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Correlation of aldo-ketoreductase (AKR) 1C3 genetic variant with doxorubicin pharmacodynamics in Asian breast cancer patients

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- Significant interindividual variation in doxorubicin efficacy and toxicity exists.
- Aldo-ketoreductase *AKR1C3* genetic variants have been identified *in vitro* to affect doxorubicin metabolism.
- Correlation between these genetic variants and doxorubicin metabolism *in vivo* will advance our understanding of pharmacogenetic influences on the complex doxorubicin disposition pathway.

WHAT THIS STUDY ADDS

- A common *AKR1C3* intronic variant, IVS4–212 C>G, correlated with doxorubicin pharmacodynamics in breast cancer patients. The variant remains significantly correlated with patient survival on multivariate analysis with known clinical prognosticators.
- This variant appears to be more common in Asians than Caucasians and may explain the previously observed greater doxorubicin-induced myelosuppression in Chinese compared with Caucasian patients.
- These findings may contribute to the use of pharmacogenetic information to tailor drug therapy in cancer patients.

AIMS

Aldo-ketoreductases have been implicated in the metabolism of doxorubicin. We sought to assess the influence of *AKR1C3* genetic variants on doxorubicin metabolism.

METHODS

We sequenced *AKR1C3* exon 5 and genotyped seven functional single nucleotide polymorphisms in *CBR3*, *ABCB1* and *SLC22A16* involved in doxorubicin pharmacology in 151 Asian breast cancer patients treated with doxorubicin-containing chemotherapy, and correlated these genotypes with doxorubicin pharmacokinetics and pharmacodynamics.

RESULTS

Two previously reported *AKR1C3* intronic variants, IVS4–212 C>G and IVS4+218 G>A, were detected. The *AKR1C3* IVS4–212 GG genotype was associated with significantly lower cycle 1 day 15 leucocyte (mean leucocytes $2.49 \pm 1.57 \times 10^9$ *vs.* $3.85 \pm 3.42 \times 10^9\ l^{-1}$, $P = 0.007$) and neutrophil counts (mean neutrophils $0.70 \pm 1.01 \times 10^9$ *vs.* $1.56 \pm 2.80 \times 10^9\ l^{-1}$, $P = 0.008$) and significant improvement of progression-free survival [PFS, mean PFS 49.0 (95% confidence interval 42.2–55.8) *vs.* 31.0 (95% confidence interval 20.7–41.2) months, $P = 0.017$] and overall survival [OS; mean OS 64.4 (95% confidence interval 58.3–70.5) *vs.* 46.3 (95% confidence interval 35.1–57.5) months, $P = 0.006$] compared with those carrying at least one C allele. There was no significant association between *AKR1C3* IVS4–212 C>G and doxorubicin pharmacokinetics. Of the other seven single nucleotide polymorphisms genotyped, *CBR3* G11A correlated with doxorubicinol area under the concentration–time curve and OS, *ABCB1* G2677T/A correlated with doxorubicin clearance and platelet toxicity, while *ABCB1* IVS26+59 T>G correlated with OS. The *AKR1C3* IVS4–212 C<G genotype remained significantly correlated with both PFS and OS on multivariate analysis with clinical prognosticators.

CONCLUSIONS

The *AKR1C3* IVS4–212 GG genotype was associated with greater haematological toxicity and longer progression-free survival and overall survival after doxorubicin-based therapy, suggesting potential interaction of this variant with doxorubicin metabolism.

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Introduction

Doxorubicin is an effective anticancer agent for a range of solid tumours, including breast cancer [1]. Interindividual variations in doxorubicin response and toxicity are well recognized [2–4]. The doxorubicin disposition pathway is complex and involves various influx and efflux transporters across the cellular membrane and metabolizing enzymes that are postulated to be responsible for the wide variability in pharmacokinetics and pharmacodynamics of doxorubicin [5].

Genetic factors have been suggested to explain the variability in therapeutic efficacy and toxicity of drugs in individual patients [6]. The influence of single nucleotide polymorphisms (SNPs) from potential functional candidate gene variants across the doxorubicin disposition pathway has been investigated [5–9]. These include influx [solute carrier family (SLCs)] and efflux drug transporters [ATP-binding cassette (ABCBs)] and drug metabolizing enzymes [carbonyl reductase (CBR) and aldo-ketoreductase (AKR)] SNPs. However, the results observed from these studies have still not been clearly elucidated, in particular for AKRs.

Aldo-ketoreductases have been implicated in the metabolism of anthracycline antibiotics, including doxorubicin [10]. Aldo-ketoreductase 1C3 plays a significant role in the deactivation of doxorubicin to doxorubicinol, a less active metabolite [11]. An *in vitro* study has demonstrated that two nonsynonymous SNPs in exon 5 of the *AKR1C3* gene (R170C and P180S) encode for enzymes with significantly reduced metabolism of doxorubicin compared with their wild-type counterparts and may contribute to the interindividual variability in anthracycline metabolism [12].

