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Prognostic value of the multidrug resistance transporter ABCG2 gene polymorphisms in Chinese patients with *de novo* acute leukaemia

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ABSTRACT

Background: Functional polymorphisms of the ABCG2 gene may contribute to individual variability in drug response and the prognosis of patients.
Methods: In the present study, the genetic polymorphisms and expression of ABCG2 were analysed in blasts cells obtained from 184 Chinese patients with *de novo* acute leukaemia to investigate their possible association with clinical outcomes.
Results: A novel synonymous ABCG2-single nucleotide polymorphism (SNP) at exon 16 (13561218 C/T) and five known SNPs at exon 2 (13608835 G/A), exon 5 (13600044 C/A), intron 10 (13576005 C/T), intron 13 (13564503 C/T) and intron 14 (13563578 A/G) were identified with occurrence rates of 1.1%, 64.1%, 30.4%, 21.2%, 39.7% and 28.8%, respectively. We found that patients with the ABCG2 34GG genotype displayed longer disease free survival (DFS) ($P < 0.001$) and overall survival (OS) ($P < 0.001$) than those with the 34GA/AA genotypes. Furthermore, the DFS and OS were significantly diminished in bone marrow transplantation (BMT) patients with the 34GA/AA genotypes relative to those with the 34GG genotype.
Conclusions: These results suggest that these highly prevalent ABCG2 34GA/AA genotypes are associated with poor prognosis of Chinese patients with acute leukaemia and BMT patients.

1. Introduction

Standard induction therapy is commonly used in conjunction with autologous or allogeneic stem cell transplantation to produce complete remission and to prolong survival in patients with acute leukaemia.[1] Although there has been significant advancement in the treatment options for acute leukaemia over the past decades, the overall prognosis of acute myeloid leukaemia (AML) remains poor.[2] For patients with acute lymphoblastic leukaemia (ALL), despite high remission rate after the initial treatment, relapses are very common and only 20–40% of patients achieve long-term survival.[3] The main obstacle to successful treatment of acute leukaemia is widely believed to be multidrug resistance (MDR), which is often associated with the increased efflux of a variety of structurally unrelated anticancer drugs by ATP-binding cassette (ABC) transporters including ABCG2.

ABCG2, also known as breast cancer resistance protein (BCRP), is the second member of the ABC transporter G subfamily.[4] It is widely expressed in many normal tissues, includ-

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ing intestine, placenta, mammary gland, brain and liver, and has been shown to play an important role in the absorption and disposition of its substrate drugs and metabolites. Moreover, high expression of ABCG2 in the maternal-foetal barrier of the placenta and the blood–brain barrier protects these crucial tissue compartments from xenobiotics.[5–7] Furthermore, ABCG2 is widely believed to be a survival factor for cancer stem cells, ultimately driving tumour growth.[8] Overexpression of ABCG2 in cancer cell lines *in vitro* has been shown to confer MDR to a variety of anticancer drugs including mitoxantrone, irinotecan, methotrexate, flavopiridol and anthracyclines.[9] Growing evidence suggests that ABCG2 also underlies the MDR of clinical samples from different cancers.[10]

Cancer stem-like cells have been identified in AML, CML and some forms of ALL.[11–13] Like normal stem cells, these leukaemic stem-like cells (LSCs) are able to self-renew, differentiate and proliferate extensively. Myeloid leukaemias originated from rare stem-like cells have been found to be tumourigenic in immunodeficient mice.[14] This suggests that LSCs surviving conventional or targeted chemotherapy may be responsible for relapse in leukaemia patients. Thus eradication of these LSCs may hold promise for the treatment of leukaemia. To this end, ABCG2 expression is conserved in stem-like cells from different sources[15,16] and it has also been suggested to be a biomarker for LSCs.[17,18] The side population (SP) phenotype, mainly mediated by ABCG2, may represent the LSC population resistant to conventional chemotherapeutic drugs, thus giving rise to tumour recurrence.[19] In fact, several studies have observed that increased ABCG2 expression is an adverse prognostic factor for either achievement of complete remission or disease-free survival and overall survival in acute leukaemia patients.[3,20–22]

Single nucleotide polymorphisms (SNPs) of ABCG2 have been extensively studied because of their relevance to patients' response to medication and/or the risk of disease.[23] It has been reported that genetic variations of ABCG2 can influence its drug efflux function and substrate specificity. In particular, two SNPs of ABCG2, G34A (V12M) in exon 2 and C421A (Q141K) in exon 5, were found to be the most prevalent in Asian population. The C421A polymorphism was associated with decreased protein expression and transport activity and altered pharmacokinetic parameters of some ABCG2 substrates *in vitro* and *in vivo*.[24–28] Polarised LLC-PK1 cells transfected with the ABCG2 34AA variant displayed a dramatic increase in cytotoxic effect of ABCG2 substrate anticancer drugs such as mitoxantrone, doxorubicin, vincristine and topoisomerase I inhibitors compared with the wild-type ABCG2 (34GG) transfected cells.[29] Interestingly, two other ABCG2 variants R482G and R482T identified in drug-resistant human cancer cell lines, which demonstrated altered substrate specificity, have not been detected in human individuals.[30,31]

In the present study, we sought to identify the positions and frequencies of ABCG2 SNPs and assess their possible prognostic impact on Chinese patients with acute leukaemia. ABCG2 expression was also measured by quantitative RT-PCR to investigate its correlation with the treatment outcomes of acute leukaemia patients.

