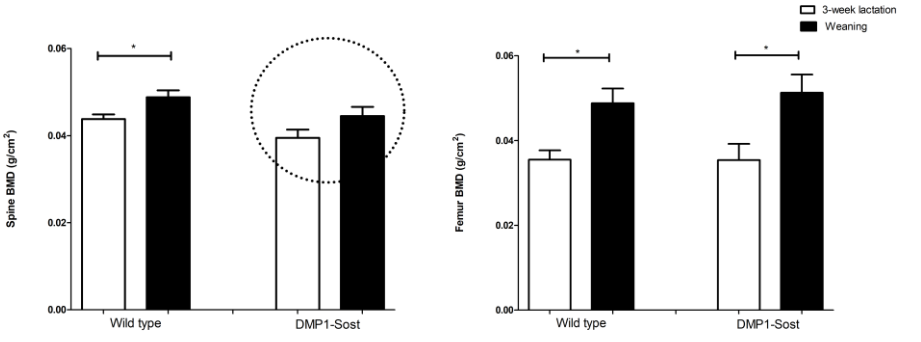


Figure 11. Effect of weaning (3 week lactation vs. weaning group)

In contrast, spine BMD of WT lactating mice did reach to that of the non-lactating control mice after weaning, average spine BMD in DMP1-Sost did not increase significantly resulting to failure to reach to BMD of non-lactating control (Fig. 12).

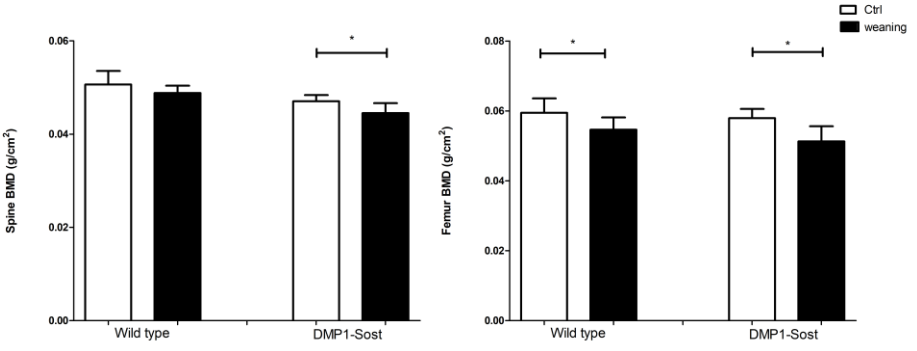


Figure 12. Effect of weaning (age-matched non-lactating vs. weaning)

We further investigated bone quality of distal femur by microCT. In WT, higher cross-sectional thickness, cross-sectional area, and higher partial moment of inertia in cortical bone compartment were observed at 2-week after weaning compared to 3-week lactation (Table 7); however, cross-sectional thickness at the mid-shaft femur remained below this parameter in age-matched non-lactating mice. Trabecular compartment in the distal femur of WT did not show the significant difference compared to non-lactating WT control mice showing the restoration of trabecular bone. In DMP1-Sost weaned mice, cortical bone compartment also remained below the compartment of non-lactating DMP1-Sost mice (table 8). Between WT and DMP1-Sost weaned

mice, only the difference of trabecular compartment was observed (lower bone volume (BV), percent bone volume (BV/TV), trabecular number (Tb.N), and higher trabecular separation (Tb.Sp) and connectivity density (Conn.D).

4-2. Serum sclerostin level

At 2 weeks after weaning, mean of sclerostin level was lower in wild type, in contrast, higher in DMP1-Sost; however there was no significance between lactating and weaned mice (Fig. 13).

