

## RESEARCH ARTICLE

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# Association of cytochrome P450 genetic polymorphisms with neoadjuvant chemotherapy efficacy in breast cancer patients

Tatyana A Seredina<sup>\*†</sup>, Olga B Goreva<sup>†</sup>, Valeria O Talaban<sup>†</sup>, Alevtina Yu Grishanova<sup>†</sup> and Vyacheslav V Lyakhovich<sup>†</sup>**Abstract**

**Background:** The enzymes of the cytochrome P450 family (CYPs) play an important role in the metabolism of a great variety of anticancer agents; therefore, polymorphisms in genes encoding for metabolizing enzymes and drugs transporters can affect drug efficacy and toxicity.

**Methods:** The genetic polymorphisms of cytochrome P450 were studied in 395 patients with breast cancer by RLFP analysis.

**Results:** Here, we studied the association of functionally significant variant alleles of CYP3A4, CYP3A5, CYP2B6, CYP2C8, CYP2C9 and CYP2C19 with the clinical response to neoadjuvant chemotherapy in breast cancer patients. A significant correlation was observed between the CYP2C9\*2 polymorphism and chemotherapy resistance (OR = 4.64; CI 95% = 1.01 – 20.91), as well as between CYP2C9\*2 heterozygotes and chemotherapy resistance in women with nodal forms of breast cancer and a cancer hereditary load (OR = 15.50; CI 95% = 1.08 – 826.12) when the potential combined effects were examined. No significant association between chemotherapy resistance and the other examined genotypes and the potential combined clinical and tumour-related parameters were discovered.

**Conclusion:** In conclusion, CYP2C9\*2 was associated with neoadjuvant chemotherapy resistance (OR = 4.64; CI 95% = 1.01 – 20.91) in the population of interest.

**Keywords:** Cytochrome P450 genetic polymorphisms, Neoadjuvant chemotherapy efficacy, Breast cancer

**Background**

Individual variability in drug efficacy and toxicity resulting in different clinical responses is common in therapeutic areas, including breast cancer. It is an important problem in clinical practice because it can lead to therapeutic failure and adverse effects. A wide variety of factors may influence drug availability and drug response, such as race, sex, diet, differences in drug pharmacokinetics and pharmacodynamics, etc. However, the importance of all of these factors is secondary to the effect of polymorphisms in drug metabolizing enzymes, drug transporters and drug targets. Polymorphisms in the genes encoding enzymes responsible for the metabolism of drugs and other xenobiotics and the

functional significance of these polymorphisms are critical for predicting clinical outcomes [1-3].

The members of the cytochrome P450 superfamily are involved in phase I of the xenobiotic metabolizing process. These enzymes catalyze the oxidation of many exogenous and endogenous compounds and are responsible for the metabolism of approximately 90% of clinically prescribed drugs. The CYPs are known to be involved in the metabolism of many anticancer drugs, including cyclophosphamide, 5-fluorouracil, adreamicin, xeloda, ifosfamide, etoposide, paclitaxel, etc. [4]. It was recently shown that the prodrug cyclophosphamide is activated by CYP2B6, CYP2C9 and CYP2C19 and is inactivated by CYP3A4 and CYP3A5 [5]. Xeloda is metabolized by CYP2B6, CYP2C8 and CYP2C9, while adreamicin and methatrexane are metabolized by CYP3A4 [4,6].

Polymorphisms in genes encoding for metabolizing enzymes and drug transporters can affect drug efficacy and

\* Correspondence: [tatyana.seredina@gmail.com](mailto:tatyana.seredina@gmail.com)

<sup>†</sup>Equal contributors

Department of Xenobiochemistry, Institute of Molecular Biology and Biophysics of Siberian Branch of the Russian Academy of Medical Sciences (RIMBB SB RAMS), 2, Timakova str, Novosibirsk 630117, Russia

toxicity. *CYP2C8* and *CYP2C9* are considered highly variable genes and have more than 14 and 34 polymorphic alleles, respectively ([www.imm.ki.se/CYPalleles](http://www.imm.ki.se/CYPalleles)). Most of the *CYP2C9* polymorphisms are known to decrease the enzymatic activity of the enzyme. The *CYP2C8\*3*, *CYP2C9\*2* and *CYP2C9\*3* polymorphic alleles frequently occur among Caucasians and lead to non-synonymous mutations, which result in decreased activity of *CYP2C8* and *CYP2C9* [7]. The *CYP2C8* enzyme is involved in the metabolism of cyclophosphamide, ifosfamide and paclitaxel, while *CYP2C9* metabolizes cyclophosphamide, ifosfamide and tamoxifen and activates tegafur [5,8].

