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ORIGINAL ARTICLE

ADRA2A Germline Gene Polymorphism is Associated to the Severity, but not to the Risk, of Breast Cancer

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Abstract Breast cancer (BC) prognosis and risk were associated to obesity, metabolic syndrome and type 2 diabetes mellitus. Two Single Nucleotide Polymorphisms (SNPs) of the adrenergic receptor-2a gene (*ADRA2A*): rs1800544 and rs553668, have been associated to these metabolic disorders. We investigated these SNPs in BC risk and prognosis. A total of 102 BC patients and 102 healthy controls were included. The rs1800544 and rs553668 were determined by real-time PCR. Genotypes and haplotypes frequencies between patients and controls, and for different clinico-pathologic parameters were compared. We found a significant association of rs1800544 GG genotype with young age at diagnosis, premenopausal status, higher tumor size, metastasis in lymph nodes, advanced TNM stages and higher Nottingham Prognosis Indicator (NPI) ($p < 0.05$). There was no association between rs1800544 and SBR stages, Her2, ER and PR statuses and the molecular classification. The rs553668 AA genotype was associated to young age at diagnosis and premenopausal status ($p < 0.05$). The haplotype GA was associated to the early age of diagnosis ($p = 0.03$), and the haplotype GG to higher tumor size, lymph node involvement, advanced TNM stages and Her2 positive status ($p < 0.05$). There was no polymorphism or haplotype association with BC risk ($p > 0.05$). *ADRA2A* polymorphism is associated with indicators BC poor prognosis but not with BC susceptibility. This is the first report suggesting that *ADRA2A* germline gene polymorphism could represent a predictor factor for BC outcome. Further investigation of other *ADRA2A* polymorphisms in BC risk or prognosis are needed and may lead to a genotype-based therapy.

Keywords *ADRA2A* · Single nucleotide polymorphism (SNP) · Breast cancer · Risk · Prognosis · Case-control study

Introduction

Breast cancer (BC) is the second cause of women cancer death in the developed regions after lung cancer, and the first one in less developed regions. With 522,000 deaths per year in the world [1], it represents a critical public health problem.

Genetics has been to the rescue of BC in the last decade, since BC prognostication is now based on both clinico-anatomical criteria and tumor gene expression or their surrogate immunohistochemical criteria [2–4]. But in addition to acquired somatic mutations of the tumor cell, different germline genetic variants have been associated to BC risk [5] and/or outcome [6–8]. However, it is estimated that these germline genetic variants explain only 28 % of the innate causes of BC [5], and much less is known about the role that patient's genetic background plays in BC prognosis [8]. Therefore, identifying genetic variants involved in BC risk and/or progression is of crucial importance.

Adrenoceptors (AR) are G protein-coupled receptors for adrenergic neurotransmitters: adrenalin and noradrenalin. AR can be divided into three types, each one having three subtypes: α1AR (α1A, α1B, α1D); α2AR (α2A, α2B, α2C); and βAR (β1, β2, β3). They are expressed by almost all organs

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in the body and encoded by separate genes. The AR α2A gene (*ADRA2A*) is an intronless gene located in chromosome 10q25.2, encoding a protein receptor of 450 amino acid residues [9]: α2A-adrenergic receptor which is implicated in several functions in the central nervous system, cardiovascular system, neurotransmitter release, platelet aggregation, blood pressure, insulin secretion and lipolysis [10]. *ADRA2A* has several SNPs. The SNP rs1800544 is located in its promoter region [11] and was associated to obesity and body fat content and distribution [12, 13]. Another SNP named rs553668 that is located in the 3′-UTR region was associated to blood pressure response to adrenergic agonists [14], to body fat accumulation in the truncal-abdominal region [15, 16] and to obesity and type 2 diabetes (T2D) [17–19].

On the other hand, it is now well established that obesity is a risk [20–22] and a prognostic factor [23] for BC. This is also valid for T2D patients who are more likely to develop BC, and those among them who have already developed this cancer get a poorer prognosis [24].