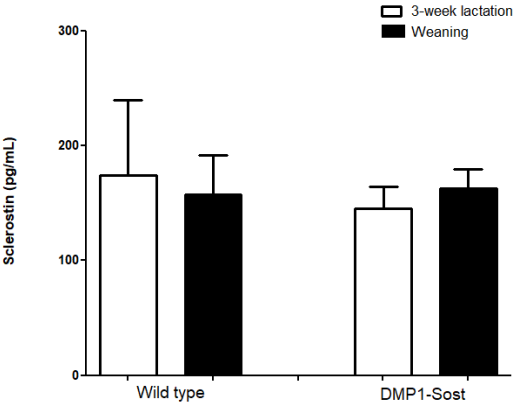


Figure 13. Effect of weaning on serum sclerostin

Table 7. Differences in femoral μCT phenotypes between 3-week lactation and weaning

	Wild type							DMP1-Sost						
	3-week lactation (n = 5)			Weaning (n = 5)				3-week lactation (n = 5)			Weaning (n = 5)			
	Mean		SD	Mean		SD	p-value	Mean		SD	Mean		SD	p-value
Distal femur														
TV, mm^3	3.34	±	0.14	2.96	±	0.26	**0.02**	3.22	±	0.30	2.86	±	0.27	0.08
BV, mm^3	0.63	±	0.19	0.60	±	0.17	0.76	0.19	±	0.18	0.13	±	0.15	0.60
BV/TV ,%	18.82	±	5.35	20.04	±	5.18	0.72	5.43	±	4.65	4.22	±	4.56	0.69
Tb.Th, μm	71.69	±	2.13	72.12	±	5.13	0.87	73.31	±	4.96	68.69	±	3.23	0.12
Tb.N,1/mm	2.62	±	0.72	2.76	±	0.59	0.75	0.77	±	0.72	0.62	±	0.68	0.74
Tb.Sp, mm	0.27	±	0.06	0.24	±	0.03	0.42	0.83	±	0.29	0.66	±	0.28	0.39
Conn.D,1/mm^3	102.91	±	41.83	111.32	±	50.77	0.18	27.55	±	20.69	15.10	±	19.70	0.36
Midshaft femur														
Cs.Th,mm	0.13	±	0.01	0.17	±	0.02	**<0.01**	0.14	±	0.01	0.15	±	0.01	**0.05**
periosteal perimeter[mm]	4.73	±	0.12	4.76	±	0.09	0.67	4.80	±	0.20	4.79	±	0.12	0.96
endosteal perimeter[mm]	4.09	±	0.15	3.90	±	0.09	**0.04**	4.15	±	0.19	3.98	±	0.15	0.16
CSA, mm^2	0.60	±	0.02	0.74	±	0.07	**<0.01**	0.63	±	0.07	0.69	±	0.04	0.17
MMI, mm^4	0.27	±	0.02	0.32	±	0.04	**0.02**	0.30	±	0.05	0.31	±	0.03	0.52

Abbreviations: TV, tissue volume; BV, bone volume; BV/TV, percent bone volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D,connectivity density; Cs.Th, crossectional thickness; CSA, crossectional area; MMI, polar moment of inertia. Values are means ± standard deviation (SD).

Table 8. Microstructures in μCT at 2-week time after weaning compared to age-matched non-lactating mice in both genotype

	Wild type						DMP1-Sost						
	Control (n = 5)			Wean (n = 5)			Control (n = 5)			Wean (n = 5)			
	Mean	±	SD	Mean	±	SD	Mean	±	SD	Mean	±	SD	p-value
Distal femur													
TV, mm^3	2.68	±	0.20	2.96	±	0.26	2.71	±	0.16	2.86	±	0.27	
BV, mm^3	0.44	±	0.12	0.60	±	0.17	0.14	±	0.09	0.13	±	0.15	*#
BV/TV ,%	16.05	±	3.46	20.04	±	5.18	5.30	±	3.24	4.22	±	4.56	*#
Tb.Th, μm	70.79	±	5.10	72.12	±	5.13	69.41	±	3.87	68.69	±	3.23	
Tb.N,1/mm	2.27	±	0.44	2.76	±	0.59	0.77	±	0.46	0.62	±	0.68	*#
Tb.Sp, mm	0.26	±	0.03	0.24	±	0.03	0.68	±	0.24	0.66	±	0.28	*#
Conn.D,$1/mm^3$	66.44	±	21.92	111.32	±	50.77	17.97	±	6.23	15.10	±	19.70	*#
Midshaft femur													
Cs.Th,mm	0.19	±	0.00	0.17	±	0.02	* 0.19	±	0.01	0.15	±	0.01	*$
periosteal perimeter[mm]	4.74	±	0.20	4.76	±	0.09	4.73	±	0.14	4.79	±	0.12	
endosteal perimeter[mm]	3.70	±	0.11	3.90	±	0.09	* 3.72	±	0.15	3.98	±	0.15	*$
CSA, mm^2	0.82	±	0.05	0.74	±	0.07	0.80	±	0.04	0.69	±	0.04	*$
MMI, mm^4	0.35	±	0.05	0.32	±	0.04	0.34	±	0.03	0.31	±	0.03	