We sought to evaluate the frequency distribution of genetic variants in and around the coding region of exon 5 in the *AKR1C3* gene and to determine the correlation between identified *AKR1C3* genetic variants with doxorubicin pharmacokinetics, haematological toxicities and antitumour efficacy in an Asian breast cancer population. The influence of SNPs from other functional candidate gene variants across the doxorubicin disposition pathway, namely *SLC22A16* (influx transporter), *ABCB1* (efflux transporter) and *CBR3* (drug metabolizing enzyme), were also examined concurrently, to assess the potential interactions of these more widely studied genetic variants with *AKR1C3* SNPs.

Methods

Study population

We studied 151 Asian breast cancer patients who participated in two preoperative chemotherapy trials conducted at the National University Cancer Institute, Singapore (n = 101 from the first study cohort [7], and n = 50 from the second study cohort). Both study populations were composed of female chemo-naïve patients with histologically or cytologically confirmed breast cancer, for whom preoperative chemotherapy was indicated. The first study cohort was treated with six cycles of preoperative alternating sequential doxorubicin (A) and docetaxel (T) every 3 weeks, and patients were randomized to start either with doxorubicin or docetaxel (A→T→A→T→A→T or T→A→T→A→T→A), followed by surgery, followed by two additional cycles of postoperative alternating docetaxel and doxorubicin. Patients from the second study cohort were randomized to either four cycles of preoperative doxorubicin (n = 25) or four cycles of docetaxel every 3 weeks (n = 25), followed by surgery, followed by four cycles of the alternative drug (docetaxel or doxorubicin). Doxorubicin was administered at 75 mg m^{-2} as a slow bolus and docetaxel at 75 mg m^{-2} over 1 h in both studies. Routine use of prophylactic colony stimulating factor was not allowed.

Genotyping of exon 5 of the AKR1C3 *gene*

Germline DNA was extracted from peripheral mononuclear cells from study participants using standard methods. Exon 5 of the *AKR1C3* gene, which encodes two known functional SNPs (R170C and P180S), was amplified by polymerase chain reaction (PCR). All PCRs were carried out in 25 µl volume with 10× PCR buffer, 25 mM magnesium chloride, 10 mM dNTP, 2 units Faststart Taq DNA polymerase, 12.5 µM each of forward and reverse primer, and 5 ng genomic DNA. The primer sequences were as follows (5′ to 3′, forward and reverse): CCCAGGTTCAATAG GAAAGAA and ACCTTCACCCATGCACTTTC. The size of the PCR product was 850 bases, which included 727 bases of intronic sequences. The PCR was performed with an initial denaturation step at 95°C for 5 min, followed by 36 cycles of denaturation at 95°C for 30 s, annealing at 57.5°C for 45 s, and extension at 72°C for 1 min, followed by a final extension step at 72°C for 5 min. The PCR products were purified and sequenced on the ABI 3100 automated sequence analyser (Applied Biosystems Inc., Foster City, CA, USA) with the forward or reverse PCR primer. Generated sequences were compared with the reference sequence for *AKR1C3* available online at http://www.ncbi.nlm.nih.gov/nuccore/NC_000010.10?from=5136568&to=5149878&report=genbank.

Genotyping of functional SNPs from ABCB1, CBR3 *and* SLC22A16

A total of seven SNPs from these three genes were chosen, based on previous reports [7, 9, 13, 14]. The *ABCB1* G2677T/A genotype has been reported to influence doxorubicin clearance and is linked to two other *ABCB1* functional variants, C1236T and C3435T, while the *ABCB1* IVS26+59 T>G variant has been reported to be associated with anthracycline-induced cardiotoxicity [13]. Both *CBR3* G11A and G730A were selected based on our previous study, which showed significant correlation with

