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Genetic determinants of aromatase inhibitor-related arthralgia: the B-ABLE cohort study

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Abstract A major side effect of aromatase inhibitor (AI) therapy is AI-related arthralgia (AIA), which often leads to therapy discontinuation. We aimed to identify genetic variants associated with AIA and therapy discontinuation in the first year of AI treatment. Our prospective cohort study included 343 postmenopausal women with early breast cancer starting AI therapy. Single nucleotide polymorphisms (SNPs) in candidate genes involved in estrogen and vitamin D signaling were selected. Univariate and multivariate linear/logistic regressions were fitted in order to asses the association between studied SNPs and AIA intensity (visual analogic scale score) at 3 and 12 months of follow-up, worsening pain, and therapy discontinuation. We also tested for a priori-defined interactions by introducing multiplicative terms in the regression equations. SNPs in *CYP17A1* and *VDR* genes appeared significantly associated with AIA ($P = 0.003$, $P = 0.012$, respectively). One SNP in *CYP27B1* gene was related to therapy discontinuation [$P = 0.02$; OR 0.29 (0.09–0.99)]. We revealed interactions between *CYP27B1* and both *CYP17A1* ($P = 0.01$) and *VDR* SNPs ($P = 0.06$). Furthermore, an additive effect on pain intensity was shown for unfavorable alleles, with two points higher mean absolute pain increase and up to 5.3-fold higher risk of worsening pain compared to favorable genotypes. SNPs in *CYP17A1*, *VDR*, and *CYP27B1* genes predict the risk of AIA. Their determination would be useful to trigger the monitoring strategies in women at risk of therapy discontinuation.

Keywords Aromatase inhibitors · Arthralgia · Breast cancer · SNP

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Introduction

Third-generation aromatase inhibitors (AIs) are first-line therapies for early-stage breast cancer (EBC) [1, 2]. Unfortunately, 25–30 % of the women treated with AIs experience AI-related arthralgia (AIA) [3], which remains the most common cause of therapy discontinuation [4]. Although, Henry et al. [3] recently described some clinical predictors of arthralgia, these same authors acknowledged their poor predictive ability and stated an urgent need for genetic studies to help us disentangle the pathophysiology of AIA. Some genetic studies have been performed to establish a genetic background of AIA [5, 6], but its total heritability remains unknown.

This study focuses on the investigation of genetic determinants of AIA and on therapy discontinuation. In order to decide a priori candidate genes for our study, two hypotheses were postulated. The first is that estrogens have an antinociceptive role through a direct effect on the opioid pain fibers [7, 8]. In addition, estrogens have a tissue-specific effect on pro-inflammatory cytokines, acting as a protective modulator of inflammation, which could account for enhanced local inflammatory activity related to the estrogen depletion caused by AI therapy [9–11].

Second, some observational data suggest that vitamin D insufficiency, which is very common in postmenopausal women with EBC [12], might also contribute to arthralgia [13].

We performed an association analysis with single nucleotide polymorphisms (SNPs) within genes of estrogen and vitamin D signaling in the B-ABLE cohort.

Methods

Study design and participants

Details on the B-ABLE study methods have been published elsewhere [14] and are outlined below.

Briefly, B-ABLE is a prospective, observational, clinical cohort study, conducted at the Breast Cancer Unit and Bone Metabolism Unit, Hospital del Mar–Parc de Salut Mar, Barcelona (Spain).

Eligible participants were excluded if they had a history of any metabolic or endocrine disease potentially affecting bone and cartilage metabolism, rheumatoid arthritis or concurrent or previous treatment with bisphosphonates, oral corticosteroids, or any other bone-active drug.

Measurements

AI-related arthralgia

A visual analogic scale (VAS) was used to score the intensity of self-reported joint pain at baseline (before starting AI therapy), 3 months and 1 year of follow-up. Score ranged from 0 (no pain) to 10 (maximum pain).

Absolute increase in pain at 3 and 12 months of AI therapy (VAS score at 3 and 12-month follow-up minus baseline, respectively) was the primary outcome for this study.

Assuming that some participants had some degree of pain at baseline, we also defined a binary pain phenotype named "*worsening pain*" (VAS score at follow-up > baseline VAS) versus "*stable/improving*" (if VAS score at follow-up ≤ baseline VAS) as a secondary outcome.

