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RESEARCH ARTICLE

Association between SNPs C1236T and C3435T of the *ABCB1* Gene and the Neoadjuvant Chemotherapy in Breast Cancer

Mouhamadou Mansour SY, Fatimata MBAYE, Binta KENEME, Pape Mbacké SEMBENE

Animal Biology Department, Cheikh Anta Diop University, Senegal.

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Corresponding Author: Mouhamadou Mansour SY, Animal Biology Department, Cheikh Anta Diop University, Senegal.

Abstract

Introduction: Breast cancer is the most common malignant tumor in women and the leading cause of cancer-related death among female populations worldwide. Neoadjuvant chemotherapy is a systematic treatment administered before radiotherapy or surgery. It remains a preferred treatment option in cancer management, given its relevant results. However, certain single-nucleotide polymorphisms (SNPs) of the *MDR1* gene are cited as factors that can induce differences between individuals in response to neoadjuvant chemotherapy.

Objectives: The aim of this study was to evaluate the possible association between SNPs of the *ABCB1* gene, specifically the polymorphisms C1236T and C3435T, and the response to neoadjuvant chemotherapy in Senegalese women with breast cancer.

Methods: Our study included 60 senegalese patients diagnosed with breast cancer and treated with four different neoadjuvant chemotherapy protocols (AC, FAC, CARBO and TXT). Wealso included 15 healthy women as a control group. The sample type was a cytological biopsy. The C1236T and C3435T polymorphisms were genotyped using the PCR-RFLP method with respectively the following primers 5′-TTTTTCTCACGGTCCTGGTAG-3′; 5′-CATCCCCTCTGTGGTCATA-3′; and 5CAAAGAAATAAAGC-3′; 5′-CTTACATTAGGCAGTGACT CG-3′. As for restriction enzymes, we used *HaeIII* for exon 12 and *DpnII* for exon 26. Genotypic data was recorded in an Excel spreadsheet, and the results were analyzed using R Studio software, version 3.4.2. The genotypic frequencies were determined using GenePop software, version 4.3. Linear regression tests were conducted to determine association between the *MDR1* gene polymorphisms and neoadjuvant chemotherapy using XLSTAT software (Lumivero, 2023), version 2023.2.0 (1411).

Results: The CC genotype was found in a proportion of 58.3% for the C1236T SNP and 61.6% for the C3435T SNP. Healthy women were exclusively (100%) of the CC genotype for exons 12 and 26. The heterozygous CT genotype was not detected in any of the participants in our study. Linear regression tests mostly showed no association between chemotherapy protocols and the C1236T and C3435T polymorphisms of the *ABCB1* gene. In the cases where the P-value was significant, the association was weak.

Conclusion: In the present study, no significant association was found between the C1236T and C3435T polymorphisms of the *MDR1* gene and neoadjuvant chemotherapy regimens in Senegalese women with breast cancer.

Keywords: *Breast Cancer, ABCB1, polymorphism, association, Senegal.*

1. Introduction

Breast cancer is the most common type of tumor found among female populations worldwide, with an estimated incidence of 2.3 million cases in 2020 [1]. In the same year, the global mortality rate from breast cancer was more than 685,000, making it the leading cause of cancer-related deaths in women [2]. The disease accounts for more than a quarter of cancers

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diagnosed in women and 16% of cancer deaths in females [3, 4].

In Senegal, the epidemiological situation is equally alarming. Breast cancer is the most prevalent type affecting Senegalese women after cervical cancer, with an incidence of 1,917 new cases and a mortality rate of 911 in 2020 [5]. This high level of mortality in developing countries such as Senegal can be attributed to the scarcity of adequate healthcare facilities, lack of qualified medical personnel, and late diagnosis of the disease [6, 7]. Therefore, the fight against breast cancer remains a common challenge for healthcare systems worldwide.

In the management of breast cancer, chemotherapy is a preferred method due to its proven effectiveness [8]. Neoadjuvant chemotherapy is a systematic treatment that is administered before surgery and radiation therapy. It involves the use of chemical molecules, administered either orally or intravenously, that have the ability to suppress or slow down the growth of cancer cells [9, 10].

Chemotherapy retains a central role in cancer treatment due to the satisfactory results it provides in many cases. Tumor regression rates can range from 10% to 50% and, in some cases, a complete response can be obtained [10, 11]. However, it must be acknowledged that there are many other cases where chemotherapy remains ineffective and does not yield the desired results [8]. "Chemo-resistance" in oncology refers to a state in which cancer cells remain unresponsive to the chemical action of anticancer drugs [8].