Abbreviations: TV, tissue volume; BV, bone volume; BV/TV, percent bone volume; Tb.Th, trabecular thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; Conn.D,connectivity density; Cs.Th, crossectional thickness; CSA, crossectional area; MMI, polar moment of inertia.
Values are means ± standard deviation (SD), *, p <0.05 (vs. wild type control), #, p <0.05 (vs. wild type wean), $, p <0.05 (vs. DMP1-Sost control).

4-3. Bone turnover markers

After stopping lactation, P1NP levels were significant lower at 2 weeks after weaning compared to the 3-week lactating point; however, CTX did not show significant difference between two time points (Fig. 14).

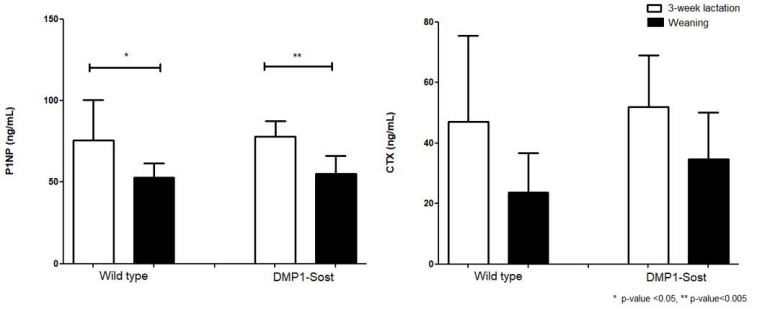


Figure 14. Effect of weaning on bone turnover markers

5. Effect of genotype

5-1. Bone density and microarchitectures

Spine bone mineral density (BMD) was significantly lower in DMP1-Sost mice, osteocyte-specific sclerostin overexpressed mice, than those in wild type mice compared by genotype (Fig. 15).

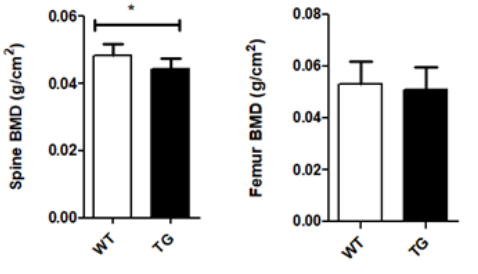


Figure 15. Effect of genotype on BMD

However, BMD of the femur between WT and TG were not significantly different. Despite of no difference in femur BMD in DXA, there were significantly different in microarchitectures of trabecular bone in distal femur between WT and TG on microCT analysis. MicroCT measurements of the lumbar vertebrae of TG mice have marked reductions in tissue volume (TV), bone volume (BV), trabecular thickness (Tb. Th), trabecular number (Tb. N), and connectivity of density, increased trabecular separation (Tb.Sp). Cortical bone parameters in mid-shaft were not significantly different (Table 9).