AI therapy discontinuation

Patients were asked at 3 and 12 months whether they were still on AI-treatment, and if not, the main reason for discontinuation, out of the following options: arthralgia, cancer-related events, concomitant disease, and personal concerns. Therapy discontinuation due to AIA was defined as a secondary outcome for these analyses.

Other assessments

Information on a large number of clinical variables was recorded at the time of enrollment, including age at recruitment, age at menarche and at menopause, lactation, parity, previous chemotherapy and radiotherapy, adjuvant treatments, weight, height, plasma levels of 25(OH)D, and smoking status.

Interventions

Participants were treated with AIs (letrozole, exemestane, or anastrozole) according to the American Society of Clinical Oncology recommendations [15], starting within 6 weeks after surgery or 1 month after the last cycle of chemotherapy, or alternatively, after 2–3 years of tamoxifen therapy.

All participants were supplemented with calcium and vitamin D tablets (1,000 mg and 800 IU daily), and those with baseline vitamin D deficiency (<30 ng/ml) received an additional dose of 16,000 IU of oral cholecalciferol every 2 weeks.

Selection of candidate genes

Three candidate genes were selected to test our estrogen hypothesis: estrogen receptor 1 (*ESR1*), aromatase (*CYP19A1*), and steroid 17-alpha-hydroxylase/17,20 lyase (*CYP17A1*) (Fig. 1).

Six genes were selected for the vitamin D hypothesis: 7-dehydrocholesterol reductase (*DHCR7*), vitamin D-binding

Fig. 1 Schematic diagram of steroid and vitamin D signaling pathway. Metabolites and selected enzymes involved in the Vitamin D$_3$ signaling [7-dehydrocholesterol reductase (encoded by DHCR7 gene), vitamin D 25-hydroxylase (encoded by CYP2R1 gene), 25-hydroxyvitamin D 1-alpha-hydroxylase (encoded by CYP27B1 gene), 1,25-dihydroxyvitamin D 24-hydroxylase (encoded by CYP24A1 gene), vitamin D-binding protein (DBP, encoded by GC gene), and vitamin D receptor (encoded by VDR gene)], and the estrogen signaling [steroid 17-alpha-monooxygenase (encoded by CYP17A1 gene), aromatase (encoded by CYP19A1 gene), and the estrogen receptor (encoded by ESR1 gene)]

protein (*GC*), vitamin D 25-hydroxylase (*CYP2R1*), 25-hydroxyvitamin D 1-alpha hydroxylase (*CYP27B1*), vitamin D3 receptor (*VDR*), and 1,25-dihydroxyvitamin D 24-hydroxylase (*CYP24A1*) (Fig. 1).

Selection of SNPs

SNPs were selected on the basis of the following criteria: (1) minor allele frequency (MAF) >0.05; (2) haplotype tagging (tag-SNPs) according to HapMap project in Caucasian population; (3) putative functional polymorphisms; and (4) previous association with other interesting phenotypes: *25(OH)D* serum levels in a Genome Wide Study [16] and bone mineral density [17–20].

DNA extraction and polymorphism genotyping

Genomic DNA was obtained from leukocytes by two procedures: Autopure LS, a Robotic workstation for automated purification of genomic DNA using Autopure chemistry (LABS Laboratory Biomedical Support Services in the IMIM), and Wizard Genomic DNA Purification Kit (Promega).

Polymorphism genotyping in 343 individuals was carried out using KASPar v4.0 genotyping systems and Kraken allele calling algorithm at the K-bioscience facilities (KBioscience, Herts, England).

To check for the quality of the genotyping, three SNPs were sequenced in 5 % of the population. The results showed a 98.5 % concordance between the two techniques.

Statistical analyses

Accepting an alpha risk of 0.0014 (corrected *P* value considering 35 included polymorphisms) and a beta risk of 0.2 in a two-sided test, at least 67 subjects were necessary in each group to recognize as statistically significant a minimum clinically relevant difference [21] of 1.8 U in VAS pain increase. The common deviation is assumed to be 2.5. It has been anticipated a drop-out rate of 5 %. Taking into account that the lowest MAF for the genotyped SNPs was 0.13, the number of individuals carrying the minor allele is approximately 75 in our cohort.

Hardy–Weinberg equilibrium (HWE) was calculated by χ^2. HWE *P* values for all the SNPs were calculated using the Tufts University website template (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls).

