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ORIGINAL ARTICLE

Predictive markers in elderly patients with estrogen receptor-positive breast cancer treated with aromatase inhibitors: an array-based pharmacogenetic study

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So far, no reliable predictive clinicopathological markers of response to aromatase inhibitors (AIs) have been identified, and little is known regarding the role played by host genetics. To identify constitutive predictive markers, an array-based association study was performed in a cohort of 55 elderly hormone-dependent breast cancer (BC) patients treated with third-generation AIs. The array used in this study interrogates variants in 225 drug metabolism and disposition genes with documented functional significance. Six variants emerged as associated with response to AIs: three located in *ABCG1*, *UGT2A1*, *SLCO3A1* with a good response, two in *SLCO3A1* and one in *ABCC4* with a poor response. Variants in the AI target *CYP19A1* resulted associated with a favourable response only as haplotype; haplotypes with increased response association were also detected for *ABCG1* and *SLCO3A1*. These results highlight the relevance of host genetics in the response to AIs and represent a first step toward precision medicine for elderly BC patients.

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INTRODUCTION

Approximately 80% of breast cancers (BCs) are estrogen receptor (ER) and/or progesterone receptor (PR) positive. In postmenopausal women, the major source of estrogen is the peripheral synthesis of estrone and estradiol through the conversion of androstenedione and testosterone by the aromatase enzyme.[1] At present, the licensed third-generation aromatase inhibitors (AIs) (i.e., anastrozole, letrozole and exemestane) are used in the treatment of this hormone-dependent BC population. Owing to their efficacy and better tolerability compared with tamoxifen, AIs have been increasingly used in elderly women with advanced ER-positive BC that for tumor stage, poor general conditions or refusal are not amenable to conventional chemotherapy.[2–6]

Response to AI treatment is highly variable and difficult to predict, with some patients exhibiting very good long-lasting response, and others rapidly progressing after an initial response or, in some cases, without ever reporting clinical response to treatment. To date, the mechanisms underlying the response to AI treatment have not been elucidated, although several factors have been considered. Clinical characteristics such as tumor subtype, grade, body mass index, Ki67 expression and circulating estrogen levels have been reported to correlate with response, but, so far, results are conflicting.[7,8] More recently, host genetic factors have also been taken into account with particular consideration for variants in the aromatase gene (*CYP19A1*), which is the therapeutic target of AIs.[9–12] Different single-nucleotide polymorphisms (SNPs) of the *CYP19A1* have been hypothesized to modify the enzyme activity or the conformational status of the protein, and thus to influence therapeutic response. However, no consensus exists on specific variants of *CYP19A1* related to AIs efficacy.[9,10,12–14] Similar inconsistencies have been reported for other genetic variants in genes involved in AI pharmacokinetics, especially *CYP3A4*, *CYP2A6*, *CYP4A11*, *CYP1A1/2* and *UGT2B17*.[15–17]

Using a pharmacogenetic array (i.e. DMET, drug metabolizing enzymes and transporters) that simultaneously interrogates 1936 polymorphic variants in 225 genes involved in drug metabolism and disposition, we looked for new host genetic predictors of response to AIs in a cohort of elderly women with either palpable and locally advanced or metastatic ER-positive BC. These patients represent a proper setting to search for putative predictive determinants of therapeutic response to AIs, given the possibility to estimate patient outcomes within a short time-lapse interval from the start of therapy.

MATERIALS AND METHODS

Patients

Elderly postmenopausal BC patients treated with AIs were enrolled from two Institutions (Veneto Institute of Oncology IOV-IRCCS, Padova and Rovigo Hospital, Rovigo), over a 3-year period from September 2010 to September 2013. Women were aged $\geqslant 60$ years with palpable ($\geqslant 20$ mm) and locally advanced or metastatic BC (defined as l-BC or m-BC throughout the text). Metastatic patients were eligible if the interval from previous adjuvant therapy was $\geqslant 12$ months. Only patients with ER-positive and epidermal growth factor receptor 2 (HER2)-negative disease were included in the study. Treatment with any third-generation AI (i.e. anastrozole, letrozole or exemestane) was admissable. Patients who received an AI for < 1 month were excluded. The study was approved by the Ethics Committee of the Veneto Institute of Oncology IOV-IRCCS; all patients

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consented to participate in this study. Data on tumor histological type, grade, Eastern Cooperative Oncology Group performance status, body mass index level and proliferation index Ki67 were recorded. Toxicity was reported and classified according to the National Cancer Institute-Common Toxicity Criteria (NCI-CTC, v.3.0). ER, HER2 and Ki67 expression were determined by our reference laboratory. Response to therapy was evaluated at 6 months according to the Response Evaluation Criteria In Solid Tumors (RECIST, v.1.1), and patients were stratified as responders (complete or $\geqslant 50\%$ response) or non-responders (response $< 50\%$, or disease stabilization, or progression).

