CBR1 and *CBR3* pharmacogenetics and their influence on doxorubicin disposition in Asian breast cancer patients

Suman Lal,[1] Edwin Sandanaraj,[1] Zee Wan Wong,[2] Peter C.S. Ang,[2] Nan Soon Wong,[2] Edmund J.D. Lee[3] and Balram Chowbay[1,4]

[1]Laboratory of Clinical Pharmacology, Division of Medical Sciences, Humphrey Oei Institute of Cancer Research, National Cancer Centre, 11 Hospital Drive, Singapore 169610; [2]Department of Medical Oncology, National Cancer Center, 11 Hospital Drive, Singapore 169610; [3]Department of Pharmacology, Yong Loo Lin School of Medicine, National University of Singapore, 10 Medical Drive, Singapore, 117597

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The present study aimed to identify polymorphic genes encoding carbonyl reductases (*CBR1*, *CBR3*) and investigate their influence on doxorubicin disposition in Asian breast cancer patients ($n = 62$). Doxorubicin (60 mg/m^2) was administered every 3 weeks for four to six cycles and the pharmacokinetic parameters were estimated using non-compartmental analysis (WinNonlin). The Mann–Whitney *U*-test was used to assess genotypic–phenotypic correlations. Five *CBR1* (*–48G>A*, c.*219G>C*, c.*627C>T*, c.693G>A, *+967G>A*) and *CBR3* (c.*11G>A*, c.*255C>T*, c.*279C>T*, c.*606G>A*, c.*730G>A*) polymorphisms were identified. The *CBR1* D2 diplotypes were characterized by the presence of at least one variant allele at the c.*627C>T* and *+967G>A* loci. Patients in the *CBR1* D1 diplotype group had significantly higher clearance (CL) normalized to body surface area (BSA) (CL/BSA[L/h/m^2]: median 25.09; range 16.44–55.66) and significantly lower exposure levels; area under curve ($AUC_{0-\infty}$/dose/BSA [h/m^5]; median 15.08; range 6.18–38.03) of doxorubicin compared with patients belonging to the *CBR1* D2 diplotype group (CL/BSA[L/h/m^2]; median 20.88; range 8.68–31.79, $P = 0.014$; and $AUC_{0-\infty}$/dose/BSA[h/m^5]; median 21.35; range 9.82–67.17, $P = 0.007$ respectively). No significant influence of *CBR3* polymorphisms on the pharmacokinetics of doxorubicin were observed in Asian cancer patients. The present exploratory study shows that *CBR1* D2 diplotypes correlate with significantly higher exposure levels of doxorubicin, suggesting the possibility of lowered intracellular conversion to doxorubicinol in these patients. Further evaluation of carbonyl reductase polymorphisms in influencing the treatment efficacy of doxorubicin-based chemotherapy in Asian cancer patients are warranted. (*Cancer Sci* 2008; 99: 2045–2054)

Drug-metabolizing enzymes play a central role in the disposition of a vast array of endogenous compounds and xenobiotics and include the phase I (cytochrome P-450 oxidoreductive enzymes, NADPH cytochrome *c* reductases, and carbonyl reductases [CBR]) and phase II (UDP-glycosyltransferases, glutathione transferases, sulfotransferases, and *N*-acetyltransferases) conjugative enzymes.[1] The phase I and II enzymes are expressed abundantly in hepatic tissues and display large interindividual variations in their metabolic capacities toward a wide array of therapeutic agents.

The CBR are ubiquitously expressed monomeric NADPH-dependent cytosolic enzymes that catalyze the reduction of chemically diverse substrates such as aldehydes, ketones, quinones, and other xenobiotics.[2,3] Apart from the metabolism of endogenous compounds and drug detoxification, CBR are also assumed to participate in cellular processes such as signal transduction,[4] apoptosis,[5] mutagenesis,[6] carcinogenesis,[7] and drug resistance.[8] Four CBR isoforms (CBR1, CBR2, CBR3, and CBR4) that were initially assigned to the aldo-keto reductase (AKR) family are now classified under the family of short-chain dehydrogenases and represent one of the largest protein families identified to date.[9] CBR1 is the major carbonyl reductase and is expressed widely in different tissues. The *CBR1* gene is mapped to chromosome 21q22.12, has three exons spanning 3.3 kb, and encodes a 30-kDa monomeric protein comprising 277 amino acids. The *CBR1* gene lacks a CAAT and TATA box and contains a GC-rich island extending into the first exon, a structure characteristic of genes having a housekeeping function.[4] The identified substrates of human CBR1 include endogenous compounds (prostaglandins and steroids) and drugs such as loxoprofen,[4] metyrapone,[10] haloperidol,[11] bromoperidol,[12] timirepone,[13] and doxorubicin.[14] *CBR2* has low sequence identity with *CBR1* and has not been identified in human tissues. The *CBR3* gene contains three exons spanning a region of 11.2 kb and has a 72% sequence similarity with *CBR1*. It is located 62 kb telomeric to the *CBR1* gene. Although widely expressed, the relative expression of CBR3 is much lower than CBR1 in most of the tissues analyzed.[15] The *CBR4* gene is located on human chromosome 4 (4q32.3) and encodes a protein composed of 237 amino acids, but its enzymatic properties and tissue distribution remain unknown.[15]

To date, there are limited studies investigating the influence of genetic polymorphisms in the *CBR* genes on the pharmacokinetics and pharmacodynamics of drugs. Avramopoulos *et al*. identified the *CBR1* 3′-untranslated region (UTR) *G>A* transition for the linkage mapping of the *CBR* gene on chromosome 21.[16] However, the effect of the polymorphism on enzyme activity and tissue expression is not known. Gonzalez-Covarrubias *et al*. reported the V88I (*262G>A*, rs1143663), L73L (*312G>C*, rs25678), A209A (*720C>T*, rs20572), and V231V (*786G>A*, rs2230192) polymorphisms by screening full-length *CBR1* cDNA samples isolated from liver donors.[17] The non-synonymous V88I (*262G>A*) polymorphism occurred at a low frequency among African-Americans ($q = 0.014$) and resulted in *CBR1* isoforms with distinct kinetic and thermodynamic properties. The V88 isoform presented 50% higher V_{max} values for the anthracycline daunorubicin and synthesized 47% higher levels of its C-13 alcohol metabolite daunorubicinol.[17] Based on earlier *in vitro* studies, it was proposed that individuals homozygous for the *CBR1* 88I genotype may exhibit a slower rate of synthesis of C-13 alcohol metabolites that may be associated with reduced risk of cardiotoxicity associated with anthracycline therapy. Lakhman *et al*. identified a *CBR3* V244M polymorphism positioned in a region critical for interactions with the NADP(H) cofactor, occurring at higher frequency among Africans ($q = 0.51$) compared to Caucasians ($q = 0.31$).[18] Kinetic experiments with the

[4]To whom correspondence should be addressed. E-mail: ctebal@nccs.com.sg

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recombinant CBR3 protein variants using the prototypical CBR quinone substrate menadione revealed that the *CBR3* M244 isoform has significantly higher V_{max} than the V244 isoform, although both had similar K_m values. *In vitro* site-directed mutagenesis experiments have also shown that genetic variations in the *CBR1* and *CBR3* genes could have significant functional consequences.[19] Substitution of highly conserved basic residues (Lys-15, Ala-37, and Arg 38) at the N-terminus of *CBR1* with neutral or acidic residues showed a reduction of the enzyme kinetic parameters by an order of several magnitudes, further highlighting their significant role in coenzyme binding and catalysis.

