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Genetic analysis of steroid pathway enzymes associated with adverse musculoskeletal effects of aromatase inhibitors

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Summary

Objetives: Identify putative functional variants in the *CYP11A1* and *CYP17A1* genes associated with musculoskeletal effects (accelerated bone mass loss and arthralgia) derived from treatment with aromatase inhibitors (AI).

Material and methods: The B-ABLE cohort is a prospective study of postmenopausal women with breast cancer undergoing AI treatment. Bone mineral density in the lumbar spine and femoral neck was measured by densitometry and joint pain using visual analogue scale. From single-nucleotide polymorphisms (SNPs) in genes *CYP11A1* (rs4077581, rs11632698 and rs900798) and *CYP17A1* (rs4919686, rs4919683, rs4919687, rs3781287, rs10786712, rs6163, rs743572), previously associated with musculoskeletal events, haplotypes were constructed for each pacient from the cohort, and those haplotypes that showed greatest phenotypic differences were chosen ($p<0.05$). Within each haplotype, patients with extreme phenotypes were chosen for the sequencing of respective genes and identifying functional genetic variants. Finally, a multiple linear regression analysis was carried out considering the models of dominant, recessive and additive genetic inheritance.

Results: No mutation was found in coding regions. A genetic variant (D15S520), in the basal promoter region of gene *CYP11A1*, was found associated with femoral neck bone loss at 24 month of AI treatment.

Conclusions: Variants in regulatory regions of the *CYP11A1* gene could modulate the expression of this gene, thus explaining part of the phenotypic variability found in bone loss of patients undergoing AI treatment.

Key words: *aromatase inhibitors, breast cancer, arthralgia, bone mineral density, CYP11A1, CYP17A1, genetic association study.*

Introduction

The use of aromatase inhibitors (AI) as adjuvant therapy after surgery, and/or radiotherapy, and/or chemotherapy, has achieved a significant increase in survival in postmenopausal women diagnosed with breast cancer with hormone receptors (estrogen and/or progesterone) positive (HR), in the initial stages[1,2].

The action of aromatase on testosterone and androstenedione produces estradiol and estrone[3]. These two components constitute the main source of estrogen in postmenopausal women. This aromatization process is performed in peripheral tissues, such as adipose tissue and muscle. Approximately two-thirds of breast tumors have been shown to have aromatase activity, locally producing estrogens in the tumor itself that stimulate the growth of breast tumor cells[4]. AI directly blocks estrogen production in the tumor and also causes a drastic reduction in circulating estrogen levels[5].

Sustained estrogen deprivation due to AI therapy causes an accelerated loss of bone mass, increasing the risk of osteoporotic fracture[6]. AIs may also produce other adverse musculoskeletal effects, such as arthralgia and muscle pain, which may hinder adherence to therapy during the years of prescribed treatment[7,8].

Furthermore, patients treated with AI reportedly present a large inter-individual variability in the appearance and intensity of musculoskeletal symptoms, suggesting that there are factors that may increase their appearance. In this sense, vitamin D levels (Vit D) have been linked to the appearance of arthralgias[9]. Likewise, there is probably also a genetic basis that modifies, in part, the effect of AI. Several studies have linked genetic variants associated with increased pain and loss of bone mass in women treated with AI of the B-ABLE cohort[10,11].

Specifically, single nucleotide polymorphisms (SNPs) in the *CYP11A1* gene: rs4077581, rs11632698 and rs900798 were associated with loss of bone mineral density (BMD) at the femoral neck (FN) at 2 years of treatment with IA[11]. The *CYP11A1* gene encodes the cholesterol side chain cleavage enzyme (alternative name: P450scc) that catalyzes the first and limiting step of steroidogenesis, converting cholesterol to pregnenolone. In addition, P450scc can also hydroxylate vitamin D2, D3 and its precursors[12,13], suggesting a broad spectrum of functions in cellular metabolism.

On the other hand, seven SNPs of the *CYP17A1* gene (rs4919686, rs4919683, rs4919687, rs3781287, rs10786712, rs6163, rs743572) were associated with increased pain at 1 year of treatment with IA[10]. *CYP17A1* (17α-hydroxylase/17,20 lyase) is a key enzyme in the steroidogenic pathway that produces progestins, mineralocorticoids, glucocorticoids, androgens, and estrogens.