Univariable and multivariable linear regressions (log-additive, dominant, and recessive models) were used to

assess the association between the studied SNPs and absolute increase in VAS at 3 and 12 months follow-up. Multivariable models were adjusted for age, body mass index (BMI), and years since menopause. Further, potential confounding for baseline and follow-up 25(OH)D concentrations was assessed. As a sensitivity analysis, all linear regression models were repeated after excluding participants with baseline 25(OH)D levels ≥30 ng/ml. To minimize false discovery due to multiple testing, we performed the false discovery rate (FDR) correction as proposed by Benjamini and Hochberg [22] accepting all predictions with q value <0.05 as significant.

We also tested for a priori-defined interactions by introducing multiplicative terms in the regression equations: (1) vitamin D levels into the effect of the tested SNPs; and (2) interactions between rs4646536 (*CYP27B1*), rs11568820 (*VDR*), and rs6163 (*CYP17A1*).

The associations between these SNPs and both secondary outcomes (*worsening pain* and therapy discontinuation) were analyzed using logistic regression.

All analyses were two-tailed. Statistical analyses were performed using SPSS for Windows version 13.0 and R for Windows version 2.13.2 (packages: SNPassoc, foreign, multtest, epicalc, and rms).

Results

Baseline characteristics

A total of 343 women were recruited from March 2006 to April 2012. Of these, 334 (97.4 %) completed at least 3 months of treatment, and 321 (93.6 %) attended the 1-year follow-up visit (Fig. 2). Baseline characteristics and pain phenotypes at 3 and 12 months follow-up for the study participants are shown in Table 1. Amongst them, 161/334 (48.2 %) and 176/321 (54.8 %) reported worsening pain at 3 and 12 months, respectively. A total of 15 (4.4 %) women discontinued treatment due to severe AIA.

Genetic association with absolute VAS increase

MAF and HWE P values for each SNP in the B-ABLE cohort are reported in Table 2.

Table 3 shows average absolute difference in VAS score for each genotype compared to the reference group (beta coefficients) and P values for SNPs significantly associated with the studied outcome. A number of these SNPs, belonging to two genes, *CYP17A1* and *VDR*, appeared significantly associated with AIA.

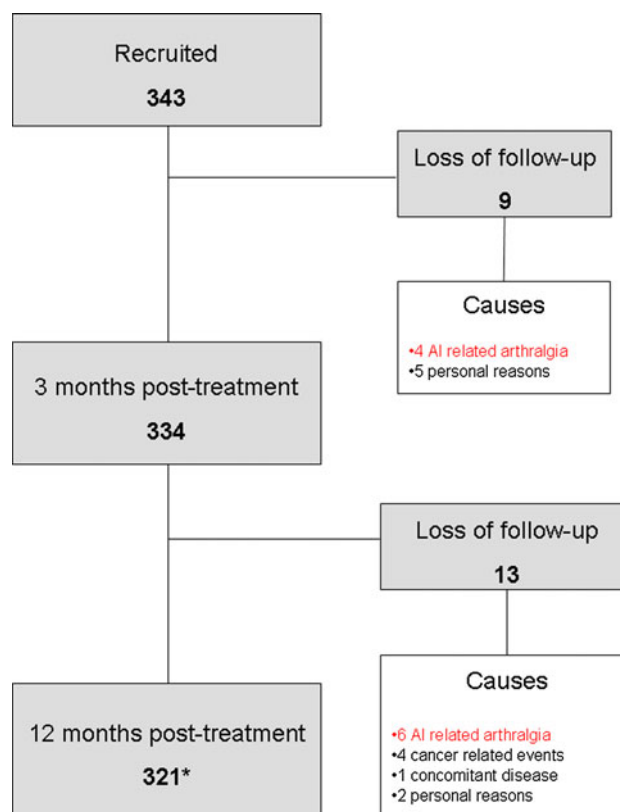Since the majority of the genotyped SNPs in *CYP17A1* reached a significant association with the outcome

Fig. 2 Flow chart of patient inclusion in the B-ABLE cohort. *5 of these 321 discontinued treatment due to AI-related arthralgia but attended follow-up visit