2. Materials and methods

2.1. Patients and treatments

A total of 184 *de novo* acute leukaemia patients during the period of 2002 to 2008 at the First Affiliated Hospital and the Tissue Typing Center of Sun Yat-Sen University were recruited in this study, including 141 acute myeloid leukaemia (AML) and 43 acute lymphoblastic leukamia (ALL). The patients were diagnosed based on French-American-British (FAB) criteria.[32] Informed consent was obtained from all patients for the research use of their specimens. The research was approved by the Regional Ethical Committee of Sun Yat-Sen University. The major characteristics of the patients are summarised in Table 1. None of them received prior chemotherapy with anticancer drugs before this study. After enrolment into this study, most of the patients were treated with uniform chemotherapy: daunorubicin or mitoxantrone in combination with Ara-C (in some cases, additional cytostatic drugs were used) for AML and VDCP (vindesine, daunorubicin, cyclophosphamide, prednisolone) for ALL. The patients achieving remission received consolidation chemotherapy and eligible patients achieving complete remission (CR) were subsequently referred for autologous or allogeneic stem-cell transplantation. Six patients with ALL underwent bone marrow transplantation (BMT) (1 autologous and 5 allogeneic), and 34 patients with AML received BMT (6 autologous and 28 allogeneic).

2.2. Cell Lines and cell culture

The mitoxantrone-resistant ABCG2-overexpressing colon cancer cell line S1-M1-80 was used as positive control for ABCG2 mRNA expression.[33] S1-M1-80 cells were maintained in DMEM medium supplemented with 10% heat-inactivated

Table 1 – Characteristics of the patients enroled in the study.

Characteristics	Data (n = 184)
Sex, No. (%)	
Male	87 (47.3)
Female	97 (52.7)
FAB classification, No. (%)	
ALL	43 (23.4)
AML	141 (76.6)
M0	16 (8.7)
M1	28 (15.2)
M2	19 (10.3)
M4	17 (9.2)
M5	50 (27.2)
M6	5 (2.7)
M7	6 (3.3)
Bone marrow transplantation, No. (%)	
Yes	40 (21.7)
No	144 (78.3)
Median age, y (range)	32 (5–70)
Median WBC,$\times 10^9$/L (range)	23.9 (0.9–249.4)
Median platelets,$\times 10^9$/L (range)	52.0 (2.0–578.0)
Median haemoglobin, g/L (range)	73.5 (40.0–140.0)

foetal calf serum (Hyclone Co., Omaha, NE), and incubated at 37 °C in an atmosphere of 5% CO_2.

2.3. DNA extraction and polymerase chain reaction

Genomic DNA was extracted from the peripheral blood using the QIAGEN (Hilden, Germany) DNA extraction kit and following the manufacturer's instructions. Concentrations of the genomic DNA were determined by an ultraviolet spectrophotometer (Beckman, DU800 type). The primers were designed to bind to sequences upstream and downstream of the different exons to cover each exon, as well as sequences at the exon–intron boundaries that are important for mRNA splicing. The sequences of primers and PCR amplification profiles are listed in Table 2. PCR amplification was performed as follows: 25 ng genomic DNAs were amplified in 25 μl reaction volume containing 2 mmol/L $MgCl_2$, 0.1 mmol/L dNTPs, 0.1 μmol/L of each primer and 0.5 unit Taq polymerase. The quality of PCR products was checked by agarose gel electrophoresis.

2.4. Genotyping and DNA sequencing

Genotypes were analysed by polymerase chain reaction (PCR)-based single-strand conformation polymorphism (SSCP) analysis and confirmed by direct DNA sequencing. Equal volume of loading buffer (95% deionised formamide, 20 mmol/L ethylenediaminetetraacetic acid and 0.05% bromophenol blue) was mixed with 10 μl PCR product, denatured at 95 °C for 10 min and quenched on ice. The denatured mixture was then loaded on a non-denaturing 10% polyacrylamide gel. DNA was visualised using silver staining. The samples representing different patterns of electrophoretic mobility were subjected to direct DNA sequencing for genotype validation. Sequences of purified PCR fragments were obtained by automated DNA sequencing on ABI377 sequencers by using BigDye Terminator cycle sequencing reactions (Perkin–ElmeryApplied Biosystems). Each result of sequencing was analysed by chromas bioanalysis software. Polymorphisms in the ABCG2 gene were then determined by comparing with the corresponding genomic sequence deposited in GenBank with accession No. AC084732 (http://www.ncbi.nlm.nih.gov/).