The anthracycline antibiotic doxorubicin is a well-established substrate of CBR enzymes that catalyzes the stereospecific reduction of the ketone on C-13, yielding dihidroxy derivatives.[4,20] Doxorubicin is used widely in the treatment of breast and other cancers and exhibits wide variations in its pharmacokinetic and pharmacodynamic profiles that may be due to functional polymorphisms in genes that encode proteins involved in its transport and metabolism.[21–23] To the best of our knowledge, there have been no studies to date investigating the influence of polymorphic variants in the *CBR1* and *CBR3* genes on the pharmacokinetics of doxorubicin and doxorubicinol in cancer patients. The aim of the present study was two fold: first, to study the pharmacogenetics of the *CBR1* and *CBR3* genes in three distinct healthy Asian populations (Chinese, Malay, and Indian); and second, to investigate the influences of *CBR1* and *CBR3* polymorphic variants on the pharmacokinetics of doxorubicin in Asian breast cancer patients.

Materials and Methods

Healthy subjects. The healthy subjects comprised three ethnic groups predominant in the Asian population: Chinese, $n = 100$; Malay, $n = 100$; and Indian, $n = 100$. The ethnicity of the study subjects were confirmed by careful screening and verified against their National Registry Identification Cards. All participants provided approved informed consent for the study. The study was approved by the ethics review committee of the National Cancer Center, Singapore.

Breast cancer patients. Patients who had histologically confirmed invasive breast cancer ($n = 62$) and received adjuvant chemotherapy with doxorubicin were recruited for the present study. Informed consent was obtained from all patients and the protocol was approved by the institutional ethics committee at the National Cancer Center, Singapore. Inclusion criteria required patients to have adequate bone marrow (absolute neutrophil count >1500/μL, platelet count >100 000/μL), hepatic function (aspartate amino transferase [AST] and alanine amino transferase [ALT] levels ≤2.5 and total bilirubin <2.0 times the upper limit of normal), and renal function (serum creatinine <140 mmol/L) documented at the time of enrollment. Patients with serious comorbidities, including poorly controlled diabetes mellitus, ischemic heart disease, uncontrolled hypertension, active infection or performance status score ≥2 on the Eastern Cooperative Oncology Group scale were excluded from the study. Pregnancy and use of growth factors during the cycle of chemotherapy were also considered as criteria for exclusion. No forms of endocrine therapy, immunotherapy, or biological response modifiers were allowed during the period of chemotherapy.

Doxorubicin administration and pharmacokinetic analysis. Doxorubicin was administered at a dose of 60 mg/m^2 intravenously over 20 min on a cycle of once every 3 weeks after standard premedications, with intravenous 10 mg dexamethasone, 50 mg diphenhydramine, 300 mg cimetidine, or 50 mg ranitidine. The adjuvant regimen also included intravenous 600 mg/m^2 cyclophosphamide administered over 30 min once every 3 weeks. Hematological parameters, including hemoglobin, total leukocyte count, platelet count, and the absolute neutrophil count after the start of treatment (days 7 and 14) and prior to the start of each cycle, were measured. New treatment cycles were started only if the absolute neutrophil count was ≥1500/μL and the platelet count >100 000/μL. Blood samples for pharmacokinetic analysis were drawn at the following time points after the start of infusion on the first day of the first cycle of chemotherapy: at predose, 5, 15, and 30 min and at 1, 4, 8, and 24 h. Blood samples were collected in plain ethylene diamine tetracetic acid-containing vacutainer glass tubes and centrifuged immediately at 1000*g* for 10 min. The plasma fraction was collected and stored at –20°C until analysis.

Plasma concentrations of doxorubicin and its major metabolite doxorubicinol were estimated by reversed-phase high-performance liquid chromatography with fluorescence detection as described previously.[24] Briefly, following a single protein precipitation step, chromatographic separation was accomplished using a C-18 column with a mobile phase consisting of 50 mmol/L sodium phosphate buffer:acetonitrile:1-propanol (65:25:2, v/v), pH 2.0. The analytes were measured by fluorescence detection with an excitation wavelength of 480 nm and emission wavelength of 560 nm. The lower limits of quantitation were 10 ng/mL for doxorubicin and 5 ng/mL for doxorubicinol. The calibration curves were linear over a concentration range of 10–2500 ng/mL for doxorubicin and 5–1250 ng/mL for doxorubicinol. The within-day and between-day coefficients of variation were less than 13%.

The pharmacokinetic parameters were determined using a non-linear regression program (WinNonLin version 2.1; Pharsight, Mountain View, CA, USA). The area under the plasma concentration–time curve (AUC) was calculated from time zero to the time (t) of the last detectable concentration ($AUC_{0\to t}$) using the trapezoidal rule. The area was extrapolated to infinity ($AUC_{0\to\infty}$) by adding $C_t/\lambda z$ to $AUC_{0\to t}$, where C_t is the last detectable plasma concentration and λz is the elimination rate constant. Peak plasma concentrations (C_{max}) were identified directly from individual subject concentration–time curves.

Identification of *CBR1* and *CBR3* variants by sequence analysis. Purified genomic DNA was isolated from peripheral blood samples (5 mL) using red cell lysis buffer and proteinase K digestion. The following primers were used for amplifying *CBR1*: exon 1, F-5′-gaggggtagggatggttcag-3′, R-5′-cctagggaggcgttatggac-3′; exon 2, F-5′-ggcagagggcactaagttttt-3′, R-5′-ggacaaagtcctgaggcaaa-3′; and exon 3, F-5′-tcacct ctctacgggattgtt-3′, R-5′-tcccttgacctttaggttga-3′. The primers used for amplifying *CBR3* were: exon 1, F-5′-atttggc ttcggacacctc-3′, R-5′-accctcctcggtcactcaag-3′; exon 2, F-5′-aggatccactttgtcatgtgag-3′, R-5′-gcacccctgagtgatttctg-3′; exon 3, F-5′-agggcgagactctgtctcaa-3′, R-5′-agcgag actccgtctcaaaa-3′. Amplification of the exonic regions was done using polymerase chain reaction and the products electrophoresed on 2% agarose gels. Unincorporated nucleotides and primers were removed by incubating the amplified polymerase chain reaction fragments with Shrimp Alkaline Phosphatase (Promega, Madison, WI, USA) and exonuclease 1 (New England Biolabs, MA, USA) for 45 min at 37°C followed by 15 min at 72°C.[25] The sequencing reactions were done using the 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and the resultant trace files were base-called using Sequencing Analysis v.5.2 (Applied Biosystems) and assembled with SeqScape v.2.5 (Applied Biosystems).

Statistical analysis. Fisher's exact test was used to assess the differences in genotype and allele distributions among the different groups in healthy subjects and cancer patients. The possible contribution of subject characteristics (ethnicity, age, height, and body surface area [BSA]) on the pharmacokinetics of doxorubicin and doxorubicinol were determined using univariate analysis. Other independent variables investigated to evaluate their relative contribution to the variability in pharmacokinetic parameters of doxorubicin and doxorubicinol included patient biochemical

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Table 1. Summary of doxorubicin pharmacokinetic parameters in Asian breast cancer patients

Pharmacokinetic parameter	Median (range)
Doxorubicin	
C_{max}/dose/BSA (/m^5)	34.01 (4.5–97.7)
$AUC_{0-\infty}$/dose/BSA (h/m^5)	17.5 (6.2–67.2)
$t_{1/2}$ (h)	15.4 (4.7–24.6)
CL/BSA (L/h/m^2)	22.9 (8.7–55.7)
V_{ss}/BSA (L/m^2)	237.5 (53.9–703.7)
Doxorubicinol	
C_{max}/dose/BSA (10^{-2}/m^5)	0.33 (0.15–2.0)
$AUC_{0-\infty}$/dose/BSA (h/m^5)	9.6 (3.7–24.3)
$t_{1/2}$ (h)	27.5 (6.3–112.4)