(Table 3), one representative SNP (rs6163), located inside the gene sequence, was chosen for interaction analyses. One SNP (rs4646536) in the *CYP27B1* gene showed a $P = 0.008$ in the association with VAS increment at 3 months post-AI treatment but did not withstand FDR correction. However, this SNP was tested for an interaction with VDR due to their potential relationship (Fig. 1): the interaction term was borderline significant at 3 months ($P = 0.06$), and consistent with this, a stratified analysis showed that the effect of rs4646536 genotypes on intensity of arthralgia was significant ($P = 0.02$) in presence of C:C genotype for rs11568820 (*VDR*), but not among participants with other genotypes. Another significant interaction was observed between rs6163 in *CYP17A1* and rs4646536 in *CYP27B1* ($P = 0.01$). The stratified analysis demonstrated different pain intensities among rs4646536 genotypes ($P = 0.0006$ at 3 months and $P = 0.003$ at 12 months follow-up) only in presence of C:C genotype for the rs6163, and no such association in A:A or A:C patients. Other interactions tested between SNPs in *CYP17A1* and *VDR* did not yield significance (data not shown). The crude effect of each genotype for the significant SNPs on arthralgia is shown in Fig. 3.

Table 1 Clinical characteristics of the B-ABLE cohort

Patient characteristic	Mean ± SD	Median (IRQ)	N (%)
Age (years)	61.96 ± 8.71		343
Age of menopause onset (years)	49.50 ± 4.19		343
BMI	29.02 ± 5.34		343
Prior tamoxifen therapy			177
Baseline joint pain (VAS score)		2.68 (5)	343
Joint pain at 3 months (VAS score)		3.74 (6)	334
Worsening pain at 3 months			161 (48.20 %)
Joint pain at 12 months (VAS score)		3.85 (5)	321
Worsening pain at 12 months			176 (54.83 %)
AI			343
Exemestane			131 (38.19 %)
Letrozole			201 (58.60 %)
Anastrozole			11 (3.20 %)
Baseline vitamin D (ng/ml)			341
<10	6.80 ± 1.66		81 (23.75 %)
10–30	18.16 ± 5.04		235 (68.91 %)
>30	40,36 ± 9.36		25 (7.33 %)
Vitamin D at 3 months (ng/ml)			332
<10	7.8 ± 1.13		2 (0.60 %)
10–30	22.93 ± 5.33		93 (27.84 %)
>30	52.90 ± 28.34		237 (71.38 %)
Vitamin D at 12 months (ng/ml)			320
<10	7.07 ± 1.66		7 (2.18 %)
10–30	22.13 ± 5.18		75 (23.44 %)
>30	54.58 ± 19.48		238 (74.38 %)

Further adjustment for baseline serum vitamin D, AI, and tamoxifen use

We adjusted the multivariable models for baseline 25(OH)D serum levels and vitamin D increments (3 month-baseline and 12 month-baseline) and tested for an interaction with the SNPs in genes related to vitamin D (*VDR* and *CYP27B1*). None of these substantially changed either the beta coefficients or the P values for the associations previously described. A sensitivity analysis looking only at patients with vitamin D deficiency at baseline gave similar results in terms of effect size and direction of the associations previously observed, but higher P values due to reduced power secondary to the exclusion of 25 (7.33 %) participants with optimal levels of 25(OH)D at baseline (data not shown). All these associations were independent of the AI prescribed to the patient (data not shown). In order to evaluate if patients treated previously with tamoxifen could have altered AI-tolerance levels, we compared baseline join pain scores and AIA at 3 and 12 month between patients with or without tamoxifen treatment. In our cohort, no differences were found and therefore, the further adjustment for previous tamoxifen therapy did not affect the association results (data not shown).

Genetic association with worsening pain

The same SNPs associated with pain intensity were also related to worsening pain: adjusted odds ratios (ORs) (95 % CI) at 3 and 12 months for rs6163 were 0.61 (0.43–0.87; $P = 0.005$) and 0.70 (0.49–0.99; $P = 0.04$), respectively. OR for worsening pain at 1 year for rs11568820 was 2.14 (1.34–3.43; $P = 0.001$) in the dominant model. An additional SNP (rs4775936) in the promoter region of *CYP19A1* gene (aromatase) was associated with worsening pain at 3 months [multivariable adjusted OR 1.62 (1.16–2.25; $P = 0.003$)].