The term "multidrug resistance mutation 1" (MDR1) refers to a specific mutation that can occur at a gene called the *MDR1* gene, also known as the *ABCB1* gene. Polymorphisms in this gene have been cited as one of the factors that may influence differences between individuals in their response to chemotherapy in cancer management [12, 13]. The *MDR1* gene codes for a 170-kilo Dalton transmembrane glycoprotein called P-glycoprotein (P-gp) [14]. P-gp is an ATP-dependent efflux pump, and one of its main roles is to remove xenobiotics of various kinds from the cell, including the active ingredients of anticancer drugs [15]. For this reason, the *MDR1* gene is considered a potential candidate in chemo-resistance [16, 17].

The *MDR1* gene contains numerous single nucleotide polymorphisms (SNPs), some of which may affect the expression and function of P-gp [18]. The SNP rs1045642 (or C3435T) in the region of the genome known as exon 26, and rs1128503 (C1236T) in exon 12, are among the most common, functional, and clinically relevant variations of the *MDR1* gene [18, 19]. They are synonymous polymorphisms that decrease mRNA expression levels and P-gp activity [15, 20].

Therefore, the aim of this study is to explore association between the C1236T and C3435T polymorphisms of the human *MDR1* gene and the anthracycline and taxane chemotherapy in Senegalese women with breast cancer. It is hoped any insights derived may provide a basis for predictive indicators in clinical treatment.

2. Methods

2.1 Study Population

In this prospective observational study, the adopted approach was a case/control design. It included a total population of 75 individuals, who were unrelated and matched based on age, ethnicity, and type of neoadjuvant chemotherapy protocol. The population consisted primarily of two groups, including:

- A case group consisting of 60 patients, all diagnosed as positive for breast cancer and treated with neoadjuvant chemotherapy using four distinct protocols, known as FAC, AC, CARBO, and TXT, described below. Each protocol involved a subgroup of 15 patients. Recruitment took place at the Joliot Curie Oncology Institute of the Aristide Le Dantec University Hospital in Dakar between March and October 2019. It included four ethnic groups: Wolof (24), Peulh (16), Serrere (14), and others (less representative ethnicities). The age range was between 25 and 80 years. Informed consent was obtained from all the patients who participated in the study.
- A control group, consisting of 15 healthy volunteers aged between 25 and 65 years, with no known familial history of cancer or any other chronic disease. The participants for this group were selected from a DNA biobank established by the GENEPOP team at the Genomics Laboratory of the Department of Animal Biology at the Faculty of Science and Technology of Cheikh Anta Diop University in Dakar, Senegal.

All candidates in our study completed a form that captured all information relevant to our project. The pathological condition of each patient had to be monitored from the beginning of cancer management until the final evaluation of clinical responses using the four distinct protocols.

The sample collection was carried out in accordance with standard rules of good practice. The samples from the "Cases" group of 60 patients were coded with the designation "CS," followed by a specific number assigned to each patient. Individuals in the "Control" group were assigned the letter "T" followed by a specific identification number. A 5 ml blood sample was taken from each patient through venous puncture. The collected blood was placed in an EDTA tube labeled with a code corresponding to the clinical record of the subject, and then stored at -20°C. All necessary measures were taken to ensure our commitment to privacy and anonymity.

Our study obtained approval from the Ethics and Research Committee of Cheikh Anta Diop University under the 0271/2018/CER/UCAD protocol.

2.2 Genetic Study

Total DNA was extracted using the standard Qiagen method (Qiagen DNeasy Blood Kit). Exons 12 and 26 of *MDR1* were amplified using the following primers: for exon 12, 5′-TTTTTCTCACGGTCCTGGTAG-3′; 5′-CATCCCCTCTGTGGTCATA-3′; and for exon 26, 5CAAAGAAATAAAGC-3′; 5′-CTTACATTAGGCAGTGACT CG-3′.

Both exons were amplified under the following conditions, with initial denaturation at 94°C for 5 min, 35 cycles (denaturation at 94°C for 30 s, hybridization at 45°C for 30 s and elongation at 72°C for 30 s), and final elongation at 72°C for 5 min. Amplifications were performed with 2 µL of DNA, 0.5 µL of dNTP, 1 µL of MgCl2, 0.2 µL of Taq polymerase, 0.25 µL of each primer, 2.5 µL of 10 × buffer, and 18.3 µL of water.