DNA extraction and array-based genotyping

Genomic DNA was extracted from peripheral blood using manual extraction according to Flexigene Kit (Qiagen, Milan, Italy) or automated extraction with Magnapure extractor (Roche, Milan, Italy). DNA quality and quantity were determined using NanoDrop 1000 spectrophotometer (Agilent Technologies, Santa Clara, CA, USA). The blood samples for genetic analysis were taken at diagnosis or at different times during AIs treatment. All 55 DNA samples were blinded genotyped for the 1931 SNPs and for the five copy number variations included in the DMET Plus GeneChip array (Affymetrix, Santa Clara, CA, USA). Pharmacogenetic profiles were generated by Affymetrix DMET Console Software (Santa Clara, CA, USA), and evaluated for appropriateness. Genotyping quality was examined by a detailed QC procedure consisting of $>95\%$ successful call rate in samples and internal positive controls. Ten DNA samples were randomly selected and reanalyzed to check the reproducibility of DMET data. After exclusion of the SNPs with minor allele frequency $\leqslant 0.05$ and those on chromosome X, 599 SNPs distributed in 199 drug metabolism and disposition genes were included in the association analysis.

Statistical analysis

Association between the response to AIs and clinicopathological variables was estimated using the Fisher's exact test for categorical and Mann–Whitney test for continuous predictors (Table 1). A Fisher's exact test was performed for all the 599 SNPs using the DMET-analyzer software.[18] The strength of association was estimated using SNPstats software (http://bioinfo.iconcologia.net/SNPstats)[19] according to the dominant model, adjusting the SNPs for clinical variables. SNPstats software was also used for haplotype construction, frequency estimation and assessment of Hardy–Weinberg equilibrium. All tests were two-sided and a $P < 0.05$ was considered statistically significant. False discovery rate correction was not applied considering the exploratory nature of this study. Using the Power of Genetic Analysis (PGA) package,[20] setting α at 0.05 and power at 80%, we calculated that with our sample size the minimum detectable odds ratio (ORs) was $\geqslant 3.5$.

RESULTS

Patient characteristics

The median age of the 55 BC patients enrolled in the study was 79 years (IQR: 73–86; Table 1). At immunohistochemistry, they were all ER-positive ($\geqslant$70%) and HER2-negative; 34 (61%) of them had a Ki67 $< 20\%$. Clinicopathological characteristics of the patient cohort are outlined in Table 1. Thirty patients (54%) had l-BC and 25 patients (46%) had m-BC. Among the m-BC patients, 14 (56%) were metastatic at the first diagnosis, whereas the other 11 (44%) progressed after previous adjuvant therapy (7 treated with tamoxifen and 4 with common chemotherapy). Overall, a good response was observed in 23 patients (42%). No statistically significant association was observed between therapeutic response and the clinical variables age at diagnosis, tumor histotype, status, grade, type of AI used, Eastern Cooperative Oncology Group performance status, body mass index, and Ki67 in the whole cohort (Table 1). No severe toxicity was reported.

Single SNP analysis

Association analysis was performed in the 599 autosomic variants with minor allele frequency >0.05 mapping in 199 drug metabolism genes, out of the total 1936 DMET variants. Six SNPs distributed in the genes *ABCC4*, *SLCO3A1*, *ABCG1* and *UGT2A1* showed a different genotype distribution between responders and non-responders (Table 2). Among them, three SNPs resulted significantly associated with a poor response: rs4148551 in *ABCC4* (OR = 8.23; 95% confidence interval (CI): 1.82–37.21) and rs2283458 (OR = 5.21; 95%CI: 1.45–18.7), and rs9604403 (OR = 4.92; 95% CI: 1.21–20.01) in *SLCO3A1* (Table 2). Conversely, three SNPs resulted associated with a good response: rs2190748 in *SLCO3A1* (OR = 0.16; 95% CI: 0.04–0.70) rs3788007 in *ABCG1* (OR = 0.08; 95% CI: 0.02–0.37) and rs4148304 in *UGT2A1* (OR = 0.10; 95% CI: 0.01–0.67) (Table 2). All the SNPs retained their statistical association

Table 1. Clinicopathological characteristics of elderly women with ER-positive BC receiving AIs