Table 9. Microstructures in femoral μCT phenotypes by genotype

	Wild type (n = 5)			**DMP1-Sost (n = 5)**			
	Mean		**SD**	**Mean**		**SD**	**p-value**
Distal femur							
TV, mm^3	3.05	±	0.28	2.88	±	0.34	**0.04**
BV, mm^3	0.62	±	0.19	0.14	±	0.12	**<0.001**
BV/TV ,%	20.24	±	5.31	4.56	±	3.40	**<0.001**
Tb.Th, mm	0.07	±	0.00	0.07	±	0.01	**<0.001**
Tb.N,1/mm	2.82	±	0.71	0.70	±	0.53	**<0.001**
Tb.Sp, mm	0.26	±	0.08	0.70	±	0.22	**<0.001**
Conn.D,$1/mm^3$	110.78	±	42.68	25.06	±	21.91	**<0.001**
Midshaft femur							
Cs.Th,mm	0.17	±	0.02	0.17	±	0.02	0.89
periosteal perimeter[mm]	4.78	±	0.14	4.79	±	0.16	0.91
endosteal perimeter[mm]	3.89	±	0.18	3.88	±	0.23	0.78
CSA, mm^2	0.75	±	0.09	0.75	±	0.08	0.97
MMI, mm^4	0.33	±	0.05	0.33	±	0.05	0.81

Abbreviations: TV,tissue volume; BV, bone volume; BV/TV, percent bone volume;Tb.Th, trabecular thickness; Tb.N, trabecular number;Tb.Sp, trabecular separation; Conn.D,connectivity density; Cs.Th, crossectional thickness; CSA, crossectional area; MMI, polar moment of inertia. Values are means ± standard deviation (SD)

5-2. serum sclerostin level

Serum sclerostin level did not show any significant difference by genotype at each matched status according to age, lactation or weaning (Table 10).

Table 10. Serum sclerostin level at each time

	Sclerostin level (pg/mL)					
	WT			TG		
1-week						
non-lactating	274.42	±	31.29	243.47	±	23.91
lactating	202.67	±	61.28	229.69	±	45.02
3-week						
non-lactating	240.04	±	45.65	231.94	±	57.54
lactating	174.09	±	65.81	145.05	±	18.98
2 week after weaning						
non-lactating	214.26	±	34.54	202.24	±	29.40
lactating	157.85	±	34.14	163.21	±	16.11

5-3. Bone turnover markers

P1NP, bone formation marker, was significantly lower in DMP1-Sost mice compared to wild type mice irrespective of lactation at 1-week lactation time; however, after 3- week lactation, serum sclerostin level was significantly lower in DMP1-Sost mice in non-lactation status. Average of CTX were slightly higher in DMP1-Sost mice without any significance (Table 11).

Table 11. Serum bone turnover markers at each time

	P1NP (ng/mL)							CTX (ng/ml)						
	WT			TG				WT			TG			
1-week														
non-lactating	90.84	±	5.58	75.08	±	9.47	*	38.83	±	10.28	39.66	±	19.02	
lactating	78.30	±	11.68	45.45	±	6.15	*	60.84	±	44.76	61.19	±	29.82	
3-week														
non-lactating	61.40	±	10.92	48.76	±	4.61	*	43.96	±	25.95	46.85	±	25.25	
lactating	75.61	±	24.94	78.16	±	9.44		47.17	±	28.31	51.99	±	17.13	
2 week after weaning														
non-lactating	44.36	±	8.43	36.60	±	5.39		29.00	±	15.34	30.54	±	16.80	
lactating	52.85	±	8.75	55.12	±	11.00		23.74	±	13.00	34.78	±	15.31	

P1NP, the amino-terminal propeptide of type 1 procollagen; CTX, cross-linked C-telopeptide;
* , p <0.05, TG vs age-matched WT mice at each condition

