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RESEARCH ARTICLE

Single-nucleotide polymorphisms and mRNA expression of *CYP1B1* influence treatment response in triple negative breast cancer patients undergoing chemotherapy

AHMAD AIZAT ABDUL AZIZ[1], MD SALZIHAN MD SALLEH[2], IBTISAM MOHAMAD[3], VENKATA MURALI KRISHNA BHAVARAJU[4], MAYA MAZUWIN YAHYA[5], ANDEE DZULKARNAEN ZAKARIA[5], SIEW HUA GAN[6] and RAVINDRAN ANKATHIL[1]*

[1]*Human Genome Centre, and* [2]*Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*
[3]*Department of Pathology, Hospital Raja Perempuan Zainab II, Kota Bharu, Kelantan, Malaysia*
[4]*Adventist Oncology Center, Penang Adventist Hospital, Pulau Pinang, Malaysia*
[5]*Department of Surgery, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia*
[6]*Pharmacy, Monash University Malaysia, Jalan Lagoon Selatan, 47500 Bandar Sunway, Selangor, Malaysia*
*For correspondence. E-mail: rankathil@hotmail.com.

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Abstract. Triple negative breast cancer (TNBC) is typically associated with poor and interindividual variability in treatment response. Cytochrome P450 family 1 subfamily B1 (*CYP1B1*) is a metabolizing enzyme, involved in the biotransformation of xenobiotics and anticancer drugs. We hypothesized that, single-nucleotide polymorphisms (SNPs), *CYP1B1* 142 C>G, 4326 C>G and 4360 A>G, and *CYP1B1* mRNA expression might be potential biomarkers for prediction of treatment response in TNBC patients. *CYP1B1* SNPs genotyping (76 TNBC patients) was performed using allele-specific polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism methods and mRNA expression of *CYP1B1* (41 formalin-fixed paraffin embedded blocks) was quantified using quantitative reverse transcription PCR. Homozygous variant genotype (GG) and variant allele (G) of *CYP1B1* 4326 C>G polymorphism showed significantly higher risk for development of resistance to chemotherapy with adjusted odds ratio (OR): 6.802 and 3.010, respectively. Whereas, *CYP1B1* 142 CG heterozygous genotype showed significant association with good treatment response with adjusted OR: 0.199. *CYP1B1* 142C-4326G haplotype was associated with higher risk for chemoresistance with OR: 2.579. Expression analysis revealed that the relative expression of *CYP1B1* was downregulated (0.592) in cancerous tissue compared with normal adjacent tissues. When analysed for association with chemotherapy response, *CYP1B1* expression was found to be significantly upregulated (3.256) in cancerous tissues of patients who did not respond as opposed to those of patients who showed response to chemotherapy. Our findings suggest that SNPs together with mRNA expression of *CYP1B1* may be useful biomarkers to predict chemotherapy response in TNBC patients.

Keywords. triple negative breast cancer; cytochrome P450 family 1 subfamily B1; single-nucleotide polymorphisms; mRNA; chemotherapy response.

Introduction

Triple negative breast cancer (TNBC) which is characterized by negative staining of oestrogen (ER), progesterone expressions and neither expression nor amplification of human epidermal growth factor receptor-2 (HER-2) (Hammond *et al.* 2010; Goldhirsch *et al.* 2011) accounts for 10–20% of breast cancer cases (Boyle 2012). TNBC is a more aggressive breast cancer that is associated with high recurrence rate, high histological grade and poorer

prognosis compared with other subtypes (Foulkes *et al.* 2010).

Chemotherapy is the mainstay of TNBC treatment as this subtype is normally sensitive to chemotherapy (Pogoda *et al.* 2013). To date, there has been no standard chemotherapy regimen specifically utilized in TNBC. However, adjuvant chemotherapy with taxanes, anthracyclines and alkylating agent are recommended (Foulkes *et al.* 2010; Goldhirsch *et al.* 2011). Recurrence or metastasis and drug resistance remain major challenges in the success of TNBC treatment. It has been estimated that most cancer deaths are caused by failure in chemotherapy due to development of drug resistance by tumour cells (Goldman 2003). Among many factors for recurrence or metastasis and drug resistance, one proposed mechanism is activation (or inactivation) of drug metabolizing enzymes including the cytochrome P450 (CYP) family (Michael and Doherty 2005; Housman *et al.* 2014). CYP is a multigene family of enzymes implicated in the metabolism of a diverse range of xenobiotics and endogenous compounds. CYP family 1 subfamily B1 (CYP1B1) is an enzyme involved in biotransformation of xenobiotics, bioactivation of procarcinogens and an anticancer drug metabolizer (Chun and Kim 2003). *CYP1B1* has been widely studied in various types of cancers including breast, ovarian, prostate and endometrium (Gajjar *et al.* 2012).