2.5. Quantitative real-time PCR

Total RNA was extracted by Trizol Reagent (Gibco-BRL, Inc.,) according to the manufacturer's instructions. Primer and Taqman probe sequences were designed with the Primer Express Software 3.0. ABCG2 forward primer: 5′-CAGTACTTCAGCATTCCACGAT-3′, Reverse primer: 5′-GGCAGAAGTTTTGTCCCAAA-3'. ABCG2 probe: 5′-FAM-CATTATGCTGCAAAGCCGTAAATCCA-TAMRA-3'. Each amplification reaction (50 μl) contained 1 μl of the cDNA product, 12.5 μl TaqMan universal PCR mastermix (Applied Biosytems, Inc.), 300 nmol/L of each primer and 200 nmol/L Taqman probe. The reaction protocol involved heating for 2 min at 90 °C, followed by 40 cycles of amplification (45 s at 94 °C and 1 min at 56 °C). Each assay included negative control without template and the standard curve for ABCG2. All tests were carried out in triplicate. The relative ABCG2 expression was compared amongst the patient samples after normalisation with GAPDH.

2.6. Statistical analysis

Statistical analysis was performed with the SPSS 13.0 software (SPSS, Chicago,IL). CR was defined as ⩽5% bone marrow blasts after recovery from induction therapy. Disease-free survival (DFS) was measured from establishment of CR until relapse or death from any cause, with observation censored for patients last known alive without report of relapse. Overall survival (OS) was measured from diagnosis until death from any cause, with observation censored for patients last known alive. DFS and OS curves were estimated using the Kaplan–Meier method and compared with the log-rank test. Univariate and multivariate analyses comprising common prognostic factors for DFS and OS were performed using the Cox regression analysis. Association between polymorphisms and the CR rate was estimated by unconditional logistic

Table 2 – Primer sequences and polymerase chain reaction conditions of sixteen exons of ABCG2 gene.

Exon	Forward primer (5′–3′)	Reverse primer (5′–3′)	Length (bp)	AT (°C)	CN
1	CCTGTGGAGGAACTGGGTAG	CCAGACACACACTCTCAGCG	251	50	32
2	AATCTCATTTATCTGGACTATCAAC	TCGATAATATTTCTTTCTCAACTG	264	55	35
3	TTCATTGAGTATATTCAGTGCCTACC	AAAAGCACATTAAAAGTGCACAG	201	49	35
4	TTGGATTCAAAGTAGCCATGAG	TTGAACTATCAGCCAAAGCACT	301	46	30
5	TCATTATGTCTCATTAAAATGCTAT	GGACACAGGGAAAGTCCTAC	246	49	35
6	CAAATGATAATGACTGGTTGTTA	TTGTTTTTCTTGATAATGCTTTT	371	43	35
7	AGCAAACAATCTAAAGGCAAGAA	CCAAAGACCAAACAGCACTCCTG	338	50	30
8	AAGTGAGTTCTCTTTGTTTTCCA	GTTGACTGGTATCAGAAGACTGC	292	45	35
9	TGTTTGTGTTTCCTTTTTATCCA	CATTGTTCCCATTTGAGTATTTC	328	45	35
10	TTATCTCTAATTGAAACTCTTCC	AAATAAACTGACTCATCCTACCC	275	50	30
11	ACGCTTCCCTGTTCCAACCAGAA	TGTAATCAGTCTAACCAATAGCC	304	50	30
12	GACAAGTCTAGCCTGCCCTGTGG	GTTTGGTTTATAGTTTTGAGAAC	302	55	30
13	AATAAGCAATCCCAAACATACGG	TTATCAGAGCAAACACAGTTCAG	252	55	30
14	AGAGGAGAAGAGTTTAGTGAGTG	ACAGTGACAGACAAGGAAGACAT	283	50	32
15	ACATTAGTTGGTTTGGTGAGACA	ATTCAGTGCCCCTGGAAGGACTC	338	50	32
16	AGGCTTGGTTCAATTTTAGGC	CTTCAATCAAAGTGCTTCTTTTTT	311	45	35

AT: Anneal temperature; CN: Cycle number.

regression. The Mann–Whitney and Kruskal–Wallis tests were used to analyse the association between ABCG2 genetic polymorphisms and expression level. Statistical significance was defined as $P < 0.05$ (two-tailed Student's *t*-test).

3. Results

3.1. Incidence of polymorphisms

In this study, genetic variations in all sixteen ABCG2 exons, covering the sequences at the exon–intron boundaries that are important for mRNA splicing were examined in 184 acute leukaemia patients. Six polymorphisms including a novel synonymous polymorphism at exon 16 were observed. The sequences, positions and frequencies of the polymorphisms are summarised in Table 3. Of the six polymorphisms, the allele frequencies of two published non-synonymous SNPs, G34A (Val12Met; exon 2) and C421A (Gln141Lys; exon 5), were 0.505 and 0.152 in our study. The polymorphism at exon 16: C13561218T was found at a 0.005 allele frequency (the occurrence rate was approximately 1.1%, heterozygous), which has not been reported before. The remaining three polymorphisms found were located in the introns: rs2231149 C/T (intron 10), rs2231162 C/T (intron 13), and rs2231164 A/G (intron 14), close to exon boundaries. Their allele frequencies were 0.106, 0.198 and 0.144, respectively (Table 3). Sequencing analysis of these SNPs is shown in Fig. 1.

3.2. Association between ABCG2 polymorphisms and survival of patients with acute leukaemia

The overall CR rate of patients was 61.4% (113/184). Patients with the 34GA/AA genotypes and the wild-type carriers (34GG) had a similar response to induction therapy. None of the other ABCG2 SNPs detected in our study were significantly associated with the CR achievement (Table 4).

