ABCC1 polymorphisms in anthracycline induced cardiotoxicity in childhood acute lymphoblastic leukemia

Agnes F. Semsei MSc[1], Daniel J. Erdelyi MD, PhD[1,2], Ildiko Ungvari MSc[1], Edit Csagoly MD[1], Marta Z. Hegyi MD[2], Petra S. Kiszel PhD[1], Orsolya Csorba MSc[1], Judit Szabolcs MD, PhD[2], Peter Masat MD[3], Gyorgy Fekete MD, DSc[2], Andras Falus PhD, DSc[1], Csaba Szalai PhD, DSc[4,5,6], Gabor T Kovacs MD, PhD[2]

[1] Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary
[2] 2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary
[3] Markusovszky Hospital of Vas county council, Szombathely, Hungary
[4] Heim Pal Children Hospital, Budapest
[5] Hungary; Inflammation biology and Immunogenomics Research Group, Hungarian Academy of Sciences - Semmelweis University, Budapest, Hungary
[6] Csertex Research Laboratory, Budapest, Hungary

Correspondence: Csaba Szalai PhD, DSc; Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary, 1089, Nagyvarad ter 4; telephone: +36-1-210-2930/56431; fax: +36-1-303-6968; email: genomika.cs@gmail.com

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Abbreviations: ABCC1, ATP-binding cassette, sub-family C, member 1; ALL, acute lymphoblastic leukemia; BFM, Berlin–Frankfurt–Münster; ECHO, echocardiography; 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.

Abstract

Anthracyclines are potent cytostatic drugs the correct dosage of which is critical to aviod possible cardiac side effects. ABCC1 (MRP1) is expressed in the heart and takes part in the detoxification and protection of the cells from toxic effects of xenobiotics, including anthracyclines. Our objective was to search for associations between left ventricular function and single nucleotide polymorphisms of the *ABCC1* gene in children who received anthracycline chemotherapy. We analyzed data of 235 pediatric patients with acute lymphoblastic leukemia. 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 found to be associated with lower left ventricular fractional shortening (LVFS) after chemotherapy. Our results suggest that genetic variants in the *ABCC1* gene could influence anthracycline induced left ventricular dysfunction.

1. Introduction and background

Childhood acute lymphoblastic leukemia (ALL) is cured in approximately 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 the treatment or become clinically evident years after the end of the therapy. Follow-up studies indicate that survivors of childhood leukemia often have cardiac problems as late side effects of the chemotherapy (Shankar et al., 2008; Mulrooney et al., 2009). These can be due to the most cardiotoxic anthracyclines but other drugs applied in the chemotherapy regimen can also damage the heart (Viale and Yamamoto, 2008).
Anthracyclines such as doxorubicin and daunorubicin are highly effective antineoplastic drugs and used in a wide range of cancers (Johnson and Richardson, 1998). Anthracycline-induced cardiotoxicity can be divided into three types. Acute anthracycline-induced cardiotoxicity occurs during treatment, often immediately after the first dose of anthracycline has been administered. 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 after treatment (Lipshultz et al., 2008). Anthracycline induced cardiotoxicity can be a mild, transient condition, caracterised by asymptomatic electrocardiographic changes, e.g., arrhythmias. More severe toxicities are left ventricular (LV) dysfunction, decreased exercise capacity, decreased left ventricular fractional shortening (LVFS), late onset cardiomyopathy, 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 far the most frequent cardiac consequence of the therapy. In a study with 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 (below 4 years), concomitant therapy (irradiation and other antineoplastic drugs), gender (female) and cumulative dose of the drug. Nevertheless, the fact that there is no safe dose of anthracyclines and the wide interpatient variability in the time and seriousness of this adverse effect suggest that genetic background is a major determinant of drug response and toxicity (Lipshultz, Alvarez, 2008).
ATP-binding cassette (ABC) 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 the energy of ATP hydrolysis. ABC transporters have been found to be expressed in the heart and some of them have anthracyclines among their substrates (Couture et al., 2007). The ABCC1 (ATP-binding cassette, sub-family C, member 1 also denoted as MRP1: multidrug
resistance-associated protein 1) transporter was first described in a doxorubicin resistant cell line and is expressed ubiquitously in the body. Tissues showing the highest level 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 defense mechanisms against oxidative stress (Bakos and Homolya, 2007). ABCC1 expression was shown to be increased after doxorubicin treatment in cardiac tissue of mice (Jungsuwadee et