This double association of obesity and T2D with *ADRA2A* polymorphisms, on the one hand, and with BC risk/prognosis at the other hand, prompted us to conduct this work aiming to investigate the possible association of *ADRA2A* polymorphisms with BC prognosis indicators and BC risk. Our approach was supplied by the fact that α2-ARs are expressed on BC cell lines [25, 26] which proliferate after stimulation by α2-adrenergic agonists [25, 27] or by catecholestrogens (estrogen metabolites) [28]. This proliferation can be reversed by α2-adrenergic antagonists [26]. Moreover, the same α2-adrenergic agonist/antagonist effects have been reproduced on mammary tumour growth in mice [29]. To our knowledge, the current study is the first investigation of *ADRA2A* germline gene polymorphism association with BC prognosis indicators and BC risk.

Patients, Materials and Methods

Patients and Controls

In this case-control study, unrelated subjects were included: a total of 102 prevalent cases of invasive BC female patients (age 48.51 ± 10.32) and 102 healthy controls (age 47.63 ± 10.31). Patients were diagnosed in the Anti-Cancer Center of Batna, between September 2012 and June 2014. Control subjects were matched to cases by age and geographical origin.

Registered data regarding: tumor size (maximum diameter), lymph node involvement, tumor-nodal-metastatic (TNM) stage, Scarff-Bloom and Richardson (SBR) histological grade at time of diagnosis, as wel as tumor expression of progesterone receptors (PR), estrogen receptors (ER) and human epidermal growth factor receptor-2 (HER2), were obtained from medical records (Table 1). We used these expressions to categorize patients into four molecular subtypes: luminal-A, luminal-B, Her-2, and basal-like according to Perou et al. [30]. The Nottingham Prognostic Index (NPI) was calculated according to Todd et al. [31] then patients were separated into three prognostic groups: Good prognostic group GPG (NPI ≤ 3.4), Moderate prognostic group MPG (3.4 < NPI ≤ 5.4) and Poor prognostic group PPG (NPI > 5.4) (Table 1). Necessary precautions to protect participant's information were taken according to the principles of the World Medical Association Declaration of Helsinki in 1964 and its later amendments. An informed written consent was obtained from all patients and controls.

DNA Extraction and Genotyping

Genomic DNA was extracted from peripheral whole blood using QIAamp DNA Mini Kit (QIAGEN, Basel, Switzerland) according to the manufacturer's protocol. The SNPs rs1800544 and rs553668 were determined by allelic discrimination with validated TaqMan probes using the TaqMan® SNP Genotyping Assays C_7611979_10 and C_996424_20, respectively (Applied Biosystems, Foster City, CA). A quantity of 20 ng DNA was used in each reaction tube with the Type-it® Fast SNP Probe PCR master mix (QIAGEN, Basel, Switzerland) to reach a final volume of 25 μL. The reactions were carried in the Rotor Gene 1.7.94 real-time cycler (QIAGEN, Basel, Switzerland). Amplification was performed according to the following cycling protocol: Initial PCR activation step at 95 °C for 5 min, followed by denaturation at 95 °C for 15 s by 45 cycles, then by annealing/extension at 60 °C for 30 s. Some samples were carried in triplicate in order to insure reproducibility of the assay.

Statistical Analysis

We used QUANTO program version 1.2.4, May 2009 (http://biostats.usc.edu/software) to estimate sample size, with a minor allele frequency data obtained from NCBI (http://www.ncbi.nlm.nih.gov/SNP/) and a study power fixed at 80 %. To test Hardy–Weinberg (HW) equilibrium of alleles frequencies we used SNPstats software [32]. This software was used also to estimate haplotype frequencies in BC cases and controls. The chi-square test or Fisher exact test (when the number of subjects was less than 5) were used to assess the significance of any difference in genotype frequency between subjects or between patient's series. Kruskal–Wallis test was used to assess whether the distribution of a categorical variable is the same between genotype groups. Patient series were stratified according to separate prognostic factors (TNM stage, SBR grade, ER, PR or HER2 receptors status and molecular subtype) or prognostic group (GPG, MPG and PPG). For the molecular subtypes, and as Ki67 expression was not available to well distinguish between luminal-A and luminal-B, these

