Cell Biology International

Cell Biol. Int. (2012) 36, 79–86 (Printed in Great Britain)

online data

Research Article

ABCC1 polymorphisms in anthracycline-induced cardiotoxicity in childhood acute lymphoblastic leukaemia

Agnes F. Semsei*, Daniel J. Erdelyi*,†, Ildiko Ungvari*, Edit Csagoly*, Marta Z. Hegyi†, Petra S. Kiszel*, Orsolya Lautner-Csorba*, Judit Szabolcs†, Peter Masat‡, Gyorgy Fekete†, Andras Falus*, Csaba Szalai[1§,¶,‖] and Gabor T. Kovacs†

* Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary
† Second Department of Pediatrics, Semmelweis University, Budapest, Hungary
‡ Markusovszky Hospital of Vas County Council, Szombathely, Hungary
§ Heim Pal Children Hospital, Budapest, Hungary
¶ Inflammation Biology and Immunogenomics Research Group, Hungarian Academy of Sciences, Semmelweis University, Budapest, Hungary
‖ Csertex Research Laboratory, Budapest, Hungary

Abstract

Anthracyclines are potent cytostatic drugs, the correct dosage being critical to avoid possible cardiac side effects. ABCC1 [ATP-binding cassette, sub-family C, member 1; also denoted as MRP1 (multidrug resistance-associated protein 1)] is expressed in the heart and takes part in the detoxification and protection of cells from the toxic effects of xenobiotics, including anthracyclines. Our objective was to search for associations between LV (left ventricular) function and single-nucleotide polymorphisms of the *ABCC1* gene in children receiving anthracycline chemotherapy. Data of 235 paediatric patients with acute lymphoblastic leukaemia was analysed. Patients were followed-up by echocardiography (median follow-up 6.3 years). Nine polymorphisms in the *ABCC1* gene were genotyped. The *ABCC1* rs3743527TT genotype and rs3743527TT–rs246221TC/TT genotype combination were associated with lower LVFS (left ventricular fractional shortening) after chemotherapy. The results suggest that genetic variants in the *ABCC1* gene influence anthracycline-induced LV dysfunction.

Keywords: ABCC1; cardiotoxicity; fractional shortening; leukaemia; pharmacogenetics; side effects

1. Introduction

Childhood ALL (acute lymphoblastic leukaemia) is cured in ~80–90% of the patients, but a significant number of survivors suffer from chemotherapy-induced side effects (Mody et al., 2008). These can appear during treatment or become clinically evident years after therapy. Follow-up studies indicate that survivors of childhood leukaemia often have cardiac problems as late side effects of chemotherapy (Shankar et al., 2008; Mulrooney et al., 2009). These can be due to the most cardiotoxic anthracyclines, but other drugs used in chemotherapy regimens can also damage the heart (Viale and Yamamoto, 2008).

Anthracyclines, such as doxorubicin and daunorubicin, are highly effective anti-neoplastic drugs used in a wide range of cancers (Johnson and Richardson, 1998). Anthracycline-induced cardiotoxicity can be divided into 3 types. Acute anthracycline-induced cardiotoxicity occurs during treatment, often immediately after the administration of the first dose. In contrast, early onset chronic progressive anthracycline-induced cardiotoxicity presents within 1 year after anthracycline administration, and late-onset chronic progressive cardiotoxicity develops years or even decades later (Lipshultz et al., 2008). Anthracycline-induced cardiotoxicity can be a mild, transient condition, characterized by asymptomatic electrocardiographic changes, e.g., arrhythmias. More severe toxicities include LV (left ventricular) dysfunction, decreased exercise capacity, decreased LVFS (left ventricular fractional shortening), late onset cardiomyopathy and congestive heart failure. Heart dysfunction is associated with cardiomyocyte loss, LV wall thinning and LV dilation (Wojtacki et al., 2000; Adams and Lipshultz, 2005; Iarussi et al., 2005).

Late-onset anthracycline-induced cardiotoxicity is by far the most frequent cardiac consequence of the therapy. In a study of 115 survivors of childhood ALL, several patients showed abnormal LV structure or function years after anthracycline treatment, while early-onset anthracycline-induced cardiotoxicity was usually experienced only in a few patients (Lipshultz et al., 2005). Subclinical reduction of heart function during or right after therapy might indicate increased susceptibility to late-onset cardiomyopathy (Kremer et al., 2002; Scully and Lipshultz, 2007).

Several risk factors for cardiotoxicity have been identified, including age at treatment (<4 years), concomitant therapy (irradiation and other anti-neoplastic drugs), gender (female) and cumulative dose of drug. Nevertheless, the fact that there is no safe dose of anthracyclines and the wide inter-patient variability in the time and seriousness of this adverse effect suggest that genetic background is a major determinant of drug response and toxicity (Lipshultz et al., 2008).

ABC (ATP-binding cassette) transporters have an important role in the protection of the body against xenobiotics (Borst et al., 2000; Sparreboom et al., 2003). These membrane-localized efflux pumps export a wide range of chemotherapeutic agents using

[1] To whom correspondence should be addressed (email genomika.cs@gmail.com).