al., 2009) and experiments with Abcc1 knockout mice demonstrated 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).
In this study our objective was to examine potential associations between subclinical reduction of cardiac function and the genetic background of childhood ALL patients investigating polymorphisms in the *ABCC1* covering all haplotype blocks in this gene in order to gain more understanding on the genetic background of the anthracycline cardiotoxicity.

2. Patients and methods

2. 1. Study population and definitions

We collected DNA retrospectively from children with ALL who underwent chemotherapy between 1990 and 2002, aged 1–18 years at diagnosis, treated according to the ALL Berlin–Frankfurt–Münster (BFM) 90 or 95 study protocols in 6 Hungarian pediatric oncology centers. We included 235 children in the analysis. Characteristics of the study population are 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 survived at least until the end of chemotherapy (Table 1). As seen, our cohort represents 70% of all cases. There is no difference in the distribution of the characteristics of the patients between the two groups. Informed consent was requested from the parents of patients. The study was approved by the Ethics Committee of the Hungarian Medical Research Council.
The patients were followed up by echocardiography (ECHO) to assess left ventricular function by measuring left ventricular end-diastolic-dimension (LVEDD) and left ventricular end-systolic dimension (LVESD). Left ventricular fractional shortening (LVFS) was calculated from these two data: LVFS % = (LVEDD-LVESD)/LVEDD*100%. We analyzed the LVFS at three time points. First at the time of diagnosis, second at the end of the treatment that is at median 2.0 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 centers so LVFS data were not present in all time points of all the children. In the case of seven patients with relapsed leukemia, we included two separate “latest” datasets. These are 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 (the latter only patients with high risk leukemia). The protocol in low risk and medium risk arms differed only in the number of anthracycline doses, the cumulative anthracycline doses were between 180-300 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 deceased before sample collection was carried out.

We extracted genomic DNA from blood using the QIAmpBlood DNA Maxi Kit (Qiagen, Hilden, Germany) according to the manufacturers' instructions; from smears and from lymphocytes with HighPure PCR Template PreparationKit (Roche Diagnostics, Roche Applied Science, Mannheim, Germany) with minor alterations (see online Supplementary data) and from Guthrie spots using Chelex 100 reagent (Bio-Rad Laboratories, München, Germany).
SNPs (single nucleotide polymorphism) were selected prioritized on the basis of their estimated functionality in this order: non-synonymous SNPs, SNPs in promoter and 3' UTR 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 one or two 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, Warrington, UK) followed by minisequencing on an ABI 310 genetic analyzer (Applied Biosystems). All other *ABCC1* SNPs were genotyped using the GenomeLab SNPstream genotyping platform (Beckman Coulter, Krefeld, Germany) according to the manufacturer's instructions. Detailed description of these procedures can be found in the online Supplementary data.

2.3. Statistical analysis

Allele frequencies were calculated by allele counting. Hardy-Weinberg equilibrium (HWE) was tested by using an on-line software (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl, significant violation of HWE was considered if $p< 0.01$). We performed the statistical analysis to assess the effect of the genetic background on cardiac parameters with and 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 center (6 centers), total anthracycline dose (mg/m^2), dexrazoxane usage (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 center and chemotherapy protocol. We analyzed the three genotype groups 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 as significant. The analyses were performed using the SPSS 15.1 (SPSS Inc., Chicago, IL, USA) and MedCalc 10.0.2 (MedCalc Software, Mariakerke, Belgium) software.

3. Results

3.1. Study population and allele frequencies

The characteristics of the study population are shown in Table 1. We performed genotyping for nine SNPs in *ABCC1* gene. The positions of the investigated SNPs in the gene can be seen in Figure 1. Minor allelic frequencies are shown in Table 2. Genotype distributions were in Hardy-Weinberg equilibrium (Table 2).