Table 1 Clinico-anatomic and molecular characteristics of BC patients

Parameter	Patients
Age, n (%)	
≤ 40 y	21 (19)
> 40 y	87 (81)
Menopausal status, n (%)	
premenopausal	59 (55)
postmenopausal	49 (45)
Tumor Size(cm), n (%)	
< 2	11 (10)
2 to 5	66 (62)
> 5	20 (19)
Unknown	10 (9)
Nodal status, n (%)	
Positive	55 (51)
Negative	43 (40)
Unknown	10 (9)
TNM stage, n (%)	
TNM_{I-II}	43 (40)
TNM_{III-IV}	50 (46)
Unknown	15 (14)
SBR grade, n (%)	
1	6 (6)
2	72 (67)
3	23 (21)
unknown	7 (6)
ER status, n (%)	
Positive	59 (55)
Negative	44 (41)
Unknown	5 (5)
PR status, n (%)	
Positive	54 (50)
Negative	47 (44)
Unknown	7 (6)
HER2 status, n (%)	
Positive	33 (31)
Negative	69 (64)
Unknown	6 (6)
Molecular subtype, n (%)	
Luminal-A	45 (42)
Luminal-B	18 (17)
HER2	15 (14)
Basal-like	23 (21)
Unknown	7 (6)
NPI, n (%)	
GPG	8 (7)
MPG	59 (53)
PPG	24 (22)
Unknown	19 (18)

subtypes were merged to form the 'luminal subtype' which is known to have better prognosis than the other subtypes [33]. Odds ratio (OR), used as a measure of association strength, and the corresponding 95 % confidence interval (CI) were calculated. Statistical significance was set at $p < 0.05$ based on a two-sided calculation. Statistical analyses were performed using Microsoft Excel 2007 (Microsoft Corporation) and GraphPad Prism version 6 (GraphPad Software, San Diego, CA).

Results

Allele and Genotype Frequencies

Allele and genotype frequencies of the rs1800544 and rs553668 in BC cases and controls are shown in Table 2. The loci were in HW equilibrium ($p > 0.05$) in both groups. There was no significant difference in the distribution of the rs1800544 or the rs553668 genotypes between BC cases and controls in different genetic models ($P > 0.05$) (Table 3).

Haplotype Analysis

Haplotype frequencies in BC cases and controls were estimated using SNPstats software. The most frequent haplotype in cases and in controls was CG (56 % and 59 %, respectively). No difference was observed between both groups regarding the distribution of all the haplotypes (Table 4).

Age and Menopausal Status at BC Diagnosis

Young age at diagnosis is often associated to more aggressive disease course and to poorer outcome [34–36], therefore we investigated the association between *ADRA2A* polymorphisms and age at BC diagnosis. We plotted the age of BC diagnosis following the genotypes of *ADRA2A* polymorphisms (Fig. 1a). There was a significant difference in the median of the age of onset in each genotype group for both rs1800544 and rs553668 ($p = 0.01$ for each loci), which means that these polymorphisms are associated with age at diagnosis (Fig. 1a). Additionally, and as there is no consensus about age cut-off for defining "young age" BC, we chose to set it at 40 years like other authors [35, 36], than we studied the distribution of the genotypes in the two age groups (≤40 y and >40 y) (Table 5). There was a significant association between the rs1800544 GG genotype and BC early age of onset in a codominant, a recessive and a log-additive genetic model ($p = 0.01$, $p = 0.01$ and $p = 0.02$, respectively). Similarly, the rs553668 AA genotype trended to be associated with BC early age of diagnosis in a codominant and a recessive model although this association did not reach statistical significance (Table 5). In agreement with these results, haplotype analysis

Table 2 Allele frequencies of *ADRA2A* polymorphisms in BC cases and controls and their association with BC risk

SNP	Allele	Cases n (%)	Controls n (%)	OR (95 % CI)	*P* value
rs1800544	C	116 (57)	121 (60)	1	
	G	88 (43)	81 (40)	0.88 (0.59–1.3)	0.53
rs553668	G	148 (76)	91 (77)	1	
	A	46 (24)	27 (23)	0.95 (0.56–1.6)	0.86

OR odds ratio, *CI* confidence interval

showed a significant association of the haplotype (GA), which involves the minor alleles, with early age of diagnosis of BC ($p = 0.03$, OR = 0.43, 95 % CI (0.20–0.94)).