Characteristics	*Total*	*Responders*[a]	*Non-responders*[b]	P-*value*[c]
	N *(%)* *55 (100)*	N *(%)* *23 (42)*	N *(%)* *32 (58)*	
Age at diagnosis (years)				
Median (IQR)	79 (73–86)	81 (77–84)	77 (66–86)	0.16
Histotype				
Ductal	47 (85)	18 (79)	29 (90)	0.41
Lobular	6 (11)	4 (17)	2 (6)	
Other	2 (4)	1 (4)	1 (4)	
Status				
l-BC	30 (54)	15 (65)	15 (47)	0.27
m-BC	25 (46)	8 (35)	17 (53)	
Grade				
G1	3 (5)	2 (9)	1 (4)	0.25
G2	29 (52)	13 (57)	16 (50)	
G3	13 (23)	3 (13)	10 (31)	
NA	10 (20)	5 (21)	5 (15)	
AI received				
Exemestane	26 (47)	13 (56)	13 (41)	0.28[d]
Letrozole	25 (45)	8 (35)	17 (53)	
Anastrozole	4 (8)	2 (9)	2 (6)	
ECOG performance status				
0–1	41 (74)	17 (74)	24 (75)	1
2–3	14 (26)	6 (26)	8 (15)	
BMI level (kg m^{-2})				
$\geqslant 25$	31 (56)	16 (70)	15 (47)	0.25
< 25	18 (33)	5 (21)	13 (41)	
NA	6 (11)	2 (9)	4 (12)	
Ki67 expression				
$< 20\%$	34 (61)	17 (74)	17 (53)	0.25
$\geqslant 20\%$	19 (36)	6 (26)	13 (41)	
NA	2 (3)	—	2 (6)	
Previous treatment				
None	39 (71)	18 (78)	21 (65)	0.74
Tamoxifen	2 (4)	1 (4)	1 (3)	
Chemotherapy	10 (18)	3 (13)	7 (22)	
NA	4 (7)	1 (5)	3 (10)	

Abbreviations: AI, aromatase inhibitor; BC, breast cancer; BMI, body mass index; ECOG, Eastern Cooperative Oncology Group; l-BC, palpable ($\geqslant$20 mm) and locally advanced BC; IQR, interquartile range; m-BC, metastatic BC; NA, not available. [a]Responders, complete response or partial response $\geqslant 50\%$. [b]Non-responders, stable disease or partial response $< 50\%$ or progression at 6 months of therapy. [c]*P*-value according to two-tailed Fisher's exact test. [d]Exemestane versus letrozole+anastrozole.

Table 2. Association between genetic polymorphisms and response to AIs in elderly ER-positive BC patients

Gene and polymorphism	*dbSNP ID*	*Genotype*	*Genotype frequencies*		P-*value*[a]	*OR (95% CI)*	P-*value*[b]	*Adjusted OR (95% CI)*	P-*value*[c]
			R N = *23* N *(%)*	*NR* N = *32* N *(%)*					
*ABCC4_c-*311G>A*	rs4148551	AA	12 (52)	7 (22)	0.025	3.90 (1.21–12.57)	0.020	8.23 (1.82–37.21)	0.003
		AG, GG	11 (48)	25 (78)					
SLCO3A1_c.1513-1102G>A	rs2283458	GG	15 (65)	8 (25)	0.005	4.80 (1.51–15.18)	0.006	5.21 (1.45–18.7)	0.008
		GA, AA	8 (35)	24 (75)					
SLCO3A1_c.1753+4399C>G	rs960440	CC	19 (83)	16 (50)	0.022	4.70 (1.32–17.11)	0.011	4.92 (1.21–20.01)	0.018
		CG, GG	4 (17)	16 (50)					
SLCO3A1_c.1513-5136A>G	rs2190748	GG	3 (13)	13 (41)	0.002	0.20 (0.05–0.78)	0.012	0.16 (0.04–0.70)	0.008
		GA, AA	20 (87)	19 (59)					
ABCG1_c.973+672G>A	rs3788007	GG	7 (30)	23 (72)	0.003	0.17 (0.05–0.55)	0.002	0.08 (0.02–0.37)	0.0002
		AG, AA	16 (70)	9 (28)					
UGT2A1_c.1171G>A (V391I)	rs4148304	GG	16 (70)	30 (94)	0.026	0.15 (0.03–0.82)	0.016	0.10 (0.01–0.67)	0.009
		AG, —	7 (30)	2 (6)					

Abbreviations: AI, aromatase inhibitor; BC, breast cancer; CI, confidence interval; ER, estrogen receptor; NR, non-responders; OR, odds ratio; R, responders; SNP, single-nucleotide polymorphism. [a]*P*-value according to two-tailed Fisher's exact test. [b]*P*-value according to crude OR. [c]*P*-value according to adjusted OR.