3.2. The effect of genotypes on LVFS

With the help of nine SNPs in the *ABCC1* gene, we investigated whether these polymorphisms influence the cardiac functions of children with ALL after anthracycline therapy. The left ventricular fractional shortening (LVFS) of the patients were measured to estimate their 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 found to be influenced by the *ABCC1* rs246221 and rs3743527 polymorphisms. The *ABCC1* rs3743527TT genotype group had decreased LVFS at the end of treatment echocardiography (34.0%) compared to 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 echocardiography 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.

Further, we found an association between harboring the rs246221 T allele and LVFS at the time of the latest echocardiography (6.6 ± 2.7 years after the diagnosis). Patients with TC and TT genotype displayed reduced LVFS (38.4% and 38.5%) compared to patients with CC 40.7%, which was, however, 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 center, chemotherapy protocol were found to be significant cofactors, while gender, total anthracycline dose, dexrazoxane usage were not (data not shown). In case of the two significant SNPs, none of these parameters influenced the effect of the genotypes.

3. 2. The effect of genotype combinations on LVFS

We analyzed the effect of genotype combinations on the LVFS. Patients harboring the rs3743527TT and rs246221TC or TT genotypes (group2) did not differ in their mean LVFS before chemotherapy from the patients with other genotypes (group1). However, after the chemotherapy the mean LVFS was significantly lower in patients with the TT-TC/TT genotype combination (34.0%) compared to other patients (39.4%) (p=0.001) (Table 3 and Figure 3).

4. Discussion

Childhood acute lymphoblastic leukemia 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. The patients survive for decades so it is particulary important to prevent these late side effects of the therapy, to identify early markers of these late problems and to identify patients with elevated susceblibility to development of late cardiac problems. In our study, we examined the association of genetic polymorphisms in the *ABCC1* gene with left ventricular function after chemotherapy.
We found that patients with the *ABCC1* genotype rs3743527TT had reduced left ventricular fractional shortening (LVFS) at the end of the treatment. Moreover the genotype combination TT-TC/TT (rs3743527-rs246221) was associated with decreased LVFS.
Possible limitations of or these findings are patients who died before the period of sample collection are underrepresented in our cohort. However, in our opininon this is not a relevant bias as late effects 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, thus these limitations are insufficient to question the results of our study.
So far, there has been only one study in the literature published by Wojnowski et al., examining the role of ABC-transporters in anthracycline-induced cardiotoxicity (Wojnowski et al., 2005), in which 206 single nucleotide polymorphisms were examined in 82 genes comparing 87 Caucasian patients with non-Hodgkin lymphoma (NHL) experiencing cardiac problems with 363 NHL patients without any cardiac malfunction.
The study found an association between chronic anthracycline-induced cardiotoxicity (ACT) and a polymorphism in the NAD(P)H oxidase subunit *NFC4* (rs1883112), and between acute ACT and the NAD(P)H oxidase subunits *CYBA* (rs4673) and *RAC2* (rs13058338); the ABC-transporter *ABCC1* Gly671Val variant (which is rs45511401) and the Val1188Glu - Cys1515Tyr (rs8187694-rs8187710) haplotype of the *ABCC2* gene.
Interestingly, in our study, 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. defined the acute anthracycline-induced cardiotoxicity with parameters we did not analyze. They defined cardiotoxicity on the basis of the following criteria: cases of arrhythmia, myocarditis, pericarditis, and acute heart failure as acute anthracycline-induced cardiotoxicity and the reduction of the ejection fraction <50%, or of the fractional shortening <25% was classified as chronic anthracycline-induced cardiotoxicity. We did not analyze their first three parameters regarding acute cardiotoxicity and we had only one patient with fractional shortening <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 left ventricular fractional shortening. In summary the important similarity between the two results is that both studies found association between cardiac problems and *ABCC1* gene variations. Naturally for a more decisive statement, both studies ought to be replicated on independent and larger populations with similar study designs and 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 any transporter to enter the cell, thus a sufficient efflux of this drug is very important in order to protect the cell (Borst, Evers, 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 defense against reactive oxygen species. Moreover, ABCC1 also requires glutathione for transport of anthracyclines (Borst, Evers, 2000; Kruh and Belinsky, 2003; Bakos and Homolya, 2007), which can also influence the response in oxidative stress induced by anthracyclines (Wojtacki, Lewicka-Nowak, 2000).