At another hand, we considered genotypes association with menopausal status (Table 6). There was a significant association between the rs1800544 GG genotype and premenopausal status in both a codominant and a recessive genetic model ($p = 0.02$, $p = 0.01$, respectively). Similarly, the rs553668 AA genotype trended to be associated premenopausal status although this association did not reach statistical significance (Table 6). This trend was observed also with the haplotype (GA) ($p = 0.05$, OR = 0.51, 95 % CI (0.26–0.99)).

Clinico-Pathological Features

We studied the association of *ADRA2A* polymorphisms with panoply of clinico-pathological features which are classical indicators of BC prognosis: tumor size, lymph node involvement, TNM stages, SBR grades [37], NPI [31], and ER, PR, Her2 statuses which are predictor factors of BC prognosis, individually [38–41] or in combination [30, 42, 43].

The rs1800544 GG genotype was significantly more frequent in tumors with larger size (> 5 cm), in a codominant and in a recessive genetic model ($p = 0.03$; $p = 0.01$, respectively) (Table 7). This genotype was more frequent also in case of positive lymph node involvement, in a codominant model although this did not reach significant level ($p = 0.05$, OR = 3.21, 95 % IC (1.01–10.22)), but the allele G of rs1800544 was significantly associated to positive lymph node involvement in a log-additive model ($p = 0.03$) (Table 7). After stratifying TNM stages by the menopausal status, the allele G of rs1800544 was found to be strongly associated to higher TNM stages (III and IV) in premenopausal patients ($p = 0.01$). The rs1800544 GG genotype was also significantly more frequent in the PPG (in a codominant model, $p = 0.04$) and the allele G of this SNP was significantly associated to PPG (in a log-additive model, $p = 0.03$) (Table 7). Moreover, there was a significant difference in the median of the NPI between rs1800544 genotype groups ($p = 0.02$), which means that this SNP is associated to the NPI (Fig. 1b). There was no association between rs1800544 and the other clinico-pathologic features: SBR stages, and ER, PR, Her2 statuses, as well as the molecular classification which is based on these statuses, even after stratification by the menopausal status. The rs553668 was not associated with all the studied clinico-pathologic features. However, hayplotype analysis showed that the haplotype GG was significantly associated with poor prognosis indicators: higher tumor size ($p = 0.03$), lymph node involvement ($p = 0.006$), advanced TNM stages ($p = 0.03$) and Her2 expression by the tumor ($p = 0.007$), (Table 8).

Discussion

Through the last decades, BC heterogeneity induced prognostication evolving from the classical clinico-pathological and histological classifications to more accurate classifications based on advances in molecular biology. However, these

Table 3 Genotype frequencies of *ADRA2A* polymorphisms in BC cases and controls and their association with BC risk

SNP	Genotype	Cases	Controls	*P* value for model of inheritance				
		n (%)	n (%)	Codominant	Dominant	Recessive	Over-dominant	Log-additive
rs1800544	C/C	36 (35)	34 (34)	0.26	0.81	0.15	0.18	0.54
	C/G	44 (43)	53 (52)					
	G/G	22 (22)	14 (14)					
rs553668	A/A	08 (08)	01 (02)	0.09	0.55	0.06	0.15	0.87
	G/A	30 (31)	25 (42)					
	G/G	59 (61)	33 (56)					

OR odds ratio, *CI* confidence interval

Table 4 Haplotype frequencies of *ADRA2A* polymorphisms in BC cases and controls and their association with BC risk

Haplotype		Frequency		OR (95 % CI)	*P* value
rs1800544	rs553668	Cases	Controls		
C	G	0.56	0.59	1	
G	A	0.23	0.25	1.01 (0.62–1.65)	0.98
G	G	0.19	0.14	0.74 (0.41–1.33)	0.31
C	A	0	0	-	-

OR odds ratio, *CI* confidence interval

classifications are mainly focusing on tumor intrinsic features [3, 4]. Yet, despite these progresses, different outcomes still exist between patients even when they belong to the same categories [44]. One of the possible explanations to such discrepancies may be the denial of the implication of tumor's growing environment, as part of patients' background genotype, in its progression. In the present study, we explored the association of two SNPs in *ADRA2A* gremline gene with BC prognostic indicators and with BC susceptibility.