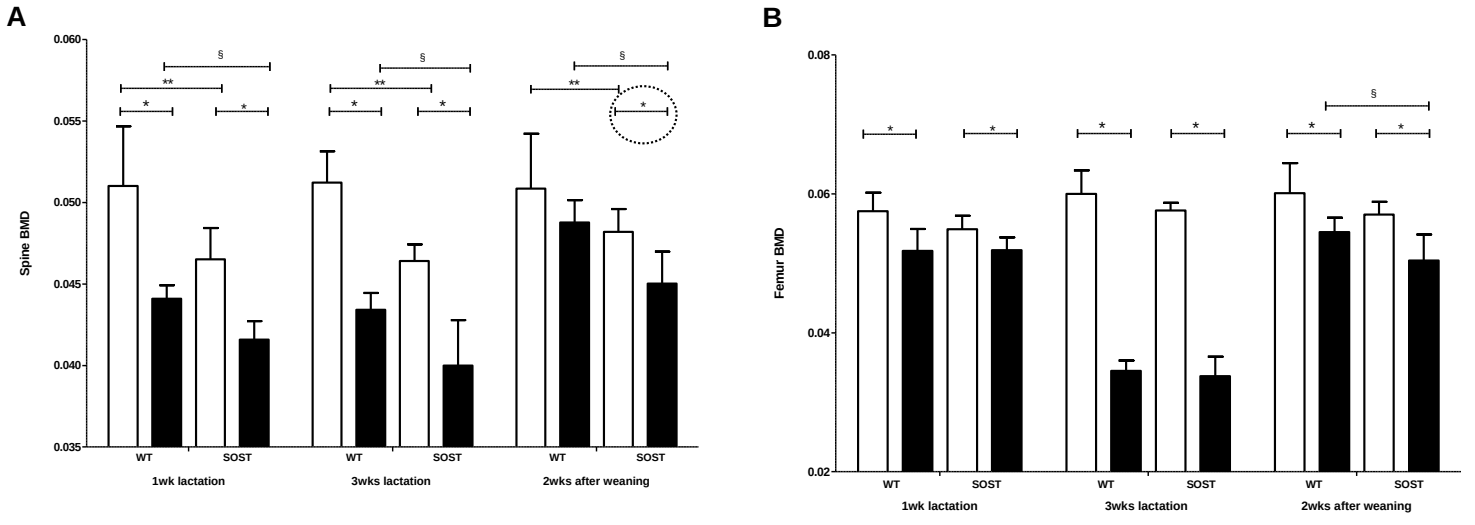


Figure 16. Comparisons in spine and femur BMD during lactation and weaning period. Lactating mice (■) were compared to age-matched non-lactating control mice (□). A, spine BMD were compared to age-matched control; $p < 0.05$; * lactating vs age-matched non-lactating group in the same genotype; ** TG vs age-matched WT in non-lactating group**,** § Gsage-matched WT in lactating group**.**