CYP1B1 is located on chromosome 2p22.2 and consists of three exons. The presence of single-nucleotide polymorphisms (SNPs) in *CYP1B1* may contribute to interindividual variation in drug efficacy and toxicity, variability in gene expression and/or functional protein resulting in therapeutic failures and adverse drug effects (Sissung *et al.* 2008; Laroche-Clary *et al.* 2010; Rizzo *et al.* 2010). Additionally, the expression of *CYP1B1* may contribute to chemoresistant phenotypes of cancer (Martinez *et al.* 2008; Sissung *et al.* 2008; Zhu *et al.* 2015). Taking into consideration the fact that *CYP1B1* is important in metabolizing many anticancer drugs, progression of tumour and also contributes to chemoresistance, determination of the association of SNPs and mRNA expression of *CYP1B1* with chemotherapy response may provide some potential clues. We hypothesized that SNPs and mRNA expression of *CYP1B1* is associated with the outcome of chemotherapy response among TNBC patients. Therefore, the objective of this study was to investigate the genetic polymorphisms and expression of *CYP1B1* in modulating chemotherapy response among TNBC patients.

Materials and methods

Study subjects

This study was approved by the Research Review Board and Ethics Committee of Universiti Sains Malaysia [USMKK/PPP/JEPeM (260.39210)] and Ministry of Health, Malaysia (NMRR-15-1200-25230) which complies with the Declaration of Helsinki. The study subjects were recruited from Hospital Universiti Sains Malaysia and Hospital Raja Perempuan Zainab II while analysis was carried out at the Human Genome Centre, Universiti Sains Malaysia, Kubang Kerian, Kelantan.

Patients who were histopathologically confirmed as TNBC by immunohistochemistry, who had undergone surgical resection and who have completed six cycles of chemotherapy with taxane, adriamycin and cyclophosphamide (TAC) were included in the study. There were two phases of the study involving blood and tissue analyses. For blood analysis, peripheral blood samples were collected from 76 TNBC patients, whereas for tissue analysis, 41 formalin-fixed paraffin embedded (FFPE) tissue blocks containing cancerous and noncancerous normal adjacent tissues were collected. Peripheral blood (3 mL) and tissue FFPE blocks of the patients were collected after obtaining written informed consent. Clinical and pathological data of the patients such as the type, stage, grade of tumour, status of lymph nodes and menopausal status were also recorded.

Evaluation of treatment response

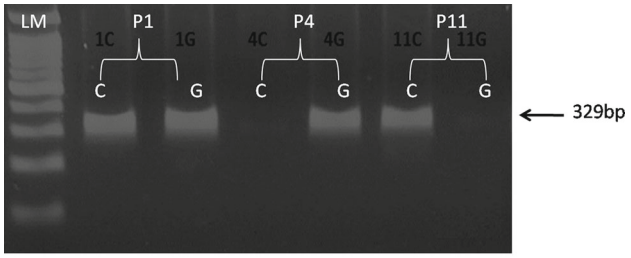
TNBC patients who had undergone surgical treatment and completed six cycles of chemotherapy with TAC were evaluated after 1 year. Patients were categorized into resistant if the patients developed disease progression, local recurrence, primary and secondary tumours at different locations. Patients who did not show any signs of above were categorized into treatment response group. The treatment response was evaluated based on ultrasound, computed tomography scan or magnetic resonance imaging findings by the treating oncologist.

DNA extraction and SNPs genotyping

Whole blood, 3 millilitres was collected from the study subjects in sterile EDTA-coated tubes. DNA was extracted using a QIAamp DNA mini kit (Qaigen, Hilden, Germany). Allele-specific polymerase chain reaction (PCR) was performed for *CYP1B1* 142 C>G (rs 10012) genotyping (figure 1) whereas for *CYP1B1* 4326 C>G (rs 1056836) and 4390 A>G (rs 1800440) genotyping, PCR-restriction fragment length polymorphism technique was employed (figures 2 and 3, respectively).

mRNA expression

Isolation of total RNA and cDNA synthesis: Total RNA was extracted from FFPE specimens using an Ambion Recover All Total Nucleic Acid Isolation kit (Ambion, Austin, USA) according to the manufacturer's protocol. The concentration and purity of the extracted total



LM : 100bp DNA ladder
P11 : Homozygous wildtype (CC)
P1 : Heterozygous (CG)
P4 : Homozygous variant (GG)

Figure 1. Gel image showing PCR (allele specific) amplicons of *CYP1B1* 142 C>G polymorphism.

