Genetic determinants of bone mineral density loss in Aromatase inhibitors treatment in the B-ABLE Cohort

Rodríguez-Sanz M[1], Garcia-Giralt N[1], Torres E[1,4], Prieto-Alhambra D[2], Servitja S[5], Grinberg D[3], Mellibovsky L[1,4], Balcells S[3], Tusquets I[5], Díez-Pérez A[1,4], and Nogués X[1,4]

1. Musculoskeletal research group, IMIM (Institut Hospital del Mar d'Investigacions Mèdiques), Red Temática de Investigación Cooperativa en Envejecimiento y Fragilidad (RETICEF), Barcelona, Spain.
2. Nuffield Department of Orthopaedics, Rheumatology and Musculoskeletal Sciences, Oxford NIHR Musculoskeletal Biomedical Research Unit, University of Oxford, Oxford, UK.
3. Departament de Genètica, Universitat de Barcelona, IBUB, Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), ISCIII, Barcelona, Spain.
4. Servei de Medicina Interna, Hospital del Mar, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain.
5. Servei d'Oncologia, Hospital del Mar, Universitat Autònoma de Barcelona (UAB), Barcelona, Spain

INTRODUCTION

Bone mineral density (BMD) loss is a consequence of Aromatase Inhibitors (AI) treatment of breast cancer. B-ABLE cohort includes 391 postmenopausal women with early breast cancer starting AI therapy. (Table 1). Participants experienced a 1.98% [95% CI 1.54–2.42% P<0.001] bone loss at lumbar spine (LS) and 1.24% [95% CI 0.81–1.67% P<0.001] bone loss at femoral neck (FN) after 1 year on AI therapy and a 3.51% [95% CI 3.00–4.03% P<0.001] bone loss at LS and 2.07% [95% CI 1.51–2.63% P<0.01] bone loss at FN after 2 years (Figure 1). We aim to identify genetic variants associated with this BMD loss during AI therapy.

METHODS

Single nucleotide polymorphisms (SNPs) in genes involved in vitamin D and estrogen pathways (Figure 2) were genotyped in the B-ABLE cohort. (Table 2). Multivariate linear regression was performed to test the association between SNPs and LS and FN BMD loss after 1 and 2 years of follow-up. All models were adjusted for age, BMI, tamoxifen, chemotherapy, 25(OH)-VITD and type of AI. P<0.05 was considered nominally significant.

Table 1. Baseline characteristics of B-ABLE patients

Patient characteristic	Mean ± SD Median (IQ)	N (%)
Age (years)	61.2 ± 8.5	391
BMI	29.5 ± 5.4	391
Age of menopause onset (years)	49.4 ± 4.5	391
Median Age of menarche	12 (3)	391
Breastfeeding (In months)	3 (11)	391
Number of life births	2 (2)	391
Prior tamoxifen therapy n (%)		159 (40.66%)
Prior chemotherapy n (%)		237 (60.61%)
Aromatase inhibitor n (%)		
Letrozole		124 (31.71%)
Exemestane		261 (66.75%)
Anastrozole		6 (1.53 %)
BMD		
Femoral neck	0.747 ± 0.086	381
Lumbar spine	0.960 ± 0.105	268

Abbreviations: BMD (Bone mineral density); SD (Standard deviation); IQ (Interquartile range). * BMD missing values are due to incorrect scans, patients who did not attend to their follow-up visit, contralateral prostheses (making normal mode hip DXA impossible) or not able to position appropriately for scanning.