None of the SNPs of the *CYP11A1* and *CYP17A1* genes, previously genotyped, cause non-synonymous changes in protein, nor are they known to have any regulatory function of gene expression.

It is possible that functional variants of the genes involved in both the coding region that would modify enzyme activity and in regulatory regions that would regulate gene expression levels could be implicated in AI side effects. Therefore, the aim of this study is to identify putatively functional variants in the *CYP11A1* and *CYP17A1* genes.

Material and methods

Study population

The B-ABLE cohort (Barcelona-Aromatase induced Bone Loss in Early breast cancer) is the population of a prospective study that includes postmenopausal patients with RH positive breast cancer and treated at the Hospital del Mar de Barcelona. Participants receive AI (letrozole, exemestane or anastrozole) over 5 years, or alternatively after 2 or 3 years of treatment with tamoxifen (3 and 2 years of AI, respectively), according to the American Society of Clinical Oncology's recommendations, starting within 6 weeks post op or 1 month after the last cycle of chemotherapy[14].

Exclusion criteria were: alcoholism, grade 3b renal insufficiency, rheumatoid arthritis, bone metabolic diseases other than osteoporosis, Paget's disease, osteomalacia, primary hyperparathyroidism, hyperthyroidism, insulin-dependent diabetes mellitus, previous or ongoing treatment with antiresorptive agents, oral corticosteroids or any other drug that could affect bone metabolism except tamoxifen.

Measurements

Bone mineral density

At the outset and every 12 months until the end of treatment, levels of BMD at the lumbar (LS L1-L4), femoral neck (FN) and total hip (TH) were measured using the dual X-ray energy densitometer (DXA) QDR 4500 SL® (Hologic, Waltham, Massachusetts, USA). The variation coefficient for this technique in our center is 1% in LS and 1.65% in FN. Densitometries with artifacts, degenerative disc disease with osteophytes, osteoarthritis with hyperostosis of the facet joints, vertebral fractures and/or aortic calcifications, and all those that could cause a false increase in BMD, were excluded as in the description of Blake et al.[15]. It was then analyzed by the relative loss of bone mass.

Visual Analogue Scale

Joint pain was measured using the visual analogue scale (VAS), at baseline, at 3 months and then every 12 months until the end of the study. Joint pain was assessed: hands, shoulders, knees, hips, ankles and feet, on a scale of 1 to 10 with decimals. Subsequently it was analyzed by means of the VAS absolute change.

Demographic variables

Data were collected from a large number of clinical variables at the time of recruitment, including age, menarche and menopause ages, lactation time, number of deliveries, previous chemotherapy and

radiotherapy, adjuvant treatments, weight, smoking habits and calcium intake through the INDI-CAD survey[16].

Construction of haplotypes

Previous studies in the B-ABLE cohort genotyped SNPs located in the *CYP11A1* and *CYP17A1*[10,11] genes. SNPs that showed a statistically significant association with the evaluated phenotypes were chosen for the construction of haplotypes (Figure 1).

To establish the relationship of the haplotypes of the *CYP11A1* gene to the SNPs rs4077581, rs11632698 and rs900798 in the B-ABLE cohort, the haplotype frequencies were calculated with the haplo.em analysis and the most common haplotypes (frequency >0.01).

The *CYP17A1* gene haplotypes were constructed in the same manner with the SNPs rs743572, rs6163, rs10786712, rs3781287, rs4919687, rs4919686 and rs4919683. Each haplotype was assigned a code to facilitate its nomenclature during the study.

DNA Extraction and Sanger Sequencing

DNA extraction was performed from peripheral blood using the Wizard® Genomic DNA Purification Kit (PROMEGA). The coding regions, 5'UTR, 3'UTR and proximal promoter (up to -601 bp for *CYP11A1* and -589 bp for *CYP17A1)* were amplified with the primers described in Table 1.

Sequencing was performed using the Sanger method. The sequences were analyzed with the Sequence Scanner program (v1.0) and alignment with the reference sequence (NCBI Reference Sequence: *CYP11A1* NG_007973.1 and *CYP17A1* NG_007955.1) was carried out through the Multiple Sequence Alignment (EMBL-EBI).