RNA was determined by using a NanoQuant Infinite M200 (Tecan, USA). The concentration of total RNA ranged from 20 to 200 ng/μL while the purity ranged from 1.8 to 1.9 (A260/280 and A260/A230). The quality of the extracted total RNA was determined by agarose gel (2%) electrophoresis. cDNA was synthesized using an Applied Biosystem High-Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, USA) based on 200 ng of total RNA and by applying RT random primer.

Quantitative reverse transcription PCR (qRT-PCR)

qRT-PCR was performed using a TaqMan gene expression assay. *CYP1B1* (Hs_00164383_m1) gene was chosen as the target gene and glyceraldehyde-3-phosphate dehydrogenase, *GAPDH* (Hs_03929097_g1), gene was chosen as a housekeeping gene. The reaction was performed using a TaqMan Advanced PCR Master Mix (Applied Biosystems) in an Applied Biosystem Step One Real Time PCR machine (Applied Biosystems) with the following thermal cycling conditions: uracil *N*-glycosylase incubation at 50°C for 2 min, enzyme activation at 95°C for 20 s, followed by 40 cycles of denaturation and annealing/extension at 95°C for 1 s and 60°C for 20 s, respectively. Each sample was prepared in triplicates and the threshold level was automatically selected using ABI Step One software ver. 2.3 (Applied Biosystems).

Statistical analysis

Genotype and allele frequencies of the SNPs *CYP1B1* 142 C>G (rs 10012), 4326 C>G (rs 1056836) and 4390 A>G (rs 1800440) were calculated and compared among chemotherapy respondents and nonrespondents by using the chi-square test (χ^2). Odds ratio (OR) with 95% confidence intervals (CI) were calculated using a binary logistic regression analysis with SPSS v.20.0 (SPSS, Chicago, USA). The association of *CYP1B1* haplotypes with treatment response was determined using Haploview v.4.2. software (Broad Institute Cambridge, USA) (Barrett *et al.* 2005). By using relative expression software tool (REST) 2009, ver. 2.0.13 (Qiagen, Germany), the relative gene expression of *CYP1B1* was determined. The mean expression level in tumour versus normal adjacent tissues was compared and their association with treatment response was determined using the Pfaffl method (Pfaffl *et al.* 2002).

Results

The clinicopathological parameters of TNBC patients

For SNP association study, a total of 76 histopathologically confirmed TNBC patients were recruited in this study. Briefly, the mean age of study subjects at diagnosis was 48.9±9.67 years. Among these 76 patients, 62 (81.6%) had ductal carcinoma and 14 (18.4%) had other histological subtypes (medullary and metaplastic). Patients diagnosed with stages I, II and III comprised of 25.0, 57.9 and 17.1%, respectively. Based on the histological grade, majority of the patients (55.3%) belonged to grade III, followed by grade II (42.1%) and grade I (2.6%), respectively. A total of 40 (52.6%) patients were positive and 47.4% were negative for axillary lymph node involvement. Fourty-nine (49) (64.5%) patients were premenopausal and 27 (35.5%) were postmenopausal females. Grouping of the patients based on chemotherapy response showed 51 patients (67.1%) to be responders and 25 (32.9%) to be nonresponders.

For the 41 TNBC patients included for mRNA expression study, the median age at diagnosis was 50.4 ± 11.45 (range 28–75) years. Among them, majority (85.4%) had infiltrating ductal carcinoma while the remaining (14.6%) had other subtypes (medullary and metaplastic). Stage-wise patients: 7.3% had stage I, 73.2% had stage II and 19.5% had stage III. Histological grading showed 63.4% to be grade III and the remaining 36.6% to be grade II. A total of 34 patients (82.9%) showed positivity for axillary lymph node involvement and 26 (63.4%) were premenopausal. Out of the 41 patients, 27 (65.9%) were responders and 14 (34.1%) were nonresponders to the treatment. The clinicopathological data of the study subjects are shown in table 1.

Genetic association of CYP1B1 polymorphisms with chemotherapy response

For *CYP1B1* 142 C>G polymorphism, the frequency of heterozygous variant genotype was significantly higher in the chemotherapy responder group (47%) compared with nonresponders (16%) ($P = 0.008$). No significant difference was observed in the frequency of alleles C and G between the nonresponder and responder groups. In

Table 1. Clinicopathological data of TNBC patients.