At least 28 *CYP2C19* variant alleles have been previously described ([www.imm.ki.se/CYPalleles](http://www.imm.ki.se/CYPalleles)), 9 of which encode for inactive enzymes. Mutations in exon 5 (*CYP2C19\*2*) and exon 4 (*CYP2C19\*3*) are the most common polymorphisms. Both *CYP2C19\*2*, which causes a 40-nucleotide deletion and a frameshift, and *CYP2C19\*3*, which leads to a premature stop codon, result in the production of a truncated protein without enzymatic activity [9]. *CYP2C19* plays a role in the metabolism of the anticancer drugs cyclophosphamide, ifosfamide, tamoxifen and thalidomide [8].

*CYP2B6* may have as many as 29 polymorphisms, but only *CYP2B6\*5* and *CYP2B6\*7* are likely to be functionally significant and result in decreased enzymatic activity [10,11]. *CYP2B6* is involved in the activation of anticancer drugs such as cyclophosphamide and ifosfamide [5].

*CYP3A* is the P450 cytochrome with the widest range of drug substrates. For the majority of people, it is also the most abundant cytochrome in the liver. *CYP3A4* activity has a wide range of individual activity, up to a 40-fold difference, which may be related to the individual's health status, environment, hormones or genetics. Over 30 *CYP3A4* alleles have been described, including 18 associated, non-synonymous mutations ([www.imm.ki.se/CYPalleles](http://www.imm.ki.se/CYPalleles)). The most common allele is *CYP3A4\*2*, which is found in Caucasians and encodes for a protein with decreased activity. A number of upstream polymorphisms have also been detected. The most extensively studied of these is *CYP3A4\*1B*. This polymorphism alters the putative transcriptional regulatory element - nifedipine oxidase specific element (NFSE), which is known to be required for the expression of *CYP3A4* [12]. Although the *CYP3A4\*1B* allele was initially shown to result in a 1.5-fold increase in transcription in vitro, subsequent reports have indicated no change in its enzymatic activity. Currently, over 11 different *CYP3A5* alleles have been identified. Individuals homozygous for the *CYP3A5\*3* allele appear to not express a functional version of the *CYP3A5* enzyme due to a cryptic splice site that results in the incorporation of intronic sequence in the mature mRNA and the production of a truncated protein due to a premature appearance of termination codon [13]. This is important in anticancer

therapies as *CYP3A* is involved in the metabolism of many drugs, including cyclophosphamide, ifosfamide, docetaxel, paclitaxel, etoposide, irinotecan, tamoxifen, imatinib, gefitinib and vinca-alkaloids [4].

We studied the association of the functionally significant variant alleles of *CYP3A4*, *CYP3A5*, *CYP2B6*, *CYP2C8*, *CYP2C9* and *CYP2C19* with the clinical response to neoadjuvant chemotherapy in breast cancer patients.

## Methods

### Patients

From 1991 to 2007, 395 women (mean age of  $52.00 \pm 9.89$ ) with a morphologically confirmed diagnosis of breast cancer at stages  $T_{1-4}N_{0-3}M_0$  were observed at the Tomsk Cancer Research Institute in the Tomsk Scientific Center of the RAMS. Of these, 261 were treated with one of the following neoadjuvant chemotherapies: CMF or CMXeloda (Cyclophosphamide, Methotrexate and either Fluorouracil or Xeloda), FAC (Fluorouracil, Adreamicin and Cyclophosphamide) and CAF or CAXeloda (Cyclophosphamide, Adreamicin and either Fluorouracil or Xeloda). The chemotherapeutic effect was estimated after 2–4 chemotherapy courses through ultrasonic scanning and mammography according to the World Health Organization criteria. Complete remission (CR) was observed when no breast tumour; partial remission (PR) when the reduction in the tumour area was 50–100%; and stable disease (SD) when the tumour area was reduced 0–50, progressive disease (PD) was recorded if the tumour area increased or if a new lesion was detected. Complete remission was achieved in 3% of the patients; the partial remission rate was 47%; the stable disease rate was 46% and 4% of the patients showed progression of the disease. The patients were divided into two groups according to the results of the neoadjuvant therapy; patients classified as having CR, PR or SD formed the positive response group, while patients classified as showing PD made up the negative chemotherapy response group. The local ethical review boards approved the study protocol (Protocol N3 from 08.06.2005), and all patients provided written, informed consent before acceptance into the study in accordance with the Declaration of Helsinki.