Genotyping was performed using restriction fragment length polymorphism (RFLP). Enzymatic digestion was performed using the PCR products incubated with a restriction endonuclease (*HaeIII* and *DpnII*). In the presence of a mutation, digestion produces different fragments. The different genotype variants and their sizes are shown in Table 1. The obtained products were visualized in the presence of ethidium bromide (safeview) on a UV transillumination, after electrophoresis on a 3% agarose gel for 45 minutes.

Table 1. *Definition of the genotypes and their sizes in function of their variants.*

	C1236T			C3435T		
Genotypes	**CC**	**CT**	**TT**	**CC**	**CT**	**TT**
Sizes	85	147	147	145	207	207
	62	85		62	145	
		62			62	
Locus	085085	147085	147147	145145	207145	207207

The RFLP conditions were those recommended by the manufacturer (37°C for 2 hours).

3. Statistical Analyses

All the data obtained were recorded in an Excel spreadsheet. Genotypic and allelic frequencies were generated using the software GenePop version 4.3. Other results were generated using the software R studio, version 3.5.3, and XLSTAT (Lumivero, 2023), version 2023.2.0 (1411).

The first statistical approach consisted of characterizing the case and control populations by describing their age composition. Age is a quantitative variable that was expressed as a mean (M) ± standard deviation (SD) using descriptive statistical tools.

For each polymorphism (SNP), the two populations were classified into three groups: homozygotes (CC), heterozygotes (CT), and homozygotes (TT). The calculation of allelic and genotypic frequencies was performed based on the number of subjects expressing the allele or genotype in question. The verification of a Hardy-Weinberg equilibrium in the distribution of polymorphisms within the control population was carried out using a Khi2 (X^2) goodness-of-fit test. This test was also used to compare the observed frequency with the expected frequency for each allele according to the laws of homogeneous distribution in the general population.

For association tests, the genotype distribution for each protocol was compared with that of the controls. Odds ratios (ORs) were calculated to estimate the strength of the association. The heterozygous genotype CT was eliminated, since it was not detected in any group. However, as the TT genotype was absent in the controls, any resulting OR would be a null or infinite value. For this reason we resorted to the Haldane-Anscombe correction. This involves adding 0.5 to the values of each cell in the contingency table before calculating the OR. The ORs were then given with a 95% confidence interval (CI).

Significance was assessed using the Fisher's exact test, with a P-value < 0.05 considered statistically

significant. A non-significant P-value or an OR equal to 1 indicates no association. If the P-value is significant and the OR is greater than 1, then the genotype would be considered a risk factor for the specific protocol. Finally, if the OR is less than 1 with a significant P-value, the genotype would be considered a protective factor.

4. Results

4.1 Describing the Population

The population consisted of two groups: patients with breast cancer, and healthy control subjects.

The patients were subdivided into four groups, each with a sample size of 15 individuals, according to the applied chemotherapy protocol. Based on their ages, the patients were divided into two categories (< 50 years and ≥ 50 years). The average age of the entire patient group was estimated to be 47.78 ± 11.72 years. Patients under the age of 50 accounted for 34 individuals, or 56.7%, while those aged 50 or older accounted for 26 individuals, or 43.3%. The statistical difference in age distribution was not significant (P > 0.05).

The analysis of the genotypic distribution in the general population of patients revealed that for exon 12, 58.3% had the CC genotype, 33.3% had the TT genotype, and 0% had the heterozygous CT genotype. For exon 26, the CC genotype accounted for 61.6%, the TT genotype for 20%, and the CT genotype for 0%. However, it should be noted that there were undetermined genotypes for exon 12 and exon 26, estimated at 8.4% and 18.4%, respectively.

Another important point to mention is the absence of the heterozygous CT genotype in both the case and control groups. The control group consisted of 15 individuals, all of whom had the CC genotype for the two exons studied.

4.2 Role of C1236T and C3435T Polymorphisms of *MDR1* in the AC Protocol

Table 2 shows the distribution of allele and genotype frequencies of exon 12 and exon 26 polymorphisms of the *ABCB1* gene in a population of Senegalese women with breast cancer treated with the AC chemotherapy protocol, compared with a healthy population (control).