Statistical analysis

The frequency of the *CYP11A1* and *CYP17A1* SNPs was estimated using the expectation-maximization algorithm. The association between haplotypes and phenotypes (change in BMD in CF and increased pain) was analyzed using the haplo.glm, based on glm regression analysis, adjusting for age, body mass index (BMI), previous tamoxifen therapy And chemotherapy. The most common haplotype was used as the reference haplotype and the additive model was assumed to obtain a p-value and the β-coefficient relative to the reference haplotype.

The potential differences between the characteristics of the patients selected according to their haplotype and with extreme phenotypes were evaluated with Student's t-test for independent samples.

The association between the genetic variants found in the sequencing and the extreme phenotypes were analyzed by multiple linear regression, contemplating dominant, recessive and additive genetic inheritance models.

All statistical analyzes were defined as significant with $P<0.05$. These were performed using the SPSS (version 22) and R for Windows (version 2.15.2) statistical programs using packages, foreign, rms, multtest, plyr, boot, haplo.stats and SNPassoc.

Ethics statement

The study protocols have been approved by the Ethical Committee for Clinical Research of the Marine Health Park (2013/5283/I). Approved protocols for obtaining DNA from blood samples were explained to potential participants, who signed an informed consent before being included in the study.

Results

Baseline characteristics of patients in the B-ABLE cohort

Table 2 shows the demographic characteristics, BMD values and the evolution of the musculoskeletal symptomatology by VAS, for the *CYP11A1* and *CYP17A1* genes, in which the haplotypes were constructed.

The scheme of the procedure to reach the final analysis of genetic association with extreme phenotypes of BMD and musculoskeletal symptomatology by VAS is shown in figure 2.

Construction of the haplotypes of the *CYP11A1* gene and the *CYP17A1* gene

Table 3 shows the constructed haplotypes and the association analysis of the *CYP11A1* and *CYP17A1* genes with the BM change in CF at 2 years and increased pain at 12 months of AI treatment, respectively.

In the *CYP11A1* gene, the haplotype that showed a major phenotypic difference with respect to the reference haplotype (11.1) was 11.2, where patients carrying haplotype 11.1 in homozygosity had a loss of BMD 4.41 times greater than haplotype carriers 11.2 in homozygosis (Table 4).

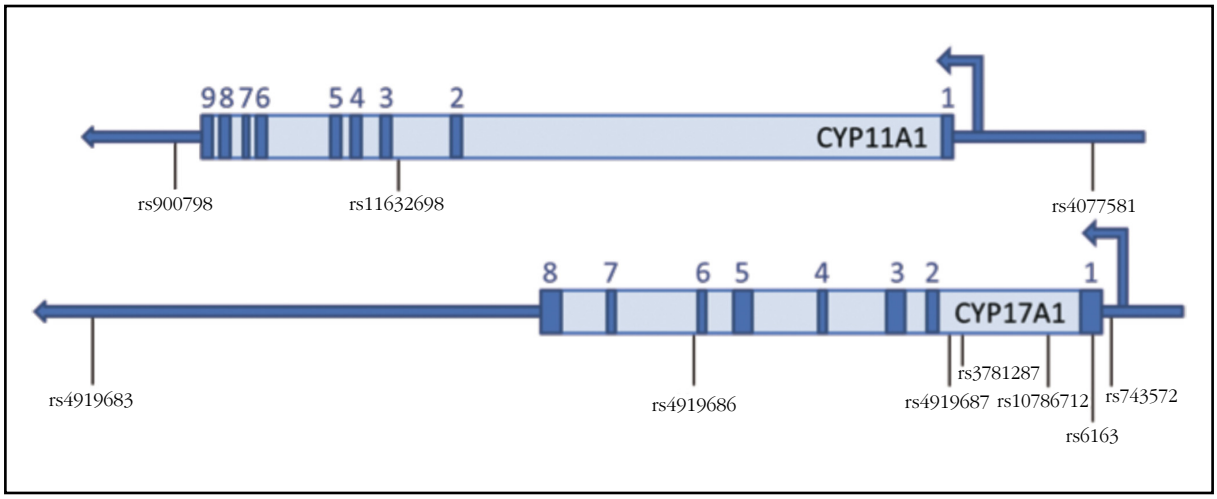
In the case of the *CYP17A1* gene, haplotypes 17.3 and 17.4 showed statistically significant differences with respect to the reference haplotype (17.1). Patients homozygous for haplotype 17.1 showed an increase in pain 3.26 times more than patients homozygous for haplotype 17.4 (Table 4).