### Genotyping

The genetic polymorphisms of cytochrome P450 were studied in 395 patients with breast cancer by RLFP analysis. Genomic DNA was kindly provided by the Tomsk Oncology Center SB RAMS. Oligonucleotide primers for the *CYP2B6\*5*, *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C19\*2* and *CYP2C19\*3* polymorphisms and restriction endonucleases were designed using the program Vector NTI 8.0. The primers for the *CYP2C9\*2*, *CYP2C9\*3*, *CYP3A4\*2*, *CYP3A4\*B* and *CYP3A5\*3* polymorphisms were described earlier [14–17]. All of the primer sequences are provided in Table 1.

**Table 1 Primers and restriction endonucleases for the CYP450 polymorphisms genotyping**

| Polymorphism               | Oligonucleotide primers         | Amplicone | Restriction endonucleases | Restriction products, b.p.* |
|----------------------------|---------------------------------|-----------|---------------------------|-----------------------------|
| <b>CYP3A5*3</b> (intron 3) | f 5'-catcagttagtagacagatga-3'   | 293       | Ssp I                     | 148 125 20                  |
|                            | r 5'-ggccaacagggaagaaata-3'     |           |                           | (168 125)                   |
| <b>CYP3A4*1B</b> (5'-NTR)  | f 5'-tccaggcataggtaaagatc-3'    | 111       | Acc 36 I                  | 111                         |
|                            | r 5'-aatctattaaatcgctctcac-3'   |           |                           | (85 26)                     |
| <b>CYP3A4*2</b> (exon 6)   | f 5'-tttttgatccattctttgtc-3'    | 124       | Bst MA I                  | 98 26                       |
|                            | r 5'-ttttaagtgatgaattacatggt-3' |           |                           | (124)                       |
| <b>CYP2C9*2</b> (exon 3)   | f 5'-cactggctgaaagactaacagag-3' | 372       | Asp S9 I                  | 179 119 74                  |
|                            | r 5'-gtgatggagtaggtcaccac-3'    |           |                           | (253 119)                   |
| <b>CYP2C9*3</b> (exon 7)   | f 5'-aggaagagattgaacgtgtga-3'   | 130       | ERh I                     | 130                         |
|                            | r 5'-ggcaggctggtgggagaagccaa-3' |           |                           | (104 26)                    |
| <b>CYP2C19*2</b> (exon 5)  | f 5'-ccagagcttgcatattgta-3'     | 230       | Sma I                     | 109 121                     |
|                            | r 5'-gaagcaatcaataaagtcaccga-3' |           |                           | (230)                       |
| <b>CYP2C19*3</b> (exon 4)  | f 5'-ctgggctgtgtccct-3'         | 147       | BamH I                    | 128 19                      |
|                            | r 5'-actggccttacctggct-3'       |           |                           | (147)                       |
| <b>CYP2B6*5</b> (exon 9)   | f 5'-aataccccaacataccacatc-3'   | 121       | Bst F5 I                  | 105 16                      |
|                            | r 5'-gctgggagtcagagccatt-3'     |           |                           | (121)                       |
| <b>CYP2C8*2</b> (exon 5)   | f 5'-aaagtaaagaacaccaagc-3'     | 167       | Kzo9 I                    | 69 65 33                    |
|                            | r 5'-aaacatccttagtaattaca-3'    |           |                           | (98 69)                     |
| <b>CYP2C8*3</b> (exon 3/8) | f 5'-aggcaattcccaatatctc-3'     | 467       | BseR I                    | 310 111 46                  |
|                            | r 5'-caggatgcgcaatgaagac-3'     |           |                           | (356 111)                   |

\* - fragments of the wild type alleles, fragments of the mutant type alleles parentheses.