Abbreviations: ABC, ATP-binding cassette; ABCC1, ABC, sub-family C, member 1; ALL, acute lymphoblastic leukaemia; BFM, Berlin–Frankfurt–Münster; ECHO, echocardiography; HWE, Hardy–Weinberg equilibrium; LV, left ventricular; LVEDD, left ventricular end-diastolic-dimension; LVESD, left ventricular end-systolic dimension; LVFS, left ventricular fractional shortening; MRP1, multidrug resistance-associated protein 1; NHL, non-Hodgkin lymphoma; SNP, single-nucleotide polymorphism; 3′-UTR, 3′-untranslated region.

the energy of ATP hydrolysis. ABC transporters are expressed in the heart, some of which have anthracyclines among their substrates (Couture et al., 2007). The ABCC1 [ATP-binding cassette, sub-family C, member 1; also denoted as MRP1 (multi-drug resistance-associated protein 1)] transporter was first described in a doxorubicin-resistant cell line and is expressed ubiquitously in the body. Tissues showing high levels of *ABCC1* expression include the heart, lung, testis, kidney and placenta. ABCC1 participates in detoxification, protects the cells from toxic effects of xenobiotics and is also involved in the defence mechanisms against oxidative stress (Bakos and Homolya, 2007). ABCC1 expression increases after doxorubicin treatment in cardiac tissue of mice (Jungsuwadee et al., 2009), and experiments with Abcc1-knockout mice have shown an important role of this protein in the efflux of drugs from the heart. Finally, several studies indicated that ABCC1 gene polymorphisms can influence the function of the transporter (Kerb et al., 2001).

Our objective has been to examine potential associations between subclinical reduction of cardiac function and the genetic background of childhood ALL patients, focusing the investigation of the polymorphisms of *ABCC1,* covering all haplotype blocks in this gene in order to gain improve our understanding on the genetic background of the anthracycline cardiotoxicity.

2. Patients and methods

2.1. Study population and definitions

DNA were collected retrospectively from children with ALL who had undergone chemotherapy between 1990 and 2002, aged 1–18 years at diagnosis, treated according to the ALL BFM (Berlin–Frankfurt–Münster) 90 or 95 study protocols in 6 Hungarian paediatric oncology centres. A total of 235 children were included in the analysis, with their characteristics being shown in Table 1. According to the data of the Hungarian Paediatric Cancer Registry, 337 cases were diagnosed with ALL in these hospitals during the same period who had survived at least until the end of chemotherapy (Table 1). The selected cohort represents 70% of all the cases. There was no difference in the distribution of the characteristics of the patients between the 2 groups. Informed consent was requested from the parents. The study was conducted according to the principles expressed in the Declaration of Helsinki (2000) and approved by the Ethics Committee of the Hungarian Medical Research Council.

The patients were followed-up by ECHO (echocardiography) to assess LV function by measuring LVEDD (left ventricular end-diastolic-dimension) and LVESD (left ventricular end-systolic dimension). LVFS was calculated from these 2 data: LVFS %=(LVEDD−LVESD)/LVEDD × 100%. We analysed the LVFS at 3 time-points, first, at the time of diagnosis, secondly at the end of the treatment, that is, at a median of 2 years after diagnosis, while the third data-point was determined at the time of the latest follow-up. ECHO was performed with varying frequency at the different centres, so LVFS data were not present at all time points for all the children. In the case of 7 patients with relapsed leukaemia, we included 2 separate 'latest' datasets. These were the last ECHO results before starting relapse chemotherapy and the latest after relapse, featured by different cumulative anthracycline doses respectively.

The chemotherapy regimen included repeated doses of intravenous vincristine, L-asparaginase, daunorubicin, doxorubicin, methotrexate, cyclophosphamide, cytosine arabinoside, oral prednisone, dexamethasone, mercaptopurine, thioguanine, intrathecal methotrexate, intrathecal prednisone and ifosfamide

Table 1 **Characteristics of the study population and all cases diagnosed with ALL between 1990 and 2002 in Hungary in the hospitals included in the study from the Hungarian Paediatric Cancer Registry**

Characteristics of patients	Study population	Whole population	*P*-value
Number of patients	235	337	
Gender *n* (%)			
Male	126 (54)	190 (56)	
Female	109 (46)	147 (44)	0.6
Age at diagnosis [means ± S.D., years (range)]	5.7 ± 3.8 (1.2–18.0)	6.2 ± 4.1 (1.0–18.0)	0.1
Risk group *n* (%)			
LR	61 (26)	69 (20)	
MR	155 (66)	231 (69)	
HR	19 (8)	37 (11)	0.2
Chemotherapy protocol *n* (%)			
ALL BFM 90 (before 1995)	83 (35)	146 (44)	
ALL BFM 95 (after 1995)	152 (65)	189 (56)	0.06
Dexrazoxane usage *n* (%)			
No	164 (70)	179 (70)	
Yes	69 (30)	76 (30)	0.9
Anthracycline dose (range, mg/m^2)			
During ALL BFM treatment	120–360	120–360	
Including cumulative doses of those treated for relapse	120–840	120–840	
LVFS (means ± S.D., %)			
At the diagnosis	39.8 ± 6.0 (*n*=133)	n.d.a.	
End of the treatment	39.0 ± 6.0 (*n*=173)	n.d.a.	
Latest ECHO	38.6 ± 5.2 (*n*=168)	n.d.a.	
Time of the ECHO from the diagnosis (median, years)			
End of the treatment (range)	2.0 (0.7–3.4)	n.d.a.	
Latest ECHO (range)	6.3 (2.4–13.7)	n.d.a.	

Abbreviations: HR, high risk; LR, low risk; MR, medium risk; n.d.a., no data available.

(the latter only in patients with high-risk leukaemia). The protocol in the low-risk and medium-risk arms differed only in the number of anthracycline doses, the cumulative anthracycline doses being between 180 and 300 mg/m^2. The high-risk arm differed considerably from the low-risk and medium-risk arms in terms of the applied therapy dosage.