Compound genotypes analysis

Table 4 shows the effect of compound genotypes on AIA. An additive effect of the unfavorable alleles for rs4646536 (*CYP27B1*) and rs6163 (*CYP17A1*) on pain intensity was observed: participants with four unfavorable alleles (13.54 and 13.59 % at 3 and 12 months, respectively) had an average increase of two points in VAS scale compared to those with only one unfavorable allele (8.61 and 8.97 % at 3 and 12 months, respectively) ($P < 0.001$). An additive

Table 2 Characteristics of the SNPs selected for genotyping

Hypothesis	Locus	SNP ID	MAF*	HWE *P* value**	Efficiency (%)
Estrogen metabolism	ESR1	rs2504063	0.41	0.8	97.40
	CYP19A1	rs4775936	0.43	0.23	95.38
		rs1062033	0.43	0.94	97.40
	CYP17A1	rs4919683	0.44	0.9	97.69
		rs4919686	0.28	0.08	97.40
		rs1004467	0.13	0.4	98.84
		rs4919687	0.29	0.22	96.25
		rs3781287	0.49	0.45	96.25
		rs10786712	0.42	0.37	96.54
		rs6163	0.43	0.11	98.84
		rs743572	0.42	0.3	95.67
		rs2486758	0.21	0.54	98.27
Vitamin D metabolism	GC	rs2282679	0.33	0.56	96.25
		rs3755967	0.33	0.66	97.69
		rs17467825	0.33	0.5	95.96
	DHCR7	rs12785878	0.33	0.23	95.38
		rs7944926	0.33	0.28	99.13
	CYP2R1	rs10741657	0.33	0.18	97.40
		rs2060793	0.33	0.31	95.38
		rs1993116	0.34	0.54	98.55
	CYP24A1	rs6013897	0.22	0.77	97.11
		rs2209314	0.3	0.7	97.11
		rs117614151	0.41	0.32	98.55
		rs11907350	0.03	0.12	98.55
		rs4809957	0.22	0.54	98.84
		rs2762934	0.23	0.78	96.25
		rs6022999	0.25	0.27	97.11
		rs2248359	0.42	0.94	98.55
	VDR	rs2544037	0.43	0.32	97.11
		rs11568820	0.26	0.056	97.69
		rs1544410	0.35	0.2	97.40
		rs17879735	0.48	0.5	97.40
		rs731236	0.34	0.39	96.25
	CYP27B1	rs10877012	0.24	0.79	97.40
		rs4646536	0.25	0.86	98.27

effect was also observed with worsening pain for participants with four unfavorable alleles: OR 3.88 (1.42–11.12) ($P < 0.01$) at 3 months and OR 5.27 (1.81–16.44) ($P < 0.001$) at 12 months.

Combination of rs6163 and rs11568820 compound genotypes also appeared associated with both absolute VAS increment and worsening pain at 12 months after therapy initiation in participants with 2 (37.94 %) and 3 (15.43 %) unfavorable alleles compared to the reference group with zero unfavorable alleles (8.36 %). The sample size of this combination was not powered to detect the same association in individuals with four unfavorable alleles (six participants).

Finally, a trend for the combination of rs4646536 and rs11568820 has also been observed.

Therapy discontinuation

Finally, the analyses of the association between these same three SNPs and therapy discontinuation showed only one significant result: 12 patients out of the 185 homozygotes (6.49 %) for the unfavorable genotype of rs4646536 discontinued AI therapy due to severe arthralgia, compared to only three out of the 133 (2.26 %) heterozygotes, and none of the remaining 19 participants [adjusted OR 0.29 (95 %CI 0.09–0.99), $P = 0.02$].

Table 3 SNPs associated with absolute VAS pain increase in linear-regression analysis for AI-induced-arthralgia; log-additive model