The PCR reactions were carried out in a 20 µl volume and contained 1x PCR buffer, 1–2 mM MgCl<sub>2</sub>, 250 µM dNTPs, 0.5 µM primers, 2 U Taq DNA polymerase (Medigen, Russia) and 30 ng of genomic DNA. Thermal cycling was performed using an initial denaturation time of 3 min at 94°C followed by 33 cycles of 15 s at 94°C, 30 s at 55°C and 30 s at 72°C. A terminal extension time of 5 min at 72°C was used. The reaction products were digested with the appropriate restriction endonucleases (SibEnzyme Ltd., Russia) (Table 1) for 6 hour at 37°C. The fragments were resolved by PAGE on 10% TBE gels and were subsequently stained with ethidium bromide (10 mg/ml) to visualize the bands using the “VersaDoc System” (Bio-Rad, USA).

#### Statistical analysis

The allelic and genotype frequencies for all examined genes were calculated for descriptive purposes, and correlation analyses were performed to investigate the relationship between the genotypes and the neoadjuvant chemotherapy outcome. The data were analyzed using EpiInfo 6.0. Differences in the distributions of the variables were analyzed by the  $\chi^2$  or Fisher's exact tests, where the cell numbers were less than 5. We also calculated the odds ratios (OR), 95% confidence intervals (CI) and the levels of significance. A *p* value less than or equal to 0.05 was considered statistically significant. All of the genotype distributions were analyzed for Hardy-Weinberg equilibrium.

## Results and discussion

### The distribution of the cytochrome P450 genotypes and allele frequencies in the breast cancer patients

The cytochrome P450 genetic polymorphisms *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3*, *CYP2C19\*2*, *CYP2C19\*3*, *CYP3A4\*1B*, *CYP3A4\*2*, *CYP3A5\*3* and *CYP2B6\*5* were investigated in 395 female breast cancer patients. Table 2 shows the distribution of the cytochrome P450 genotypes and allele frequencies in the patients. The distribution of the genotype frequencies in breast cancer patients is in agreement with the expected frequencies for the majority of the investigated polymorphisms. Significant deviation from Hardy-Weinberg equilibrium was observed for *CYP2C19\*2* (*p* = 0.003) and *CYP2B6\*5* (*p* = 0.0008). This may be indicative of the functional significance of this locus or the variants being in non-equilibrium, linking this locus to breast cancer.

The frequencies of the *CYP2C8\*2* and *CYP2C8\*3* mutant alleles were 0.39% and 8.33% in the investigated group of breast cancer patients. According to the literature, the *CYP2C8\*2* allele is known to be found only in African Americans, which carry the allele at a frequency of 18%, while the *CYP2C8\*3* mutant allele occurs primarily in Caucasians at a frequency of 13%. Neither allele has so far been identified in Asians [18,19].

In this group of breast cancer patients the frequency of the *CYP2C9\*2* and *CYP2C9\*3* mutant alleles was 10.36%

**Table 2 Genotype and allele frequencies of the CYP P450 genes in breast cancer patients**

| Nomenclature     | rs                     | Nucleotide changes | Effect          | n   | Genotype frequencies, % |       |       | Allele frequencies, % |       |
|------------------|------------------------|--------------------|-----------------|-----|-------------------------|-------|-------|-----------------------|-------|
|                  |                        |                    |                 |     | wt                      | ht    | vt    | p                     | q     |
| <b>CYP2C8*2</b>  | rs11572103             | T805A              | I269F           | 383 | 99.22                   | 0.78  | 0.00  | 99.61                 | 0.39  |
| <b>CYP2C8*3</b>  | rs11572080+ rs10509681 | G416A+ A1196G      | R139K + K399R   | 390 | 83.85                   | 15.64 | 0.51  | 91.67                 | 8.33  |
| <b>CYP2C9*2</b>  | rs1799853              | C430T              | R144C           | 391 | 79.54                   | 20.20 | 0.26  | 89.64                 | 10.36 |
| <b>CYP2C9*3</b>  | rs1057910              | A1075C             | I359L           | 394 | 81.22                   | 17.77 | 1.02  | 90.10                 | 9.90  |
| <b>CYP2C19*2</b> | rs4244285              | G681A              | Splicing defect | 393 | 58.52                   | 39.19 | 2.29  | 78.12                 | 21.88 |
| <b>CYP2C19*3</b> | rs4986893              | G636A              | W212X           | 388 | 97.94                   | 2.06  | 0.00  | 98.97                 | 1.03  |
| <b>CYP3A4*1B</b> | rs2740574              | A-392 G            | 5'NTO change    | 389 | 94.86                   | 5.14  | 0.00  | 97.43                 | 2.57  |
| <b>CYP3A4*2</b>  | rs55785340             | T664C              | S222P           | 391 | 100.00                  | 0.00  | 0.00  | 100.00                | 0.00  |
| <b>CYP3A5*3</b>  | rs776746               | A6986G             | R487C           | 392 | 0.26                    | 11.99 | 87.76 | 6.25                  | 93.75 |
| <b>CYP2B6*5</b>  | rs3211371              | C1459T             | Splicing defect | 390 | 85.64                   | 12.56 | 1.79  | 91.92                 | 8.08  |