Table 3. Haplotype distribution and association with response to AIs in elderly ER-positive BC patients

		Total BC patients				
Gene and polymorphism	*Haplotype*	*Frequency R/NR*	*OR (95% CI)*	P-*value*[a]	*Adjusted OR (95% CI)*	P-*value*[b]
CYP19A1, rs1062033 and rs10046	GT	0.21/0.06	0.18 (0.04–0.7)	0.021	0.12 (0.02–0.68)	0.021
CYP19A1, rs1062033 and rs700518	GG	0.15/0.03	0.15 (0.03–0.8)	0.040	0.12 (0.02–0.81)	0.036
CYP19A1, rs1062033, rs10046, rs700518	GTG	0.15/0.03	0.13 (0.02–0.7)	0.027	0.08 (0.01–0.67)	0.024
SLCO3A1, rs2283458 and rs960440	AG	0.08/0.28	7.44 (1.75–31.65)	0.009	5.92 (1.53–22.95)	0.014
ABCG1, rs3788007 and rs914189	AC	0.36/0.10	0.09 (0.02–0.4)	0.003	0.05 (0.01–0.35)	0.004

Abbreviations: AI, aromatase inhibitor; BC, breast cancer; CI, confidence interval; NR, non-responders; OR, odds ratio; R, responders. [a]*P*-value according to crude OR. [b]*P*-value according to adjusted OR.

with response also after adjustment for the clinical variables histology, stage, Ki67 expression and type of AI used. No association with response was found for the four *CYP19A1* SNPs included in the analysis (Supplementary Table S1).

Haplotype construction

Since SNPs with small individual effects may show an association with phenotype when considered together, we performed a haplotype analysis for the AIs target *CYP19A1*, although no relevant single SNP was detected. We found that rs1062033, either in combination with rs10046 (haplotype GT) (OR = 0.12; 95% CI: 0.02–0.68) or rs700518 (haplotype GG) (OR = 0.12; 95% CI: 0.02–0.81) was associated with a favorable response. Moreover, the copresence of the three *CYP19A1* SNPs rs1062033, rs10046 and rs700518 (haplotype GTG) further increased the strength of association with response (OR = 0.08; 95% CI: 0.01–0.67) (Table 3).

We also carried out haplotype analyses for the genes that emerged from the single SNP analyses, namely *ABCC4*, *SLCO3A1*, *ABCG1* and *UGT2A1*. We used all the informative SNPs encompassed in DMET array for these genes excluding those in linkage disequilibrium. We found one haplotype for *SLCO3A1* (haplotype AG), composed of rs2283458 and rs960440, which exibited a stronger association with a poor response (OR = 5.92; 95% CI: 1.53–22.95), and the haplotype AC in *ABCG1* composed of rs3788007 and rs914189, which had an increased strength of association with a good response (OR = 0.05; 95% CI: 0.01–0.35). No haplotypes came out for *ABCC4* and *UGT2A1* genes (Table 3).

DISCUSSION

Third-generation AIs are recommended for elderly postmenopausal women with ER-positive and HER2-negative BC. Great variability has been observed concerning the response to AIs and, frequently, patients who experience an initial response become resistant and progress.[21] Predictive markers of response to AIs that can be translated into clinical practice are still missing, although many clinical and genetic factors have been taken into account.[7,8,15–17,22,23]

In this study, using a pharmacogenetic array, we searched for host genetic variants predictive of response to AIs in a cohort of elderly women with ER-positive l-BC or m-BC. The clinical management of these elderly patients is complex, primarily because of their age-related frailty and the few alternative therapeutic choices, thus making them a unique setting to discover putative predictive determinants of response to AIs. Few studies have specifically enrolled elderly BC patients and, to the best of our knowledge, no studies on the host genetic susceptibility to AI treatment have been performed in this setting. Our cohort consisted of 55 elderly patients with ER-positive l-BC or m-BC treated with third-generation AIs as either neoadjuvant or first-line