Both groups were in Hardy-Weinberg equilibrium. In the patient group, the genotypic frequencies of exon 12 were distributed as follows: 80% (CC) and 20% (TT), while for exon 26, the frequencies were 86.6% (CC) and 13.4% (TT). In the control group, only the CC genotype was identified in 100% of individuals for both exons.

The heterozygous genotype CT was not detected in either group. A discrepancy was also observed between the observed and expected genotypic frequencies.

The allelic frequencies were identical to the genotypic frequencies, which is expected since all genotyped individuals were homozygous, without exception. The C allele was predominant in the patients for both exons studied, and was the only allele detected in the controls. The fact that the Fis coefficients (inbreeding) were close to 1, suggests an exclusivity of homozygotes.

A statistically significant difference was found between patients treated with the AC protocol and the controls regarding the C1236T and C3435T polymorphisms (P < 0.05).

Table 2. *Distribution of allelic and genotypic frequencies of exon 12 and 26 of MDR1 in patients treated with the AC protocol.*

	Patients		Controls	
Genotype	Observed	excepted	Observed	Excepted
Exon 12	N (%)	N (%)	N (%)	N (%)
CC	12 (80)	9,5 (63,4)	15 (100)	15 (100)
CT	0 (0)	4,9 (33,1)	0 (0)	0
TT	3 (20)	0,6 (3,5)	0 (0)	0
P-value	= 0.0009 W&C = 1		= 10^{-9} R&H = 1.0714	
Exon 26				
CC	13 (86,6)	11,2 (74,7)	15 (100)	15 (100)
CT	0 (0)	3,5 (23,9)	0 (0)	0 (0)
TT	2 (13,4)	0.2 (10,4)	0 (0)	0 (0)
P-value	= 0.0038 W&C = 1		= 10^{-9} R&H = 1.0714	

N = number, % = percentage, P = P-value, W&C= index of Weir and Cockerham, R&H = index of Robertson and Hill. W&C and R&H are blood-relationship parameters that indicate a high presence of homozygotes genotypes when their value is close to 1.

4.3 Role of the C1236T and C3435T polymorphisms of *MDR1* in the CARBO protocol.

Table 3 represents the distribution of allelic and genotypic frequencies of exon 12 and exon 26 of the *ABCB1* gene in Senegalese women with breast cancer treated with the CARBO chemotherapy protocol, compared with a healthy population (control).

The genotypic frequencies were 38.4% CC, 61.6% TT, and 0% CT for exon 12. Meanwhile, exon 26 had 87.5% CC, 14.3% TT, and 0% CT. In the genotypic distribution of the exons, we also observed 13.3% and 53.3% of indeterminate genotypes for exon 12 and exon 26, respectively. A discrepancy was also noted between the expected and actually obtained frequencies. The statistical difference was significant only for exon 12.

Table 3. *Distribution of allelic and genotypic frequencies of exon 12 and 26 of the MDR1 gene in patients treated with the CARBO protocol.*

	Patients		Controls	
Genotype	Observed	excepted	Observed	Excepted
Exon 12	N (%)	N (%)	N (%)	N (%)
CC	5 (38,4)	1,8 (13,8)	15 (100)	15 (100)
CT	0 (0)	6,4 (49,2)	0 (0)	0 (0)
TT	8 (61,6)	4,8 (3,5)	0 (0)	0 (0)
P-value	=0.0003 W&C = 1		$=10^{-9}$ R&H = 1.0833	
Exon 26				
CC	6 (85,7)	5,1 (72,8)	15 (100)	15 (100)
CT	0 (0)	1,8 (25,8)	0 (0)	0 (0)
TT	1 (14,3)	0.1 (10,5)	0 (0)	0 (0)
P-value	=0.00747 W&C = 1		$=10^{-9}$ R&H = 1.0667	

4.4 Role of C1235T and C3435T Polymorphisms in the *MDR1* Gene in the FAC Protocol.

Table 4 represents the distribution of allelic and genotypic frequencies of exon 12 and exon 26 of the *ABCB1* gene in a population of Senegalese women with breast cancer treated with the FAC chemotherapy protocol, compared with a healthy population (control group).

The genotypic frequencies were 71.4% CC, 28.6% TT, and 0% CT for exon 12. By contrast, for exon 26 they were 76% CC, 23.1% TT, and 0% CT. A deviation was also observed between the actual obtained frequencies and the expected frequencies. For both exons, the statistical difference was significant ($P < 0.05$). From the analysis of these results, we observed percentages of undetermined genotypes of 6.6% for exon 12, and 13.3% for exon 26.