Locus	SNP	Months	Unadjusted		Multivariable[a]	
			Beta coefficient (95 %CI)	P value	Beta coefficient (95 %CI)	P value
CYP27B1	rs4646536	3	−0.61 (−1.06 to −0.16)	0.008	−0.56(−1.02 to −0.10)	0.017
		12	−0.53 (−1.00 to −0.06)	0.028	−0.54 (−1.01 to −0.06)	0.027
	rs10877012	3	−0.60 (−1.05 to −0.15)	0.009	−0.58 (−1.04 to −0.12)	0.015
CYP17A1	rs4919686	12	−0.53 (−1.01 to −0.05)	0.032	−0.60 (−1.08 to −0.12)	0.015
	rs4919683	3	−0.41 (−0.83 to −0.00)	0.05	−0.52 (−0.94 to −0.11)	0.014
		12	−0.58 (−1.01 to −0.15)	0.009	−0.67 (−1.10 to −0.23)	**0.003**
	rs4919687	12	−0.65 (−1.12 to −0.18)	0.007	−0.71 (−1.18 to−0.24)	**0.003**
	rs3781287	12	−0.55 (−0.97 to −0.19)	0.013	−0.63 (−1.06 to −0.20)	**0.004**
	rs10786712	12	−0.57 (−1.00 to −0.14)	0.009	−0.66 (−1.09 to −0.23)	**0.003**
	rs6163	12	−0.58 (−1.01 to −0.15)	0.009	−0.65 (−1.09 to −0.22)	**0.003**
	rs743572	12	−0.54 (−0.97 to −0.11)	0.014	−0.62 (−1.06 to −0.19)	**0.005**
VDR	rs11568820	12	0.53 (0.08–0.99)	0.021	0.5838 (0.13–1.04)	0.012 (**0.005**)[b]

In bold, P values that withstand for multiple testing corrections. Beta coefficients are equivalent to the average difference in VAS pain score increment between each genotype versus reference (AA) group, in an additive manner (e.g. pain in Aa = pain in AA + beta; pain in aa = pain in AA + 2× beta)

[a] Adjusted for age, BMI, and years since menopause

[b] Dominant model
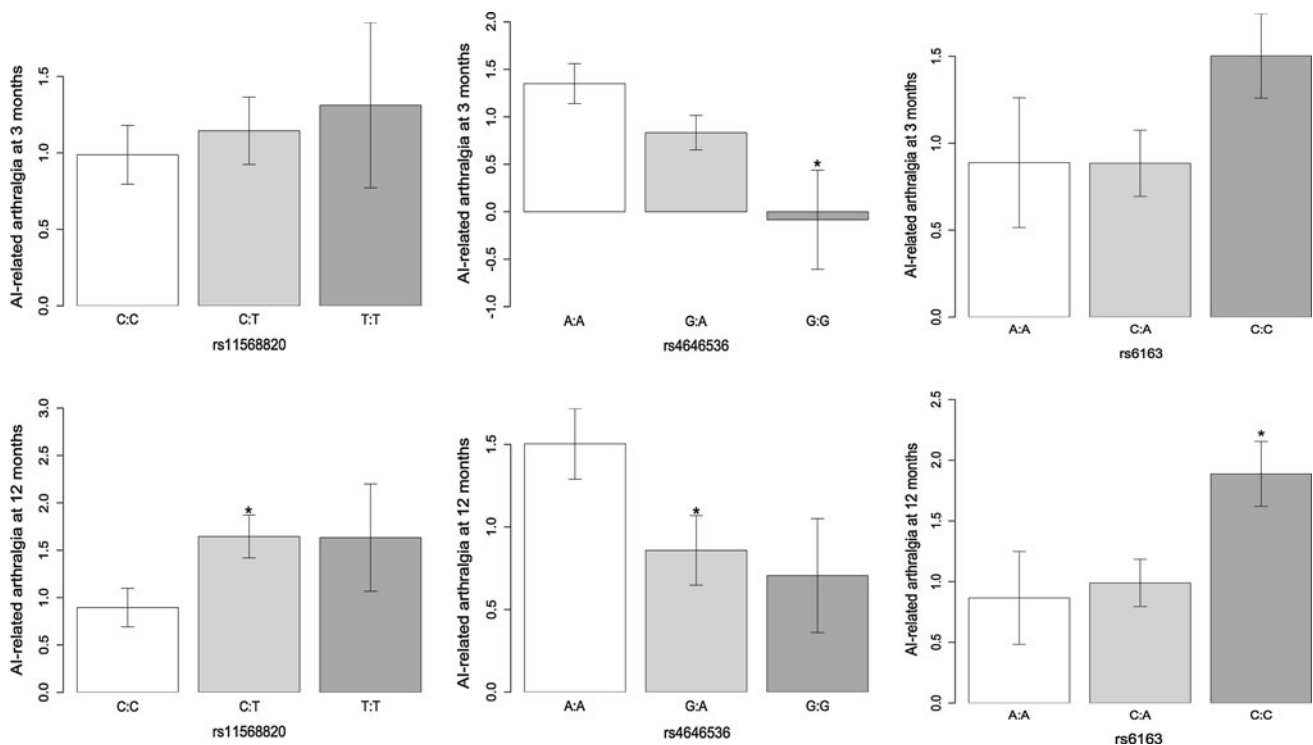
Fig. 3 Association between genotypes of significant SNPs and AI-related arthralgia measured as absolute increase in visual analogic scale (at 3 and 12 months follow-up). *Error bars* show 95 % confidence intervals. *P < 0.05; **P < 0.01