therapy, respectively. No correlation was found between any of the patient clinicopathological characteristics and the response to AIs. Results of the genetic association analysis showed that none of the *CYP19A1* polymorphisms analyzed was significantly associated with response as single variant. This result could be because of the fact that polymorphisms with a relatively low impact cannot be detected as single variants when a limited number of patients is analyzed. Haplotype analysis helped to highlight low-risk SNP associations. Indeed, by performing haplotype construction, we found that *CYP19A1* rs1062033 in combination with either rs10046 (haplotype GT) or rs700518 (haplotype GG) was associated with a good response (OR = 0.12; 95% CI: 0.02–0.68; OR = 0.12; 95% CI: 0.02–0.81, respectively). In our study, rs700518 in *CYP19A1* only emerged when paired with rs1062033, whereas alone it was found to be associated with clinical benefit in an overdominant model by Park *et al.*[10] in a cohort of 109 pre- and postmenopausal Korean patients with metastatic hormone receptor-positive BC treated with letrozole. In the study by Colomer *et al.*[9] involving 65 patients with metastatic hormone receptor-positive BC, median age of 62 years, and evaluated for treatment efficacy in terms of time to progression (TTP), rs4646 in *CYP19A1* was the only variant associated with a favorable therapeutic outcome out of the three analyzed. Although rs4646 has been correlated with AIs efficacy in a few other studies,[11,24] no association was found in our study. In agreement with our results, Ferraldeschi *et al.*[13] reported no significant association with AI treatment outcome within 37 *CYP19A1* variants, which include the variants of this study, in a cohort of 309 BC patients (90% of Caucasian ethnicity). More recently, Leyland-Jones *et al.*[14] found no statistically significant association between six *CYP19A1* polymorphisms, encompassing the variants included in DMET, and letrozole treatment outcomes in tumor specimens of 4861 postmenopausal BC women enrolled in the Breast International Group 1-98 trial. Unfortunately, no *CYP19A1* haplotype data are available from these studies.[13,14] The conflicting results among studies may be because of different response parameters and the end points considered, or to ethnic differences among patient populations (Caucasians[9,11,13] vs Asians[10,24]) as genetic variability of *CYP19A1* and response to AIs have been reported as putatively affected by ethnicity.[25]

Interestingly, some new genetic variants predictive of response to AIs emerged from our study. In particular, rs2190748 in *SLCO3A1*, rs3788007 in *ABCG1* and rs4148304 in *UGT2A1* were found to be associated with a good response, according to the dominant model. For *ABCG1*, it was also possible to define a haplotype, given by the combination of rs3788007 and rs914189. Beyond genetic variants associated with a favorable outcome, two in the above-mentioned *SLCO3A1* and one in *ABCC4* were found to be associated with a poor response. Whereas for *ABCC4* only rs4148551 came out, for *SLCO3A1*, rs2283458 and rs960440 resulted significantly associated with a poor response as single variants and they increased their association when present as haplotype. All these variants also retained their statistical association with response when adjusted for histology, stage, Ki67 expression and type of AI used.

To our knowledge, the seven SNPs distributed in *UGT2A1, SLCO3A1*, *ABCG1* and *ABCC4* genes have not been previously associated with response to AIs. However, these genes that encode membrane transporters can be reasonably implicated in AI metabolism. Indeed, membrane transporters have a critical role in drug response, serving as drug targets and facilitating drug absorption, metabolism and elimination. As reported in the online genomics and genetics databases PharmGKB (http://www.pharmgkb.org) and GeneCards (http://www.genecards.org): (i) *UGT2A1* is involved in the same steroid hormone biosynthesis superpath of *CYP19A1*, and participates in the elimination of estrone and estradiol and their metabolites by glucuronation; (ii) *SLCO3A1* is involved in the transport of organic anions such as estrone-3-sulfate; (iii) *ABCG1* regulates cellular lipid homeostasis; (iv) *ABCC4* has a role in the transport of estradiol glucuronide. Unfortunately, no data about the impact of the SNPs that emerged from our analysis on phenotype are available in PharmGKB and GeneCards databases, and in the literature.

We are aware that the relatively small number of patients included as well as the short follow-up did not allow testing the correlation of these variants with survival outcomes and is a limitation of this study. Furthermore, by using DMET array, which specifically tests drug metabolism and disposition genes, we may lose the putative involvement of variants mapping in genes of other pathways. However, we believe that a pathway-based array such as DMET represents, at present, the best approach to look inside the yet unexplored impact of host genetics in the pharmacokinetics and pharmacodynamics of AIs.

As our BC patient cohort mainly received exemestane or letrozole, the results suggest that the emerged response-related gene variants are most likely those shared by the two types of third-generation AIs (steroidal vs nonsteroidal), justifying somehow the interchangeable use of the AIs in current clinical practice.

In conclusion, through pharmacogenetic DMET array, which interrogates variants in genes specifically involved in drug metabolism and disposition, we described the role of host genetics in the response to AIs. Moreover, we also identified new genetic determinants putatively predictive of therapeutic response in elderly women with ER-positive l-BC or m-BC. These results might be helpful to tailor AI-based therapy and encourage larger studies on AI pharmacogenetics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)