doxorubicin pharmacokinetics and pharmacodynamics [7]. Genotypes *SLC22A16* T312C, T755C and T1226C were included because they were present at significant frequencies in our Asian breast cancer population, but there is a paucity of data on their influence on doxorubicin pharmacokinetics and pharmacodynamics [14]. Genotype data for *CBR3* G11A were obtained from our previous report of the first study cohort (n = 99) [7], while genotyping for the other six SNPs was performed in multiplexed reactions using the MassArray iPLEX Gold platform (Sequenom, San Diego, CA, USA) for both study cohorts (n = 151). In brief, PCR amplification was carried out using 20 ng of DNA in a 5μl reaction that contained 0.5 units Taq polymerase, 10× PCR buffer, 4 mM magnesium chloride, 500 μM dNTPs and 0.1 μM of primers, in the following conditions: 95°C for 2 min, 45 cycles of 95°C for 30 s, 56°C for 30 s, 72°C for 1 min and a final extension step of 72°C for 5 min. Unincorporated dNTPs were removed using 0.5 units of shrimp alkaline phosphatase dissolved in the shrimp alkaline phosphatase buffer provided. Single base extension was carried out in a 9μl reaction that contained iPLEX GOLD buffer, termination mix, extend primer mix and enzyme, using the following two cycling loop programme: 94°C for 30 s, 40 cycles of 94°C for 5 s, 52°C for 5 s and 80°C for 5 s. Within the 40 cycles, the annealing and extension step was repeated five times (i.e. $40 \times 5 = 200$ short cycles), before a final extension step of 3 min at 72°C. Reactions were desalted using 6 mg of clean resin and diluted with 16 μl water. A 10 nl sample of each reaction was spotted onto the 384-spot SpectroChipII using the Nanodispenser (Sequenom), followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) analysis using the MassARRAY Compact system (Sequenom). The mass spectra analysis and genotype calls were generated using the TYPER 4.0.22 software (Sequenom).

Pharmacokinetics analyses

Whole blood (5 ml) was collected from the contralateral arm from chemotherapy administration for analysis of doxorubicin and doxorubicinol levels at 0, 1, 2, 4, 7 and 24 h after the first doxorubicin treatment. Analytical grade doxorubicin hydrochloride was purchased from Woo Shin Medics Co. (Seoul, South Korea) and doxorubicinol hydrochloride was purchased from Toronto Research Chemicals Inc. (Canada). Plasma concentrations of doxorubicin and its major metabolite, doxorubicinol, were determined in all samples by a reverse-phase high-performance liquid chromatography method with fluorescence detection modified from a published method [15]. The limits of quantification for doxorubicin and doxorubicinol were 2 and 1 ng ml^{-1}, respectively. The calibration curves were linear over a concentration range of 2–200 ng ml^{-1} for doxorubicin and 1–100 ng ml^{-1} for doxorubicinol. The average recoveries were greater than 84% for both analytes. The intraday and interday precision coefficients of variation ranged from 1.1 to 14% for doxorubicin and from 2.3 to 13% for doxorubicinol. Accuracy was 97–110% for doxorubicin and 92–105% for doxorubicinol.

Doxorubicin pharmacokinetic parameters were analysed with two-compartmental and noncompartmental methods using Kinetica software, version 4.4 (Thermo Fisher Scientific, Inc., Waltham, MA, USA). All pharmacokinetic parameters for doxorubicin were derived from two-compartmental analysis. The noncompartmental method was used to analyse the area under the concentration–time curve (AUC) of doxorubicinol because it was a metabolite. The AUC, maximal concentration (C_{max}), clearance, elimination half-life and volume of distribution at steady state were calculated.

Statistical analysis

Identified genetic variants were correlated with antitumour efficacy, haematological toxicity and doxorubicin and doxorubicinol pharmacokinetic (PK) data. The PK data, which included doxorubicin and doxorubicinol AUC, doxorubicin C_{max}, clearance and terminal half-life, were available only for 98 patients recruited into the first trial. Efficacy parameters of treatment included the objective clinical response rate after completing four to six cycles of preoperative chemotherapy, which was classified according to the World Health Organization criteria [16], progression-free survival (PFS) and overall survival (OS). Analysis of the objective clinical response rate was limited to the 125 patients who were treated with four to six cycles of preoperative chemotherapy containing doxorubicin, excluding the 25 patients in the second study cohort who were randomized to receive doxorubicin postoperatively. The haematological parameters evaluated included weekly complete blood counts, nadir blood counts and febrile neutropenia rates during the first cycle of doxorubicin.

The Mann–Whitney U-test was used to compare means of continuous variables, while the chi-squared or Fisher's exact test was used to compare categorical variables. Differences in survival distributions between different genotype variants in each assessed gene were compared using the Kaplan–Meier method and the logrank test. In addition, multivariate analysis using Cox regression was performed by including genetic variants and known clinical parameters that influenced survival at $P < 0.1$ in univariate analysis. These prognostic clinical parameters include metastatic status, histology grade and hormonal receptor status. All tests were two sided at the 5% level of significance.

The Lewontin's D′ was used to determine linkage disequilibrium between two genetic variants and calculated using the software PowerMarker (version 3.25, http://statgen.ncsu.edu/powermarker/).

Results

The median age of the entire study cohort was 51 years (range 26–74 years), and the study population mainly comprised of Chinese (57%) and Malay women (34%). Forty-seven patients (31%) had metastatic disease at diagnosis.