$AUC_{0-\infty}$/dose/BSA, area under plasma concentration–time curve from time zero to infinity normalized by dose and body surface area; BSA, body surface area; CL/BSA, plasma clearance normalized by body surface area; C_{max}/dose/BSA, peak plasma concentration normalized by dose and body surface area; $t_{1/2}$, half-life; V_{ss}/BSA; volume of distribution at steady state normalized by body surface area.

parameters such as albumin, total protein and markers of hepatic function (AST, ALT, alkaline phosphatase, and bilirubin) and renal function (serum creatinine). The non-parametric Mann–Whitney *U*-test was used for pairwise comparisons between genotype groups. Pharmacokinetic parameters are reported as median values unless otherwise indicated. Univariate and multivariate analysis was carried out to identify the significant association of patient covariates and genotypes with pharmacokinetic parameters. Backward exclusion regression analysis was carried out to identify the independent influence of genetic covariates in the present study compared to previously reported classes in our patient cohort. The minimum level of statistical significance was set at $P = 0.05$ for all statistical comparisons. Linkage analysis of the *CBR1* and *CBR3* polymorphisms was carried out using Helixtree software (HelixTree Genetics Analysis Software Golden Helix, MT, USA). All statistical analysis was carried out using Stata Statistical Software release 7.0 (Stata Corporation, TX, USA).

Results

Patient demographics. The majority of the breast cancer patients belonged to the Chinese ethnic group (74%), followed by Malays (18%) and Indians (8%). The median age, height, and BSA of the patients were 51 years (range 29–73 years), 154 cm (range 144–168 cm), and 1.52 m^2 (range 1.23–1.95 m^2) respectively.

Doxorubicin pharmacokinetics. Plasma samples for pharmacokinetic analysis were available in 52 of the 62 patients and a high degree of interpatient variability was observed with regard to the pharmacokinetic parameters of doxorubicin and doxorubicinol (Table 1). Approximately six-fold variation in clearance (median 22.9 [L/h/m^2]; range 8.7–55.7 [L/h/m^2]) and 13-fold variation in the volume of distribution at steady state (V_{ss}) of doxorubicin (median 237.5 [L/m^2]; range 53.9–703.7 [L/m^2]) were observed among the cancer patients. Interpatient variability was also high with regard to doxorubicinol pharmacokinetic parameters with more than six-fold variation observed in its $AUC_{0-\infty}$ (median 9.6 [h/m^5]; range 3.7–24.3 [h/m^5]). Univariate analysis revealed the patients' heights and weights to be significant covariates influencing the $AUC_{0-\infty}$ ($P < 0.015$ and $P < 0.0001$, respectively) and C_{max} of doxorubicin ($P < 0.028$ and $P < 0.0001$ respectively). Age and ethnicity were identified as significant covariates in relation to the half life ($t_{1/2}$; h) of doxorubicin ($P < 0.018$). The relationship between the half life of doxorubicin and age was further analyzed following stratification according to age (below and above 60 years). The $t_{1/2}$ of doxorubicin was significantly higher in patients above 60 years of age (median 15.73 years; range 4.7–24.6 years) compared with patients below 60 years of age (median 12.54 years; range 8.1–17.8 years; $P < 0.02$). No significant influences of other covariates were observed on the pharmacokinetics of doxorubicin and doxorubicinol.

***CBR1* polymorphisms in healthy subjects and breast cancer patients.** Five polymorphisms were identified by direct sequencing of the exonic regions of the *CBR1* gene (Table 2). Two identified polymorphisms were in the 5′-UTR (*–48G>A*) and 3′-UTR (*+967G>A*) regions. Among the coding region polymorphisms, one synonymous transversion (c.*219G>C*) in exon 1, and two synonymous transitions (c.*627C>T* and c.*693G>A*) in exon 3 were identified.

The genotype and allele frequencies of the five *CBR1* polymorphisms identified in the Asian ethnic groups and breast cancer patients are listed in Table 2. The *–48G>A* polymorphism was monomorphic in the Indian population but was present at low frequencies among the Chinese (0.02) and Malays (0.01). No significant differences in the allele and genotype frequencies of the c.*219G>C* polymorphism were observed between different Asian ethnic groups. The allele frequency of the c.*627C>T* polymorphism was significantly higher among the Chinese (0.26) when compared with the Indians (0.18, $P = 0.05$). The c.*693G>A* polymorphism was monomorphic in the Chinese population but was present at low frequencies among Malays and Indians (0.01 in each case). The *+967G>A* polymorphism was present at a significantly higher frequency among Chinese and Malays (0.23 in each case) when compared with the Indians (0.17, $P = 0.01$ in each case). The allele frequencies of the *CBR1* polymorphisms among the breast cancer patients conformed to the frequencies found among the local population.

***CBR1* linkage disequilibrium analysis.** Pairwise analysis showed moderate linkage of c.*219G>C* with the c.*627C>T* and *+967G>A* polymorphisms among Chinese and Malays ([measure of disequilibrium] $|D'| > 0.7$ in all groups), whereas the degree of linkage was weak among Indians (c.*219G>C* and c.*627C>T*; [$|D'|$~0.57] and c.*219G>C* and *+967G>A* [$|D'|$~0.41]). The degree of linkage between the c.*627C>T* and *+967G>A* polymorphisms was high in all ethnic groups ($|D'| > 0.8$ in all cases).

Table 3 shows the *CBR1* haplotypes and diplotypes identified in the healthy Asian ethnic groups and breast cancer patients. A total of eight *CBR1* haplotypes were determined in the Chinese population and four high-frequency haplotypes (H1, H2, H3, and H4) accounted for a cumulative frequency of 95%. Among the seven haplotypes inferred in the Malay population, three were identified as high-frequency haplotypes (H1, H2, and H3) and had a cumulative frequency of 90%. There were seven haplotypes identified in the Indian population and four high-frequency haplotypes (H1, H2, H3, and H5) constituted 93% of the total haplotype frequencies. The major diplotypes (>5%) among the Chinese (H1-H1, H1-H2), Malays (H1-H1, H1-H2, H1-H3, H1-H6, and H3-H3), and Indians (H1-H1, H1-H2, and H1-H4) comprised 71, 74, and 77% of the total diplotypes, respectively, in each ethnic group.

The linkage pattern between the c.*219G>C*, c.*627C>T*, and *+967G>A* polymorphisms ($|D'|$ ~0.7, in all groups) in the breast cancer patients was similar to those of the healthy populations from which they were recruited (Fig. 1a). A total of seven haplotype groups were identified and the major haplotypes (>5%) were $H1_b$ (*G-C-G*), $H2_b$ (*C-T-A*), and $H4_b$ (*C-T-G*) with a cumulative frequency of 83% (Table 3). Among the 12 diplotypes inferred, the major diplotypes were $H1_b$-$H1_b$ (56%), $H1_b$-$H2_b$ (8%), $H2_b$-$H3_b$ (7%), $H2_b$-$H2_b$ (5%), and $H3_b$-$H4_b$ (5%), which represented a cumulative frequency of 81%.