Selection of patients for the genetic study by Sanger sequencing

Based on the results of the haplotype-association analysis, we selected patients from the B-ABLE cohort who had haplotypes (with a 99% probability) showing greater phenotypic differences: for the *CYP11A1* gene, The haplotypes 11.1 and 11.2 in homozygosis. For the *CYP17A1* gene, we selected patients with haplotypes 17.3 and 17.4, both in homozygosis and in heterozygosity. In addition, patients with haplotype 17.1 and any other haplotype (with the exception of 17.3 and 17.4) were selected (Figure 2 and Table 3).

Later, within each *CYP11A1* gene haplotype group, patients who showed an extreme phenotype in CF BMD (greater or less loss of BMD at 24 months of treatment) (n=40) were selected. The same procedure was performed for the haplotype groups of the *CYP17A1* gene in which patients with the extreme phenotype for arthralgia (greater or lesser pain increase at 12 months of treatment) (n=39) were selected (Table 5).

Figure 1. SNPs selected for the construction of haplotypes



Identification of genetic variants and analysis of association with extreme phenotypes

Following sequencing of the *CYP11A1* and *CYP17A1* genes, several SNPs were found in both genes. None of them corresponded to a non-synonymous change, or in splicing sites and, therefore, a change in the protein sequence was ruled out.

However, in the basal promoter region of the *CYP11A1* gene, a genetic variant (D15S520) associated with the BMD variation in CF at 24 months was found (Coefficient β=-6.32; 95% confidence interval (CI): [-8.55, -4.09], p=3.71e-06).

The D15S520 polymorphism is a microsatellite in the -373 bp position that is used as a genetic marker (Sequence Tagged Sites, STS) and consists of the tandem repeat of pentanucleotide (TAAAA) n. In our patients, the number of repetitions observed was 4, 6, 8 and 9.

The haplotype 11.1 was found to correlate with the allele of 4 replicates of the pentanucleotide. In contrast, patients carrying haplotype 11.2 had different alleles of the microsatellite that could be homozygosis or heterozygous, but never the allele with 4 replicates.

Discussion

AIs have a number of side effects, including the onset or increase of arthralgias and loss of bone mass, thus increasing the risk of fractures. All this can affect compliance with therapy, decrease the quality of life of patients and increase the risk of breast tumor recurrence.

In previous studies, genetic variants of the *CYP11A1* and *CYP17A1* genes were associated with loss of BMD in FN[11] and increased joint pain[10], respectively. None of the SNPs associated with these events produced a change in the protein structure and, therefore, a possible functionality of these SNPs in the determination of the event was discarded.

In order to identify putative functional genetic variants that explain the association of these genes with musculoskeletal effects, the coding and regulatory regions of the *CYP11A1* and *CYP17A1* genes were sequenced.

No variant was found in the coding region that would cause a change in the amino acid sequence of the protein and, therefore, could involve a structural change of the enzyme. However, a genetic variant, D15S520, located in the regulatory region of *CYP11A1*, was found to be associated with loss of bone mass.

The D15S520 is a microsatellite based repeating pentanucleotide (TAAAA) n located in the *CYP11A1* promoter, at 528 bp upstream from the start of gene translation. In our study, this polymorphism was found to be significantly associated with loss of bone mass at 24 months of AI treatment. It has been observed that all patients carrying the 11.1/11.1 haplotype were also carriers of the 4/4 genotype. In the B-ABLE cohort, these patients had a greater predisposition to lose bone mass (-3.014%) than those with haplotypes 11.2/11.2 (-0.683%).