AKR1C3

Genetic variants identified in exon 5 of *AKR1C3* and their allelic frequencies are listed in Table 1. Both coding region variants of interest (R170C and P180S) were not identified in this study cohort. However, two common intronic variants were detected, IVS4–212 C>G and IVS4+218 G>A, which have been previously reported [National Center for Biotechnology Information, Reference SNP (ref. SNP) cluster report: rs1937840; National Center for Biotechnology Information, ref. SNP cluster report: rs1937841]. The IVS4–212 C>G variant was common in this Asian population, with an overall variant frequency of 0.86; 76% of the study population was homozygous for the variant. The IVS4+218 G>A variant was less common, with an overall variant frequency of 0.11 in this population. Strong linkage dysequilibrium was observed between *AKR1C3* IVS4–212 C and IVS4+218 A (D′ = 0.9274, $P < 0.001$) using Lewontin's D′ measure. There was no ethnic difference (Chinese *vs.* Malay *vs.* Indian *vs.* others) in the distribution for IVS4+218 G>A ($P = 0.63$) and IVS4–212 C>G ($P = 0.23$).

Correlation of AKR1C3 *intronic variants with doxorubicin pharmacokinetics and pharmacodynamics*

IVS4–212 C>G After completing four to six cycles of preoperative chemotherapy, patients who were homozygous for *AKR1C3* IVS4–212 GG ($n = 95$; Table 2) had a higher objective response rate than patients who carried at least one C allele ($n = 30$), although the difference was not statistically significant (85 *vs.* 77%, $P = 0.27$). At a median follow-up of 36 months, patients who were IVS4–212 GG homozygotes had significantly longer PFS compared with those who carried at least one C allele {mean PFS 49.0 [95% confidence interval (CI) 42.2–55.8] *vs.* 31.0 [95% CI 20.7–41.2] months, $P = 0.017$; Figure 1A}. Overall survival was statistically longer in patients who were IVS4–212 GG homozygotes compared with those who carried at least one C allele [mean OS 64.4 (95% CI 58.3–70.5) *vs.* 46.3 (95% CI 35.1–57.5) months, $P = 0.006$; Figure 1B].

After the first cycle of doxorubicin, patients who were IVS4–212 GG homozygotes developed significantly lower absolute leucocyte and neutrophil counts on day 15 compared with those who carried at least one C allele (mean leucocyte count $2.49 \pm 1.57 \times 10^9$ *vs.* $3.85 \pm 3.42 \times 10^9\ l^{-1}$, $P = 0.007$; mean neutrophil count $0.70 \pm 1.01 \times 10^9$ *vs.* $1.56 \pm 2.80 \times 10^9\ l^{-1}$, $P = 0.008$). There was no significant difference in other haematological parameters between the two groups, including febrile neutropenia rates. No significant difference in doxorubicin pharmacokinetics were observed between the two genotype groups, including doxorubicin and doxorubicinol AUC, doxorubicin C_{max} and doxorubicin terminal half-life.

IVS4+218 G>A Despite the significant linkage dysequilibrium between *AKR1C3* IVS4–212 CC or CG and IVS4+218 GA or AA, the only significant association for this intronic variant with doxorubicin pharmacokinetics or pharmacodynamics was lower absolute neutrophil counts on day 15 after the first cycle of doxorubicin for IVS4+218 GG compared with those who carried at least one A allele (mean neutrophil count $0.76 \pm 1.12 \times 10^9$ *vs.* $1.53 \pm 2.93 \times 10^9\ l^{-1}$, $P = 0.038$). Otherwise, there was no statistically significant correlation observed between this intronic variant with

Table 1

Frequency distribution of *AKR1C3* genetic variants ($n = 151$)

Genetic variants (SNP)	Genotype frequency			Allele frequency	
	C/C	C/T	T/T	C	T
Exon 5 508 C>T (rs35575889)	100%	0%	0%	1.00	0
Exon 5 538 C>T (rs34186955)	100%	0%	0%	1.00	0
IVS4–212 C>G (rs1937840)	**C/C**	**C/G**	**G/G**	**C**	**G**
Chinese	0% (0/86)	20% (17/86)	80% (69/86)	0.10	0.90
Malay	8% (4/52)	21% (11/52)	71% (37/52)	0.18	0.82
Indian	11% (1/9)	33% (3/9)	56% (5/9)	0.28	0.72
Others	0% (0/4)	0% (0/4)	100% (4/4)	0	1.00
Total	3% (5/151)	21% (31/151)	76% (115/151)	0.14	0.86
IVS4+218 G>A (rs1937841)	**G/G**	**G/A**	**A/A**	**G**	**A**
Chinese	79% (68/86)	21% (18/86)	0% (0/86)	0.90	0.10
Malay	77% (40/52)	21% (11/52)	2% (1/52)	0.88	0.12
Indian	89% (8/9)	11% (1/9)	0% (0/9)	0.94	0.06
Others	100% (4/4)	0% (0/4)	0% (0/4)	1.00	0
Total	79% (120/151)	20% (30/151)	1% (1/151)	0.89	0.11