***CBR1* polymorphisms and doxorubicin pharmacokinetics.** The influence of the major *CBR1* polymorphisms (c.*219G>C*, c.*627C>T*, and *+967G>A*) on the pharmacokinetics of doxorubicin in Asian breast cancer patients is summarized in Table 4. There was no

Table 2. Genotype and allele frequency of *CBR1* polymorphisms in Asian ethnic groups and breast cancer patients

Polymorphisms	Location	*n*	Genotype frequencies			Allele frequency	
–48G>A [rs11542168]	5′ UTR		GG	GA	AA	G	A
Healthy subjects							
Chinese		100	95 (95)	5 (5)	0	0.98	0.02
Malays		100	99 (99)	1 (1)	0	0.99	0.01
Indians		100	100 (100)	0	0	1	0
Pooled Asian population		300	294 (98)	6 (2)	0	0.99	0.01
Asian cancer patients		62	58 (93.5)	4 (6.5)	0	0.97	0.03
c.*219G>C* [rs25678]	Exon 1		GG	GC	CC	G	C
Healthy subjects							
Chinese		100	70 (70)	27 (27)	3 (3)	0.84	0.16
Malays		100	77 (77)	16 (16)	7 (7)	0.85	0.15
Indians		99	69 (69.7)	21 (21.2)	9 (9.1)	0.80	0.20
Pooled Asian population		299	216 (72.2)	64 (21.4)	19 (6.4)	0.83	0.17
Asian cancer patients		60	36 (60)	18 (30)	6 (10)	0.75	0.25
c.*627C>T* [rs20572]	Exon 3		CC	CT	TT	C	T
Healthy subjects							
Chinese		100	62 (62)	25 (25)	13 (13)	0.74	0.26
Malays		100	66 (66)	17 (17)	17 (17)	0.75	0.25
Indians		100	68 (68)	27 (27)	5 (5)	0.82	0.18
Pooled Asian population		300	196 (65.3)	69 (23)	35 (11.7)	0.77	0.23
Asian cancer patients		60	38 (63.4)	10 (16.6)	12 (20)	0.72	0.28
c.*693G>A* [rs2230192]	Exon 3		GG	GA	AA	G	A
Healthy subjects							
Chinese		100	100 (100)	0	0	1	0
Malays		100	99 (99)	1 (1)	0	0.99	0.01
Indians		100	99 (99)	1 (1)	0	0.99	0.01
Pooled Asian population		300	298 (99.3)	2 (0.7)	0	0.997	0.003
Asian cancer patients		62	62 (100)	0	0	1	0
+967G>A [rs9024]	3′ UTR		GG	GA	AA	G	A
Healthy subjects							
Chinese		100	63 (63)	28 (28)	9 (9)	0.77	0.23
Malays		100	66 (66)	21 (21)	13 (13)	0.77	0.23
Indians		100	72 (72)	23 (23)	5 (5)	0.84	0.17
Pooled Asian population		300	201 (67)	72 (24)	27 (9)	0.79	0.21
Asian cancer patients		60	37 (61.7)	14 (23.3)	9 (15)	0.73	0.27

rs, SNP database ID.

significant influence of the c.*219G>C* polymorphism on doxorubicin and doxorubicinol pharmacokinetics. The genotypic effect of the c.*627C>T* and *+967G>A* polymorphisms on the pharmacokinetics of doxorubicin and doxorubicinol were similar (Table 4). Patients carrying the reference c.*627CC* genotype had significantly higher clearance (median 24.07 CL/BSA[L/h/m^2]; range 16.44–49.40 CL/BSA[L/h/m^2]) of doxorubicin when compared to patients who were heterozygous (median 20.13 CL/BSA[L/h/m^2]; range 8.68–31.79 CL/BSA[L/h/m^2], $P = 0.028$). Breast cancer patients carrying the reference c.*627CC* genotype showed a trend toward lower exposure levels (median 15.44 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 6.18–38.03 $AUC_{0-\infty}$/dose/BSA[h/m^5]) of doxorubicin compared with patients who were heterozygous (median 20.36 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 9.82–67.17 $AUC_{0-\infty}$/dose/BSA[h/m^5], $P = 0.066$) or homozygous for the variant allele (median 20.74 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 15.15–30.16 $AUC_{0-\infty}$/dose/BSA[h/m^5], $P = 0.60$). Significantly lower peak plasma concentrations of doxorubicinol (median 0.32 C_{max}/dose/BSA[h/m^5]; range 0.16–2.0 C_{max}/dose/BSA[h/m^5]) were observed in patients with the reference c.*627CC* genotypes compared to heterozygotes (median 0.49 C_{max}/dose/BSA[h/m^5]; range 0.18–1.49 C_{max}/dose/BSA[h/m^5], $P = 0.030$).

With regard to the *+967G>A* polymorphism, breast cancer patients carrying the reference *+967GG* genotype also had significantly higher clearance (median 24.42 CL/BSA[L/h/m^2]; range 16.44–49.40 CL/BSA[L/h/m^2]) of doxorubicin when compared with patients who were heterozygous (median 20.43 CL/BSA[L/h/m^2]; range 8.68–31.79 CL/BSA[L/h/m^2]) for the polymorphism ($P = 0.009$). Patients carrying the reference *+967GG* genotype had significantly lower exposure levels (median 15.62 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 6.18–29.33 $AUC_{0-\infty}$/dose/BSA[h/m^5]) of doxorubicin when compared with patients who were heterozygous (median 22.43 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 9.82–67.17 $AUC_{0-\infty}$/dose/BSA[h/m^5]) ($P = 0.024$). The *+967GG* genotypes were also associated with a significantly lower peak plasma concentration of doxorubicinol (median 0.32 C_{max}/dose/BSA[h/m^5]; range 0.16–2.0 C_{max}/dose/BSA[h/m^5]) when compared with the patients harboring the heterozygous genotypes (median 0.48 C_{max}/dose/BSA[h/m^5]; range 0.22–1.49 C_{max}/dose/BSA[h/m^5]) ($P = 0.026$).

No major influences of individual *CBR1* haplotypes on doxorubicin pharmacokinetics were observed in the present study. We examined the phenotypic associations of linked high-frequency *CBR1* polymorphisms with the pharmacokinetics of doxorubicin by considering diplotypes that included composite genetic information from all of the loci of interest. Sequence clustering using the complete linkage algorithm identified two major groups (D1 and D2) among the 12 *CBR1* diplotypes in Asian breast cancer patients (Fig. 2). The D2 diplotype group was characterized by the presence of at least one variant allele at both the c.*627* and *+967* loci. Significant differences were observed in the doxorubicin pharmacokinetic parameters between the two diplotype groups. Patients belonging to the D1 diplotype group had significantly higher clearance of doxorubicin (median 25.09 CL/BSA[L/h/m^2];

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Table 3. *CBR1* haplotypes and diplotypes in Asian ethnic groups and breast cancer patients

	Chinese (n = 100)				Malays (n = 100)				Indians (n = 100)				Asian breast cancer Patients (n = 62)				
Haplotypes[†]				Frequency	Haplotypes				Frequency	Haplotypes				Frequency	Haplotypes				Frequency
H1	G	C	G	0.70	H1	G	C	G	0.69	H1	G	C	G	0.71	$H1_b$	G	C	G	0.65
H2	C	T	A	0.13	H2	C	T	A	0.11	H2	C	T	A	0.09	$H2_b$	C	T	A	0.13
H3	G	T	A	0.07	H3	G	T	A	0.10	H3	C	C	G	0.08	$H3_b$	G	T	A	0.10
H4	G	T	G	0.05	H4	G	T	G	0.03	H4	G	T	A	0.05	$H4_b$	C	T	G	0.05
H5	C	C	G	0.02	H5	C	C	G	0.03	H5	C	T	G	0.03	$H5_b$	C	C	G	0.04
H6	G	C	A	0.02	H6	G	C	A	0.03	H6	G	C	A	0.03	$H6_b$	C	C	A	0.03
H7	C	C	A	0.01	H7	C	T	G	0.01	H7	G	T	G	0.01	$H7_b$	G	T	G	<0.01
H8	C	T	G	<0.01															
Diplotypes																			
H1-H1	GG	CC	GG	0.56	H1-H1	GG	CC	GG	0.57	H1-H1	GG	CC	GG	0.59	$H1_b$-$H1_b$	GG	CC	GG	0.56
H1-H2	GC	CT	GA	0.15	H1-H2	GC	CT	GA	0.07	H1-H2	GC	CT	GA	0.13	$H1_b$-$H2_b$	GC	CT	GA	0.08
					H2-H2	CC	TT	AA	0.06	H1-H4	GG	CT	GA	0.05	$H2_b$-$H3_b$	GC	TT	AA	0.07
					H1-H3	GG	CT	GA	0.05						$H2_b$-$H2_b$	CC	TT	AA	0.05
					H1-H6	GG	CC	GA	0.05						$H3_b$-$H4_b$	GG	TT	GA	0.05
					H3-H3	GG	TT	AA	0.05										

Minor *CBR1* diplotypes (frequency <5%) are not shown. [†]Haplotype represents the single sequence of genotype information derived from the expectation maximization algorithm in the population-based studies.