Table 2. Characteristics of the SNPs selected for genotyping

Hypotheses	Locus	SNP ID	Alleles (R/A)	MAF (ma)	HWE p-value	Genotyping efficiency (%)
ESTROGEN PATHWAY	CYP19A1	rs1062033	C/G	0.45 (G)	0.472	98.7
		rs4775936	C/T	0.47 (T)	0.837	96.9
	CYP17A1	rs4919687	G/A	0.30 (A)	0.179	98.5
		rs6163	C/A	0.43 (A)	0.098	99.2
	CYP11A1	rs2959008	A/G	0.41 (A)	0.752	97.7
		rs7174179	A/G	0.34 (A)	0.648	98.5
		rs8039957	G/A	0.20 (A)	0.638	99.0
	HSD3B2	rs2854964	A/T	0.33 (T)	0.910	99.2
		rs3765948	T/C	0.15 (C)	0.845	99.7
	HSD17B3	rs408876	G/A	0.14 (A)	0.538	99.5
		rs2066474	G/A	0.18 (G)	0.165	99.2
		rs2183009	A/G	0.35 (A)	0.071	98.0
	CYP2C19	rs12248560	C/T	0.19 (T)	0.631	99.5
		rs3758581	G/A	0.07 (A)	**0.000**	97.7
		rs4244285	G/A	0.14 (A)	1.000	98.7
		rs4917612	C/G	0.09 (G)	0.355	99.7
		rs4986894	T/C	0.14 (C)	0.516	99.0
	CYP2C9	rs28371674	C/T	0.16 (T)	0.573	98.0
	ESR1	rs2504063	G/A	0.44 (A)	0.149	98.5
VITAMIN D PATHWAY	CYP27B1	rs10877012	G/T	0.23 (T)	1.000	99.7
		rs4646536	G/A	0.24 (A)	1.000	99.5
	GC	rs3755967	C/T	0.31 (T)	0.551	99.2
	DHCR7	rs7944926	G/A	0.34 (A)	0.433	98.0
	CYP2R1	rs1993116	G/A	0.35 (A)	1.000	99.5
		rs6013897	T/A	0.22 (A)	0.381	97.7
	CYP24A1	rs11907350	G/A	0.04 (A)	0.081	98.7
		rs4809957	A/G	0.24 (G)	0.261	99.7
		rs2544037	A/G	0.39 (G)	0.201	99.0
	VDR	rs11568820	C/T	0.23 (T)	0.774	98.7
		rs1544410	G/A	0.35 (A)	0.434	98.0
		rs17879735	C/A	0.47 (A)	1.000	98.5

Abbreviations: R (Reference allele); A (Alternative allele); ma (Minor allele), HWE (Hardy-Weinberg equilibrium). In bold, HWE p-values deviated from HWE.

RESULTS

Two SNPs in CYP11A1 (rs2959008 and rs7174179) were associated with FN BMD loss at one (*P*=0.003 and *P*=0.012) and two years (*P*=0.004 and *P*=0.002). For LS BMD loss, SNPs in HSD3B2 (rs2854964), CYP2C19 (rs12248560) and CYP2C9 (rs28371674) were associated at one year of follow-up (*P*=0.026, *P*=0.019 and *P*=0.011 respectively). The rs12248560 remained significant at 2 years (*P*=0.014) (Table 3.1)

The rs11907350 in CYP24A1 was associated with FN BMD loss at one year. For LS BMD loss, one SNP in GC (rs11907350) at one year (*P*=0.020) and one in VDR (rs2544037) at two years (*P*=0.024) reached significant p-values. Only the rs7174179 in CYP11A1 for FN BMD loss association at 2 years remained significant after Bonferroni correction (Table 3.2).

Figure 1. Cumulative individual percent change (Mean and CI) in bone mineral density (BMD) of the lumbar spine and femoral neck up to 2 years on AI-treatment. Paired T-test * (P<0.001); **(P<0.01).**

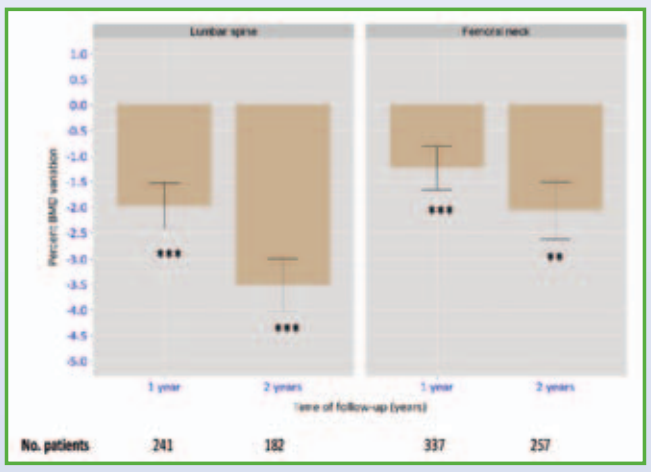

Figure 2. Schematic diagram of metabolites and enzymes of steroid and vitamin D signaling pathways. Selected genes for the association analyses are marked in grey boxes and bold font.