The association between the ABCG2 polymorphisms with patient survival and other clinical parameters was also analysed. In a separate univariate analysis, ABCG2 G34A SNP (HR = 2.48, $P < 0.01$), C13564503T SNP (HR = 1.57, $P < 0.05$) and BMT (HR = 2.00, $P < 0.01$) were shown to be statistically significant prognostic factors for DFS whereas ABCG2 G34A SNP (HR = 4.06, $P < 0.01$), C421A SNP (HR = 1.56, $P < 0.05$), C13564503T SNP (HR = 2.11, $P < 0.01$) and BMT (HR = 1.63, $P < 0.05$) were found to be significant prognostic factors for OS (Table 5). Multivariate analysis was further performed by including all the significant variables in univariate analysis. Interestingly, the presence of ABCG2 G34A SNP was found to be an independent prognostic factor for DFS (HR = 2.31, $P < 0.01$) and OS (HR = 6.05, $P < 0.01$) in the 144 patients treated with standard induction therapy (Table 6).

Kaplan–Meier results showed that patients with the ABCG2 34GA/AA genotypes had significantly shorter DFS and OS than those with the wild-type genotype (34GG) (median DFS: 8 versus 18 months, $P < 0.001$; median OS: 15 versus 30 months, $P < 0.001$) (Fig. 2a). Amongst the patients treated with chemotherapy only, patients with the 34GG genotype displayed significantly longer DFS and OS than those carrying the 34GA/AA genotypes (median DFS: 15 versus 6 months, $P < 0.001$; median OS: 28 versus15 months, $P < 0.001$) (Fig. 2b). In the subgroup of patients with the 34GG genotype, the combined effects of ABCG2 C421A and 13564503 C/T SNPs on survival were then examined. Compared with patients carrying the CC+CC genotype, those with the CA+CT genotype displayed the worst DFS ($P < 0.001$) and OS ($P < 0.001$), while the CA+CC and CC+CT combinations showed an intermediate survival (Fig. 2c).

3.3. Effect of ABCG2 G34A polymorphism on survival of acute leukaemia patients with BMT

Amongst the 40 transplant recipients, 14 were found to carry the wild-type ABCG2 genotype (34GG) whereas 6 were heterozygous (34GA) and 20 were homozygous (34AA) for the G34A SNP. According to the multivariate analysis, ABCG2 G34A polymorphism remained an independent prognostic factor for DFS (HR = 3.11, $P < 0.05$) and OS (HR = 4.00, $P < 0.05$) of patients undergoing BMT (Table 6). Kaplan–Meier analysis showed that the DFS and OS were significantly diminished in BMT patients with the 34GA/AA genotypes relative to those with the 34GG genotype (median DFS: 9 versus 36 months, $P < 0.05$; median OS: 15 versus 41 months, $P < 0.01$) (Fig. 3).

3.4. Impact of ABCG2 expression on survival

Amongst the 184 patient blast cell samples, ABCG2 mRNA expression varied from 0.944 to 1.26×10^{-11} copies. The ABCG2 expression level of all patient samples is shown in Fig. 4a,

Table 3 – Positions, sequences and frequencies of ABCG2 SNPs in 184 patients.

Position		Location	Change		% Genotype frequency(n)			Allele	% Allele frequency
NT-016354.18	dbSNP (NCBI)		Nucleotide	Amino acid	Wildtype	Heterozygote	Homozygote		
13608835	rs2231137	Exon 2	TCCCAG/ATGTCA	Val12Met	35.9 (66)	27.2 (50)	36.9 (68)	A	50.5
13600044	rs2231142	Exon 5	ACTTAC/AAGTTC	Gln 141Lys	69.6 (128)	30.4 (56)	0	A	15.2
13561218[a]	N.D.	Exon 16	GGCATC/TGATCT	Ile619Ile	98.9 (182)	1.1 (2)	0	T	0.5
13576005	rs2231149	Intron10	TCAAGC/TTTATT	N/A	78.8 (145)	21.2 (39)	0	T	10.6
13564503	rs2231162	Intron13	TGACTC/TTTAGT	N/A	60.3 (111)	39.7 (73)	0	T	19.8
13563578	rs2231164	Intron14	TTCTTA/GAAATT	N/A	71.2 (131)	28.8 (53)	0	G	14.4

NCBI, National Cancer Center for Biotechnology Information; N.D., not determined; N/A, not applicable.
[a] Novel variation detected in this study.