This microsatellite was previously associated with the risk of breast cancer[17,18], although there is some controversy concerning the results[19,20]. The study by Sakoda et al.[18] suggested that women with 4 repetitions in homozygosis would have a lower risk of breast cancer. One hypothesis would be that the allele of 4 replicates would affect the expression of the *CYP11A1* gene by decreasing estrogen production. As a consequence, lower estrogen exposure would reduce the risk of breast cancer[21], but during treatment with AI, the remaining estrogen levels may be lower than those of the carriers of the other alleles, thus increasing the loss of bone mass.

The detection of genetic variants that partly explain the action of AIs on the musculoskeletal system would allow for the development of personalized therapies in order to avoid, or at least anticipate, the side effects of AI. This could improve adherence to the treatment of these patients, which currently stands between 75.5-78.5%, thus avoiding relapses and a new contralateral breast cancer[22].

Table 1. Pairs of primers used

CYP11A1	Promoter	F'	5'-CAACCAGATTTGCCAAGGTC-3'
		R'	5'-GGGCCAAGATTATAACTACCAGC-3'
	5'UTR y EXÓN 1	F'	5'-GCACAGGCAGATATTTCAGGA-3'
		R'	5'-GGGGACTACAGCAGGGCTAC-3'
	EXÓN 2	F'	5'-CCTATTGTCTTGTCCTTCAGCA-3'
		R'	5'-AGGTGGGACTCAGTGAGCAA-3'
	EXÓN 3	F'	5'-GTGAGAGGCAGAGGGTGCT-3'
		R'	5'-CAGAGCAAGGGGTCTCACTC-3'
	EXÓN 4	F'	5'-GTTGCCAGAGGTCAGCTTTC-3'
		R'	5'-CAACAGCCAGCCTTCCAT-3'
	EXÓN 5	F'	5'-CCCCAAGAATTCGATGAAAA-3'
		R'	5'-TGACCCCACCATCTTAGGAG-3'
	EXÓN 6	F'	5'-CAAGTGCTGCCCTGAATGTT-3'
		R'	5'-TGTGTGGCATCTCAGCCCTA-3'
	EXÓN 7	F'	5'-GAGGTTGGAAGCAGGAAGTG-3'
		R'	5'-CTCAGACCCAGGCAAATCAT-3'
	EXÓN 8	F'	5'-AAGGGTGGGACAATCATCCT-3'
		R'	5'-AACTGTGGGAGAGAGCGAGA-3'
	EXÓN 9 y 3'UTR	F'	5'-CAACCACTCATCACCCACTG-3'
		R'	5'-GATTCTGCTGGCTCCTGAAC-3'
CYP17A1	Promoter 1.1	F'	5'-GGTTCCCCCAGTACGCTAGT-3'
		R'	5'-GCCTTGTGGAAAGATTCTCCT-3'
	Promoter 1.2	F'	5'-TGACCCTCCTGAATCTGTCA-3'
		R'	5'-TTGGGCCAAAACAAATAAGC-3'
	5'UTR y EXÓN 1	F'	5'-GTTTGCCCTGGAGTTGAGC-3'
		R'	5'-TCTGAAGACCTGAACAATCCCA-3'
	EXÓN 1.1	F'	5'-AAGGGCAAGGACTTCTCTGG-3'
		R'	5'-TGTGAGCCTGAGTAGCTGGA-3'
	EXÓN 1.2	F'	5'-GAAAATGGGGGCAGTGACTA-3'
		R'	5'-GAGCCGCCTCCTCCTAGA-3'
	EXÓN 1.3	F'	5'-CAGGGTCAGGAAATGGAAAA-3'
		R'	5'-GCGATACCCTTACGGTTGTT-3'
	EXÓN 2 y 3	F'	5'-CCAGAGGTGTAAGGGCAAGA-3'
		R'	5'-AAAGGAAGGAAGATTGGGGAC-3'
	EXÓN 3	F'	5'-GTGGACCTAGTCCCCTGGTT-3'
		R'	5'-AGGGTTTTGTTGGGGAAAAT-3'
	EXÓN 4 y INTRÓN 4	F'	5'-CCGCCTCCAGGAGAGACT-3'
		R'	5'-GTGCAATGGCATGATCTCAG-3'
	INTRÓN 4.2 y EXÓN 5	F'	5'-CCTGCCCAGACTTGCTCTAC-3'
		R'	5'-GGGTCAAAGCCAACTACTGC-3'
	INTRÓN 5, EXÓN 6 y INTRÓN 6.1	F'	5'-CACAATCCTCAGGTGTGCTT-3'
		R'	5'-TCTTGAACCCCTGACCTCAT-3'
	INTRÓN 6.2	F'	5'-GCTGGCCAACCTAAAGTCAG-3'
		R'	5'-GCCCTTTACTCCCTCATTCC-3'
	EXÓN 7 y INTRON 7.1	F'	5'-ACAGAAGCGCCTGTTAGGAG-3'
		R'	5'-AGCCCTTAACGACACAGAGG-3'
	EXÓN 8 y 3'UTR	F'	5'-TCTCTTTTCCATCCTTCCTGA-3'
		R'	5'-CGGTGTTGAAAGAATGAGTGAG-3'