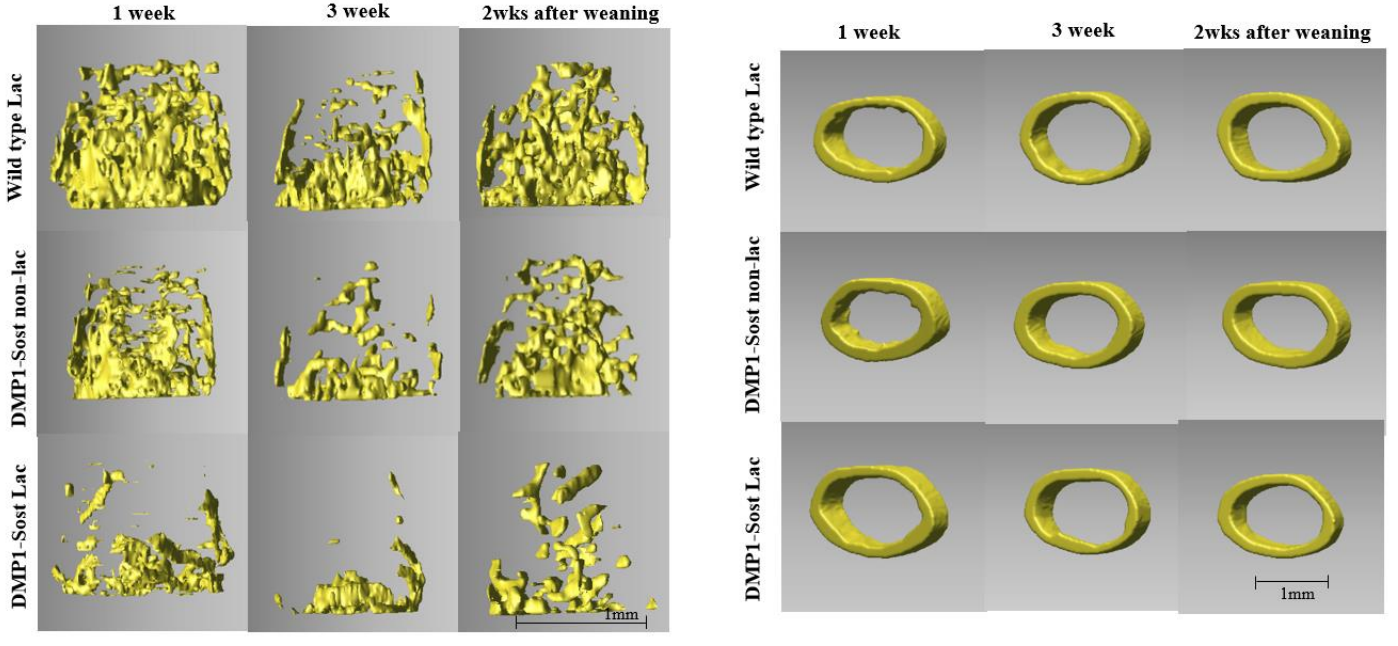


Figure 17. 3D reconstruction of distal and mid-shaft femur during lactation and weaning period

IV. DISCUSSION

In the present study, we found *SOSTDC1*, *LRP5*, *LRP6* pathogenic variants related Wnt signaling pathway in pregnancy and lactation associated osteoporosis (PLO) patients. We also revealed sclerostin, Wnt signaling inhibitor and secreted by osteocyte, has important influence on controlling bone mineral metabolism during lactation and weaning.

Canonical Wnt signaling is initiated by binding of Wnt ligands to dual receptor complex comprised of frizzled and either LRP5/6, resulting to bone formation[31]. Sclerostin, secreted primarily from osteocytes, inhibits Wnt signaling by binding to LRP5 and the related LRP4 and LRP6 receptors[21,32]. In our whole exome sequencing study, pathogenic variants related Wnt pathway were detected in 7/12 cases. Especially, observed variants of LRP5 or LRP6 encode the extracellular domain of LRP5/6, important for sclerostin as a ligand to bind the receptor and regulate the bone formation[30,33]. These findings could infer that mutations of *SOSTDC1*, *LRP5*, *LRP6* genes would be one of genetic risk factors for PLO in women in pregnancy. In addition, HGMD based candidate gene analysis showed all 12 patients have common variant, *CALCR* p.Pro447Leu and *C17orf53* p.Thr126Pro[26] related calcium sensing receptor suggesting calcium metabolism and bone mineral density, respectively. *TNFRSF11A*, *TNFRSF11B* were also nonsynonymous SNPs, associated with the regulation to activate osteoclast, which is important to maintain the balance of bone remodeling. In addition these nonsynonymous SNPs, PR domain zinc

finger protein 2 (*PRDM2*), interacting with estrogen receptor alpha suggests bone resorption and mineral metabolism are also importantly involved in PLO women.

With association of sclerostin-Wnt signaling to PLO patients, to further investigate sclerotin-Wnt signaling related bone deterioration during lactation and weaning, we evaluated and compared bone density and microarchitectures among non-lactating and lactating mice in wild type and osteocyte-specific sclerostin overexpressed transgenic mice (DMP1-Sost mice). By genotype, only trabecular-dominant lumbar spine and trabecular compartment of distal femur was affected suggesting the osteocyte-specific sclerostin overexpression in DMP1-Sost mice deteriorates trabecular bone dominantly. This bone phenotype of DMP1-Sost mice was consistent the previous study[34].

Lactation dramatically increases skeletal bone turnover to provide calcium to pups through milk. Increased bone turnover uncoupled remodeling results to bone resorption. Previous studies revealed that lactating mice resorb bone mineral primarily from trabecular-rich vertebrae, to a lesser extent from trabecular bone of the femora and tibiae, and much less from purely cortical bones [19,35-38]. In our study, both lower spine and femur aBMD by DXA and deteriorated cortical compartments and bone strength (polar moment of inertia) in distal femur revealed active bone resorption in both trabecular and cortical bones of wild type and DMP1-Sost mice during lactation. In addition, significant reduction of cortical site in lactating mice compared to age-matched non-lactating mice could be explained by osteocytic osteolysis as well as

osteoclast-mediated bone resorption is important in lactating mice. Even though osteocyte-specific sclerostin overexpression deteriorated only trabecular compartment in distal femur in mice, lactation resulted to trabecular and cortical compartment bone in both WT and DMP1-Sost mice. This result could be explained by that lactation is strong stimuli to deteriorate bone mineral metabolism. Serum sclerostin level, which was not affected by genotype, was suppressed in lactating status compared to non-lactating status, suggesting osteocytes work by controlling sclerostin in response to bone loss during lactation[35].