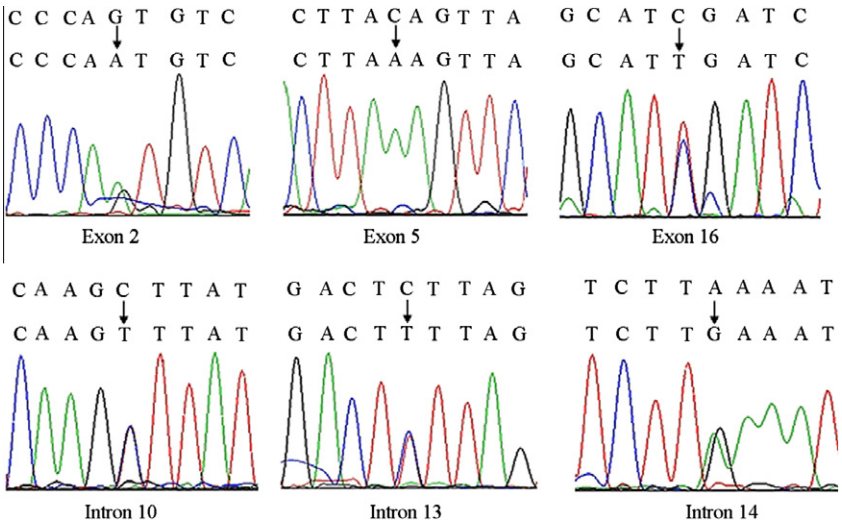


Fig. 1 – Sequencing analysis of ABCG2 polymorphisms identified in the study. Sites of nucleotide change were indicated by arrows and at these sites overlapping peaks were noted in the direct DNA sequencing results.

Table 4 – Logistic regression analysis of ABCG2 SNPs for CR achievement in 184 patients.

SNPs	CR rate (%)	OR	95%CI	*P*
13608835 G/A (Exon 2)	58.5/66.7	0.467	0.316–1.009	0.053
13600044 C/A (Exon 5)	71.4/57.0	2.029	0.968–4.253	0.061
13576005 C/T (Intron 10)	51.3/64.1	0.747	0.339–1.646	0.469
13564503 C/T (Intron 13)	58.9/63.1	0.599	0.294–1.220	0.158
13563578 A/G (Intron 14)	66.0/59.5	1.662	0.808–3.419	0.167

CR, complete remission; OR, odds ratio for achieving a complete remission; CI, confidence interval.

Table 5 – Univariate analyses of factors with potential prognostic impact on survival in 184 acute leukaemia patients.

Variables	DFS		OS	
	HR (95%CI)	*P*	HR (95%CI)	*P*
Age, years (⩽32 versus >32)	1.01 (0.99–1.02)	0.253	0.85 (0.60–1.20)	0.355
Sex (female versus male)	1.13 (0.78–1.64)	0.531	1.06 (0.72–1.55)	0.770
SNPs				
13608835 G/A (Exon 2)	2.48 (1.68–3.64)	<0.01	4.06 (2.69–6.15)	<0.01
13600044 C/A (Exon 5)	1.11 (0.77–1.61)	0.574	1.56 (1.06–2.30)	<0.05
13576005 C/T (Intron 10)	1.40 (0.95–2.07)	0.087	1.30 (0.87–1.93)	0.204
13564503 C/T (Intron 13)	1.57 (1.10–2.22)	<0.05	2.11 (1.46–3.04)	<0.01
13563578A/G (Intron 14)	1.27 (0.89–1.83)	0.191	1.44 (0.99–2.07)	0.052
BMT (without versus with)	2.00 (1.31–3.06)	<0.01	1.63 (1.06–2.49)	<0.05

OS, overall survival; DFS, disease-free survival; CI, confidence interval; BMT, bone marrow transplantation.

relative to that in ABCG2-overexpressing drug-resistant S1-M1–80 cells. None of the patient samples showed higher expression than the positive control S1-M1–80 cells (2.44 copies). Given the significant impact of ABCG2 polymorphisms on survival, the association between polymorphisms and the ABCG2 expression level was also analysed. However, our data did not show any correlation between the G34A and C421A polymorphisms and the expression of ABCG2 (Fig. 4b).

As shown in Fig. 4a, the ABCG2 expression of patient samples was automatically divided into two separate cluster groups. To investigate the association between the ABCG2 expression level and survival, patients were divided into two groups with the middle expression of patient samples (1.66×10^{-6} copies, indicated with arrow) as cut-off for high and low level. In the 144 patients without BMT, the median DFS was 9 months (95% CI, 2.36–15.64) in the low-expression group and 10 months (95% CI, 4.34–15.66) in the high-expression group with no significant association between ABCG2 mRNA expression level and DFS ($P = 0.125$). The median OS was 17 months (95% CI, 10.78–23.21) and 19 months (95% CI, 16.69–21.30) in the low and high-expression group respectively. No significant association was found between ABCG2

Table 6 – Multivariate analyses of ABCG2 SNPs associated with survival.

Patients	SNPs	DFS		OS	
		HR (95%CI)	*P*	HR (95%CI)	*P*
Without BMT					
(*n* = 144)	13608835 G/A (Exon 2)	2.31 (1.52–3.52)	<0.01	6.05 (3.58–10.21)	<0.01
	13600044 C/A (Exon 5)	0.82 (0.54–1.24)	0.340	1.00 (0.64–1.58)	0.988
	13564503 C/T (Intron 13)	1.48 (0.97–2.27)	0.070	1.33 (0.87–2.04)	0.191
With BMT					
(*n* = 40)	13608835 G/A (Exon 2)	3.11 (1.13–8.60)	<0.05	4.00 (1.30–12.34)	<0.05
	13600044 C/A (Exon 5)	1.08 (0.33–3.52)	0.901	1.41 (0.46–4.37)	0.548
	13564503 C/T (Intron 13)	0.82 (0.23–2.91)	0.757	0.75 (0.23–2.45)	0.636

OS, overall survival; DFS, disease-free survival; CI, confidence interval; BMT, bone marrow transplantation.