F: forward; R: reverse.

Table 2. Baseline characteristics of patients genotyped for the *CYP11A1* and *CYP17A1* genes

	Patients *CYP11A1* (n=391)	**Patients *CYP17A1* (n=532)**
Age (years), mean ± SD	61.3 ± 8.5	61.9 ± 8.5
BMI, mean ± SD	29.5 ± 5.4	28.9 ± 5.2
Age at onset of menopause (years), mean ± SD	49.3 ± 4.5	49.4 ± 4.3
Age of menarche (years), median (IR)	12 (3)	12 (3)
Lactation (months), median (IR)	3 (11)	3 (10)
Number of children, median (IR)	2 (2)	2 (2)
Previous therapy with tamoxifen, n (%)	159 (40.7%)	227 (42.7%)
Previous chemotherapy, n (%)	235 (60.1%)	319 (60.0%)
Aromatase inhibitor, n (%)		
Letrozole	262 (67.0%)	348 (65.4%)
Exemestane	124 (31.7%)	173 (32.5%)
Anastrozole	5 (1.3%)	11 (2.1%)
BMD LS (g/cm^2), mean ± SD	0.961 ± 0.109	0.916 ± 0.132
BMD FN (g/cm^2), mean ± SD	0.747 ± 0.085	0.718 ± 0.100
BMD TH (g/cm^2), mean ± SD	0.895 ± 0.096	0.850 ± 0.112
VAS, mean ± SD	2.435 ± 2.525	2.434 ± 2.469

SD: standard deviation; BMI: body mass index; IR: interquartile range, BMD LS, FN and TH: bone mineral density of the lumbar spine, femoral neck and total hip; VAS: visual analogue scale.

The main limitation of this study is that it does not prove that this microsatellite is really a functional variant, since there are no functional studies of the *CYP11A1* promoter that validate this hypothesis. However, the fact that no functional variable was found in the coding regions of any of the genes studied seems to indicate that the observed association between these genes and the phenotypes has to be caused by genetic variants located in regulatory regions. Another limitation of the study is the use of the EVA parameter for the evaluation of the musculoskeletal symptomatology. EVA assumes that pain is a one-dimensional experience that can be measured on a single-point intensity scale. However, the toxicity reported by the patient more comprehensively captures the side effects of therapies (ie, pain) in daily experience and is more consistent with the patient's quality of life than the clinician-verified toxicity, Thus, being appropriate for the investigation of the musculoskeletal symptomatology. Likewise, the VAS scale ratio allows detecting the percentage differences between the VAS measurements obtained at multiple points in time. Other advantages of the VAS are its ease and brevity of punctuation, minimal intrusiveness and conceptual simplicity.

In conclusion, the D15S520 variant of the *CYP11A1* gene promoter could modulate the expression of this gene, thus explaining some of the phenotypic variability found in the loss of bone mass of patients under treatment with AI. Furthermore, no variant has been found in *CYP17A1* to explain the increase or decrease in joint pain observed in patients receiving AI. The promoter regions of these genes should be further studied to detect possible genetic variants that could be involved in the regulation of their expression.

Conflict of interest: The authors declare that they have no conflicts of interest in relation to this work.