Fig. 1. Pairwise linkage disequilibrium among (a) *CBR1* and (b) *CBR3* polymorphisms in Asian breast cancer patients.

Fig. 2. *CBR1* diplotype groups observed in Asian breast cancer patients.

Table 4. *CBR1* polymorphisms and pharmacokinetics of doxorubicin in Asian breast cancer patients

Pharmacokinetic parameters	CBR1 genotypes			Pairwise *P*-values*		
c.219G>C	GG (*n* = 30)	GC (*n* = 15)	CC (*n* = 5)	GG versus GC	GC versus CC	GG versus CC
Doxorubicin						
$AUC_{0-\infty}$/dose/BSA (h/m^5)	15.85 (6.18–67.17)	18.76 (9.82–38.03)	15.15 (12.96–20.74)	0.139	0.191	0.944
C_{max}/dose/BSA (/m^5)	33.12 (4.55–97.70)	41.59 (12.80–81.22)	29.92 (17.59–42.38)	0.126	0.191	0.689
CL/BSA (L/h/m^2)	23.30 (8.68–55.66)	21.57 (13.80–31.79)	26.09 (19.57–28.53)	0.094	0.163	0.906
Doxorubicinol						
$AUC_{0-\infty}$/dose/BSA (h/m^5)	9.473 (3.679–21.71)	11.64 (6.48–24.34)	7.77 (6.3–16.8)	0.064	0.278	0.923
C_{max}/dose/BSA (/m^5)	0.31 (0.1483–2.004)	0.39 (0.18–1.49)	0.38 (0.28–0.49)	0.152	0.541	0.556
c.627C>T	CC (*n* = 31)	CT (*n* = 10)	TT (*n* = 9)	CC *versus* CT	CT *versus* TT	CC *versus* TT
Doxorubicin						
$AUC_{0-\infty}$/dose/BSA (h/m^5)	15.44 (6.18–38.03)	20.36 (9.82–67.17)	20.74 (15.15–30.16)	0.066	0.968	0.060
C_{max}/dose/BSA (/m^5)	31.08 (4.55–97.70)	41.81 (12.80–81.22)	37.98 (27.76–80.72)	0.187	1.000	0.164
CL/BSA (L/h/m^2)	24.07 (16.44–49.40)	20.13 (8.68–31.79)	23.66 (13.80–28.53)	0.028	0.315	0.348
Doxorubicinol						
$AUC_{0-\infty}$/dose/BSA (h/m^5)	9.47 (3.68–21.71)	12.87 (6.59–24.34)	10.52 (6.3–16.8)	0.054	0.328	0.357
C_{max}/dose/BSA (/m^5)	0.32 (0.16–2.0)	0.49 (0.18–1.49)	0.38 (0.18–0.57)	0.030	0.182	0.650
+967G>A	GG (*n* = 30)	GA (*n* = 13)	AA (*n* = 7)	GG *versus* GA	GA *versus* AA	GG *versus* AA
Doxorubicin						
$AUC_{0-\infty}$/dose/BSA (h/m^5)	15.62 (6.18–29.33)	22.43 (9.816–67.17)	18.76 (15.15–31.63)	0.024	0.526	0.126
C_{max}/dose/BSA (/m^5)	32.03 (4.55–97.70)	40.96 (12.80–81.22)	42.04 (29.92–73.53)	0.239	0.751	0.126
CL/BSA (L/h/m^2)	24.42 (16.44–49.40)	20.43 (8.675–31.79)	24.93 (15.04–28.53)	0.009	0.178	0.473
Doxorubicinol						
$AUC_{0-\infty}$/dose/BSA (h/m^5)	9.5 (3.68–24.34)	10.40 (6.48–16.79)	10.87 (6.25–18.71)	0.402	0.880	0.484
C_{max}/dose/BSA (/m^5)	0.32 (0.16–2.0)	0.48 (0.22–1.49)	0.39 (0.18–0.57)	0.026	0.178	0.862

$AUC_{0-\infty}$/dose/BSA, area under plasma concentration-time curve from time zero to infinity normalized by dose and body surface area; BSA, body surface area; CL/BSA, plasma clearance normalized by body surface area; C_{max}/dose/BSA, peak plasma concentration normalized by dose and body surface area. Values are expressed as median (range).
*$P < 0.05$ considered statistically significant.

range 16.44–55.66 CL/BSA[L/h/m^2]) compared with patients belonging to the D2 diplotype group (median 20.88 CL/BSA[L/h/m^2]; range 8.68–31.79 CL/BSA[L/h/m^2]) ($P = 0.014$) (Fig. 3a). Patients belonging to the D1 diplotype group also had significantly lower exposure levels of doxorubicin (median 15.08 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 6.18–38.03 $AUC_{0-\infty}$/dose/BSA[h/m^5]) compared to patients belonging to the D2 diplotype group (median 21.35 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 9.82–67.17 $AUC_{0-\infty}$/dose/BSA[h/m^5]) ($P = 0.007$) (Fig. 3b). The peak plasma concentrations of doxorubicin in patients belonging to the D1 diplotype group were also significantly lower (median 25.49 C_{max}/dose/BSA[/m^5]; range 4.55–77.67 C_{max}/dose/BSA[/m^5]) compared with patients belonging to the D2 diplotype group (median 41.50 C_{max}/dose/BSA[/m^5]; range 12.80–81.22 C_{max}/dose/BSA[/m^5]) ($P = 0.038$). With regard to doxorubicinol, patients harboring the D1 diplotype family had lower exposure levels of doxorubicinol (median 8.99 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 3.68–20.63 $AUC_{0-\infty}$/dose/BSA[h/m^5]) when compared with patients belonging to the D2 diplotype family (median 11.28 $AUC_{0-\infty}$/dose/BSA[h/m^5]; range 6.25–18.71 $AUC_{0-\infty}$/dose/BSA[h/m^5]) ($P = 0.057$).

***CBR3* polymorphisms in healthy subjects and breast cancer patients.** Direct sequencing of the 5′-UTR, 3′-UTR, and coding regions of the *CBR3* gene identified a total of five polymorphisms. One non-synonymous transition (c.*11G>A*; C4Y) and two synonymous transitions (c.*255T>C* and c.*279C>T*) were identified in exon 1. A synonymous (c.*606G>A*) and a non-synonymous (c.*730G>A*; V244M) polymorphism were identified in exon 3 of the *CBR3* gene.