2.2. Laboratory methods

DNA was extracted from peripheral blood taken in remission (n=206) from the survivors. DNA was obtained from bone marrow smears (n=19), from neonatal Guthrie spots (n=4), or from stored buffy coats (n=6) from patients who were deceased before sample collection was carried out. We extracted genomic DNA from blood using the QIAmpBlood DNA Maxi Kit (Qiagen) according to the manufacturers' instructions; from smears and from lymphocytes with HighPure PCR Template Preparation Kit (Roche Diagnostics) with minor alterations (see the Supplementary online data available at http://www.cellbiolint.org/cbi/036/cbi0360079add.htm): and from Guthrie spots using Chelex 100 reagent (Bio-Rad Laboratories).

SNPs (single nucleotide polymorphisms) were selected prioritized on the basis of their estimated functionality in this order: non-synonymous SNPs, SNPs in promoter and 3′-UTR (3′-untranslated region) region, synonymous SNPs and intronic SNPs. Our goal was to cover every haplotype block in the gene defined by the Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview/) (Barrett et al., 2005) with 1 or 2 SNPs. Detailed information on the selected SNPs is shown in Table 2.

The *ABCC1* rs45511401 genotypes were determined by multiplex single base extension using a SNaPshot Multiplex Kit (Applied Biosystems) followed by mini-sequencing on an ABI 310 genetic analyser (Applied Biosystems). All other *ABCC1* SNPs were genotyped using the GenomeLab SNPstream genotyping platform (Beckman Coulter) according to the manufacturer's instructions. Detailed description of these procedures can be found in the Supplementary online data.

2.3. Statistical analysis

Allele frequencies were calculated by allele counting. The HWE (Hardy–Weinberg equilibrium) was tested by using on-line software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl, and significant violation of HWE was considered where $P<0.01$). We performed the statistical analysis to assess the effect of the genetic background on cardiac parameters with multi-adjusted general linear model procedures. The multi-adjusted models included the following potential confounders: gender (male–female), age at the time of diagnosis (years), clinical centre (6 centres), total anthracycline dose (mg/m^2), dexrazoxane administration (yes–no), and chemotherapy protocol (ALL BFM 90–ALL BFM 95). Regarding the data at the time of diagnosis, the multi-adjusted models included only gender, age at the time of diagnosis, clinical centre and chemotherapy protocol. Three genotype groups were analysed separately when the number of patients was sufficient in each group.

Alpha levels of $P<0.005$, that is, 0.05 after Bonferroni correction considering multiple testing for 9 SNPs, were considered significant. The analyses were performed using the SPSS 15.1 (SPSS Inc.) and MedCalc 10.0.2 (MedCalc Software) software.

3. Results

3.1. Study population and allele frequencies

As mentioned above, the characteristics of the study population are shown in Table 1. Genotyping for 9 SNPs in *ABCC1* gene was performed. The positions of the investigated SNPs in the gene can be seen in Figure 1, and minor allelic frequencies in Table 2. Genotype distributions were in HWE (Table 2).

3.2. The effect of genotypes on LVFS

With the help of the 9 SNPs in the *ABCC1* gene, we investigated whether these polymorphisms influence cardiac functioning in children with ALL after anthracycline therapy, LVFS being used to measure cardiac function.

There were no significant differences in the LVFS in the genotype groups at the time of diagnosis (Table 2; LVFS dg.). At later time points, however, LVFS was influenced by *ABCC1* rs246221 and rs3743527 polymorphisms. The *ABCC1* rs3743527TT genotype group had decreased LVFS at the end of treatment ECHO (34.0%) compared with patients with CC (39.5%) or CT (39.3%) genotype (P=0.001; Table 2 and Figure 2). LVFS was 35.3, 38.9 and 38.7% at the time of the latest ECHO of the patients with TT, CT and CC genotypes respectively, although due to the low number of patients being homozygote for the rare allele, this difference was not statistically significant.

Furthermore, we found an association between harbouring the rs246221 T allele and LVFS at the time of the latest ECHO (6.6$\pm$2.7 years after the diagnosis). Patients with TC and TT genotype displayed reduced LVFS (38.4 and 38.5% respectively) compared with patients with CC 40.7%, which was only nominally significant (P=0.027; Table 2 and Figure 2).

Among the clinical factors included in the analysis of LVFS, the age at the time of diagnosis, clinical centre, chemotherapy protocol were significant cofactors (data not shown). In the case of the 2 significant SNPs, neither of these parameters influenced the effect of the genotypes.

3.3. The effect of genotype combinations on LVFS

Because rs3743527TT, rs246221TT and TC genotypes were associated with lower mean LVFS, we were interested in the LVFS data of patients who had both the rs3743527TT and rs246221TC or rs246221TT genotypes, and therefore analysed the effect of these genotype combinations on the LVFS. We compared the LVFS data of patients with both the rs3743527TT and rs246221TT or rs246221TC genotypes to the LVFS data of patients with any other genotype for these 2 SNPs. Patients harbouring the rs3743527TT and rs246221TC or TT genotypes (group 2) did not differ in their mean LVFSs before chemotherapy from the patients with other genotypes (group 1). However, after the chemotherapy the mean

Table 2 Genotyped SNPs and LVFS in the three time points in different genotype groups