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Figure 2. General outline of the association analysis process performed in the study

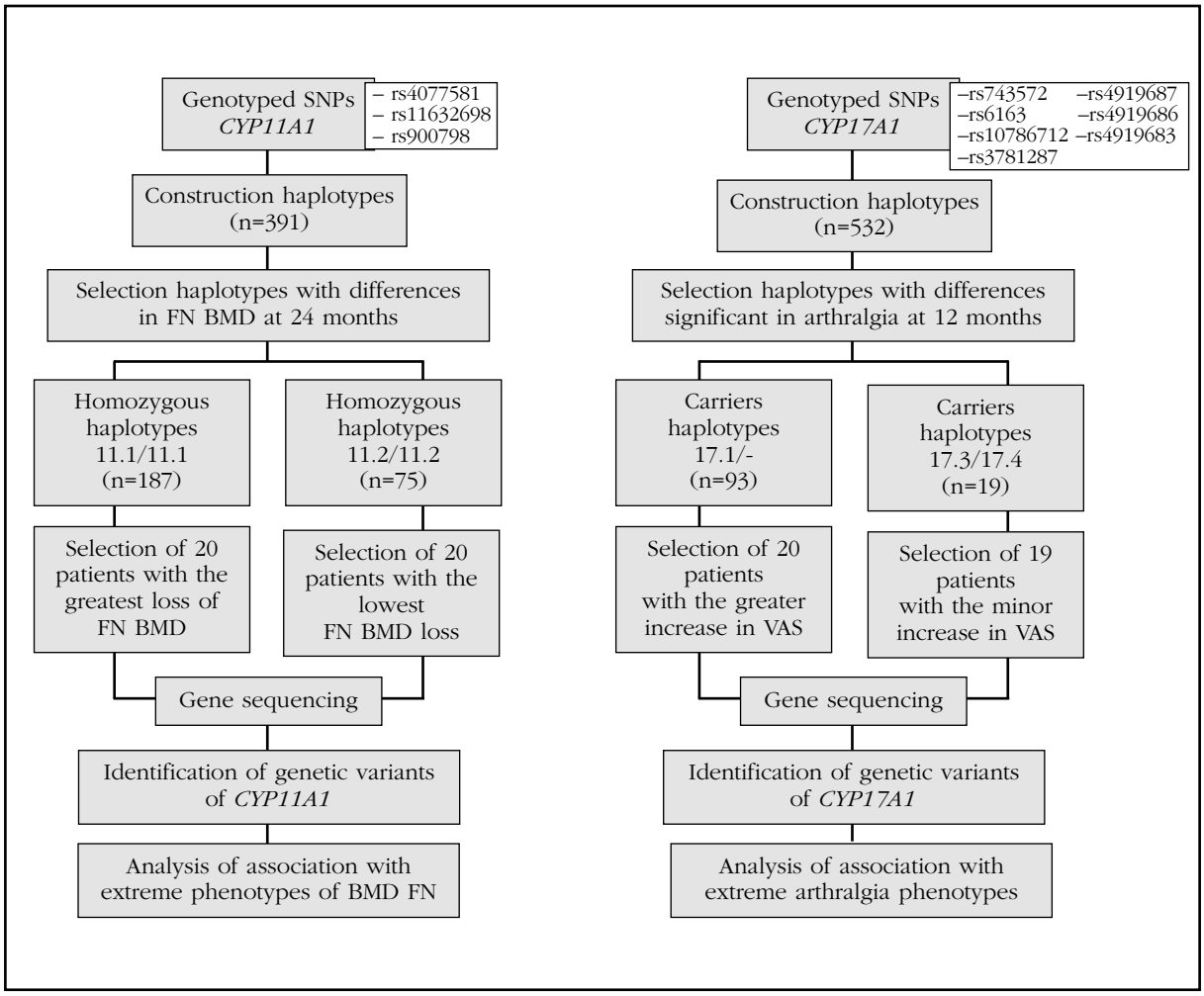

Table 3. Association between haplotypes of *CYP11A1* and *CYP17A1* genes, with loss of BMD in FN at 2 years and changes in pain at 12 months of treatment with AI, respectively