Characteristics	SNPs study ($n = 76$)	mRNA study ($n = 41$)
Age		
(Mean ± SD)	48.9 ± 9.67	50.4 ± 11.5
Type		
Infiltrating ductal carcinoma	62 (81.6%)	35 (85.4%)
Other (medullary and metaplastic)	14 (18.4%)	6 (14.6%)
Histological grade		
I	2 (2.6%)	0 (0.0%)
II	32 (42.1%)	15 (36.6%)
III	42 (55.3%)	26 (63.4%)
Stage		
I	19 (25.0%)	3 (7.3%)
II	44 (57.9%)	30 (73.2%)
III	13 (17.1%)	8 (19.5%)
Lymph node status		
Negative	36 (47.4%)	7 (17.1%)
Positive	40 (52.6%)	34 (82.9%)
Menopausal status		
Premenopausal	49 (64.5%)	26 (63.4%)
Postmenopausal	27 (35.5%)	15 (36.6%)
Treatment status		
Responder	51 (67.1%)	27 (65.9%)
Nonresponder	25 (32.9%)	14 (34.1%)

SD, standard deviation.

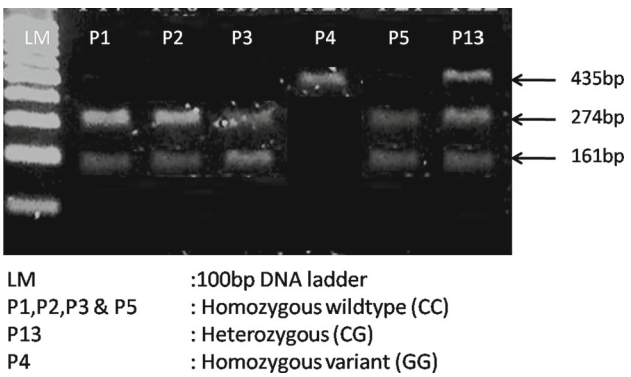


Figure 2. Gel image showing different genotype patterns of *CYP1B1* 4326 C>G polymorphism.

case of *CYP1B1* 4326 C>G polymorphism, the frequency of homozygous variant genotype was significantly higher among nonresponders (36%) when compared with the responder group (12%) ($P = 0.012$). The frequency of allele G was significantly higher in nonresponder (48%) compared with the responder groups (28%) ($P = 0.019$). For *CYP1B1* 4390 A>G polymorphism, only homozygous genotype was observed in both nonresponder and responder groups and hence excluded from further analysis.

Binary logistic regression analysis was performed to establish the association of the investigated SNPs with treatment response. Heterozygous variant (CG) genotype of *CYP1B1* 142 C>G polymorphism was significantly associated with good chemotherapy response (OR: 0.250, 95% CI: 0.073–0.858, $P = 0.028$). Significant good chemotherapy response with OR: 0.199, 95% CI: 0.048–0.817, $P = 0.025$ was observed even after adjusted for age, histological grade, stage, lymph node and menopausal status. The homozygous variant (GG) genotype of *CYP1B1* 4326 C>G polymorphism showed significantly higher risk for chemotherapy resistance with OR: 4.200, 95% CI: 1.191–14.812, $P = 0.026$ and even after adjustment (OR: 6.802, 95% CI: 1.213–38.148, $P = 0.029$). The allele G of *CYP1B1* 4326 C>G polymorphism showed significantly higher risk for poor treatment response with OR: 2.324, 95% CI: 1.151–4.689, $P = 0.019$ and even after adjustment (OR: 3.010, 95% CI: 1.214–7.464, $P = 0.017$) (table 2). However, combination of *CYP1B1* 142 C>G and 4326 C>G genotypes did not show any significant higher association with chemotherapy response ($P>0.05$) (table 3).

Haplotype analysis

Haplotype analysis showed that the frequency of *CYP1B1* 142C and 4326G haplotypes to be significantly higher in the nonresponder group (24.3%) compared with the responder (11.4%) group ($P = 0.040$). When the risk association was performed, patients who carried combination of *CYP1B1* 142C and 4326G haplotypes showed a significant higher risk for chemotherapy resistance with OR:2.579, 95% CI:1.153–5.771, $P = 0.019$ (table 4).

Table 2. Genetic association of *CYP1B1* polymorphisms with chemotherapy response in TNBC patients.