SNP	Alleles on the forward strand	Position according to NCBI Genome Build 36.3	B*	Function	MAF	Deviation from the HWE (χ^2 test)	LVFS time	*N* all	LVFS 11†	S.D.	N 11	LVFS 12†	S.D.	N 12	LVFS 22†	S.D.	N 22	*P*-value
rs215060	A/G	15984788	4	Intron	0.14	0.51	LVFS dg.	130	39.1	5.7	93	41.5	6.7	37	–	–		0.1
							LVFS e.t.	168	38.8	6.0	118	39.6	5.9	50	–	–		0.8
							LVFS l.	163	38.4	4.8	126	39.7	5.4	37	–	–		0.2
rs246219	C/T	15993136	5	Intron	0.14	0.02	LVFS dg.	128	39.2	6.0	91	40.9	6.2	37	–	–		0.1
							LVFS e.t.	166	38.7	6.0	119	39.8	6.3	47	–	–		1
							LVFS l.	161	38.3	5.1	123	39.1	5.1	38	–	–		0.6
rs246221	T/C	16045823	10	V275V	0.35	0.08	LVFS dg.	132	40.0	6.0	57	39.3	6.1	65	42.5	5.0	10	0.1
							LVFS e.t.	171	39.4	6.5	69	38.4	5.6	84	40.0	6.4	18	0.2
							LVFS l.	164	38.4	4.8	62	38.5	5.2	86	40.7	5.6	16	0.027
rs45511401	G/T	16080733	12	G671V	0.05	0.41	LVFS dg.	129	39.7	6.2	114	41.3	5.2	15	–	–		0.3
							LVFS e.t.	164	39.0	6.1	147	39.2	5.3	17	–	–		1
							LVFS l.	155	38.7	5.0	140	38.1	5.8	15	–	–		0.3
rs4148358	C/T	16094676	13	Intron	0.2	0.76	LVFS dg.	130	39.6	6.1	80	40.2	6.0	50	–	–		0.8
							LVFS e.t.	168	39.3	6.4	103	38.7	5.5	65	–	–		0.3
							LVFS l.	162	38.5	5.3	112	38.5	4.5	50	–	–		0.9
rs11864374	A/G	16109386	14	Intron	0.24	0.56	LVFS dg.	129	39.0	6.1	72	41.1	5.7	49	39.3	6.8	8	0.1
							LVFS e.t.	165	39.0	5.7	95	39.0	6.5	57	39.4	6.6	13	0.5
							LVFS l.	162	38.5	4.6	97	38.3	5.2	53	41.5	6.1	12	0.2
rs6416666	A/G	16121463	15	Intron	0.07	0.28	LVFS dg.	131	39.7	6.2	112	39.9	5.3	19	–	–		0.7
							LVFS e.t.	170	39.1	6.0	144	38.5	6.4	26	–	–		0.9
							LVFS l.	166	38.7	5.1	148	37.4	5.7	18	–	–		0.3
rs3743527	C/T	16143182	16	3′-UTR	0.22	0.49	LVFS dg.	131	39.7	5.6	79	40.1	7.1	43	38.5	4.5	9	0.5
							LVFS e.t.	168	39.5	5.8	103	39.3	6.4	54	34.0	4.4	11	0.001
							LVFS l.	161	38.7	4.9	108	38.9	5.1	47	35.3	3.6	6	0.2
rs212097	A/G	16151630	16	3′-UTR	0.47	0.43	LVFS dg.	131	39.9	6.0	38	39.6	6.2	69	40.0	5.9	24	0.9
							LVFS e.t.	170	38.0	6.3	45	39.4	6.0	87	39.5	5.7	38	0.2
							LVFS l.	166	37.8	5.8	40	38.8	4.9	88	38.8	5.2	38	0.8

* B: haplotype blocks determined by Haploview 4.1 using the HapMap data.

† Mean LVFS of the patients in the different genotype groups: 11, homozygote for the frequent allele; 12, heterozygote; 22, homozygote for the rare allele. If there were not enough data for the rare 22 genotype groups, the 12 and 22 data were merged for the analysis. This is indicated by a dash in column LVFS 22.; LVFS is indicated in mean %; LVFS dg., LVFS at the time of diagnosis; LVFS e.t., LVFS at the end of the treatment; LVFS l., LVFS at the time of the latest echocardiography; N, number of patients in the genotype group.

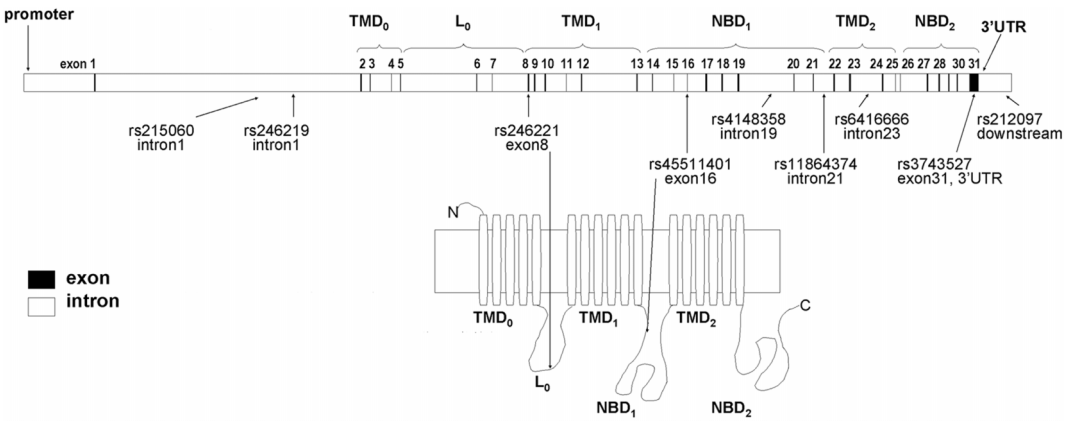

Figure 1 **The genotyped SNPs and their position in the *ABCC1* gene**
The exons, introns and SNPs of *ABCC1* gene are indicated in this illustration. Locations of the exonic SNPs are also indicated in the schematic structure of the protein. Abbreviations: L, linker region; NBD, nucleotide binding domain; TMD, transmembrane domain.