We found that rs1800544 GG genotype is significantly associated with poor prognosis indicators: young age at the onset of the disease, premenopausal status, larger tumor size, positive lymph node involvement, advanced TNM stages and higher NPI. The rs553668 AA genotype was significantly associated to young age at the onset of BC and to the premenopausal status, but not to any of the other studied prognosis indicators although many trends to such association have been observed. By haplotype analysis, we found that the haplotype GA is associated to the early onset of the disease and the haplotype GG is associated to poor prognosis indicators: larger tumor size, positive lymph node involvement, advanced TNM stage and Her2 positive status.

The SNP rs1800544 is located in the promoter region of ADRA2A gene [11] and this may lead to its implication in gene expression. Interestingly, Small et al. found that cell-lines that are transfected with different *ADRA2A* haplotypes showed a diffential expression of both mRNA and protein [45]. Another study conducted on human subjects showed that different *ADRA2A* haplotypes are associated to different responses to an α2-AR-agonist: dexmedetomidine [14]. Thus, it is possible that the association of both GA and GG haplotypes with poor prognosis indicators of BC could be explained by a differential expression α2A-AR. It has been shown that the α2A-AR receptors mediate adrenergic suppression of insulin secretion, and that human pancreatic islets having a lower expression of this receptor show a higher insulin secretion [17]. Insulin is known to enhance cell proliferation, angiogenesis and estradiol bioavailability [23]. Therefore, we can hypothesize that the observed association of the *ADRA2A* haplotype GA and GG with poor prognosis indicators could be explained by the elevated