Table 2

Comparison of tumour efficacy, haematological toxicity parameters and doxorubicin pharmacokinetic parameters in patients with *AKR1C3* IVS4–212 C>G genotype

	GG	GC/CC	*P* value	
Objective response rate (%; *n* = 125)	84	76	0.25	
Progression-free survival (months; *n* = 151) **[mean (95% CI)]**	49.0 (42.2–55.8)	31.0 (20.7–41.2)	0.017*	
Overall survival (months; *n* = 151) **[mean (95% CI)]**	64.4 (58.3–70.5)	46.3 (35.1–57.5)	0.006*	
Haematological toxicities after cycle 1 doxorubicin (*n* = 142†; mean ± SD)			***P* value**	**95% CI of difference**
Day 8 neutrophils ($\times 10^9$ l^{-1})	3.76 ± 1.79	3.53 ± 2.27	0.19	–0.60 to 1.07
Day 15 neutrophils ($\times 10^9$ l^{-1})	0.70 ± 1.01	1.56 ± 2.80	0.008*	–1.80 to 0.10
Nadir neutrophils ($\times 10^9$ l^{-1})	0.41 ± 0.52	0.49 ± 0.48	0.23	–0.26 to 0.11
Day 8 leucocytes ($\times 10^9$ l^{-1})	5.34 ± 1.87	5.04 ± 2.48	0.21	–0.60 to 1.21
Day 15 leucocytes ($\times 10^9$ l^{-1})	2.49 ± 1.57	3.85 ± 3.42	0.007*	–2.54 to –0.18
Nadir leucocytes ($\times 10^9$ l^{-1})	1.93 ± 1.05	2.05 ± 1.12	0.58	–0.54 to 0.32
Day 8 haemoglobin (g dl^{-1})	11.43 ± 1.59	11.48 ± 1.94	0.54	–0.76 to 0.68
Day 15 haemoglobin (g dl^{-1})	10.78 ± 1.41	11.01 ± 1.93	0.33	–0.93 to 0.46
Nadir haemoglobin (g dl^{-1})	10.11 ± 1.51	10.19 ± 1.88	0.73	–0.78 to 0.61
Day 8 platelets ($\times 10^9$ l^{-1})	240 ± 76	252 ± 92	0.58	–46.65 to 21.49
Day 15 platelets ($\times 10^9$ l^{-1})	264 ± 139	303 ± 141	0.06	–92.96 to 14.55
Nadir platelets ($\times 10^9$ l^{-1})	189 ± 70	198 ± 69	0.41	–36.22 to 17.45
Febrile neutropenia (%)	18.3	13.5	0.51	—
Pharmacokinetic parameters (*n* = 98) Median doxorubicin dose 115 mg (range 90–140 mg)	**Mean ± SD (range)**	**Mean ± SD (range)**	***P* value**	**95% CI of difference**
Doxorubicin AUC (mg l^{-1} h)	1.42 ± 0.55 (0.67–3.86)	1.47 ± 0.63 (0.58–3.60)	0.73	–0.34 to 0.24
Doxorubicin clearance (l h^{-1})	90.02 ± 26.83 (28.12–178.84)	88.55 ± 38.88 (36.13–214.46)	0.49	–15.94 to 18.87
Doxorubicin C_{max} (mg l^{-1})	0.57 ± 1.36 (0.09–10.26)	0.37 ± 0.32 (0.09–1.12)	0.67	–0.14 to 0.54
Doxorubicin half-life (terminal phase; h)	16.24 ± 5.78 (6.70–35.45)	14.13 ± 3.73 (9.81–24.35)	0.09	0.04 to 4.17
Doxorubicinol AUC (mg l^{-1} h)	1.99 ± 1.66 (0.83–12.08)	1.74 ± 0.68 (0.88–3.43)	0.88	–0.22 to 0.72

Abbreviations: AUC, area under the concentration–time curve; CI, confidence interval; C_{max}, maximal plasma concentration. *Significant. †*n* = 142 because nine patients who were randomized to start with docetaxel in the first cycle (T→A→T→A→T→A) in the first study cohort (*n* = 2) or to postoperative doxorubicin in the second study cohort (*n* = 7) did not receive doxorubicin due to study withdrawal.

other doxorubicin pharmacokinetics or pharmacodynamic parameters, including the objective clinical response rate, PFS or OS.