and 9.90%, which was not significantly different from the number found in the literature for Caucasians, i.e., 8-19% and 0-8.5% [1,7,20-23] for *CYP2C9\*2* and *CYP2C9\*3*, respectively, but it is significantly higher than that found in African Americans, who carry the alleles at a frequency of 1% and 0.5%, respectively. The *CYP2C9\*2* allele has not been detected in a Chinese population, and the frequency

of the *CYP2C9\*3* allele was 2-2.6% [22,24]. The *CYP2C19\*2* mutant allele was found at a frequency of 21.9% in this study, which is not significantly different from previous reports in the literature for Caucasians (15 20%) [1,7] and Japanese (26.7%) [25]. The *CYP2C19\*3* mutant allele frequency was 1.03% in our group of breast cancer patients, which was not different from the value found in

**Table 3 Distribution of CYP2 genotypes and risk of neoadjuvant chemotherapy resistance developing in breast cancer patients**

| Polymorphism                   | Genotype  | Positive neoadjuvant chemotherapy response |       | Negative neoadjuvant chemotherapy response |        | OR          | CI (95%)       | p           |
|--------------------------------|-----------|--|-------|--|--------|-------------|----------------|-------------|
|                                |           | n  | %     | n  | %      |             |                |             |
| <b>CYP2B6*5 C1459T</b>         | <b>CC</b> | <b>205</b>                                 | 82.66 | <b>9</b>                                   | 90.00  | 1.89        | 0.25 - 84.64   | 0.99        |
|                                | <b>CT</b> | <b>37</b>                                  | 14.92 | <b>1</b>                                   | 10.00  | 1.13        | 0.54 - 2.40    | 0.99        |
|                                | <b>TT</b> | <b>6</b>                                   | 2.42  | <b>0</b>                                   | 0.00   | 0.63        | 0.01 - 4.82    | 0.99        |
| <b>CYP2C8*2 T805A</b>          | <b>TT</b> | <b>243</b>                                 | 99.59 | <b>10</b>                                  | 100.00 | 0.04        | 0.01 - 3.55    | 0.08        |
|                                | <b>TA</b> | <b>1</b>                                   | 0.41  | <b>0</b>                                   | 0.00   | 24.30       | 0.28 - 1914.18 | 0.08        |
|                                | <b>AA</b> | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *           |                |             |
| <b>CYP2C8*3 G416A + A1196G</b> | <b>GG</b> | <b>203</b>                                 | 82.19 | <b>6</b>                                   | 60.00  | 0.33        | 0.07 - 1.64    | 0.09        |
|                                | <b>GA</b> | <b>44</b>                                  | 17.81 | <b>4</b>                                   | 40.00  | 3.08        | 0.61 - 13.53   | 0.09        |
|                                | <b>AA</b> | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *           |                |             |
| <b>CYP2C9*2 C430T</b>          | <b>CC</b> | <b>204</b>                                 | 82.26 | <b>5</b>                                   | 50.00  | <b>0.22</b> | 0.05 - 0.99    | <b>0.02</b> |
|                                | <b>CT</b> | <b>44</b>                                  | 17.74 | <b>5</b>                                   | 50.00  | <b>4.64</b> | 1.01 - 20.91   | <b>0.02</b> |
|                                | <b>TT</b> | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *           |                |             |
| <b>CYP2C9*3 A1075C</b>         | <b>AA</b> | <b>211</b>                                 | 84.40 | <b>8</b>                                   | 80.00  | 0.74        | 0.14 - 7.41    | 0.66        |
|                                | <b>AC</b> | <b>36</b>                                  | 14.40 | <b>1</b>                                   | 10.00  | 0.66        | 0.01 - 5.03    | 0.99        |
|                                | <b>CC</b> | <b>3</b>                                   | 1.20  | <b>1</b>                                   | 10.00  | 9.15        | 0.16 - 124.95  | 0.15        |
| <b>CYP2C19*2 G681A</b>         | <b>GG</b> | <b>136</b>                                 | 54.62 | <b>8</b>                                   | 66.67  | 3.32        | 0.64 - 32.61   | 0.19        |
|                                | <b>GA</b> | <b>109</b>                                 | 43.78 | <b>2</b>                                   | 16.67  | 0.32        | 0.03 - 1.66    | 0.20        |
|                                | <b>AA</b> | <b>4</b>                                   | 1.61  | <b>2</b>                                   | 16.67  | 6.13        | 0.11 - 69.01   | 0.20        |
| <b>CYP2C19*3 G636A</b>         | <b>GG</b> | <b>241</b>                                 | 97.97 | <b>10</b>                                  | 100.00 | 0.21        | 0.02 - 10.78   | 0.23        |
|                                | <b>GA</b> | <b>5</b>                                   | 2.03  | <b>0</b>                                   | 0.00   | 4.82        | 0.09 - 48.97   | 0.23        |
|                                | <b>AA</b> | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *           |                |             |