LVFS was significantly lower in patients with the TT-TC/TT genotype combination (34.0%) compared with other patients (39.4%) (P=0.001; Table 3 and Figure 3).

4. Discussion

Childhood ALL is highly curable today, but the survivors may suffer from severe late side effects of the chemotherapy. One of the most important late adverse effects is anthracycline cardiotoxicity. The development and progression of this toxicity varies between patients, which suggests that drug toxicity might be influenced by genetic background. Patients can survive for decades, so it is particularly important to prevent these late side effects of therapy, identify early markers of these late problems, and identify patients with elevated susceptibility to development of late cardiac problems. We accordingly examined the association of genetic polymorphisms in the *ABCC1* gene with LV function after chemotherapy.

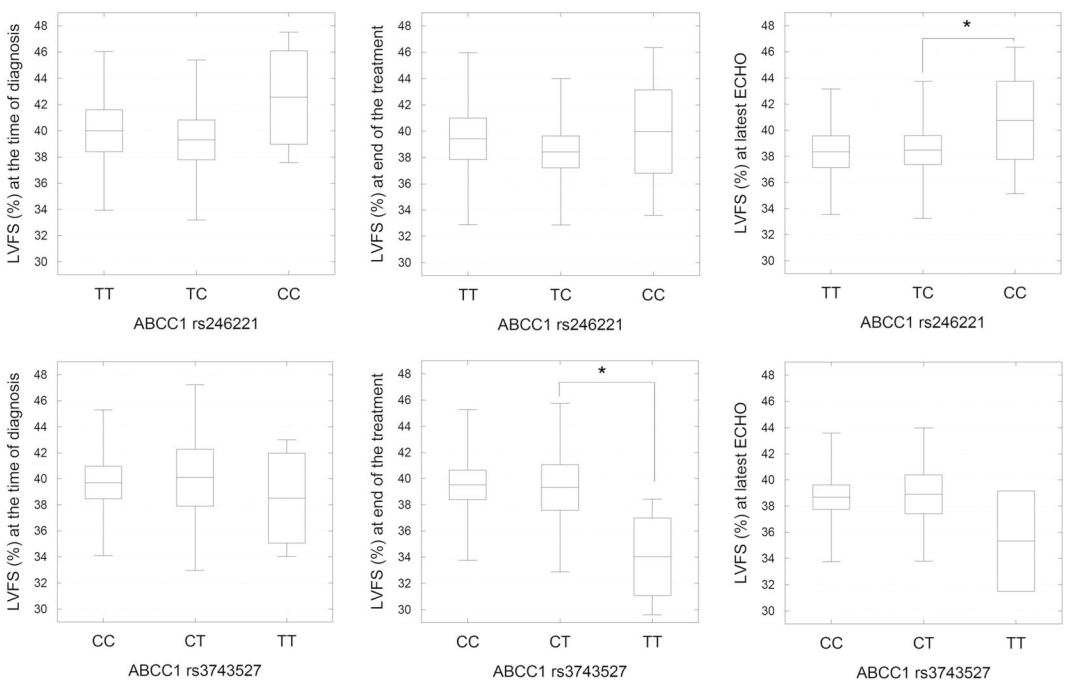

Figure 2 **LVFS in the 3 time points in different genotypes of *ABCC1* rs3743527 and rs246221 SNPs**
LVFS data of patients with rs3743527 and rs246221 genotypes are indicated in box plots. LVFS at the time of diagnosis, at the end of treatment and at the time of the latest ECHO are on separate graphs. LVFS is indicated in box plots, box is mean ±95% CI, whiskers are means±S.D.; *statistically significant differences.

Table 3 LVFS in patients with rs3743527TT and rs246221TC or rs246221TT genotypes

LVFS time	Patients involved	LVFS group 1*	S.D.	Number of patients in group 1	LVFS group 2†	S.D.	Number of patients in group 2	*P*-value
LVFS dg.	131	39.8	6.2	122	38.5	4.5	9	0.4
LVFS e.t.	169	39.4	6	158	34.0	4.4	11	0.001
LVFS 1.	163	38.7	4.9	158	35.6	4	5	0.1

* Group 1: patients with genotype other than group 2 patients.
† Group 2: patients with rs3743527TT and rs246221TC or TT genotypes; LVFS is indicated in mean %; LVFS dg., LVFS at the time of diagnosis; LVFS e.t., LVFS at the end of the treatment; LVFS 1., LVFS at the time of the latest echocardiography.

Patients with the *ABCC1* genotype rs3743527TT had reduced LVFS at the end of the treatment. Moreover, the genotype combination TT-TC/TT (rs3743527–rs246221) was associated with decreased LVFS.

A possible limitation of these findings is that patients who died before the period of sample collection were under-represented in our cohort. However, in our opinion, this is not a relevant bias, as late effects are only manifest and have relevance in survivors. Furthermore, according to the data stored at the Hungarian Paediatric Cancer Registry, these patients did not die due to cardiac-related events, and thus these limitations are insufficient to question the results of our study.