	Treatment status ($n = 76$)						
	Nonresponder ($n = 25$)	Responder (n=51)	P value	Crude OR (95% CI)	P value	Adjusted OR (95% CI)[c]	P value
CYP1B1 142 C>G							
Genotype							
CC	16 (64%)	24 (47%)	0.165	1.000 (reference)[a]		1.000 (reference)[a]	
CG	4 (16%)	24 (47%)	0.008[b]	0.250 (0.073–0.858)	0.028[b]	0.199 (0.048–0.817)	0.025[b]
GG	5 (20%)	3 (6%)	0.105	2.500 (0.523–11.956)	0.251	2.349 (0.325–16.982)	0.398
Allele							
C	36 (72%)	72 (71%)	0.857	1.000 (reference)[a]		1.000 (reference)[a]	
G	14 (28%)	30 (29%)		0.933 (0.441–1.976)	1.000	0.809 (0.341–1.920)	0.630
CYP1B1 4326 C>G							
Genotype							
CC	10 (40%)	28 (55%)	0.222	1.000 (reference)[a]		1.000 (reference)[a]	
CG	6 (24%)	17 (33%)	0.405	0.988 (0.304–3.209)	0.984	1.635 (0.357–7.486)	0.527
GG	9 (36%)	6 (12%)	0.012[b]	4.200 (1.191–14.812)	0.026[b]	6.802 (1.213–38.148)	0.029[b]
Allele							
C	26 (52%)	73 (72%)	0.019[b]	1.000 (reference)[a]		1.000 (reference)[a]	
G	24 (48%)	29 (28%)		2.324 (1.151–4.689)	0.019[b]	3.010 (1.214–7.464)	0.017[b]
CYP1B1 4390 A>G							
Genotype							
AA	25 (100%)	51 (100%)	–	NA	–	NA	–
Allele							
A	50 (100%)	102 (100%)	–	NA	–	NA	–

[a]Genotype served as a reference category. [b]$P < 0.05$, statistically significant. [c]Adjusted OR by age, histological grade, stage, lymph node and menopausal status.
OR, odds ratio; CI, confidence interval; NA, not available.

Table 3. Genetic association of genotype combinations of *CYP1B1* polymorphisms with chemotherapy response in TNBC patients.

CYP1B1 142 C>G+ *CYP1B1* 4326 C>G		Treatment status ($n = 76$)					
		Nonresponder ($n = 25$)	Responder ($n = 51$)	Crude OR (95% CI)	*P* value	Adjusted OR (95% CI)[b]	*P* value
CC	CC	8	16	1.000 (reference)[a]		1.000 (reference)[a]	
CC	CG	2	5	0.800 (0.126–5.070)	1.000	1.492 (0.100–22.309)	0.813
CC	GG	6	3	4.000 (0.788–20.317)	0.122	10.332 (0.859–124.243)	0.066
CG	CC	0	12	NA	—	NA	—
CG	CG	2	10	0.400 (0.070–2.277)	0.438	0.405 (0.037–4.419)	0.458
CG	GG	2	2	2.000 (0.236–16.929)	0.601	4.949 (0.350–69.925)	0.239
GG	CC	2	0	NA	—	NA	—
GG	CG	2	2	2.000 (0.236–16.929)	0.601	6.942 (0.349–118.102)	0.211
GG	GG	1	1	2.000 (0.110–36.308)	1.000	0.397 (0.005–34.920)	0.686

[a]Genotype served as a reference category. [b]Adjusted OR by age, histological grade, stage, lymph node and menopausal status. OR, odds ratio; CI, confidence interval; NA, not available.

Table 4. Genetic association of *CYP1B1* haplotypes with chemotherapy response in TNBC patients.

CYP1B1 haplotype 142C>G/4326 C>G	Frequency (%)	Non responder (%)	Responder (%)	*P* value	OR (95% CI)	*P* value
CC	52.1	45.7	55.3	0.269	1.000 (reference)[a]	—
GC	26.2	24.3	27.1	0.708	1.085 (0.553–2.128)	0.733
CG	15.6	24.3	11.4	0.040[b]	2.579 (1.153–5.771)	0.019[b]
GG	6.1	5.7	6.2	0.905	1.113 (0.334–3.704)	0.862

[a]Genotype served as a reference category. [b]$P < 0.05$, statistically significant. OR, odds ratio; CI, confidence interval.