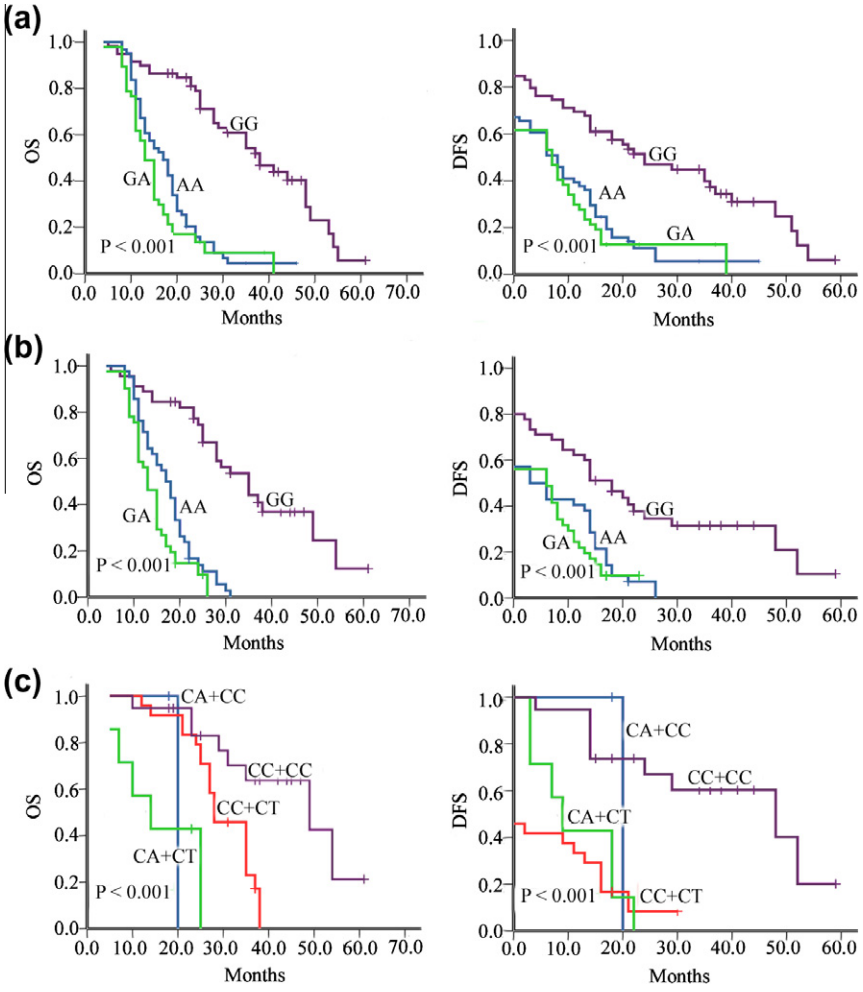


Fig. 2 – Kaplan–Meier analyses of OS and DFS for acute leukaemia patients according to ABCG2 polymorphisms. (a) OS and DFS were significantly longer in patients with the 34GG genotype than those with the 34GA/34AA genotypes in all 184 patients. (b) OS and DFS were significantly longer in patients with the 34GG genotype than those with the 34GA/34AA genotypes in patients without BMT. (c) In the subgroup of patients with the 34GG genotype, compared with those carrying the CC+CC genotype, patients with the CA+CT genotype displayed the worst survival, while the CA+CC and CC+CT combinations showed the intermediate survival.

mRNA expression level and OS (*P* = 0.502) (Fig. 4c). Moreover, no significant association was detected between the ABCG2 expression level and the CR achievement of patients (Fig. 4d).

4. Discussion

Functional polymorphisms of ABC transporters have been shown to alter their protein expression, transport activities

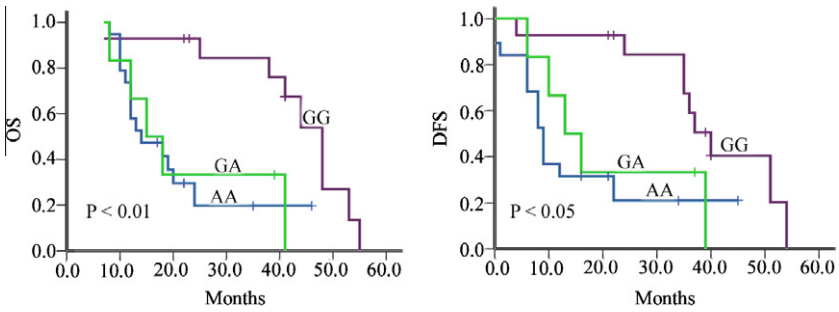


Fig. 3 – Kaplan–Meier analyses of OS and DFS for patients undergoing BMT. OS and DFS were significantly longer in patients with the 34GG genotype than those with the 34GA/34AA genotypes in patients with BMT.