It has also to be mentioned that patients with rs3743527TT genotype have rs246221TT or rs246221TC genotypes, except for one patient with rs246221CC genotype. The reason for this is the low number of patients ($n=11$) with rs3743527TT genotype, but this may also cause some bias.

So far, there has been only one study by Wojnowski et al. (2005) examining the role of ABC transporters in anthracycline-induced cardiotoxicity, in which 206 SNPs were examined in 82 genes comparing 87 Caucasian patients with NHL (non-Hodgkin lymphoma) experiencing cardiac problems with 363 NHL patients without any cardiac malfunction. This study found an association between chronic anthracycline-induced cardiotoxicity and a polymorphism in the NAD(P)H oxidase subunit *NFC4* (rs1883112), and between: acute anthracycline-induced cardiotoxicity and the NAD(P)H oxidase subunits *CYBA* (rs4673) and *RAC2* (rs1305 8338); and the ABC transporter *ABCC1* Gly671Val variant (which is rs45511401) and the Val1188Glu–Cys1515Tyr (rs8187694–rs8187710) haplotype of the *ABCC2* gene.

Interestingly, we did not find an association between *ABCC1* rs45511401 and reduced LVFS, a fact that must be interpreted carefully, as there were differences in the chemotherapy protocols and the age of the study populations; and also because this SNP has a low minor allele frequency, which, together with the investigated number of patients, makes this comparison under-powered. In addition, Wojnowski et al. (2005) defined the acute anthracycline-induced cardiotoxicity with parameters we did not analyse. They defined cardiotoxicity on the basis of the following criteria: cases of arrhythmia, myocarditis, pericarditis, and acute heart failure as acute anthracycline-induced cardiotoxicity. Reduction of the ejection fraction to $<50\%$, or of fractional shortening to $<25\%$, was classified as chronic anthracycline-induced cardiotoxicity. We did not analyse their first 3 parameters regarding acute cardiotoxicity and we had only one patient with fractional shortening of $<25\%$. They found no association with ABCC1 rs45511401 and chronic anthracycline-induced cardiotoxicity which is similar to our study as we examined the reduction in LVFS. In summary, the important similarity between the 2 results is that they found an association between cardiac problems and *ABCC1* gene variations. Naturally for a more decisive

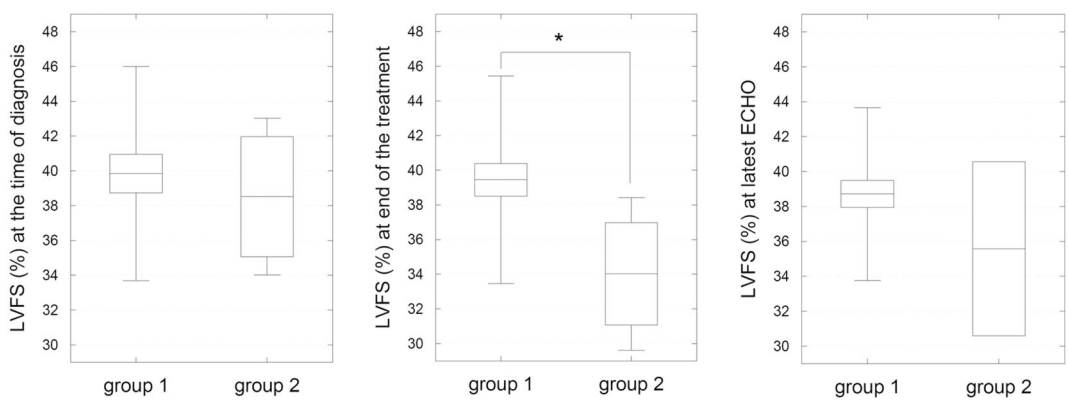

Figure 3 LVFS at three time points in rs3743527 and rs246221 genotype combinations
LVFS data of patients with genotype combination determined from rs3743527 and rs246221 SNPs are indicated in the box plots. We compared patients with rs3743527TT and rs246221TC or rs246221TT genotype combination (group 2) to patients with any other genotype combination (group 1). LVFS at the time of diagnosis, at the end of treatment and at the time of the latest ECHO are on separate graphs. Abbreviations: group 1, patients with genotype other than group 2; group 2, genotype rs3743527TT and rs246221TC/TT; LVFS is indicated in box plots, box is mean $\pm$ 95% CI, whiskers are means $\pm$ S.D.; *statistically significant differences.

statement, both studies ought to be repeated on independent and larger populations with more SNPs in the ABCC1 gene involved in the analysis.

The ABCC1 transporter is important in the protection of the cell from distinct types of chemical stress. In most polarized cells, it is localized in the basolateral membrane, contralateral to the other ABC transporters involved in the detoxification. This suggests that the main role of ABCC1 is the protection of the cells against xenobiotics (Bakos and Homolya, 2007). The anthracycline doxorubicin does not require a transporter to enter the cell; thus a sufficient efflux of this drug is very important to protect the cell (Borst et al., 2000). This fact is in agreement with our observation regarding the role of ABCC1 in the protection of cardiomyocytes.

Also the ABCC1 transporter plays an important role in oxidative stress. It is involved in the maintenance of sufficient levels of glutathione, which is necessary for the defence against reactive oxygen species. Moreover, ABCC1 also requires glutathione for transport of anthracyclines (Borst et al., 2000; Kruh and Belinsky, 2003; Bakos and Homolya, 2007), which can also influence the response in oxidative stress induced by anthracyclines (Wojtacki et al., 2000).