Correlation between CBR3, ABCB1 *and* SLC22A16 *genetic variants with doxorubicin pharmacokinetics and pharmacodynamics*

The distribution of the genotypes is summarized in Table 3. Patients who carried at least one A allele for *CBR3* G11A had significantly lower doxorubicinol AUC (mean AUC 1.78 ± 1.53 *vs.* 2.18 ± 1.37 mg l^{-1} h, *P* = 0.004) and longer OS [mean OS 64.4 (95% CI 56.9–71.9) *vs.* 51.2 (95% CI 40.4–62.0) months, *P* = 0.05]. Patients carrying at least one A allele for *CBR3* G730A had significantly higher doxorubicinol AUC (mean AUC 2.15 ± 1.79 *vs.* 1.56 ± 0.60 mg l^{-1} h, *P* = 0.031) compared with GG homozygotes, but there was no correlation with pharmacodynamics parameters, including survival. For *ABCB1* G2677T/A, patients who carried at least one T allele had significantly higher doxorubicin clearance (93.29 ± 29.22 *vs.* 84.46 ± 30.86 l h^{-1}, *P* = 0.027) and significantly higher day 8 and nadir platelet counts compared with those who did not have T alleles (mean day 8 platelet count 255 ± 79 × 10^9 *vs.* 227 ± 79 × 10^9 l^{-1}, *P* = 0.031; mean nadir platelet count 201 ± 66 × 10^9 *vs.* 176 ± 74 × 10^9 l^{-1}, *P* = 0.031) after the first cycle of doxorubicin. Patients who carried at least one G allele for *ABCB1* IVS26+59 T>G had significantly higher day 8 neutrophil and leucocyte counts (neutrophil count 4.03 ± 2.10 × 10^9 *vs.* 3.14 ± 1.41 × 10^9 l^{-1}, *P* = 0.019; leucocyte count 5.56 ± 2.22 × 10^9 *vs.* 4.74 ± 1.55 × 10^9 l^{-1}, *P* = 0.032) and significantly longer OS than those who did not [mean OS 64.6 (95% CI 58.0–71.2) *vs.* 50.6 (95% CI 41.5–59.6) months, *P* = 0.026].

Regarding the *SLC22A16* T312C variant, patients carrying at least one C allele had lower day 15 platelet counts (mean platelet count 250 ± 107 × 10^9 *vs.* 307 ± 171 × 10^9 l^{-1}, *P* = 0.036) than patients with the TT genotype, while patients with the *SLC22A16* T1226C TT genotype had lower