Table 4. *Distribution of allelic and genotypic frequencies of exon 12 and 26 of MDR1 in patients treated with the FAC protocol.*

	Patients		Controls	
Genotype	Observed	excepted	Observed	Excepted
Exon 12	N (%)	N (%)	N (%)	N (%)
CC	10 (71,4)	7 (50)	15 (100)	15 (100)
CT	0 (0)	6 (42,8)	0 (0)	0 (0)
TT	4 (28,6)	1 (7,2)	0 (0)	0 (0)
P-value	=0.0004 W&C = 1		$=10^{-9}$ R&H = 1.0769	
Exon 26				
CC	10 (76,9)	7,6 (58,4)	15 (100)	15 (100)
CT	0 (0)	4,8 (36,9)	0 (0)	0 (0)
TT	3 (23,1)	0.6 (2)	0 (0)	0 (0)
P-value	=0.0010 W&C = 1		$=10^{-9}$ R&H = 1.833	

4.5 Role of the C1236T and C3435T Polymorphisms of *MDR1* in the TXT Protocol.

Table 5 represents the distribution of allelic and genotypic frequencies of exon 12 and exon 26 of the *ABCB1* gene in patients treated with the chemotherapy protocol TXT, compared with a healthy population (control).

The populations were in Hardy-Weinberg equilibrium, with a P-value less than 0.05. The genotypic frequencies for exon 12 were 61.5% CC, 38.5% TT, and 0% CT, and for exon 26 were 53.8%, 46.2%, and 0%, respectively. The percentages of indeterminate genotypes were 13.3% for both exons.

The CC genotype was the one most frequently found among the patients, regardless of the protocol used, and it was the only genotype found in the control group.

Table 5. *Distribution of allelic and genotypic frequencies of exon 12 and 26 of MDR1 in patients treated with the TXT protocol.*

	Patients		**Controls**	
Genotype	Observed	excepted	Observed	Excepted
Exon 12	N (%)	N (%)	N (%)	N (%)
CC	8 (61,5)	4,8 (36,9)	15 (100)	15 (100)
CT	0 (0)	6,4 (49,2)	0 (0)	0 (0)
TT	5 (38,5)	1,8 (13,9)	0 (0)	0 (0)
P-value	=0.0003 W&C = 1		=10^{-9} R&H = 1.0833	
Exon 26				
CC	7 (53,8)	3,6 (27,7)	15 (100)	15 (100)
CT	0 (0)	6,7 (51,5)	0 (0)	0 (0)
TT	6 (46,2)	2.7 (20,8)	0 (0)	0 (0)
P-value	=0.0001 W&C = 1		=10^{-9} R&H = 1.833	

4.6 Study of the Association between the C1236T and C3435T Polymorphisms of *MDR1* and Various CAN Protocols

The analysis of results for the C1236T and C3435T polymorphisms of the *MDR1* gene and their association with neoadjuvant chemotherapy protocols was performed solely based on genotypic distributions, rather than allelic distributions. As mentioned earlier, the correlation between patients treated with different protocols and control populations based on genotypic frequency distributions mostly followed the principle of the Hardy-Weinberg equilibrium, which states that, in the absence of disturbing factors, the genetic variation in a population will remain constant from one generation to the next. However, this equilibrium was never observed, regardless of the protocol, if the correlation between patients and controls was assessed based on allelic frequencies.

Table 6 illustrates the results of the univariate linear regression tests between the genotypes of exon 12 of the *MDR1* gene and the different chemotherapy protocols applied to the patients involved in our study. Statistical significance was assessed by the Fischer exact test, with a P-value considered significant if less than 0.05.

The statistical differences were not significant for the AC and FAC protocols. However, for the CARBO and TXT protocols, the differences found were statistically significant, with respective P-values of 0.002 and 0.033. A non-significant statistical value suggested no association between the CC genotype of exon 12 of the *MDR1* gene and the chemotherapy protocol.

Table 6. *Linear regression test between the genotypes of exon 12 and the different protocols.*

	AC		CARBO		FAC		TXT	
Genotypes	P-Value	OR (IC 95%)	P-Value	OR (IC 95%)	P-Value	OR (IC 95%)	P-Value	OR (IC 95%)
CC TT	0.335	0.115 (0.011–0.161)	0.002	0.021 (0.002–0.202)	0.085	0.075 (0.008–0.736)	0.033	0.05 (0.005–0.482)

Separately, Table 7 below represents the association tests between the genotypes of exon 26 and the different chemotherapeutic protocols applied to the patients included in our study. Fisher's exact test was performed, with a significant P-value below 0.05.