\*- OR, CI (95%) and p-values were not applicable to these samples.

**Table 4 Distribution of CYP3 genotypes and risk of neoadjuvant chemotherapy resistance developing in breast cancer patients**

| Polymorphism             | Genotype  | Positive neoadjuvant chemotherapy response |        | Negative neoadjuvant chemotherapy response |        | OR    | CI (95%)       | p    |
|--------------------------|-----------|--|--------|--|--------|-------|----------------|------|
|                          |           | n  | %      | n  | %      |       |                |      |
| <b>CYP3A4*1B A-392 G</b> | <b>AA</b> | <b>234</b>                                 | 94.74  | <b>9</b>                                   | 90.00  | 0.04  | 0.01 - 3.47    | 0.08 |
|                          | <b>AG</b> | <b>13</b>                                  | 5.26   | <b>1</b>                                   | 10.00  | 24.90 | 0.29 - 1961.25 | 0.08 |
|                          | <b>GG</b> | <b>0</b>                                   | 0.00   | <b>0</b>                                   | 0.00   | *     |                |      |
| <b>CYP3A4*2 T664C</b>    | <b>TT</b> | <b>249</b>                                 | 100.00 | <b>10</b>                                  | 100.00 | *     |                |      |
|                          | <b>TC</b> | <b>0</b>                                   | 0.00   | <b>0</b>                                   | 0.00   | *     |                |      |
|                          | <b>CC</b> | <b>0</b>                                   | 0.00   | <b>0</b>                                   | 0.00   | *     |                |      |
| <b>CYP3A5*3 A6986G</b>   | <b>AA</b> | <b>0</b>                                   | 0.00   | <b>0</b>                                   | 0.00   | *     |                |      |
|                          | <b>AG</b> | <b>33</b>                                  | 13.31  | <b>1</b>                                   | 10.00  | 0.72  | 0.02 - 5.53    | 0.99 |
|                          | <b>GG</b> | <b>215</b>                                 | 86.69  | <b>9</b>                                   | 90.00  | 1.38  | 0.18 - 62.36   | 0.99 |

\*- OR, CI (95%) and p-values were not applicable to these samples.

the literature data for Caucasians (0.0%) or Asians (3.75%) [26]. This allele occurs only in heterozygosity. None of the investigated patients were homozygotes for the *CYP2C19\*3* allele. The *CYP2B6\*5* mutant allele frequency was 8.1%, which was not significantly lower than the published value for Caucasians (9.1%) and higher than that published for Asians (4.2%).

The frequency of the *CYP3A4\*1B* mutant allele was 2.57% in this group of breast cancer patients; the literature reports significant interethnic variations in this allele (2-9% for Caucasians, 35-66% for African Americans and 0% for Taiwanese and Chinese [27]). The *CYP3A4\*2* mutant allele was not present in the investigated group of breast cancer

patients, while in its frequency in Caucasians ranges from 1.1% in a German population to 4.5% in a Portuguese population [28].