The association of CYP1B1 mRNA expression with clinicopathological data and treatment response

CYP1B1 mRNA expression was determined in cancerous ($n = 41$) and normal adjacent tissues ($n = 17$) of TNBC patients. Overall, the relative expression of *CYP1B1* was downregulated (0.592) in cancerous tissues when compared with normal adjacent tissues. However, no significant difference in the expression level of *CYP1B1* was observed between tumorous and normal adjacent tissues. In addition, the associations of *CYP1B1* expression with clinicopathological data and chemoresistance were also evaluated. *CYP1B1* showed a relatively higher level of expression (2.494) in cancerous tissues than in normal adjacent tissue in medullary and metaplastic subtypes. However, in infiltrating ductal carcinoma, the level of *CYP1B1* expression was relatively lower (0.546) in cancerous tissues than in normal adjacent tissues, although the difference was not statistically significant. When other clinicopathological data such as histological grade, stage, lymph node and menopausal status were taken into account, a lower *CYP1B1* gene expression level was observed in cancerous tissues compared with normal adjacent tissues, but not significant ($P > 0.05$). Interestingly, the expression of *CYP1B1* was significantly upregulated by 3.256-fold in cancerous tissues of patients who did not respond compared with those of patients who showed response to treatment (table 5).

Discussion

Drug resistance and recurrence or metastasis remain major clinical obstacles for successful management in TNBC patients. Chemotherapy has been known to be effective and adjuvant chemotherapy has generally been shown to give a better treatment outcome in TNBC patients compared with other breast cancer subtypes (Liedtke *et al.* 2008). Moreover, adjuvant chemotherapy has been reported to reduce the recurrence and mortality rates in breast cancer (Foulkes *et al.* 2010). Recent study showed adjuvant chemotherapy using TAC regimens increased the disease-free survival (DFS) and overall survival (OS) rates of TNBC patients compared with other regimens (Buyukhatipoglu *et al.* 2015). The TNBC patients in the present study had also undertaken TAC chemotherapy regimen.

Of the 76 TNBC patients who completed six cycles of TAC regimens in the present study, 25 (32.9%) did not respond to treatment. In the study by Pogoda *et al.* (2013), 35.0% of TNBC patients developed recurrence whereas

Table 5. The relative gene expression of *CYP1B1* with clinicopathological data and chemotherapy response in TNBC patients.

	Relative expression	SE	*P* value
Gene	Tumour ($n = 41$) versus normal adjacent tissues ($n = 17$)		
CYP1B1	0.592	0.048–6.630	0.297
Type			
Infiltrating ductal carcinoma ($n = 35$)	0.546	0.044–6.794	0.254
Other (medullary and metaplastic) ($n = 6$)	2.494	0.492–13.741	0.357
Stage			
Stage II ($n = 33$)	0.547	0.045–6.712	0.341
Stage III ($n = 8$)	0.652	0.051–6.021	0.640
Histological grade			
Grade II ($n = 15$)	0.572	0.055–6.156	0.370
Grade III ($n = 26$)	0.572	0.044–7.110	0.302
Menopausal status			
Postmenopausal ($n = 15$)	0.809	0.079–7.835	0.706
Premenopausal ($n = 26$)	0.469	0.033–6.135	0.203
Lymph node status			
Negative ($n = 7$)	0.797	0.100–12.790	0.764
Positive ($n = 34$)	0.535	0.044–6.237	0.238
	Nonresponder ($n = 14$) versus responder ($n = 27$)		
CYP1B1	3.256	0.383–21.833	0.039[a]

[a] $P < 0.05$, statistically significant. SE, standard error.

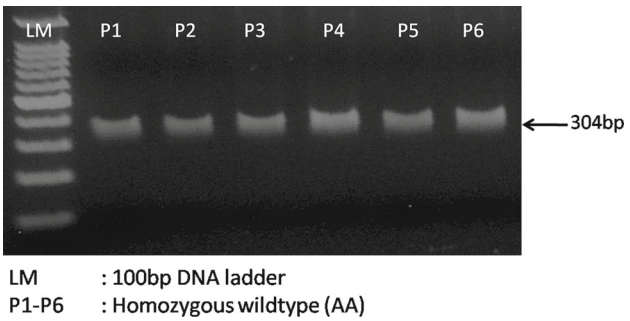


Figure 3. Gel image showing the homozygous wild-type genotype of *CYP1B1* 4360 A>G polymorphism.