The analysis of the table reveals a significant difference only for patients treated with the TXT protocol, with a P-value of 0.04. By contrast, no association was observed between the three other protocols and the exon 26. Our analyses were conducted by calculating only the probabilities of finding the genotype CC compared with the genotype TT because the opposite yields inadequate results with an overestimation of odds ratios.

Table 7. *Linear regression test between the genotypes of exon 26 and the different protocols.*

	AC		CARBO		FAC		TXT	
Genotypes	P-Value	OR (IC 95%)	P-Value	OR (IC 95%)	P-Value	OR (IC 95%)	P-Value	OR (IC 95%)
CC TT	0.351	0.121 (0.014–1.173)	0.343	0.118 (0.012–1.168)	0.079	0.077 (0.010–0.784)	0.04	0.043 (0.004–0.439)

5. Discussion

The *ABCB1* gene may be responsible for reducing intracellular drug levels through ATP-dependent efflux [15]. Indeed, the functioning of the efflux pump causes the expulsion of the medicinal principles out of the cell, thereby rendering the treatment ineffective [21]. Various reports on the association between *MDR1* gene polymorphisms and chemotherapy response have shown conflicting results. While some researchers have found no association between *MDR1* and chemotherapy [22], others have argued the opposite [23].

Following this line of inquiry, we conducted a case/control study aimed at investigating a possible association between chemotherapy protocols and *MDR1* gene polymorphisms in exon 12 and exon 26. Samli et al. (2020) have mentioned rs1045642 (C3435T) in exon 26 and rs1128503 (C1236T) in exon 12 as among the most common, functional, and clinically relevant SNPs [18].

To carry out our work, we started by determining the distribution of allelic and genotypic frequencies of variants in our population in order to compare them with those obtained in other studies.

Firstly, it is important to mention that in our statistical analysis, the genotype frequencies were equal to the allele frequencies. This is due to the absence of the heterozygous genotype CT in all patients. Therefore, all individuals included in the study were homozygous, including the controls. All subgroups were in Hardy-Weinberg equilibrium ($P < 0.05$), including the control subgroup, except for the CARBO protocol in exon 26.

The C1236T polymorphism showed variation in the distribution of genotype frequencies. Among patients treated with the AC protocol, 80% had the CC genotype and 20% had the TT genotype. For those treated with the CARBO protocol, the distribution was 38.4% CC and 61.6% TT. In patients treated with the FAC protocol, 71.4% had the CC genotype and 28.6% had the TT genotype. Lastly, for those treated with the TXT protocol, the distribution was 61.5% CC and 38.5% TT.

We observed that the CC genotype was the most common overall, except for the CARBO protocol, where the TT genotype was slightly more prevalent. However, it is important to note that for this protocol 13.4% of individuals had indeterminate genotypes, presumed to be CC. These results differ from those of Mutlu et al. (2020), who, in their study on the association between breast cancer and *MDR1* gene polymorphisms in Turkish patients, were not only able to identify the heterozygous genotype CT but also found that the genotype TT was more frequent than the genotype CC (40% TT, 25% CT, and 35% CC) [24]. Our results also showed discrepancies with those of Chen et al. (2021) in their study on the correlation between *MDR1* and resistance in Hui patients treated with percutaneous coronary intervention. Their data on the distribution of genotypic frequencies of the C1236T polymorphism attributed 25.5% CC, 40.2% CT, and 34.3% TT to populations of African origin. They concluded that the C1236T polymorphism exists not only in all regions but also in all ethnic groups, with genotypes CC, TT, and CT [25].

Regarding the C3435T polymorphism, the genotype frequencies were 86.6% CC and 13.4% TT for patients treated with the AC protocol, 85.7% CC and 14.3% TT for those treated with the CARBO protocol, 76.9% CC and 23.1% TT for those treated with the FAC protocol, and 53.8% CC and 46.2% TT for patients treated with the TXT protocol.