The frequency of the *CYP3A5\*3* polymorphic allele in the total population sampled was 93.75%. This agrees with the previously published data, which reports a total frequency of the *CYP3A5\*3* allele of 91.7- 94.2% in Caucasians [14,27,29] and 66.7-75% in Asians [30].

#### The cytochrome P450 polymorphisms and the chemotherapy efficacy in the breast cancer patients

Table 3 shows the distribution of the polymorphic variants of the analyzed genes in the breast cancer patients along

**Table 5 Risk of chemotherapy resistance in breast cancer patients with different CYP2C9\*2 genotypes and tumour characteristics**

| Group                                      | Allele/Genotype CYP2C9*2 C430T | Positive neoadjuvant chemotherapy response |       | Negative neoadjuvant chemotherapy response |        | OR    | CI (95%)      | p            |
|--|--------------------------------|--|-------|--|--------|-------|---------------|--------------|
|  |                                | n  | %     | n  | %      |       |               |              |
| <b>Cancer hereditary load</b>              | <b>C</b>                       | <b>167</b>                                 | 91.76 | <b>7</b>                                   | 70.00  | 4.77  | 0.71 - 23.47  | <b>0.055</b> |
|  | <b>T</b>                       | <b>15</b>                                  | 8.24  | <b>3</b>                                   | 30.00  |       |               |              |
|  | <b>CC</b>                      | <b>76</b>                                  | 83.52 | <b>2</b>                                   | 40.00  | 0.13  | 0.01 - 1.29   | <b>0.04</b>  |
|  | <b>CT</b>                      | <b>15</b>                                  | 16.48 | <b>3</b>                                   | 60.00  | 7.60  | 0.78 - 95.24  | <b>0.04</b>  |
|  | <b>TT</b>                      | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *     |               |              |
| <b>Nodal form</b>                          | <b>C</b>                       | <b>371</b>                                 | 91.83 | <b>10</b>                                  | 71.43  | 4.50  | 0.97 - 16.60  | <b>0.027</b> |
|  | <b>T</b>                       | <b>33</b>                                  | 8.17  | <b>4</b>                                   | 28.57  |       |               |              |
|  | <b>CC</b>                      | <b>169</b>                                 | 83.66 | <b>3</b>                                   | 42.86  | 0.15  | 0.02 - 0.92   | <b>0.019</b> |
|  | <b>CT</b>                      | <b>33</b>                                  | 16.34 | <b>4</b>                                   | 57.14  | 6.83  | 1.09 - 48.12  | <b>0.019</b> |
|  | <b>TT</b>                      | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *     |               |              |
| <b>Cancer hereditary load + nodal form</b> | <b>C</b>                       | <b>136</b>                                 | 91.89 | <b>3</b>                                   | 50.00  | 11.33 | 1.32 - 91.23  | <b>0.01</b>  |
|  | <b>T</b>                       | <b>12</b>                                  | 8.11  | <b>3</b>                                   | 50.00  |       |               |              |
|  | <b>CC</b>                      | <b>62</b>                                  | 83.78 | <b>0</b>                                   | 0.00   | 0.06  | 0.01 - 0.91   | <b>0.02</b>  |
|  | <b>CT</b>                      | <b>12</b>                                  | 16.22 | <b>3</b>                                   | 100.00 | 15.50 | 1.08 - 826.12 | <b>0.02</b>  |
|  | <b>TT</b>                      | <b>0</b>                                   | 0.00  | <b>0</b>                                   | 0.00   | *     |               |              |

\*- OR, CI(95%) and p-values were not applicable to these samples.