17.9% of TNBC failed treatment in the study by Dogra *et al.* (2014). In a Dutch cohort study, 12 (16.0%) TNBC patients had regional, local and distant recurrence (van Roozendaal *et al.* 2016). A recent study reported that, the frequency of recurrence or metastasis in TNBC patients was higher than in nonTNBC patients (27.95 *vs* 13.38%, respectively) (Qiu *et al.* 2016). These results which are in agreement with ours indicated that TNBC patients have higher rate of recurrence or metastasis compared with nonTNBC patients.

This is the first study from Malaysia to associate the genetic polymorphisms and mRNA expression levels of *CYP1B1* in TNBC patients undergoing TAC chemotherapy regimen. *CYP1B1* affects tumour response to anticancer drugs (Gehrmann *et al.* 2008; Rizzo *et al.* 2010; Chang *et al.* 2015). Polymorphisms in *CYP1B1* have been linked to response to taxanes and anthracyclines. In the present study, the SNP *CYP1B1* 4390 A>G was excluded from further analysis as only homozygous wild-type genotype was observed in all patients. The homozygous variant genotype (GG) and allele G of *CYP1B1* 4326 C>G were associated with significantly higher risk for development of chemotherapy resistance. On the contrary, the heterozygous variant genotype 142 CG of *CYP1B1* was associated with good response. *CYP1B1* 142 C>G and 4326 C>G polymorphisms are located in the coding region leading to amino acid substitution from arginine to glycine, and leucine to valine, respectively (Gajjar *et al.* 2012).*CYP1B1* 142 C>G substitution has been reported to result in increased *CYP1B1* gene expression without alteration of catalytic properties unless when present in combination with other functional alleles (McLellan *et al.* 2000; Landi *et al.* 2005) whereas, 4326 C>G has been associated with increased catalytic activity of *CYP1B1* (Shimada *et al.* 1999; Landi *et al.* 2005).

When the association between various combinations of *CYP1B1* 142 C>G and 4326 C>G polymorphisms was evaluated, no significant higher risk for the development of resistance was observed for any of the combination. A study conducted by Dumont *et al.* (2015) reported that the combination of *CYP1B1* 432 CC and GG genotypes significantly increased the pathological complete response (pCR) rate with OR: 3.26 when compared with a CG genotype. Moreover, in their study, combination of *CYP1B1*

homozygous wild-type (CC) and *ERCC1* heterozygous (CT) genotypes showed a significantly increased pCR rate with OR: 8.5 (Dumont *et al.* 2015). However, the presence of *CYP1B1**3 allele, either as homozygous or heterozygous variant genotype, leads to decreased risk of hypersensitivity to the drug (Rizzo *et al.* 2010). Few researchers reported that, the presence of *CYP1B1* 4326G allele was associated with a lower response rate, shorter progression-free-survival (PFS) and decreased overall-survival (OS) in breast and prostate cancer patients treated with taxanes (Marsh *et al.* 2007; Sissung *et al.* 2008; Pastina *et al.* 2010). Moreover, the variant allele (G) was associated with a reduced sensitivity to chemotherapeutic agents including DNA interacting agents, alkylators, camptothecins, topoisomerase II inhibitors and antimetabolites (Laroche-Clary *et al.* 2010). In a study by Tulsyan *et al.* (2014), combination of heterozygous and homozygous variant genotypes of *CYP1B1* 4326 C>G showed higher risk (1.5-fold) of non-response to the treatment among breast cancer patients undergoing neo-adjuvant chemotherapy with taxanes. A recent study showed that the presence of heterozygous and homozygous genotypes of *CYP1B1* 4326 C>G posed a lower risk for the lack of treatment response in TNBC patients undergoing FAC regimens (Tecza *et al.* 2016) although this result was not statistically significant.

In the present study, *CYP1B1* 142C-4326G haplotypes showed significantly higher risk for chemoresistance with OR: 2.579, 95% CI: 1.153–5.771, $P = 0.019$ in TNBC patients. This could be due to adverse impacts of functional alterations when these alleles are together in combinations as well as in combination with other functional alleles. There are no other reports available on the impact of 142C/4326G haplotype on chemotherapy response in TNBC patients. However, in a study on colorectal cancer susceptibility risk, the haplotype 142C/119 T/4326G of *CYP1B1* was found to be at higher risk of developing colorectal cancer (OR: 21.4, 95% CI: 1.2–365.56, $P = 0.0019$) (Trubicka *et al.* 2010). There is evidence that the *CYP1B1* variants C142G, G355 T and C4326G all exhibit greater catalytic 4-hydroxylation activity than the wild-type enzyme (Hanna *et al.* 2000) and thereby increase the DNA single-strand breaks as well as nuclear levels of 8-hydroxyguanosine. *CYP1B1* metabolizes 17β-oestradiol (E_2) to produce 4-hydroxyoestradiol ($4-OH-E_2$) (Tsuchiya *et al.* 2005). E_2 contributes to the growth and development of ER-dependent cancer such as breast cancer, whereas the presence of $4-OH-E_2$ in hormone response tissues may enhance carcinogenicity, promote formation of DNA adducts, generate reactive oxygen species and finally alter and interfere with drug's microtubule stabilizing action as well as gene expression (Cavalieri 1996; Sissung *et al.* 2008; Takemura *et al.* 2010; Yager 2012).