Gen	Haplotypes	Haplotype code	Frequency	Coefficient[c] [95% CI] each copy of the haplotype	P value
CYP11A1[a]	TGG*	11.1	0.517	Ref.	Ref.
	CAT	11.2	0.368	0.99 [0.29 ; 1.69]	0.006
	TAG	11.3	0.087	0.26 [-0.96 ; 1.47]	0.676
	TAT	11.4	0.026	1.03 [-0.96 ; 1.47]	0.342
CYP17A1[b]	ACCTGAC*	17.1	0.555	Ref.	Ref.
	ACCGGAA	17.2	0.014	-0.32 [-1.45 ; 2.09]	0.723
	GATGAAA	17.3	0.014	-1.67 [-3.24 ; -0.10]	0.037
	GATGACA	17.4	0.278	-0.61 [-1.03 ; -0.19]	0.005
	GATGGAA	17.5	0.123	-0.30 [-0.82 ; 0.22]	0.26

*Reference haplotype; [a]Haplotypes built by: rs4077581, rs11632698 and rs900798; [b]Haplotypes constructed by: rs743572, rs6163, rs10786712, rs3781287, rs4919687, rs4919686 and rs4919683; [c]Adjusted for: age, body mass index, chemotherapy, and previous tamoxifen.
BMD: bone mineral density; FN: femoral neck; CI: confidence interval.

Table 4. Mean of phenotypes (loss of BMD in FN in CYP11A1 and increase in pain in CYP17A1) of patients in the cohort B-ABLE carrying the haplotypes in homozygosis

Gen	Haplotype code	N patients homozygotes	Mean phenotype homozygotes patients
CYP11A1	11.1	187	-3.01%
	11.2	75	-0.683%
	11.3	3	-2.42%
	11.4	1	-
CYP17A1	17.1	93	1.76
	17.2	0	-
	17.3	1	-
	17.4	18	0.54
	17.5	0	-

BMD: bone mineral density; FN: femoral neck.

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Table 5. Characteristics of patients with selected extreme phenotypes for genetic analysis

Patients *CYP11A1*	**11.2/11.2 (n=20)**	**11.1/11.1 (n=20)**
Age (years), mean ± SD	60.6 ± 10.8	59.1 ± 9.9
BMI, mean ± SD	27.31 ± 4.6	27.91 ± 4.6
Age at onset of menopause (years), mean ± SD	47.8 ± 3.6	48.2 ± 4.9
Age of menarche (years), median (IR)	13 (2)	12 (2)
Lactation (months), median (IR)	5 (15)	2,5 (9)
Number of children, median (IR)	2 (2)	2 (2)
Previous therapy with tamoxifen, n (%)	0 (0%)	0 (0%)
Previous chemotherapy, n (%)	13 (65.0%)	13 (65.0%)
Aromatase inhibitor, n (%)		
Letrozole	10 (50.0%)	9 (45.0%)
Exemestane	10 (50.0%)	11 (55.0%)
BMD FN (g/cm^2) (basal), mean ± SD	0.763 ± 0.104	0.777 ± 0.073*
Change in BMD FN (2 years), relative mean (%) ± SD	2.330 ± 3.203	-7.858 ± 3.684**
Patients *CYP17A1*	**17.3/17.4 (n=19)**	**17.1/- (n=20)**
Age (years), mean ± SD	61.79 ± 9.13	61.15 ± 7.85
BMI, mean ± SD	29.22 ± 7.29	31.01 ± 6.23
Age at onset of menopause (years), mean ± SD	48.63 ± 3.99	48.65 ± 5.02
Age of menarche (years), median (IR)	12 (3)	12 (3)
Lactation (months), median (IR)	3 (12)	6 (14)
Number of children, median (IR)	2 (1)	2 (1)
Previous therapy with tamoxifen, n (%)	14 (73.7%)	15 (75.0%)
Previous chemotherapy, n (%)	12 (63.2%)	14 (70.0%)
Aromatase inhibitor, n (%)		
Letrozole	11 (57.9%)	8 (40.0%)
Exemestano	7 (36.8%)	11 (55.0%)
Anastrozole	1 (5,3%)	1 (5,0%)
VAS (basal), mean ± SD	2.750 ± 0.097	0.825 ± 1.270*
Change in VAS (1 year), mean ± SD	-0.078 ± 2.264	6.290 ± 1.032**

SD: standard deviation; BMI: body mass index; IR: interquartile range, BMD FN: bone mineral density of the femoral neck; VAS: visual analogue scale; *p<0.01; **p<0.001.