Depending on duration of lactation, longer lactation more deteriorated mid-shaft femoral architectures in both genotype. At 3-week lactation in DMP1-Sost, serum sclerostin was significantly lower and bone formation marker, P1NP was significantly higher than the level at 1-week lactation or age-matched non-lactating mice. Endogenous sclerostin would be suppressed more significantly in DMP1-Sost mice by compensatory response to prolonged lactation resulting to devastating bone loss, and subsequent overexpression of osteocyte-specific sclerostin[39]. In microCT, increased bone formation in DMP1-Sost at 3-week lactation contributed higher trabecular microarchitecture in distal femur despite of more reduction in femur BMD at 3-week lactation. This finding could expect that decreased sclerostin could activate Wnt signaling for bone formation, especially affecting trabecular microarchitectures at distal femur[24,40].

Weaning also leads to activation of bone formation, a rapid process of remineralization to the pre-pregnancy level in the maternal skeleton[1,5,6,20,41,42]. Bone turnover increases and is uncoupled in the opposite direction from lactation, finally to regain bone mass. Previous studies revealed that C57BL/6 mice regain their BMC in whole body, lumbar spine within 14 days of weaning[20]. Consistent to study of Liu et. al[38], trabecular-dominant lumbar spine BMD and trabecular microarchitectures at distal femur recovered to the level of non-lactating mice in WT. However, deterioration of trabecular architectures in weaned DMP1-Sost mice compared to weaned WT mice. At 2-week after weaning, incomplete recovery of trabecular microarchitectures at distal femur in DMP1-Sost mice would also explain that sclerostin would affect bone formation, especially in trabecular compartment during weaning period. Lastly, in correlation analysis, serum sclerostin level is positively related with spine and both BMD in wild type mice consistent to results of healthy human studies[43]. In DMP1-Sost mice, detection of serum sclerostin could be differed by amount of human Sost-gene expression resulting to different serum sclerostin level.

In our PLO women, all women had vertebral fracture. The phenotype of vertebral compression fractures could be inferred to severe bone loss during pregnancy and lactation, or failure of recovery after weaning. In the light of our animal study, failure of osteocyte-associated compensatory response for devastating bone loss due to lactation in regulating sclerostin level related with Wnt signaling, could result to pregnancy and lactation associated osteoporosis.

Our study has several limitations. For genetic analysis, our cases were only twelve. Whole exome-sequencing has limitations itself because of lack of sequence coverage of the variants, variant calling issues, possibility to miss the cause of the disease if located outside the coding sequences[44]. We could not validate the genetic mutation detected in our women by functional study. Our animal study is limited by a cross-sectional study design. We could not measure parameters of the mice during the period from pregnancy to weaning longitudinally. Lastly, we could not measure milk production, dietary calcium intake. Number of pups, change of weight during from pregnancy to weaning was not adjusted to analyze the difference of BMD.

Despite of several limitations, to our knowledge, this is the first study that bone contents and density by DXA and microarchitectures by micro CT together with serum sclerostin and bone turnover markers have been evaluated in DMP1-Sost transgenic mice during lactation and weaning compared to WT mice. In addition, we also revealed the novel candidate genes, *LRP6* and *SOSTDC1* related to Wnt signaling suggesting that sclerostin-Wnt signaling would be important to control bone formation to adapt bone and mineral metabolism during lactation and weaning.

V. CONCLUSION

Whole-exome sequencing allowed for confirming the involvement Wnt signaling related sclerostin and LRP5/6 in PLO women with vertebral compression fractures. Osteocyte-specific sclerostin-overexpressed DMP1-sost mice study also revealed that sclerostin controlled by osteocyte would be important to adapt bone mineral metabolism to bone loss during lactation and activate Wnt signaling for bone formation to achieve the recovery to pre-pregnancy level during weaning. More functional studies of genes discovered by our study would be needed. However, these findings would be important clues to identify vulnerability to pregnancy and lactation associated osteoporosis and treat the future treatment, such as anti-sclerostin antibody.