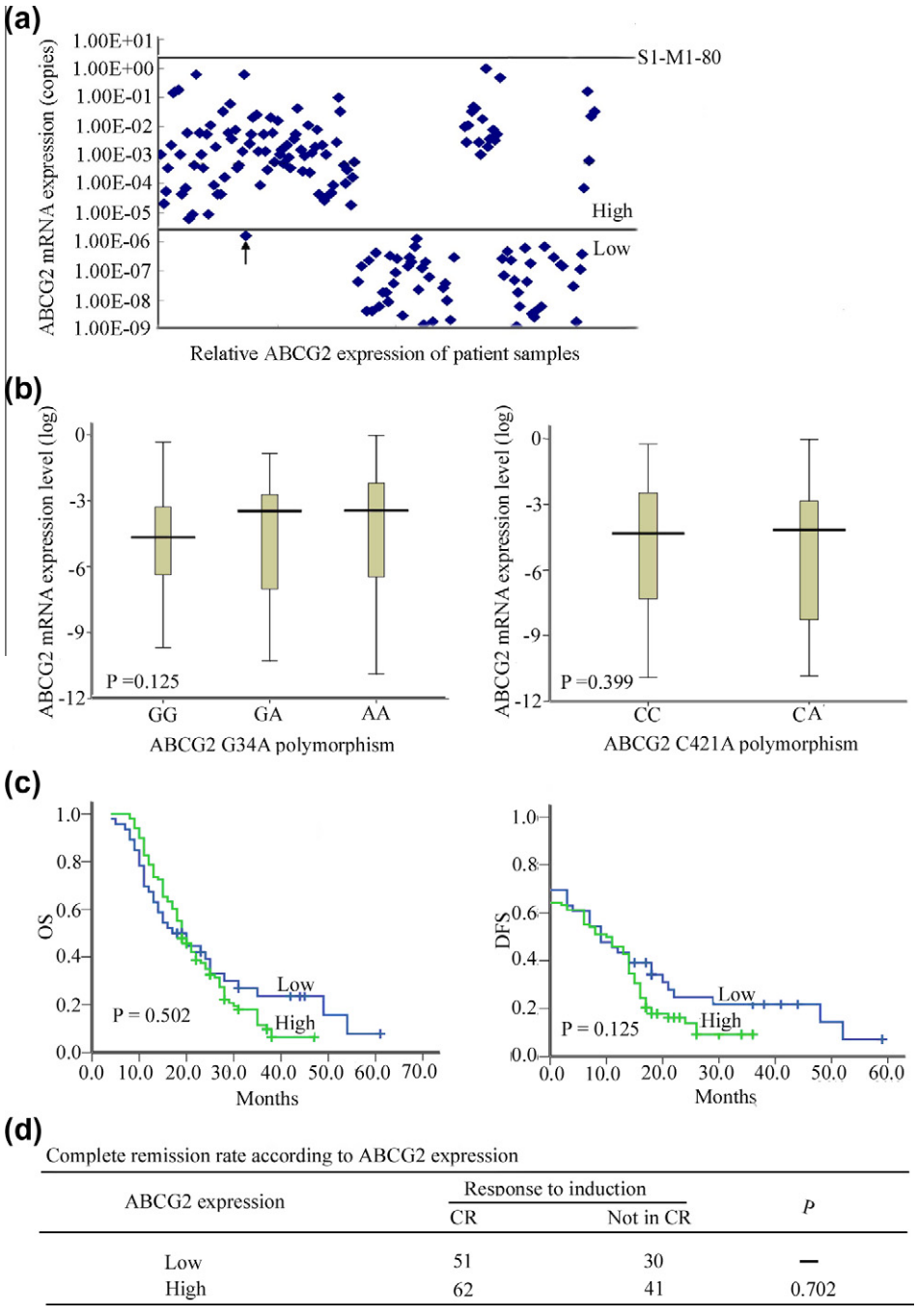
and substrate specificity.[34,35] To date, more than 40 naturally occurring ABCG2 SNPs, within the promoter, exon and intron sequences, have been reported in various ethnic populations.[36–38] In the present study, genetic polymorphisms of ABCG2 in 184 Chinese patients with acute leukaemia were analysed. As shown in Table 3, six polymorphisms including a novel synonymous SNP were detected. The G34A (V12 M) polymorphism was found to occur at the highest frequency in the acute leukaemia patients (allele frequency 50.5%). The polymorphism at exon 16: 13561218 C/T was detected in two patients, resulting in an occurrence rate of 1.1% (2/184). Since it could not be found in the ABCG2-SNP database, this mutation is an unreported new SNP of ABCG2.

Naturally occurring mutations that alter ABCG2 expression and/or transport activity could affect the pharmacokinetic properties of its substrate drugs. Given the relatively high frequency of the ABCG2 G34A polymorphism identified in study, we evaluated its prognostic value in acute leukaemia patients. Interestingly, we found that the ABCG2 G34A polymorphism was associated with significantly decreased survival in the present study population including BMT recipients. Patients carrying the 34GA/34AA genotypes displayed worse overall survival ($P < 0.001$) and shorter disease free survival ($P < 0.001$) than those with the wild-type 34GG genotype. Subgroup analysis showed that in patients with the ABCG2 34GG genotype, ABCG2 C421A and 13564503 C/T polymorphisms also showed adverse effects on survival. Patients carrying the CA+CT genotype displayed the worst prognosis ($P < 0.001$).