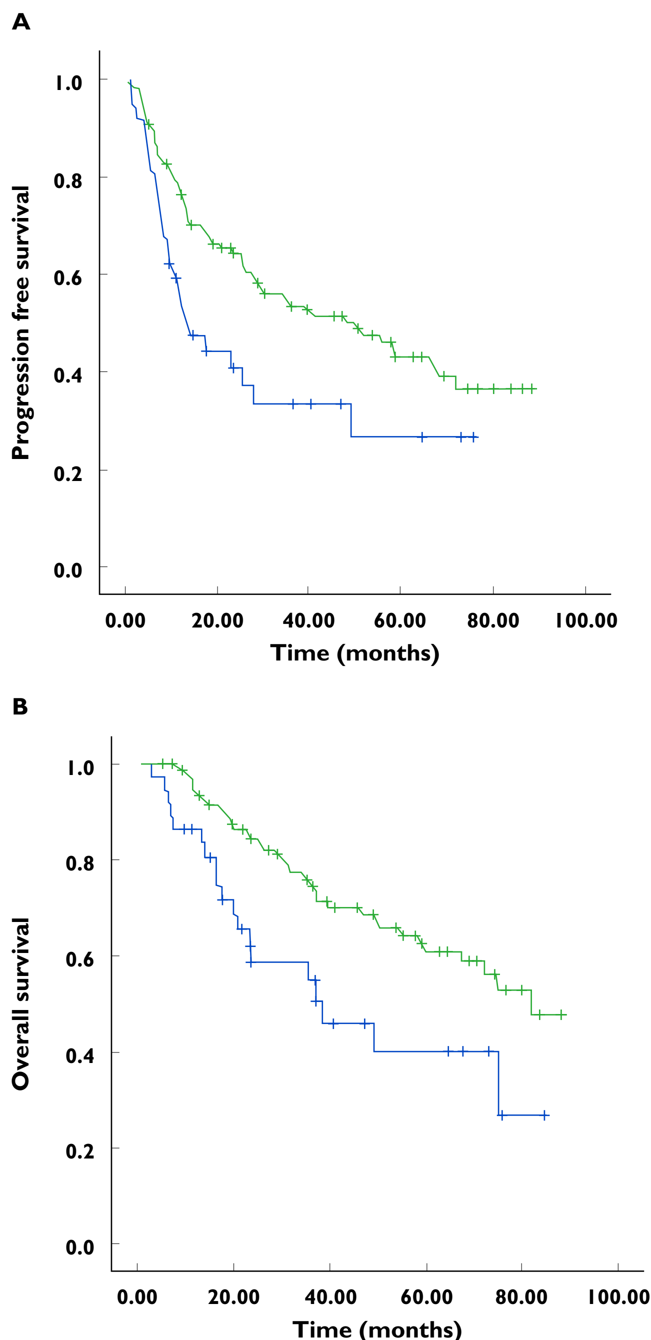

Figure 1

(A) Kaplan–Meier estimate of progression-free survival. —, IVS4–212 GG, mean progression-free survival 49.0 (42.2–55.8) months; —, IVS4–212 GC/CC, mean progression-free survival 31.0 (20.7–41.2) months; $P = 0.017$. (B) Kaplan–Meier estimate of overall survival. —, IVS4–212 GG, mean overall survival 64.4 (58.3–70.5) months; —, IVS4–212 GC/CC, mean overall survival 46.3 (35.1–57.5) months; $P = 0.006$

nadir platelet counts (mean platelet count $189 \pm 70 \times 10^9$ *vs.* $244 \pm 36 \times 10^9\ l^{-1}$, $P = 0.044$) than patients with at least one C allele. However, there was no correlation between these two variants with other doxorubicin pharmacokinetics or pharmacodynamics parameters. There was also no significant correlation between the *SLC22A16* T755C variant with doxorubicin pharmacokinetics and pharmacodynamics.

Multivariate analysis

Three genetic variants, namely *AKR1C3* IVS4–212 C>G ($P = 0.006$), *ABCB1* IVS26+59 T>G ($P = 0.026$) and *CBR3* G11A ($P = 0.05$), and two clinical parameters, namely metastatic status (metastatic *vs.* nonmetastatic, $P < 0.001$) and histological grade (grade 1 and 2 *vs.* 3, $P = 0.005$), influenced OS on univariate analysis and were included in multivariate Cox regression analysis. All five factors remained significantly predictive of OS (*AKR1C3* IVS4–212 C>G, $P = 0.017$; *ABCB1* IVS26+59 T>G, $P = 0.036$; *CBR3* G11A, $P = 0.031$; metastatic status, $P < 0.001$; histological grade, $P = 0.000$). Both *AKR1C3* IVS4–212 GG ($P = 0.017$) and metastatic status ($P < 0.001$) significantly influenced PFS on univariate analysis, and both remained significant on multivariate analysis ($P = 0.044$ and $P < 0.001$, respectively).

Discussion

Aldo-ketoreductases play an important role in the metabolism of a broad array of carbonyl-containing compounds, including anthracycline antibiotics, and functional *AKR1C3* genetic variants have been previously described to affect doxorubicin metabolism [11]. The two previously described functional SNPs in the *AKR1C3* gene were not present in this Asian population. However, two common *AKR1C3* intronic variants were identified, and we report, for the first time, correlations between the *AKR1C3* IVS4–212 GG genotype and doxorubicin pharmacodynamics. Patients with the GG genotype developed significantly lower day 15 leucocyte and neutrophil counts after one cycle of doxorubicin, and had significantly longer progression-free and overall survival following doxorubicin-containing chemotherapy treatment. In concordance, these patients had slightly higher objective response rates, although the difference was not statistically significant. Given these observations, we postulate that the *AKR1C3* IVS4–212 GG intronic variant may influence AKR1C3 activity and thus doxorubicin metabolism. Indeed, a previous *in vitro* study had demonstrated a high proportion of noncoding intronic SNPs to contribute towards chemotherapeutic drug sensitivity [17]. Influence of intronic SNPs in polymorphic expression of cytochrome P450 3A5 has also been reported to cause variability in drug metabolisms [18, 19]. Postulated molecular mechanisms include alteration of the upstream regulatory regions, resulting in alternative splicing and protein truncation of gene products.

The insignificant pharmacokinetics correlation that was demonstrated in our study in contrast to the significant pharmacodynamics findings may be related to sample size, because pharmacokinetic data were available in only a subset of patients; furthermore, the relationship

Table 3

Frequency distribution of *ABCB1*, *CBR3 and SLC22A16* genetic variants (n = 151)