In our study, 2 SNPs, rs3743527 and rs246221, influenced cardiac function after anthracycline treatment. Unfortunately, there is no data on the potential function of these SNPs. There are studies investigating the role of other known *ABCC1* SNPs (Wang et al., 2006; Huang, 2007), but not of these ones. Rs246221 is an exonic synonymous polymorphism, which does not influence the amino acid sequence. It is located in the linker region of the ABCC1. The rs3743527 SNP is located in the 3′-UTR of the *ABCC1* gene. In the literature, there is only one study involving this SNP. Studying the susceptibility to lung cancer in Chinese patients, Wang et al. (2009) investigated SNPs located in the 3′-UTR of *ABCB1* and *ABCC1*, including rs3743527. They found no association with *ABCC1* rs3743527, but with *ABCC1* rs212090 and *ABCB1* rs3842. The *ABCC1* rs212090 is only 300 bp distance away from rs3743527.

Further studies are important to reveal the exact role of these polymorphisms. Haplotype analysis may be useful to discover possible linked functional SNPs, because not these but others in the same haplotype may block or influence the function of ABCC1. Also it is possible that there are some as yet unknown regulatory elements in this region that could be affected by this SNP. According to the miRDB microRNA database (http://mirdb.org/miRDB/), there are predicted hsa-miR-185, hsa-miR-548o and hsa-miR-1254 binding sites in a 500 bp region around rs3743527 that might have regulatory roles. Other polymorphisms in the same haplotype block with rs3743527 can influence the binding sequence of the regulatory elements. The ENCODE project (Birney et al., 2007) identified regulatory sites in the genome also in the proximity of SNPs in the *ABCC1* gene. There are predicted transcription factor binding sites near rs3743527 and rs246221. The CTCF transcription factor binds 1235 bp downstream from rs3743527 and BAF155 binds 893 bp downstream from rs246221. DNase hypersensitivity assays also show regulatory regions within 3000 bp to these SNPs.

In our study, the anthracycline dose did not influence LVFS. This is probably because the dose range was relatively narrow (180–240 mg/m^2) in ~80% of the samples of the cohort. The threshold for LVFS associated with LV systolic dysfunction is LVFS <30% according to the National Cancer Institute Common Terminology Criteria for Adverse Events [v.3.0] (http://ctep.info.nih.gov/protocolDevelopment/electronic_applications/ctc.htm). It must be noted that the reduced LVFS values in this study associated with certain genetic background were in the 'normal' range. According to several studies, normal range or subclinical reduction of LVFS in patients after anthracycline therapy might have prognostic value for late onset more serious cardiotoxicity (Kremer et al., 2002; Lipshultz et al., 2008).

It is also important to note that some drugs used in the chemotherapy protocols, like *Vinca* alkaloids and methotrexate, may be cardiotoxic (Floyd et al., 2005; Simbre et al., 2005). They are also substrates of ABCC1 and might also have contributed to the associations identified in our study. However, it is generally accepted that the cardiotoxic effect of anthracyclines considerably exceeds that of these other drugs.

5. Conclusions

Our results indicate that certain genotypes of the *ABCC1* rs3743527 or rs246221 SNPs might influence the development of cardiotoxicity after anthracycline treatment in the patient population studied. Common genetic polymorphisms have weak effects; each one contributes only slightly to the susceptibility to a disease. The effect might be stronger if genotype associations or haplotypes are investigated. According to these and earlier findings the ABCC1 transporter is important in limiting the anthracycline exposure of cardiomyocytes, and this protective mechanism may be influenced by genetic polymorphisms. Further studies with longer follow-up involving other genes and more patients are necessary to examine the exact genetic background of anthracycline-induced late onset cardiomyopathy. Such studies are needed for establishing possible individualized chemotherapies.

Author contribution

Agnes Semsei performed the genotyping, evaluated the results and wrote the paper. Daniel Erdelyi organized the whole project, and organized and collected patients. Ildiko Ungvari performed statistical evaluation. Edit Csagoly and Marta Hegyi collected the clinical data. Petra Kiszel evaluated the results. Orsolya Lautner-Csorba performed the genotyping. Judit Szabolcs and Peter Masat performed the clinical measurements. Gyorgy Fekete evaluated the clinical data. Andras Falus organized the project. Csaba Szalai organized the laboratory work and wrote the paper. Gabor Kovacs collected and evaluated the clinical data. All of the authors read the paper, and contributed to the evaluation of the results.

Acknowledgements

We are grateful to all the nurses, physicians and patients who took part in the study. We thank Anna Zalka, Eniko Kamory, Bela

Csokay, Andras Kiss, Dora Lippai, Xenia Majorosi, Melinda Racz, Veronika Galasz, Erika Toth, Gabor Varadi, Krisztina Toth and Judit Bali for technical assistance and Zoltan Pos for help with writing the article prior to submission.

Funding

The majority of the SNP genotyping was carried out in the Semmelweis University-SNP Core Facility (http://www.dgci.sote.hu/en/snpcorefacilityen) which is supported by the Richter Gedeon PLC Company. This study was funded by the Hungarian Scientific Research Fund [grant number T042500], the Economic Competitiveness Operational Programme, Hungary [grant number GVOP 3.1.1-2004-05-0022/3.0] and NKTH (National Research and Technology) [grant number TECH_08-A1/2-2008-0120].