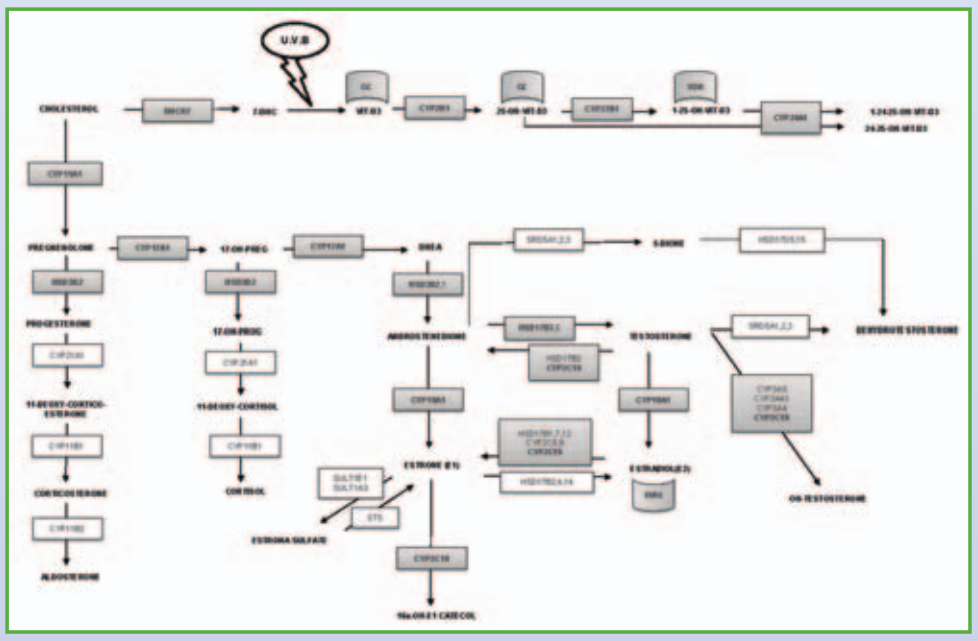

Table 3.1: Estrogen pathway associated SNPs with LS and FN BMD variation in linear-regression analysis.

Site	Time of follow-up	Locus	SNP	Genotype groups	N	Beta Coefficient§ [95%CI]	p-value
FN	One year	CYP11A1	rs2959008	G/G G/A-A/A	111 218	1.36 [0.48 to 2.25]	0.003[D]
			rs7174179	G/G G/A A/A	144 152 36	0.81 [0.18 to 1.44]	0.012[A]
	Two years	CYP11A1	rs2959008	G/G-G/A A/A	209 43	2.18 [0.71 to 3.65]	0.004[R]
			rs7174179	G/G-G/A A/A	224 29	2.60 [0.97 to 4.24]	**0.002**[R]
LS	One year	HSD3B2	rs2854964	A/A A/T T/T	105 103 31	-1.34 [-0.09 to 0.026]	0.026[A]
		CYP2C19	rs12248560	C/C C/T-T/T T/T	158 73 8	-0.94 [-1.73 to -0.16]	0.019[A]
		CYP2C9	rs28371674	C/C C/T-T/T	160 75	1.19 [0.28 to 2.11]	0.011[D]
	Two years	CYP2C19	rs12248560	C/C C/T-T/T T/T	122 50 8	-1.07 [-1.91 to -0.23]	0.014[A]

Table 3.2: Vitamin D associated SNPs with LS and FN BMD variation in linear-regression analysis.

Site	Time of follow-up	Locus	SNP	Genotype groups	N	Beta Coefficient§ [95%CI]	p-value
FN	One year	CYP24A1	rs11907350	G/G G/A-A/A	310 24	-1.84 [-3.54 to -0.23]	0.026[D]
LS	One year	GC	rs3755967	A/A-A/G G/G	212 23	1.72 [0.28 to 3.17]	0.020[D]
	Two years	VDR	rs2544037	A/A-A/G G/G	150 27	-1.50 [-2.79 to -0.20]	0.024[R]

§Adjusted by: Age, BMI, previous chemotherapy treatment and previous tamoxifen treatment. Abbreviations: LS (Lumbar spine); FN (Femoral neck); A (Additive model); D (Dominant model); R (Recessive model). In bold, p-values that withstand for multiple testing corrections

CONCLUSION

Several genes in estrogen and vitamin D signaling appeared involved in BMD loss in AI-treated women, suggesting a complex regulation of this outcome.

THERE IS NO CONFLICT OF INTEREST

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