Genetic variants (SNPs)	Genotype frequency						Allele frequency		
ABCB1									
G2677T/A*	GG 37 (25%)	GT 56 (37%)	GA 18 (12%)	TT 23 (15%)	AA 4 (3%)	TA 12 (8%)	G 0.49	T 0.38	A 0.13
IVS26+59 T>G	TT 56 (37%)	TG 64 (42%)	GG 31 (21%)				T 0.37	G 0.63	
CBR3									
G11A†	GG 36 (37%)	GA 30 (30%)	AA 33 (33%)				G 0.52	A 0.48	
G730A*	GG 31 (21%)	GA 56 (37%)	AA 63 (42%)				G 0.39	A 0.61	
SLC22A16									
T312C*	TT 63 (42%)	TC 65 (43%)	CC 22 (15%)				T 0.64	C 0.36	
T1226C*	TT 145 (97%)	TC 5 (3%)	CC 0 (0%)				T 0.98	C 0.02	
T755C*	TT 123 (82%)	TC 27 (18%)	CC 0 (0%)				T 0.91	C 0.09	

*Genotype data are available for only 150 patients because of failed assays in one patient. †Genotype data are available for only 99 patients of the first study cohort.

between drug pharmacokinetics and pharmacodynamics is often complex and nonlinear, and pharmacokinetics correlations may be difficult to demonstrate clinically with analysis of only the parent compound and selected metabolites [20–22]. For doxorubicin, factors contributing to the differences in pharmacokinetics end-points and the clinical pharmacodynamics outcomes may be attributed to tumour heterogeneity, pre-existing physiological conditions, age, co-morbid disease states and co-medications [23]. This phenomenon of inconsistency observed in our study between the biological effects and pharmacological parameters is likely to be multifactorial and needs to be explored further.

Other SNPs in the genes for the CBR3 drug metabolizing enzyme as well as SLC22A16 influx and ABCB1 efflux transporters may potentially affect doxorubicin pharmacokinetics and pharmacodynamics [5]. Thus, we took further steps to examine their effects in the context of *AKR1C3* genotype variants. As previously reported, both *CBR3* G11A and G730A variants influenced doxorubicinol AUC, with the former variant also correlating with overall survival. The well-studied *ABCB1* G2677T/A correlated with both doxorubicin clearance and platelet counts but not survival in our study, while an *ABCB1* intronic variant previously reported to be associated with cardiotoxicity was found to influence overall survival and white cell toxicity, although not in a concordant way, because survival was longer and white cell toxicity lower for patients with the G allele. Two of three *SLC22A16* variants correlated with only platelet toxicities, with no other pharmacokinetics and pharmacodynamics effects observed. Interestingly, *ABCB1* IVS26+59 T>G and *CBR3* G11A variants correlated only with OS but not with PFS, contrary to what was observed with *AKR1C3* IVS4–212 GG genotype, which prolonged both PFS and OS on both univariate and multivariate analysis that included traditional prognostic clinical parameters, such as metastatic status and histological grade of tumour. The discrepancy between the effects on OS and PFS for *ABCB1* IVS26+59 T>G and *CBR3* G11A variants suggested that the observed effects on OS were unlikely to be contributed solely by their influence on the doxorubicin disposition pathway but may be related to potential effects on subsequent lines of treatment or even tumour biology.

Another interesting finding from our study is the higher frequency of *AKR1C3* IVS4–212 C>G GG homozygotes in our study population compared with those who carried at least one C allele (76 *vs.* 24%), which is in contrast with Caucasian populations, which have higher frequency of individuals carrying at least one C allele, ranging from 55.5 to 85% [National Center for Biotechnology Information, Reference SNP (ref. SNP) cluster report: rs1937840]. These interpopulation differences in genotype frequencies of SNPs may exert effects on drug outcomes in different populations. For doxorubicin pharmacodynamics, there are data suggesting interethnic differences, with Asians being more susceptible to doxorubicin-induced myelosuppression compared with Caucasians [24, 25]. These findings are consistent with the significantly lower day 15 absolute leucocyte and neutrophil counts in patients who were IVS4–212 GG homozygotes, which is commoner in our Asian study population.

The strength of our study was its prospective nature, with systematic and prospective collection of efficacy and toxicity data. In addition, pharmacokinetics data were available for a significant proportion of the study

cohort, allowing pharmacogenetics correlation for both doxorubicin pharmacokinetics and pharmacodynamics. However, as all the patients in the study cohort received docetaxel in addition to doxorubicin in the preoperative or postoperative setting, and many went on to receive other forms of anticancer therapy, such as surgery, radiotherapy and endocrine therapy, while doxorubicin contributed to the survival data, they could not be attributed solely to it.

Conclusions

We found a positive correlation between an *AKR1C3* intronic variant with doxorubicin-induced haematological toxicity and survival, suggesting potential interaction with doxorubicin metabolism. These findings underpin the importance of pharmacogenetic factors on doxorubicin metabolism in explaining the interindividual variation of its efficacy and toxicity across various ethnic populations worldwide. Such findings could potentially translate into a better personalized treatment for different individuals in the future.

Competing Interests

All authors have completed the Unified Competing Interest form and declared that there was no support from any organization for the submitted work, no financial relationships with any organization that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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