References

Adams MJ, Lipshultz SE. Pathophysiology of anthracycline- and radiation-associated cardiomyopathies: implications for screening and prevention. Pediatr Blood Cancer 2005;44:600–6.

Bakos E, Homolya L. Portrait of multifaceted transporter, the multidrug resistance-associated protein 1 (MRP1/ABCC1). Pflugers Arch 2007;453:621–41.

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.

Birney E, Stamatoyannopoulos JA, Dutta A, Guigo R, Gingeras TR, Margulies EH et al. Identification and analysis of functional elements in 1% of the human genome by the ENCODE pilot project. Nature 2007;447:799–816.

Borst P, Evers R, Kool M, Wijnholds J. A family of drug transporters: the multidrug resistance-associated proteins. J Natl Cancer Inst 2000;92:1295–302.

Couture L, Nash JA, Turgeon J. Role of ATP-binding cassette transporters in drug distribution to the heart and protection from toxic compounds. Heart Metab 2007;35:16–21.

Floyd JD, Nguyen DT, Lobins RL, Bashir Q, Doll DC, Perry MC. Cardiotoxicity of cancer therapy. J Clin Oncol 2005;23:7685–96.

Huang Y. Pharmacogenetics/genomics of membrane transporters in cancer chemotherapy. Cancer Metastasis Rev 2007;26:183–201.

Iarussi D, Indolfi P, Casale F, Martino V, Di Tullio MT, Calabro R. Anthracycline-induced cardiotoxicity in children with cancer: strategies for prevention and management. Paediatr Drugs 2005;7:67–76.

Johnson SA, Richardson DS. Anthracyclines in haematology: pharmacokinetics and clinical studies. Blood Rev 1998;12:52–71.

Jungsuwadee P, Nithipongvanitch R, Chen Y, Oberley TD, Butterfield DA, St Clair DK et al. Mrp1 localization and function in cardiac mitochondria after doxorubicin. Mol Pharmacol 2009;75:1117–26.

Kerb R, Hoffmeyer S, Brinkmann U. ABC drug transporters: hereditary polymorphisms and pharmacological impact in MDR1, MRP1 and MRP2. Pharmacogenomics 2001;2:51–64.

Kremer LC, van der Pal HJ, Offringa M, van Dalen EC, Voute PA. Frequency and risk factors of subclinical cardiotoxicity after anthracycline therapy in children: a systematic review. Ann Oncol 2002;13:819–29.

Kruh GD, Belinsky MG. The MRP family of drug efflux pumps. Oncogene 2003;22:7537–52.

Lipshultz SE, Lipsitz SR, Sallan SE, Dalton VM, Mone SM, Gelber RD et al. Chronic progressive cardiac dysfunction years after doxorubicin therapy for childhood acute lymphoblastic leukemia. J Clin Oncol 2005;23:2629–36.

Lipshultz SE, Alvarez JA, Scully RE. Anthracycline associated cardiotoxicity in survivors of childhood cancer. Heart 2008;94:525–33.

Mody R, Li S, Dover DC, Sallan S, Leisenring W, Oeffinger KC et al. Twenty-five-year follow-up among survivors of childhood acute lymphoblastic leukemia: a report from the Childhood Cancer Survivor Study. Blood 2008;111:5515–23.

Mulrooney DA, Yeazel MW, Kawashima T, Mertens AC, Mitby P, Stovall M et al. Cardiac outcomes in a cohort of adult survivors of childhood and adolescent cancer: retrospective analysis of the Childhood Cancer Survivor Study cohort. Br Med J 2009;339:b4606.

Scully RE, Lipshultz SE. Anthracycline cardiotoxicity in long-term survivors of childhood cancer. Cardiovasc Toxicol 2007;7:122–8.

Shankar SM, Marina N, Hudson MM, Hodgson DC, Adams MJ, Landier W et al. Monitoring for cardiovascular disease in survivors of childhood cancer: report from the Cardiovascular Disease Task Force of the Children's Oncology Group. Pediatrics 2008;121:e387–96.

Simbre VC, Duffy SA, Dadlani GH, Miller TL, Lipshultz SE. Cardiotoxicity of cancer chemotherapy: implications for children. Paediatr Drugs 2005;7:187–202.

Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD. Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Update 2003;6:71–84.

Viale PH, Yamamoto DS. Cardiovascular toxicity associated with cancer treatment. Clin J Oncol Nurs 2008;12:627–38.

Wang H, Jin G, Liu G, Qian J, Jin L, Wei Q et al. Genetic susceptibility of lung cancer associated with common variants in the 3′ untranslated regions of the adenosine triphosphate-binding cassette B1 (ABCB1) and ABCC1 candidate transporter genes for carcinogen export. Cancer 2009;115:595–607.

Wang Z, Sew PH, Ambrose H, Ryan S, Chong SS, Lee EJ et al. Nucleotide sequence analyses of the MRP1 gene in four populations suggest negative selection on its coding region. BMC Genomics 2006;7:111.

Wojnowski L, Kulle B, Schirmer M, Schluter G, Schmidt A, Rosenberger A et al. NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity. Circulation 2005;112:3754–62.

Wojtacki J, Lewicka-Nowak E, Lesniewski-Kmak K. Anthracycline-induced cardiotoxicity: clinical course, risk factors, pathogenesis, detection and prevention – review of the literature. Med Sci Monit 2000;6:411–20.

Received 2 May 2011/7 July 2011; accepted 20 September 2011

Published as Immediate Publication 20 September 2011, doi 10.1042/CBI20110264