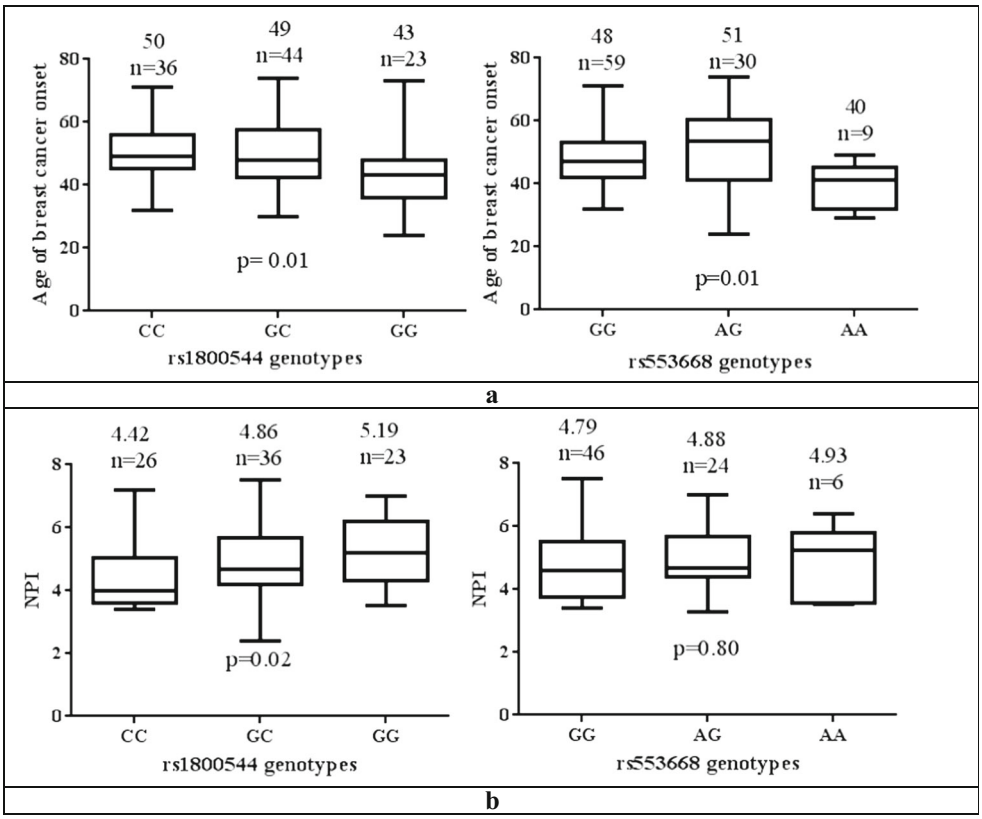


Fig. 1 Distribution of age of breast cancer diagnosis and NPI with respect to genotype. Distribution of BC age of onset (1a) and NPI (1b) with respect to *ADRA2A* polymorphism genotypes (rs1800544 and rs553668). The interquartile range is indicated by the boxes, and the maximum and minimum values are represented by the bars. The *horizontal bar* indicates median values. The number of subjects and the mean value of age (**a**) or NPI (**b**) are mentioned above the each genotype group. *P* value for Kruskal-Wallis test is indicated. *NPI* Nottingham Prognostic Index

Table 5 Genotype association with early age of breast cancer diagnosis

SNP	rs1800544					rs553668				
Model	Genotype	≤40y	>40y	OR (95 % CI)	*P*-value	Genotype	≤40y	>40y	OR (95 % CI)	*P*-value
Codominant	C/C	5 (23.8 %)	31 (38.3 %)	1.00	-	G/G	10 (50 %)	49 (63.6 %)	1.00	-
	G/C	7 (33.3 %)	37 (45.7 %)	0.85 (0.25–2.95)	0.80	A/G	6 (30 %)	24 (31.2 %)	0.82 (0.27–2.51)	0.72
	G/G	9 (42.9 %)	13 (16.1 %)	**0.23 (0.06–0.83)**	**0.01**	A/A	4 (20 %)	4 (5.2 %)	**0.20 (0.04–0.96)**	0.05
Dominant	C/C	5 (23.8 %)	31 (38.3 %)	1.00	0.21	G/G	10 (50 %)	49 (63.6 %)	1.00	0.27
	G/C-G/G	16 (76.2 %)	50 (61.7 %)	0.50 (0.17–1.51)		A/G-A/A	10 (50 %)	28 (36.4 %)	0.57 (0.21–1.54)	
Recessive	C/C-G/C	12 (57.1 %)	68 (84 %)	1.00	**0.01**	G/G-A/G	16 (80 %)	73 (94.8 %)	1.00	0.05
	G/G	9 (42.9 %)	13 (16.1 %)	**0.25 (0.09–0.73)**		A/A	4 (20 %)	4 (5.2 %)	**0.22 (0.05–0.97)**	
Overdominant	C/C-G/G	14 (66.7 %)	44 (54.3 %)	1.00	0.3	G/G-A/A	14 (70 %)	53 (68.8 %)	1.00	0.92
	G/C	7 (33.3 %)	37 (45.7 %)	1.68 (0.61–4.60)		A/G	6 (30 %)	24 (31.2 %)	1.06 (0.36–3.08)	
Log-additive	—	—	—	**0.47 (0.24–0.92)**	**0.02**	—	—	—	0.53 (0.26–1.09)	0.08

OR odds ratio, *CI* confidence interval, statistically significant results are emphasized in bold

insulin secretion in patients who harbour these haplotypes. However, and as we did not investigate other *ADRA2A* SNPs concomitantly with rs1800544 and rs553668, further investigations are needed to confirm or infirm such hypothesis especially because in Small et al. study [45], other *ADRA2A* haplotypes with the same alleles G (rs1800544) and G (rs553668) but accompanied with alleles of other SNPs, showed an enhanced α2A-AR expression. On the other hand, it has been shown by using cDNA microarray technology for assessing gene expression profile in BC tumors, that *ADRA2A* gene expression is lower in the tumors of patients with poor clinical outcome [46–49]. Aditionnaly, Du et al. showed, by using immunohistochemistry on paraffin-embedded samples, that α2a AR expression is associated with Her-2 status (p = 0.048) [50]. Hence, we can speculate that the different expression of α2a AR between tumors with distinct outcome could be explained by differential mutations in tumor genes or by differential genetic background of mammary tissue in the patient before malignancy occurrence.