We observed that the CC genotype was predominant in exon 26, accounting for over 75% of the cases, except

in the presence of indeterminate genotypes as seen in the TXT protocol. These results are consistent with those of Elmagid and colleagues (2021) regarding the dominance of the CC genotype. They found a frequency of the CC genotype equivalent to 55% in their study on the association between the C3435T polymorphism of *MDR1* and epilepsy in Egyptian children. However, unlike us, they also identified the heterozygous CT genotype [26]. Our results had even more similarities with those of Chen et al. (2021) in their study on the correlation between *MDR1* and resistance in Hui patients treated with percutaneous coronary intervention. The frequencies of the T allele for the C3435T polymorphism were 17% among Ghanaians and Kenyans, and 27% among Sudanese. The TT genotype was present in 4% of Kenyans and 6% of Sudanese, in contrast with the CC genotype, which was predominant among individuals of African origin, according to their conclusions [25].

Bezerra and colleagues (2020), in their study on the association between breast cancer and the C3435T SNP in Brazilian patients, found the following genotype distribution: CC (31.2%), CT (43.8%), and TT (25%). They were able to identify the heterozygous CT genotype, which was the most common, contrary to our results [27]. Like Bezerra et al., Priyadarshini and her colleagues (2019) identified a more widespread presence of the heterozygous CT genotype in exon 26 of the *ABCB1* gene in a population from southern India [28]. By comparison, a Turkish study on the association between breast cancer and the C3435T SNP has found a fairly balanced genotypic distribution, with 37.1% CC, 34.3% TT, and 28.6% CT [24].

Our statistical analyses also involve univariate linear regression tests. These tests were aimed at determining whether there is an association between the mononucleotide polymorphisms of the *MDR1* gene (C1236T for exon 12 and C3435T for exon 26) and the chemotherapy protocols applied to the patients included in our study (AC, FAC, CARBO TAXOL, and TXT).

Analysis of exon 12 shows that in patients treated with the AC protocol, the probability of finding genotype CC was 0.115 times that of genotype TT compared to controls, with a statistically non-significant power (P = 0.335, OR = 0.115, and 95% CI = 0.011–1.161). For those treated with the CARBO protocol, the probability of genotype CC was 0.021 times that of genotype TT compared to controls, with a statistically significant difference (P = 0.002, OR = 0.021, and 95% CI = 0.002–0.202). In patients treated with the FAC protocol, the odds of finding the CC genotype were 0.075 times that of the TT genotype, with a statistically non-significant difference (P = 0.085, OR = 0.075, and 95% CI = 0.008–0.736).

Regarding the C1236T polymorphism, patients treated with the TXT protocol had a 0.05 times chance of having the CC genotype compared with the TT genotype, in relation to the controls, with a statistically significant difference (P = 0.033, OR = 0.05, and 95% CI = 0.005–0.482). These results are consistent with those of Haque et al. (2020), who found an association between the platinum-based chemotherapy protocol (CB/TX) and exon 12 in their study on the association between *MDR1* and the type of chemotherapy in Saudi women with ovarian cancer. However, they also established a lack of association between the C1236T polymorphism and Carboplatin and 5FU protocols [29]. In our own results, only the CARBO and TXT protocols appeared to have a weak association with exon 12 of the *MDR1* gene.

A study conducted in Asia (Zhou and al., 2015) on the polymorphisms of *ABCB1* and the chemotherapy response in female patients with malignant tumors showed no association between the C1236T polymorphism of *MDR1* and chemotherapy [30]. Another study by Alsaif et al. (2013) also demonstrated a very weak or nonexistent association between the C1236T polymorphism and chemotherapy in Saudi women with breast cancer [31].

The results of association tests between the protocols of the CAN and exon 26 revealed that in patients treated with the AC protocol, there was a probability of 0.121 times of finding the CC genotype compared to the TT genotype, with a statistically non-significant difference (P = 0.351, OR = 0.121, and 95% CI = 0.014–1.173). In those treated with the CARBO protocol, the probability of finding the CC genotype was 0.118 times that of finding the TT genotype, with a statistically non-significant difference (P = 0.343, OR = 0.118, and 95% CI = 0.012–1.168). In those treated with the FAC protocol, the probability of finding the CC genotype was 0.077 times that of finding the TT genotype, with a statistically non-significant power (P = 0.079, OR = 0.077, and 95% CI = 0.010–0.784). The probability of finding the CC genotype in patients treated with the TXT protocol was 0.043 times that of finding the TT genotype, with a significant statistical difference (P = 0.04, OR = 0.043, and 95% CI = 0.004-0.439).