Discussion

We demonstrate that AIA is genetically determined: SNPs located in genes encoding key factors for the metabolism of estrogens and vitamin D (*CYP17A1*, *VDR*, and *CYP27B1*) are associated with self-reported arthralgia during AI therapy. We report a major interaction between *CYP17A1* and *CYP27B1* in our study. Accordingly, patients who carry a bad–bad genotype for both genes have the worst clinical response, with two points higher increase in pain at

Table 4 Effect of compound genotypes in absolute VAS pain increase and worsening pain

SNP interaction	Months	N unfavorable alleles	N individuals	Absolute pain increase Mean (95 % CI)	Worsening pain OR (95 %CI)
Rs6163 × Rs11568820	12	0	26 (8.36 %)	0.36 (−0.77 to 1.49)	Ref
		1	113 (36.33 %)	0.78 (0.28–1.28)	1.24 (0.51–3.05)
		2	118 (37.94 %)	1.50* (1.08–1.93)	2.58* (1.06–6.43)
		3	48 (15.43 %)	2.00** (1.18–2.86)	3.08* (1.12–8.62)
		4	6 (1.93 %)	2.00 (−0.57 to 4.57)	3.02 (0.48–25.32)
Rs6163 × Rs4646536	3	0	No individual		No individual
		1	28 (8.61 %)	0.50 (−0.31 to 1.30)	Ref
		2	106 (32.62 %)	0.83 (0.33–1.32)	1.48 (0.62–3.63)
		3	147 (45.23 %)	0.93 (0.52–1.34)	1.19 (0.52–2.84)
		4	44 (13.54 %)	2.50*** (1.72–3.27)	3.88** (1.42–11.12)
	12	0	No individual		No individual
		1	28 (8.97 %)	0.70 (−0.15 to 1.55)	Ref
		2	102 (32.69 %)	0.76 (0.26–1.25)	1.10 (0.46–2.67)
		3	139 (44.55 %)	1.15 (0.73–1.59)	1.26 (0.52–2.85)
		4	43 (13.39 %)	2.70*** (1.84–3.55)	5.27*** (1.81–16.44)
Rs11568820 × Rs4646536	12	0	10 (3.23 %)	0.45 (−0.61–1.51)	Ref
		1	81 (26.21 %)	0.47 (−0.03–0.98)	0.86 (0.22–3.74)
		2	129 (41.75 %)	1.28 (0.81–1.75)	1.72 (0.45–7.23)
		3	75 (24.27 %)	1.87 (1.27–2.46)	2.84 (0.76–12.41)
		4	14 (4.53 %)	1.59 (−0.19 to 3.36)	1.87 (0.35–10.89)

Reference group (0/1 unfavourable alleles)

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Adjusted for age, BMI, and years since menopause

3 and 12 months follow-up, compared to participants with the most favorable genotype for these same SNPs. Stronger effects were detected when worsening pain was the outcome, with an OR above five for the most unfavorable genotypes. This suggests that both metabolic hormone pathways might be AIA determinants.

CYP17A1 is a key enzyme in the steroidogenic pathway [23]. Assuming that aromatase would maintain some minimum activity during AI therapy, the amount of substrate available might be crucial for this process and would therefore determine the levels of final product. Hence, this estrogen background could contribute to the prevention of AIA.

The other two genes associated with AIA in our data are involved in vitamin D signaling. The potential non-skeletal effects of vitamin D include the synovium and muscle, with putative consequences in the development of arthritis and arthralgia [13]. Consistent with our data, a correlation between musculoskeletal pain intensity and lower vitamin D plasma concentrations has been shown among breast cancer survivors on AI therapy [24].

The SNP rs11568820 is in a cdx-2-binding site in the VDR promoter and can modulate gene expression levels. Arai et al. [25] demonstrated in an in vitro study that rs11568820 alleles have different transcriptional activities.

Interestingly, other genotyped SNPs in the VDR gene did not appear associated to the outcome. This would suggest a possible functionality of rs11568820 in the AIA pathogenesis.