The genotype and allele frequencies of the five *CBR3* polymorphisms identified in the Asian healthy ethnic groups and the breast cancer patients are listed in Table 5. The allele frequency of the c.*11G>A* polymorphism was significantly higher among the Chinese (0.38) and Malays (0.41) when compared to Indians (0.28) (Chinese vs Indians; $P = 0.03$, Malays vs Indians; $P = 0.01$). The c.*255T>C* polymorphism was observed to be monomorphic in the Chinese healthy subjects. The allele frequency of the c.*279C>T* polymorphism was significantly higher among the Chinese (0.39) when compared to Indians (0.29, $P = 0.03$). The c.*606G>A* polymorphism was monomorphic in the Chinese and Malay healthy subjects but was present at an allele frequency of 0.03 among the Indians. The allele frequency of the c.*730G>A* polymorphism was also significantly higher among the Indians when compared to the Chinese and Malays (Indians vs Chinese, $P < 0.0001$; Indians vs Malays, $P = 0.003$). The genotype and allele frequencies of *CBR3* polymorphisms among cancer patients conformed to the frequency in healthy study subjects.

***CBR3* linkage disequilibrium analysis.** Strong linkage of the c.*11G>A* polymorphism with the c.*255T>C*, c.*279C>T*, and c.*730G>A* polymorphisms was observed in all of the ethnic groups ($|D'| > 0.9$ in all cases). The c.*11G>A* and c.*606G>A* polymorphisms were strongly linked in the Indian healthy population ($|D'| > 0.9$). The linkage between the c.*255T>C* and c.*730G>A* polymorphisms were strong among Chinese and Malays ($|D'| > 0.9$) but weak among Indians ($|D'|$~0.3). Strong linkage was also observed between the c.*279C>T* and c.*730G>A* polymorphisms among all of the ethnic groups ($|D'| > 0.9$ in all groups). A total of six *CBR3* haplotypes were determined in the Chinese population and three high-frequency haplotypes (H1, H2, and H3) accounted for a cumulative frequency of 92% (Table 6). Among the nine haplotypes inferred in the Malay population, four were identified as high-frequency haplotypes (H1, H2, H3, and H4) and had a cumulative frequency of 94%. There were nine haplotypes identified in the Indian population, of which the H1, H2, and H3 haplotypes constituted 90% of the total haplotype frequencies. The H1 haplotype was tagged by the c.*730G>A* polymorphism, H2 haplotype by the linked c.*11G>A* and c.*279C>T* polymorphisms, and the H3 haplotype by the c.*255T>C* polymorphism in all ethnic groups studied.

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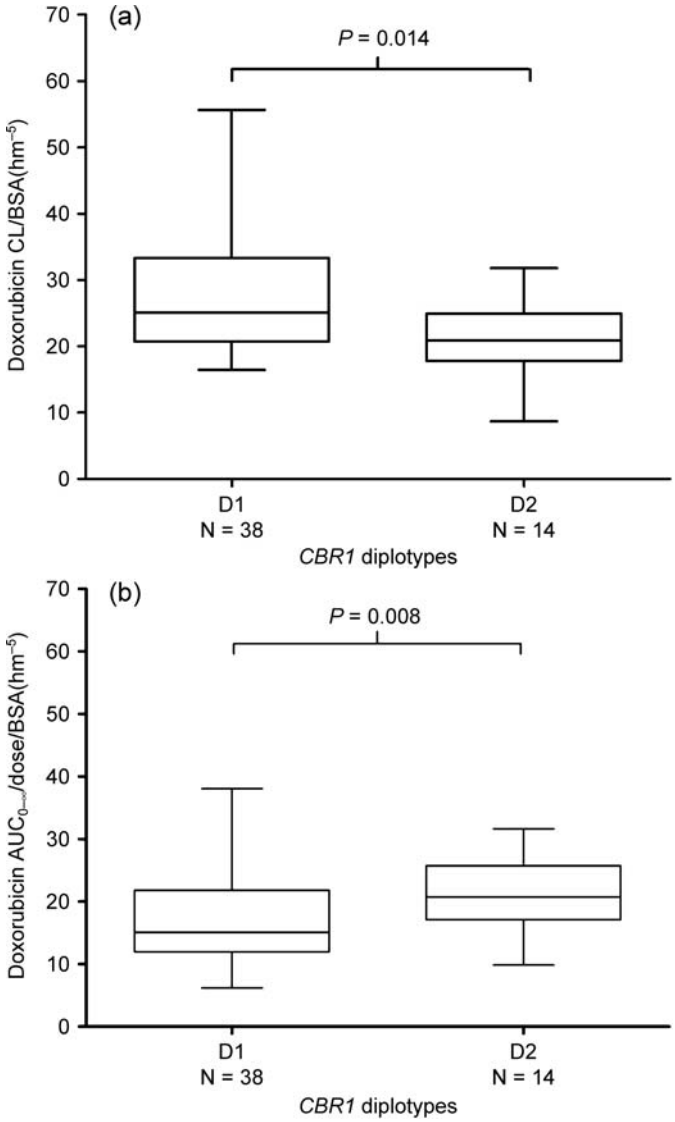

Fig. 3. Influence of *CBR1* diplotypes on (a) clearance and (b) exposure levels of doxorubicin in Asian breast cancer patients. N, number of subjects.

Pairwise analysis of *CBR3* polymorphisms in cancer patients showed a high degree of linkage between the c.*11G>A*, c.*255T*>C, c.*279C>T*, c.*606G>A*, and c.*730G>A* polymorphisms ($|D'| > 0.9$ in each case) (Fig. 1b). Haplotype analysis revealed a total of nine haplotype groups, of which the $H1_b$ (*G-T-C-G-A*), $H2_b$ (*A-T-T-G-G*), and $H3_b$ (*G-C-C-G-G*) haplotypes had a cumulative frequency of 91% (Table 6). Similar to the healthy subjects, the $H1_b$, $H2_b$, and $H3_b$ haplotypes in breast cancer patients were tagged by the c.*730G>A*, c.*11G>A*, and c.*279C>T*, and c.*255T>C* polymorphisms, respectively. A total of 14 diplotypes were inferred by the expectation–maximization algorithm and the major diplotypes ($H1_b$-$H1_b$ [23%], $H2_b$-$H2_b$ [18%], $H1_b$-$H3_b$ [16%], $H2_b$-$H3_b$ [11%], $H1_b$-$H2_b$ [11%], and $H3_b$-$H3_b$ [5%]) accounted for a cumulative frequency of 84%.

No significant influences of the *CBR3* polymorphisms (c.*11G>A,* c.*255T>C*, c.*279C>T*, c.*606G>A*, and c.*730G>A*) on doxorubicin and doxorubicinol pharmacokinetics were observed in the present study (data not shown). Examination of the influence of the major *CBR3* haplotype and diplotype structures also failed to reveal any significant association with doxorubicin disposition in Asian breast cancer patients.

Discussion

Polymorphisms in the coding or regulatory regions of genes encoding CBR drug-metabolizing enzymes may have significant phenotypic influences due to their critical roles in the phase I metabolism of both endogenous and xenobiotic compounds.[26] The alterations in tissue-specific expression and activity of CBR could also contribute to interindividual variations in the pharmacokinetics and pharmacodynamics of several putative drug substrates. The present study is the first report on the pharmacogenetic profiling of the human CBR genes (*CBR1* and *CBR3*) in three distinct Asian ethnic groups (Chinese, Malays, and Indians) and their impact on the pharmacokinetics of doxorubicin and doxorubicinol in Asian breast cancer patients.