In acute leukaemia patients, the occurrence of treatment-related toxicity is an important limitation for the success of treatment and is the primary cause of interruption or discontinuation of chemotherapy, leading to increased relapse risk.[39–41] The existence of functional polymorphism in ABCG2 is of tremendous clinical importance due to the involvement of this widely expressed transporter in protection against environmental toxins, pharmacokinetics of commonly used drugs and multidrug resistance. ABCG2 protects hematopoietic stem cells under hypoxic conditions by preventing the accumulation of haem that causes mitochondrial death.[42] Both of the G34A and C421A polymorphisms have been associated with lower expression and activity of ABCG2. It has been reported that diffuse large B-cell lymphoma (DLBCL) patients with the ABCG2 34AA genotype displayed worse survival compared with those carrying the 34G allele. Considering the extremely low IC_{50} values for the LLC-PK1 cells with 34AA variant, the author proposed that anticancer compounds might confer much higher toxic side-effect to those with 34A allele and thus result in the intolerance to the treatment and worse prognosis.[43] On the other hand, the ABCG2 C421A polymorphism has been reported to cause increased plasma concentrations of rosuvastatin and diflomotecan and is associated with diarrhoea, an adverse event related to oral gefitinib administration.[44,45] Polymorphisms in ABCG2 are also associated with irinotecan-induced severe myelosuppression.[46] Therefore, although it has not been demonstrated *in vitro*, we hypothesise that decreased level/activity of ABCG2 in carriers with the 34GA/34AA genotypes may lead to decreased clearance and, therefore, higher plasma concentration of substrate drugs, leading to increased drug exposure to the various organs. However, we did not find an evident association between ABCG2 polymorphisms and expression level in leukaemia patient samples. This may due to the relative low expression level in most of the patient samples.

Immunosuppressive drugs such as cyclosporine A and methotrexate are to prevent graft versus host disease.[47] Organ toxicities after bone marrow transplantation are common and potentially life threatening. Patients with BMT carrying the ABCG2 G34A polymorphism may be more prone to toxicity from anticancer drugs because of the use of immunosuppressants. Although our data did not show any correlation between the G34A polymorphism and the treatment efficacy of the induction chemotherapy (i.e. in terms of complete remission (CR)), the shorter duration of patient survival associated with the ABCG2 G34A genotype may suggest that the G34A genotype is related to lower ABCG2 level/transport activity in the vital organs and, therefore, less protection.

Several studies have demonstrated the significant inter-individual variation of ABCG2 expression in different patients.[10,38] It has been reported that increased expression of ABCG2 is associated with poor treatment outcome in AML patients with either decreased rates of remission or decreased disease-free survival and overall survival.[48] In the present study, ABCG2 mRNA expression varied from 0.944 to 1.26×10^{-11} copies in patient samples and high ABCG2 expression levels were relatively uncommon. Van den Heuvel-Eibrink et al. have shown that ABCG2 mRNA expression was significantly higher in refractory/relapsed AML than in *de novo* AML patients, suggesting that ABCG2 is associated with clinically resistant disease in AML.[49] Since patients recruited into



(d)

Complete remission rate according to ABCG2 expression

ABCG2 expression	Response to induction		*P*
	CR	Not in CR	
Low	51	30	—
High	62	41	0.702

Fig. 4 – ABCG2 mRNA expression levels in patient blast cells and their association with survival. (a) The ABCG2 mRNA expression levels of 184 acute leukaemia patients were measured by real-time RT-PCR and normalised for GAPDH expression. Data are expressed relative to the ABCG2 mRNA expression level of S1-M1-80 cells. (b) No correlation was found between the G34A and C421A polymorphisms and the expression of ABCG2. (c) The middle expression level (indicated with arrow) was used as cut off for the high and low levels. No significant association was found between ABCG2 mRNA expression and survival. (d) No significant association was detected between the ABCG2 expression level and the CR achievement of patients.

our study were mostly newly diagnosed with *de novo* AML without prior chemotherapy, our data suggest that ABCG2 levels in their blast cells remain relatively low and are not high enough to lead to drug resistance. It implies that repeated administration of anticancer drugs may induce ABCG2 expression and lead to drug resistance in the refractory/relapsed cases.

Synonymous SNPs do not produce altered coding sequences or amino acid substitution, so they are not expected to change the function of the protein in which they occur.

However, Kimchi-Sarvaty et al. have demonstrated that a synonymous SNP in the *MDR*1 gene unexpectedly results in changed substrate selectivity.[50] Thus, the function of synonymous SNPs deserves further investigation. It is also possible that the new polymorphism at exon 16 of ABCG2 detected in our study may have functional and phenotypic effects. Further studies are necessary to give a conclusive answer to the significance of such an observation.

In conclusion, six polymorphisms were identified in ABCG2, including one novel synonymous polymorphism at exon 16 with a low allele frequency (0.5%). Our data also showed that ABCG2 G34A polymorphism could be a potential prognostic factor for acute leukaemia patients. This ABCG2 G34A genotype may be an important factor contributing to the interindividual differences in patient responses to ABCG2 substrate drugs. Its screening may be useful for predicting the safety, toxicity and efficacy of therapies to avoid adverse reactions and therapeutic failure.

Conflict of interest statement

None declared.

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