CYP27B1 catalyzes the conversion of calcidiol $(25(OH)D)$ to calcitriol $(1,25(OH)_2D)$ [26] which binds to VDR. Two tag SNPs were genotyped: rs10877012 in the promoter and rs4646536 in the intronic region. Both SNPs reached a $P < 0.05$ in the association with AIA at 3 months of treatment, but did not withstand for FDR correction. However, the fact that this CYP acts in a previous step of VDR signaling would suggest that this association is biologically plausible. Supporting this suggestion, we have also shown an association between these SNPs and therapy discontinuation in our cohort, stressing the main role of vitamin D in the occurrence of arthralgia. We hypothesize that genetic variations in CYP27B1 gene reduce the efficiency of the hydroxylation of 25(OH)D to $1,25(OH)_2D$ resulting in suboptimal concentrations of the active ligand.

There is observational [13] and experimental data [27] suggesting a potential effect of vitamin D supplementation in preventing AIA. All the participants in our study were supplemented with vitamin D at the time of AI initiation according to current guidelines [28, 29], with a 71.38 % of

patients achieving 25(OH)D levels >30 ng/ml. The mean (SD) was of 44.24 (27.75) ng/ml after 3 months of treatment. Low drop-out rates in our cohort (<7 % after 1 year of follow-up) compared to those described by other AIA studies could be explained by this supplementation with vitamin D amongst other factors.

No significant differences in 25(OH)D serum levels were found among genotypes for any SNP at 3 or 12 months of follow-up. Furthermore, the observed associations between selected genetic variants and AIA remained significant after adjusting for both baseline and increments in vitamin D serum levels. This finding suggests that the effect of these genetic determinants on pain intensity is independent of serum 25(OH)D. Accordingly, the two genes related to vitamin D response, which are associated to AIA in our study, work downstream of 25(OH)D.

We hypothesize that some patients with normal vitamin D (25(OH)D) serum levels, but with a less functional genotype for the described SNPs of CYP27B1 and VDR, within a context of reduced estrogen levels, would be more likely to experience AIA. Thus, vitamin D-supplemented women who still experience pain have a genetic background that could explain this response. Some studies have demonstrated that the conversion of 25(OH)D to 1,25(OH)$_2$D by CYP27B1 depends on the substrate concentration [30]. Hence, higher supplementation of 25(OH)D may help to compensate the lack of function of the enzyme, achieving higher serum concentrations of the active ligand (1,25(OH)$_2$D), which in turn would benefit a less functional VDR, contributing to decrease pain levels. However, this intervention may be also insufficient, and therefore alternative therapies should be implemented.

We also describe a borderline significant interaction between CYP27B1 (rs4646536) and VDR (rs11568820). Our study was not powered to detect this interaction. However, the significant differences among rs4646536 genotypes are detected only in a CC context of the rs11568820, suggesting that this interaction could constitute a true finding. This allows us to speculate on the existence of a complex regulatory mechanism of vitamin D response where all proteins and factors involved in the signal pathway are tightly coupled. Therefore, the addition of small functionality changes in different proteins of the pathway drive to determine the final vitamin D response. However, further research is required in order to clarify the genetic architecture underlying 1,25(OH)$_2$D serum concentrations, and to unravel the mechanisms of action responsible for these associations and interactions.

Finally, the additive effect of the alleles observed suggests that the combination of functional genetic variants in CYP17A1, VDR, and CYP27B1 genes are decisive for the occurrence and/or magnitude of arthralgia.

Moreover, CYP19A1 has been associated to worsening pain in our study, replicating previous work by Mao et al. [6]. Interestingly, both studies defined AIA as a binary outcome.

The main limitation of our study is that the sample size available (343 women) does not allow us to study rare genotypes or to examine the interactions with a low effect size. Hence, our results need further replication in larger studies. Nonetheless, our cohort is the largest source of prospective observational data on women with adjuvant AI treatment to our knowledge. Furthermore, the low drop-out rates can be considered as a potential strength allowing the continual follow-up of the patients with AI-treatment.

Conclusion

Genetic variants in the CYP17A1, VDR, and CYP27B1 genes lead to assess the risk of AIA. The determination of these SNPs could be useful in clinical practice to identify women at high risk of AIA and therapy discontinuation, who could then be targeted for higher dose vitamin D supplementation and/or monitoring strategies to improve quality of life and compliance.

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