Additionally, in vitro, in vivo and clinical studies have shown that the long-term effects of stress promote tumor growth and progression [51]. Adrenalin and noradrenalin are at the centre of the adrenergic stress response and are recognized by the adrenergic receptors. Activation of α2A-AR in normal tissues decreases intracellular cyclic AMP (cAMP) by downregulating the synthesis of adenylate cyclase [52] and contribute to the regulation of blood flow in different tissues including mammary gland [53], they also may inhibit milk secretion in this gland [54]. *In vitro* and in vivo studies on BC cell lines showed that activation of alpha adrenergic receptors induced chemoresistance and cell proliferation [25, 55, 56]. Hence, adrenergic receptors may be the leaders of stress effect on BC outcome. Interestingly, in a murine

Table 6 Genotype association with menopausal status

SNP	rs1800544					rs553668				
Model	Genotype	Pre M	M	OR (95 % CI)	*P*-value	Genotype	Pre M	M	OR (95 % CI)	*P*-value
Codominant	C/C	18 (31 %)	18 (40 %)	1.00		G/G	31 (57.4 %)	28 (63.6 %)	1.00	
	G/C	22 (37.9 %)	22 (48.9 %)	1.00 (0.41–2.41)	1	A/G	14 (25.9 %)	16 (36.4 %)	1.27 (0.52–3.05)	0.60
	G/G	18 (31 %)	5 (11.1 %)	**0.28 (0.08–0.91)**	**0.02**	A/A	9 (16.7 %)	0 (0 %)	0.05 (0.003–1.0)	0.007
Dominant	C/C	18 (31 %)	18 (40 %)	1.00	0.34	G/G	31 (57.4 %)	28 (63.6 %)	1.00	0.53
	G/C-G/G	40 (69 %)	27 (60 %)	0.68 (0.30–1.53)		A/G-A/A	23 (42.6 %)	16 (36.4 %)	0.77 (0.34–1.74)	
Recessive	C/C-G/C	40 (69 %)	40 (88.9 %)	1.00	**0.01**	G/G-A/G	45 (83.3 %)	44 (100 %)	1.00	0.02
	G/G	18 (31 %)	5 (11.1 %)	**0.28 (0.09–0.82)**		A/A	9 (16.7 %)	0 (0 %)	0.08 (0.004–1.5)	
Overdominant	C/C-G/G	36 (62.1 %)	23 (51.1 %)	1.00	0.26	G/G-A/A	40 (74.1 %)	28 (63.6 %)	1.00	0.27
	G/C	22 (37.9 %)	22 (48.9 %)	1.57 (0.71–3.45)		A/G	14 (25.9 %)	16 (36.4 %)	1.63 (0.69–3.88)	
Log-additive	—	—	—	0.59 (0.34–1.01)	0.05	—	—	—	0.57 (0.30–1.09)	0.08

OR odds ratio, *CI* confidence interval, *Pre-M* premenopausal patients; *M* postmenopausal patients. Statistically significant results are emphasized in bold

Table 7 Association of rs1800544 genotypes with clinico-pathologic parameters

Parameter	Genetic Model	Genotype	Parameter category n (%)		OR (95 % CI)	*P*-value
			≤ 5 cm	**> 5 cm**		
Tumor size (*n* = 103)	Codominant	C/C	27 (37)	5 (26.3)	1.00	
		G/C	35 (48)	6 (31.6)	0.93 (0.26–3.36)	0.90
		G/G	11 (15.1)	8 (42.1)	**3.93 (1.05–14.69)**	**0.03**
	Recessive	C/C-G/C	62 (84.9)	11 (57.9)	1.00	**0.01**
		G/G	11 (15.1)	8 (42.1)	**4.10 (1.35–12.49)**	
			Negative	**Positive**		
Nodal involvement (*n* = 103)	Codominant	C/C	18 (42.9)	12 (23.5)	1.00	
		G/C	17 (40.5)	24 (47.1)	2.12 (0.81–5.52)	0.15
		G/G	7 (16.7)	15 (29.4)	**3.21 (1.01–10.22)**	0.05
	Log-additive	—	—	—	**1.82 (1.02–3.24)**	**0.03**
			Stage I-II	**Stage III-IV**		
TNM stage (*n* = 103)	Dominant	C/C	18 (45)	12 (25)	1.00	
		G/C-G/G	22 (55)	36 (75)	2.45 (1.00–6.05)	0.04
			Stage I-II	**Stage III-IV**		
TNM stage (premenopausal status, *n* = 58)	Dominant	C/C	9 (45)	4 (14.3)	1.00	**0.01**
		G/C-G/G	11 (55)	24 (85.7)	**4.91 (1.24–19.46)**	
			GPG/MPG	**PPG**		
NPI (*n* = 103)	Codominant	C/C	24 (39.3)	4 (17.4)	1.00	
		G/C	26 (42.6)	11 (47.8)	2.54 (0.71–9.06)	0.23
		G/G	11 (18)	8 (34.8)	**4.36 (1.08–17.63)**	**0.04**
	Log-additive	—	—	—	**2.06 (1.05–4.07)**	**0.03**