These results are consistent with those of Haque et al. (2020). Their conclusions on the study of the

association between chemotherapy protocols in ovarian cancer among Saudi subjects and MDR1 polymorphisms established no association between C3435T and the 5FU and Carboplatin protocols. Even so, the authors suggest that the C1236T and C3435T polymorphisms of *MDR1* could play a role in inducing resistance to certain drugs [29]. However, Lévy and colleagues (2013) in their study on the impact of the C3435T polymorphism on the efficacy of docetaxel chemotherapy in women with breast cancer demonstrated that patients carrying the CC genotype had an average docetaxel value, which was statically lower than those carrying the CT and TT genotypes [32]. Another Chinese study (Ji et al., 2012) demonstrates a poorer response for carriers of the TT genotype compared with those carrying the CC genotype in breast cancer patients treated with anthracyclines [33].

The results of our current study suggest a certain homogeneity in the distribution of genotype and allele frequencies of the *MDR1* gene's single nucleotide polymorphisms (SNPs) for exons 12 and 26 in Senegalese women. Specifically, the CC genotype dominates, with an average frequency of 60% for the C1236T SNP and slightly higher for the C3435T SNP, regardless of the ethnic group considered.

Regarding our linear regression tests, the results suggest no association between neoadjuvant chemotherapy protocols and the *MDR1* gene's single nucleotide polymorphisms in exon 12 and 26, as most of the statistical differences are non-significant. In the few cases where we found an association between a polymorphism and a CAN protocol, it was not only weak, but also the CC genotype seemed to be a protective factor against the protocol. Regarding the C1236T polymorphism, there appeared to be an association between exon 12 and the CARBO and TXT protocols, with P-values of 0.002 and 0.033, respectively. However, the chances of finding CC genotypes compared to TT genotypes were only 0.021 times and 0.05 times, respectively. The only statistically significant difference found with the C3435T polymorphism was in the TXT protocol, where the chance of finding the CC genotype compared to the TT genotype was only 0.043 times.

When compared with the highly controversial findings reported in the literature, our results do not allow us to categorically exclude a possible association between the *MDR1* gene polymorphisms C1236T and C3435T and neoadjuvant chemotherapy protocols in Senegalese women with cancer. A different study sample may also reveal a different distribution of genotype and allele frequencies from those revealed by our results. These noted contradictions could be the result of variations in allele frequencies and genotypic polymorphisms among different study populations. They could also be a consequence of variability in environmental factors. Additionally, beside the limited representativeness of our sample size, there were undetermined genotypes in our analyses. These factors, along with the lack of investigations into haplotype effects, can be named as limitations of our study. In support of our findings, Haque and his colleagues (2020) did not find any association between the individual polymorphisms C1236T and C3435T and chemo-resistance. However, they concluded that the combined effect of C1236T and C3435T could have an impact on drug pharmacokinetics [29].

6. Conclusion

Breast cancer is the most common form of malignant tumor and the leading cause of cancer deaths worldwide among women. Neoadjuvant chemotherapy has become an essential component in the treatment of breast tumors due to its effectiveness, although in many other cases tumors can be resistant to chemotherapy. The *MDR1* gene has been cited in several epidemiological studies as one of the main causes of this chemo-resistance.

In this context, the purpose of our work was to study the impact of the mononucleotide polymorphisms of the *MDR1* gene rs1128503 (C1236T) and rs1045642 (C3435T) in neoadjuvant chemotherapy in Senegalese women with breast cancer. These two SNPs of the *ABCB1* gene are frequently mentioned in the literature as being among the most common, functional, and clinically relevant polymorphisms.

Our results indicate an absence of significant association between the choice of neoadjuvant chemotherapy protocols and the C1236T mononucleotide polymorphism in exon 12 and the C3435T polymorphism in exon 26 among Senegalese women with breast cancer. The CC genotype is the most common (approximately 60%) for both polymorphisms, while the heterozygous CT genotype is absent in all patients and even in the controls.

In light of the results revealed by our study and those reported in the literature, it would be important not only to expand the sample size of our study population but also to explore the combined effect of *MDR1* polymorphisms, in addition to studying them individually, in order to obtain more evidential results.

Conflicts of Interest and Funding

The authors declare no conflicts of interest. This study did not receive any funding.

Ethics Committee Statement

Our study received approval from the ethics and research committee of Cheikh Anta Diop University under the 0271/2018/CER/UCAD protocol.

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