Screening the exon and exon–intron boundaries of the *CBR1* gene in 300 healthy Asian subjects identified five polymorphisms (*–48G>A*, c.*219G>C*, c.*627C>T*, c.*693G>A*, and *+967G>A*) that exhibited significant interethnic variations in genotype and allele frequencies. The allele frequency of the c.*219G>C* polymorphism among the Chinese (0.16), Malay (0.15), and Indian (0.20) ethnic groups were similar to the frequency reported in the Caucasian population (0.15).[17] The non-synonymous c.*262G>A* (V88I) polymorphism was absent in the Asian health subjects and patients. This polymorphism is present at a low frequency among African-Americans (<0.2%) and has been postulated to be associated with higher intracellular prostaglandin E2 levels.[17] The allele frequency of the c.*627C>T* polymorphism in the Indian population (0.18) was similar to the Caucasians (0.15) but lower compared to the Chinese subjects (0.26, $P = 0.05$). The c.*693G>A* polymorphism reported in Caucasians (0.02)[17] was monomorphic among the Chinese and occurred at a low frequency in both Malays and Indians (0.01 in each ethnic group).

Earlier reports by Lakhman *et al.* showed wide interethnic variation in the allele frequency of the *CBR3* c.*730G>A* (V244M) polymorphism (0.07 among Pacific origin and 0.67 among Indo-Pakistanis) that was proposed to cause structural variation and alterations in dinucleotide binding interactions, leading to higher catalytic efficiency of the enzyme.[18] In the present study, the c.*730G>A* polymorphism occurred at a significantly higher allele frequency among the Indians (0.57) compared to the Chinese (0.38) and Malays (0.42) ($P < 0.0001$ and $P = 0.003$, respectively). The significantly lower allele frequency of the c.*11G>A* and c.*279C>T* polymorphism among Indians when compared to the rest of the ethnic groups and the absence of c.*255T>C* and c.*606G>A* polymorphisms in the Chinese subjects suggests the existence of wide interethnic differences in the frequency of *CBR3* polymorphisms.

Association studies with polymorphic data from linked loci suggest that diplotype constitution, which characterizes the subset of every single-locus genotype in a gene and is representative of the type of chromosome pairs in an individual,[27,28] could be major determinants of phenotypes.[29] In the present study, homozygosity for the reference allele at the *CBR1c.627C>T* and *+967G>A* loci was observed to be associated with increased clearance and reduced exposure levels of doxorubicin in Asian breast cancer patients. The reasons for the lack of influence of the homozygous variant alleles at the *CBR1* c.*627C>T* and *+967G>A* loci on doxorubicin pharmacokinetic parameters are unknown and could possibly be due to a smaller cohort of breast cancer patients studied. Because the *CBR1* c.*219G>C*, c.*627C>T*, and *+967G>A* polymorphisms were observed to be in linkage disequilibrium, further analysis was done to delineate the influences of the inferred *CBR1* diplotypes on doxorubicin pharmacokinetics. Pairwise comparisons revealed that patients belonging to the *CBR1* D1 diplotype group had 20% higher clearance ($P = 0.007$) and 29% lower exposure ($AUC_{0-\infty}$) of doxorubicin ($P = 0.014$) compared to patients belonging to the

Table 5. Genotype and allele frequency of *CBR3* polymorphisms in Asian ethnic groups and breast cancer patients

Polymorphisms	Location		Genotype frequencies			Allele frequency	
c.*11G>A* [rs8133052]	Exon 1	*n*	GG	GA	AA	G	A
Healthy subjects							
Chinese		100	40 (40)	44 (44)	16 (16)	0.62	0.38
Malays		100	33 (33)	52 (52)	15 (15)	0.59	0.41
Indians		100	51 (51)	42 (42)	7 (7)	0.72	0.28
Pooled Asian population		300	124 (41.3)	138 (46)	38 (12.7)	0.64	0.36
Asian Cancer patients		62	32 (51.6)	18 (29)	12 (19.4)	0.66	0.33
c.255T>C [rs881711]	Exon 1		TT	TC	CC	T	C
Healthy subjects							
Chinese		97	63 (64.9)	34 (35.1)	0	0.82	0.18
Malays		100	72 (72)	25 (25)	3 (3)	0.84	0.16
Indians		100	75 (75)	24 (24)	1 (1)	0.87	0.13
Pooled Asian population		297	210 (70.7)	83 (27.9)	4 (1.3)	0.85	0.15
Asian cancer patients		61	41 (67.2)	17 (27.9)	3 (4.9)	0.81	0.19
c.279C>T [rs881712]	Exon 1		CC	CT	TT	C	T
Healthy subjects							
Chinese		98	38 (38.8)	44 (44.9)	16 (16.3)	0.61	0.39
Malays		100	39 (39)	46 (46)	15 (15)	0.62	0.38
Indians		100	50 (50)	42 (42)	8 (8)	0.71	0.29
Pooled Asian population		298	127 (42.6)	132 (44.3)	39 (13.1)	0.65	0.35
Asian cancer patients		61	33 (54.1)	16 (26.2)	12 (19.7)	0.67	0.33
c.606G>A [rs17849671]	Exon 3		GG	GA	AA	G	A
Healthy subjects							
Chinese		100	100 (100)	0	0	1	0
Malays		99	99 (100)	0	0	1	0
Indians		97	92 (94.8)	5 (5.2)	0	0.97	0.03
Pooled Asian population		296	291 (98.3)	5 (1.7)	0	0.99	0.01
Asian cancer patients		61	59 (96.7)	2 (3.3)	0	0.98	0.02
c.730G>A [rs1056892]	Exon 3		GG	GA	AA	G	A
Healthy subjects							
Chinese		99	37 (37.4)	47 (47.5)	15 (15.1)	0.61	0.38
Malays		98	31 (31.6)	51 (52.0)	16 (16.4)	0.58	0.42
Indians		98	22 (22.4)	40 (40.8)	36 (36.8)	0.43	0.57
Pooled Asian population		295	90 (30.5)	138 (46.8)	67 (22.7)	0.54	0.46
Asian cancer patients		62	24 (38.7)	23 (37.1)	15 (24.2)	0.57	0.43

rs, SNP database ID.

CBR1 D2 diplotype group. The peak plasma concentration of doxorubicin was also 38% lower among patients belonging to the *CBR1* D1 diplotype group ($P = 0.038$). This suggests that the increased clearance of doxorubicin in patients harboring the *CBR1* D1 diplotype cohort may probably be due to the increased CBR activity, resulting in increased metabolic conversion of doxorubicin to doxorubicinol among these patients. The *CBR1* D2 diplotypes were characterized by the presence of at least one variant allele at the c.*627* and 3′-UTR *+967* loci. There have been no studies to date that examined the functional roles of the synonymous c.*627C>T* polymorphism. Recent reports investigating polymorphic variations in drug transporters have indicated that synonymous polymorphisms can alter mRNA stability and translation efficiency, contributing to alterations in drug disposition.[30] The 3′-UTR regions have several *cis*-acting binding sites as several transcription factors and mutations in these regions have been associated with alterations in protein expression and disease phenotypes.[31,32] Future studies are required to delineate the functional roles of the synonymous c.*627C>T* polymorphism and the 3′-UTR *+967G>A* polymorphism in affecting the pharmacokinetics of doxorubicin and doxorubicinol.