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ABSTRACT (IN KOREAN)

골세포 특이 스클레로스틴 유전자 변형 마우스 모델에서 수유 및 이유에 따른 골대사 및 미세구조 변화

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이 수 진

출산 후 수유시에는 아이에게 칼슘 및 무기질을 전달하기 위해 모체에서는 뼈 소실이 일어난다. 하지만, 수유를 중단한 후에는 빠른 속도로 재무기질화가 일어나게 된다. SOST 유전자로 발현되는 스클레로스틴 (sclerostin) 은 골세포 (osteocyte) 에서 주로 분비되는데, LRP5/6 의 세포외 도메인에 결합하여 윈트 신호를 억제함으로서 골재형성을 조절하는 중요한 역할을 한다. 하지만, 스클레로스틴과 윈트 신호가 수유와 이유기간 동안 골재형성을 조절하는데 어떤 역할을 하는지는 아직 연구된 바가 없다.

이번 연구에서, 저자들은 드물게 발생하는 다발성 척추 골절이 있는 임신 및 수유 관련 골다공증은 윈트 신호경로의 돌연변이와 관련이 있을 것이며, 윈트 신호와 관련된 스클레로스틴이 수유와 이유기간 동안 골대사 및 미세구조에 영향을 미칠 것이다라고 가설을 세웠다. 저자들은 전장 엑솜 분석기법을 이용하여 다발성 척추 골절이 있는 임신 및 수유 관련 골다공증 환자에서 유전자 변이와 임신 및 수유

관련 골다공증과의 관련성을 알아보고자 하였으며, 스클레로스틴의 이중에너지 X 선 흡수계측법과 미세컴퓨터단층촬영시스템을 이용하여 정상 마우스 및 골세포 특이 스클레로스틴 과발현 유전자 도입 (DMP1-Sost) 마우스의 골량 및 미세구조에 미치는 영향을 확인하고자 하였다. 결과를 보면, 전장 엑솜 분석에서는 다발성 척추 골절이 있는 임신 및 수유 관련 골다공증 환자 12 명 중 8 명에서 LRP5/6 에서 스클레로스틴이나 윈트 신호 수용체 작용제가 결합하는 세포외 도메인을 코딩하는 유전자에 돌연변이가 있었다. 동물 연구 결과에서는 수유는 1 주 및 3 주 수유했을 때 척추 및 대퇴부 골밀도를 유의하게 감소시켰고, 특히, 대퇴간부의 피질골 미세구조(단면적 및 단면 두께)가 손상되었다. 이유 후 2 주 후에는 결과는 대퇴부 골밀도 및 대퇴간부 피질골 미세구조는 정상 및 DMP1-Sost 마우스에서 모두 회복이 되지 않았다. 하지만, 정상 마우스에서는 척추 골밀도 및 대퇴골 원위부는 회복이 된 반면에 DMP1-Sost 는 두 부위 모두 회복되지 않은 결과로 미루어보다 이는 스클레로스틴 과발현이 특히 해면골이 주로 차지하는 뼈부분에 영향을 끼치는 것으로 사료된다. 요약하자면, 윈트 시그널 억제자인 스클레로스틴이 결합하는 LRP5/6 세포외 도메인의 유전자 돌연변이가 수유 및 이유 중 대한 모체의 골대사 적응기전의 실패로 인한 임신 및 수유 관련 골다공증 질병을 일으키는 유전적 위험 요인 중의 하나일 수 있겠으며, 골세포에 의한 스클레로스틴의 레벨 조절은 수유 중 골소실을 보상하고, 이유 후에 골밀도 회복을 달성하는데 중요한 역할을 할 것이다.

핵심되는 말: 윈트 신호 경로, DMP1-hSOST 유전자 도입 마우스, 스클레로스틴, LRP5/6, 전장 엑솜 분석, 임신 수유 관련 골다공증