The present study also investigated the expression level of *CYP1B1* mRNA. Our results indicated that the expression level of *CYP1B1* was higher in normal adjacent tissues when compared with cancerous tissues. Our finding was parallel with previous studies which reported low *CYP1B1* mRNA expression in breast cancer tissues (Modugno *et al.* 2003; Vaclavikova *et al.* 2007). In a few other cancers such as in melanoma (Muthusamy *et al.* 2006), endometrium (Hevir *et al.* 2011; Singh *et al.* 2008; Lepine *et al.* 2010) and oral (Pradhan *et al.* 2011), a lower *CYP1B1* mRNA expression was observed. Nevertheless, an explanation for the relative lower expression of *CYP1B1* in cancerous tissues compared with normal adjacent tissues as found in the present study could only be speculated. *CYP1B1* is regulated by aryl hydrocarbon receptors (AhRs) and aryl hydrocarbon receptor nuclear translocators. The transcription induced by AhRs has been reported to be expressed by an aryl hydrocarbon receptor repressor that acts as a tumour suppressor that governs the downregulation of *CYP1B1* in cancerous tissues (Haarmann-Stemmann *et al.* 2007; Evans *et al.* 2008). Epigenetic modifications such as microRNA (miRNA) expression and promoter methylation at multiple CpG sites in *CYP1B1* have been reported to result in gene silencing (Muthusamy *et al.* 2006; Tsuchiya *et al.* 2006). Therefore, it is reasonable to suggest that some of the miRNAs might also be contributing for the lower expression of *CYP1B1* in tumour tissues of TNBC patients.

When the mRNA expression level was compared between the chemotherapy responders and nonresponders, high level of *CYP1B1* expression was observed in those who did not respond to treatment. This result is in agreement with a previous study, where a higher *CYP1B1* expression level was found to increase drug resistance of cells exposed to docetaxel and antagonizes, the anticancer effects of docetaxel in *in vitro* study (McFadyen *et al.* 2001). *CYP1B1* was also reported to accelerate the degradation of anticancer drugs in resistant target cells (Rochat 2005). In phase 1 clinical trial, low expression level of *CYP1B1* was found to significantly improve response to therapy (Gribben *et al.* 2005). In a few *in vivo* and *in vitro* studies also, *CYP1B1* expression was found to enhance resistance of ovarian cancer cells (Zhu *et al.* 2015) and overexpression of *CYP1B1* was found to cause resistance of renal cancer carcinoma cells to docetaxel (Chang *et al.* 2015). McFadyen *et al.* (2001) suggested that by binding and sequestrating docetaxel, CYP1B1 reduces its available concentration and influences its activity. CYP1B1 has also been reported to produce ER metabolites which could limit docetaxel efficacy by inhibition of tubulin polymerization (Sissung *et al.* 2008). Thus, it is reasonable to suggest that CYP1B1 might modulate response to taxane treatment by increased mRNA expression and catalytic activity.

The present study is limited by a small number of TNBC patients especially for the genetic association of polymorphic variants of the three SNPs studied. This has been reflected in our results on association with recurrence risk where wide 95% CI ranges were observed. But, the fact that TNBC is the rarest among all other breast cancer subtypes

need to be considered. Further study involving a large sample size may increase the power of the study. Additionally, epigenetics study on the involvement of mRNAs in modulating *CYP1B1* mRNA expression is also warranted.

In conclusion, SNPs 142 C>G and 4326 C>G and mRNA expression of *CYP1B1* were found to be associated with treatment response in TNBC patients undergoing TAC chemotherapy. Expression levels of *CYP1B1* in tumour tissues could be a predictive maker for recurrence risk. Therefore, SNPs and mRNA expression of *CYP1B1* could be useful prognostic indicators of chemotherapy response in TNBC patients undergoing TAC chemotherapy regimens.

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