The increased clearance of doxorubicin in breast cancer patients belonging to the *CBR1* D1 diplotype group was, however, not associated with increased exposure levels of doxorubicinol. Possible reasons that may have accounted for this observation may include further metabolism of doxorubicinol to its aglycone, doxorubicinolaglycone,[32] thereby rendering it more favorable for excretion. In addition, the activities of other carbonyl-group reducing enzymes such as aldehyde reductases (*AKR1A1*) and dihydrodiol dehydrogenase 2 (*AKR1C2*) have been identified to be important in contributing to the intracellular formation of doxorubicinol,[10,33] and may also influence its clearance. Doxorubicinol is also a substrate for the efflux transporter ABCB1 substrate and its clearance may hence be affected by its affinity toward ABCB1 and the variable ABCB1 expression levels in the biliary canalicular membrane of hepatocytes.[34] We have previously shown that homozygosity for the reference allele at the *ABCB1* c.*1236C>T*, c.*2677G>T/A*, and c.*3435C>T* loci was associated with significantly lower peak plasma concentrations of doxorubicin and doxorubicinol.[35] To examine whether the observed influence of *CBR1* diplotypes is independent of the influence of *ABCB1* homozygosity at the respective loci, we carried out a backward exclusion regression incorporating the published *ABCB1* classes and *CBR1* diplotypes. It was observed that the influence of *CBR1* diplotypes was retained as significant despite the exclusion of *ABCB1* classes in the model. Regression analysis showed that the observed contribution of *CBR1* diplotypes to the doxorubicin clearance was highly significant and independent of *ABCB1* classes ($R^2 = 40.4\%$; $P = 0.008$). We also carried out a multivariate analysis to examine the *CBR1* diplotypic effect on the increased exposure of doxorubicin by incorporating the significant patient covariates (age, ethnicity, height, and weight), which were

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Table 6. *CBR3* haplotypes and diplotypes in Asian ethnic groups and breast cancer patients

Haplotypes	Chinese (n = 100)					Frequency	Haplotypes	Malays (n = 100)					Frequency	Haplotypes	Indians (n = 100)					Frequency	Haplotypes	Asian breast cancer patients (n = 62)					Frequency
H1	G	T	C	G	A	0.39	H1	G	T	C	G	A	0.40	H1	G	T	C	G	A	0.53	$H1_b$	G	T	C	G	A	0.41
H2	A	T	T	G	G	0.36	H2	A	T	T	G	G	0.34	H2	A	T	T	G	G	0.26	$H2_b$	A	T	T	G	G	0.31
H3	G	C	C	G	G	0.17	H3	G	C	C	G	G	0.15	H3	G	C	C	G	G	0.11	$H3_b$	G	C	C	G	G	0.19
H4	G	T	C	G	G	0.04	H4	A	T	C	G	G	0.05	H4	G	T	C	A	G	0.03	$H4_b$	G	T	C	G	G	0.03
H5	G	T	T	G	G	0.02	H5	G	T	T	G	G	0.02	H5	G	C	C	G	A	0.02	$H5_b$	A	T	C	G	G	0.03
H6	A	T	C	G	G	0.01	H6	A	T	T	G	A	0.01	H6	G	T	T	G	G	0.02	$H6_b$	G	T	T	G	A	0.01
							H7	G	T	C	G	G	0.01	H7	G	T	C	G	G	0.01	$H7_b$	G	T	C	A	G	0.01
							H8	G	C	T	G	A	<0.01	H8	G	T	T	G	A	0.01	$H8_b$	G	T	C	A	A	0.01
							H9	A	C	T	G	G	<0.01	H9	A	T	C	G	G	0.01	$H9_b$	G	T	T	G	G	<0.01
Diplotypes																											
H1-H2	GA	TT	CT	GG	GA	0.24	H1-H2	GA	TT	CT	GG	GA	0.29	H1-H1	GG	TT	CC	GG	AA	0.31	$H1_b$-$H1_b$	GG	TT	CC	GG	AA	0.23
H1-H3	GG	TC	CC	GG	GA	0.18	H1-H1	GG	TT	CC	GG	AA	0.16	H1-H2	GA	TT	CT	GG	GA	0.28	$H2_b$-$H2_b$	AA	TT	TT	GG	GG	0.18
H1-H1	GG	TT	CC	GG	AA	0.15	H1-H3	GG	TC	CC	GG	GA	0.11	H2-H3	GA	TC	CT	GG	GG	0.10	$H1_b$-$H3_b$	GG	TC	CC	GG	GA	0.16
H2-H2	AA	TT	TT	GG	GG	0.15	H2-H2	AA	TT	TT	GG	GG	0.11	H1-H3	GG	TC	CC	GG	GA	0.09	$H2_b$-$H3_b$	GA	TC	CT	GG	GG	0.11
H2-H3	GA	TC	CT	GG	GG	0.15	H2-H3	GA	TT	CT	GG	GG	0.11	H2-H2	AA	TT	TT	GG	GG	0.06	$H1_b$-$H2_b$	GA	TT	CT	GG	GA	0.11
							H1-H4	GA	TT	CT	GG	GA	0.07								$H3_b$-$H3_b$	GG	CC	CC	GG	GG	0.05

Minor *CBR3* diplotypes (frequency <5%) are not shown.

significantly associated with $t_{1/2}$ and $AUC_{0-\infty}$ of doxorubicin in the univariate analysis. Multivariate analysis revealed that doxorubicin exposure was significantly associated with patients' weights and *CBR1* diplotype groups ($P < 0.0001$ and $P = 0.022$ respectively). Age and ethnicity had no significant influence on doxorubicin exposure levels. With regard to *CBR3* pharmacogenetics, the *CBR3* c.*730G>A* polymorphism was previously reported to alter the catalytic efficiency of the enzyme.[18] However, no influence of the c.*730G>A* polymorphism on doxorubicin pharmacokinetics was observed in the present study. The lack of association of C*BR3* polymorphic variants with doxorubicin disposition could be due to the relatively low level of hepatic *CBR3* expression.[15]

Similar to the roles played by ATP-binding cassette (ABC) transporters, CBR polymorphisms could also contribute to the mechanisms of drug resistance, treatment efficacy, and toxicity during cancer chemotherapy. CBR expression in K562 cells resulted in protection against cytotoxicity when treated with doxorubicin,[36] whereas cells selected for doxorubicin resistance showed a 60% increase in CBR mRNA levels.[37] Taxanes, such as paclitaxel, have been shown to increase the intracellular conversion of doxorubicin to doxorubicinol by binding to the regulatory allosteric and catalytic sites of CBR enzymes, thereby influencing the efficacy and toxicity of combination chemotherapy regimens.[38] CBR-mediated intracellular conversion of doxorubicin to doxorubicinol has also been proposed to be important in the development of anthracycline cardiotoxicity.[39] *In vitro* studies have shown a higher potency of doxorubicinol than doxorubicin in depressing myocardial contractility and inhibiting ion channel pumps,[40] whereas studies on transgenic mice overexpressing *CBR1* in the heart showed higher intracardiac levels of doxorubicinol and increased signs of myocardial damage after doxorubicin administration.[39] Although the precise molecular mechanisms that mediate anthracycline cardiotoxicity remain to be identified, alterations in intracellular concentrations of doxorubicin and doxorubicinol by polymorphic CBR could be assumed to play significant roles in the incidence of cardiotoxicity observed in a subset of patients on chemotherapy.

In conclusion, the present study identified novel *CBR1* and *CBR3* polymorphisms and characterized their haplotype and diplotype structures in three distinct Asian ethnic groups and breast cancer patients. The study is the first report on the contribution of CBR polymorphisms on doxorubicin pharmacokinetics in Asian breast cancer patients. The present exploratory study shows that *CBR1* D2 diplotypes tagged by at least one variant allele at the *CBR1* c.*627* and *+967* loci correlate with significantly higher exposure levels of doxorubicin, suggesting the possibility of lowered intracellular conversion to doxorubicinol in these patients. Given the modest sample size of the patients analyzed, the findings should be considered exploratory in nature, and larger studies with a higher sample size must be carried out to arrive at definitive conclusions and to further evaluate the role of CBR polymorphisms in influencing treatment efficacy in Asian cancer patients on doxorubicin-based adjuvant chemotherapy.

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