with the known efficacy of the neoadjuvant chemotherapy for the CYP2 family. The study of the association of the *CYP2B6\*5*, *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*2*, *CYP2C9\*3*, *CYP2C19\*2* and *CYP2C19\*3* variants with a negative response to neoadjuvant chemotherapy in breast cancer patients included an odds ratio evaluation indicating the probability of poor chemotherapeutic efficacy in individuals with certain genotypes. The observed distribution of the most common genotypes in breast cancer patients are in Hardy-Weinberg equilibrium with the exception of *CYP2B6\*5* ( $p = 0.0198$ ) and *CYP19\*2* ( $p = 0.0003$ ). The enrichment of the *CYP2B6\*5* and *CYP19\*2* alleles in this group may be due to the population possessing its own pool of alleles, resulting in different frequencies of unfavorable alleles. The risk of an insufficient response to breast cancer neoadjuvant chemotherapy in *CYP2C9\*2* heterozygotes was 4.64-fold higher ( $OR = 4.64$ ,  $p = 0.02$ ) than in patients with the wild type allele. The impaired efficacy of neoadjuvant chemotherapy in patients containing the *CYP2C9\*2* mutant allele might be a result of the enzymatic activity of CYP2C9, which is involved into the chain reaction responsible for the conversion of the cyclophosphamide prodrug into an active metabolite [29,30].

The risk of poor neoadjuvant chemotherapy efficacy is much higher in *CYP2C8\*2* heterozygotes ( $OR = 24.30$ ) and slightly higher in *CYP2C8\*3* heterozygotes ( $OR = 3.08$ ) at a level of statistical significance close to the proposed level ( $p = 0.08$  and  $p = 0.09$ , respectively, versus  $p < 0.05$ ). Furthermore, the *CYP2C9\*3* ( $OR = 9.15$ ) and *CYP2C19\*2* ( $OR = 6.13$ ) mutant type genotypes and heterozygotic *CYP2C19\*3* ( $OR = 4.82$ ) genotype are associated with a low efficacy of neoadjuvant chemotherapy; however, these associations were not found to be statistically significant ( $p > 0.05$ ). It appears that the *CYP2B6\*5*, *CYP2C8\*2*, *CYP2C8\*3*, *CYP2C9\*3*, *CYP2C19\*2* and *CYP2C19\*3* mutant alleles are not a factor in resistance to neoadjuvant chemotherapies in breast cancer patients.

Table 4 shows the distribution of the polymorphic variants of the genes studied here in breast cancer patients along with the known efficacy of the neoadjuvant chemotherapy for the CYP3 family. Gene polymorphisms association with neoadjuvant chemotherapy efficacy were analyzed for the *CYP3A4\*1B* and *CYP3A5\*3* polymorphisms. *CYP3A4\*2* was not included in the study because no heterozygotes or homozygotes containing the *CYP3A4\*2* mutant allele were found in the population. For *CYP3A4\*1B* heterozygotes, the risk of a negative response to neoadjuvant chemotherapy is 24.9-fold higher than in carriers of the wild type allele, which has a level of significance ( $p = 0.08$ ) that approaches the significance threshold ( $p = 0.05$ ). No significant association between *CYP3A5\*3* genotypes and chemotherapy resistance were discovered.

The analyses performed here cover the distribution of cytochrome genetic polymorphisms and their association with the known efficacy of neoadjuvant chemotherapy in breast cancer patients known to have a cancer hereditary load (cancer cases in patient's relatives), a clinical form of cancer, degree of malignancy and histological type of tumour. In *CYP2C9\*2* heterozygotes with a high hereditary load, the risk of tumour resistance to neoadjuvant chemotherapy was 7.6-fold higher than wild type homozygotes ( $OR = 7.6$ ,  $p = 0.04$ ) (Table 5). A similar association was observed in patients with the nodal form of breast cancer. The risk of tumour resistance to chemotherapy for *CYP2C9\*2* heterozygotes was 6.83-fold higher than in wild type homozygotes (Table 5;  $OR = 6.83$ ,  $p = 0.019$ ). In *CYP2C9\*2* heterozygotes with nodal form of cancer and a cancer hereditary load, the risk of resistance to chemotherapy was 15.5-fold higher than those with the wild type genotype ( $OR = 15.5$ ,  $p = 0.02$ ). Patients with other clinical features and genetic variants of the investigated cytochromes showed no statistically important association with neoadjuvant chemotherapy efficacy.

## Conclusions

*CYP2C9\*2* polymorphism is associated with neoadjuvant chemotherapy efficacy in breast cancer patients ( $OR = 4.64$ ;  $CI\ 95\% = 1.01 - 20.91$ ).

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

TAS, OBG, VOT carried out the molecular genetic studies, performed the statistical analysis and drafted the manuscript. AYUG and VWL conceived of the study, and participated in its design and coordination and conducted data acquisition. All authors read and approved the final manuscript.

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