OR odds ratio, *CI* confidence interval, *GPG* Good prognostic group (NPI ≤ 3.4), *MPG* Moderate prognostic group (3.4 < NPI ≤ 5.4), *PPG* Poor prognostic group (NPI > 5.4). Only clinico-pathologic features and genetic models showing significant differences are shown. Significant results are emphasized in bold

Table 8 Haplotype association with clinico-pathologic parameters

Parameter	Haplotype rs1800544/ rs553668		Haplotype frequency in parameter's sub-groups		Total haplotype frequency	OR (95 % CI)	*P*-value
			≤ 5 cm	**> 5 cm**			
Tumor size (*n* = 92)	C	G	0.60	0.42	0.57	1.00	—
	G	A	0.23	0.26	0.24	1.62 (0.68–3.84)	0.27
	G	G	0.15	0.31	0.18	**2.58 (1.11–6.02)**	**0.03**
			Negative	**Positive**			
Nodal involvement (*n* = 93)	C	G	0.63	0.47	0.54	1.00	—
	G	A	0.27	0.24	0.26	1.25 (0.65–2.40)	0.51
	G	G	0.09	0.27	0.19	**3.64 (1.46–9.10)**	**0.006**
			Stage I-II	**Stage III-IV**			
TNM stage (*n* = 88)	C	G	0.65	0.50	0.56	1.00	—
	G	A	0.23	0.26	0.25	1.45 (0.73–2.88)	0.29
	G	G	0.11	0.23	0.18	**2.59 (1.08–6.24)**	**0.03**
			Negative	**Positive**			
Her2 status (*n* = 97)	C	G	0.59	0.45	0.55	1.00	—
	G	A	0.25	0.20	0.24	1.07 (0.51–2.25)	0.87
	G	G	0.14	0.34	0.20	**2.92 (1.35–6.29)**	**0.007**

OR odds ratio, *CI* confidence interval, *GPG* Only clinic-pathologic features and genetic models showing significant differences are shown. Significant results are emphasized in bold

model of BC, selective alpha adrenergic receptors antagonists (rauwoscine), reduced tumor volume [29]. Hence, further detection of *ADRA2A* polymorphisms that are associated to poor BC prognosis may open the door for genotype-based treatment testing in this disease.

Conclusion

Our study showed that *ADRA2A* polymorphism rs1800544, alone and in a haplotype combination with rs553668, is associated with indicators of BC poor prognosis: young age at the onset of the disease, premenopausal status, larger tumor size, positive lymph node involvement, advanced TNM stages, higher NPI and Her2 positive status. These data suggest that *ADRA2A* germline polymorphism could represent a predictor factor for BC prognosis. The two studied SNPs were not associated to BC risk. However, further investigation of other *ADRA2A* polymorphisms, either in BC risk or BC prognosis, are needed.

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Authors' Contributions GB conceived of the study, obtained financial support for the project, carried out genotyping, carried out the statistical analyses, managed the project, coordinated data collection and wrote the manuscript. BK carried out genotyping, retrieved the clinical data and wrote the manuscript. WB and KH retrieved the clinical data. WT and MS carried out genotyping. HB co-ordinated data collection.

All authors read and approved the final manuscript.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in this study are in accordance with the ethical standards of the Thematic Agency for Research in Health Sciences (ATRSS, ex-ANDRS) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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