In this present study, two SNPs, rs3743527 and rs246221 were found to influence the 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 but not of these (Wang et al., 2006; Huang, 2007). 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'untranslated region (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. 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 (Wang et al., 2009).

Further studies are important to reveal the exact role of these polymorphisms. Haplotype analysis may be useful to discover possible linked functional SNPs, because maybe not these but others in the same haplotype block influence the function of ABCC1. Also possible is the existence of some, 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 500bp region around rs3743527, which 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 1235bp downstream from rs3743527, the BAF155 binds to 893bp downstream from rs246221. DNase hypersensitivity assays also show regulatory regions within 3000bp to these SNPs.

In our study the anthracycline dose applied did not influence LVFS. This is probably because the dose range was relatively narrow (180-240mg/m^2) in approximately 80% of the samples of the cohort.

The threshold for LVFS associated with left ventricular 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, van der Pal, 2002; Lipshultz, Alvarez, 2008).
It is also important to note that some drugs used in the chemotherapy protocols, like vinca alkaloids and methotrexate, may also exert cardiotoxicity (Floyd et al., 2005; Simbre et al., 2005). They are also substrates of ABCC1. These 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 studied patient population. It is well known that 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 are necessary to examine the exact genetic background of anthracycline induced late onset cardiomyopathy. Such studies are needed for establishing possible individualized chemotherapies.

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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.

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

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.

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. BMJ. 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 Updat. 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.

Figure legends

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

Figure 2. Left ventricular fractional shortening in the three time points in different genotypes of *ABCC1* rs3743527 and rs246221 SNPs. Abbreviations: LVFS, Left ventricular fractional shortening; LVFS is indicated in boxplots, box is mean±95%CI, whiskers is mean±SD; * statistically significant differences.

Figure 3. LVFS at three time points in rs3743527 and rs246221 genotype combinations. Abbreviations: LVFS, Left ventricular fractional shortening; group1, patients with genotype other than group2; group2, genotype rs3743527TT and rs246221TC/TT; LVFS is indicated in boxplots, box is mean±95%CI, whiskers is mean±SD; *statistically significant differences.

Figure 1.

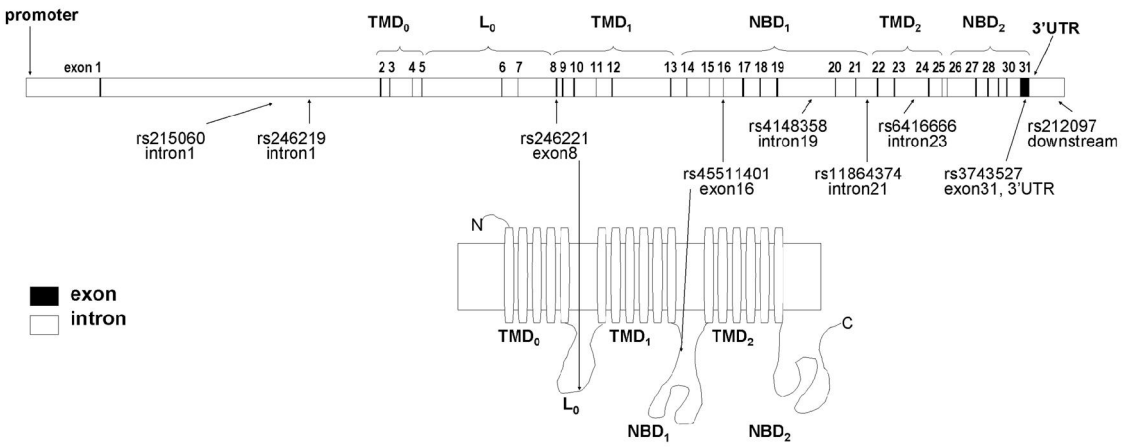

Figure 2.

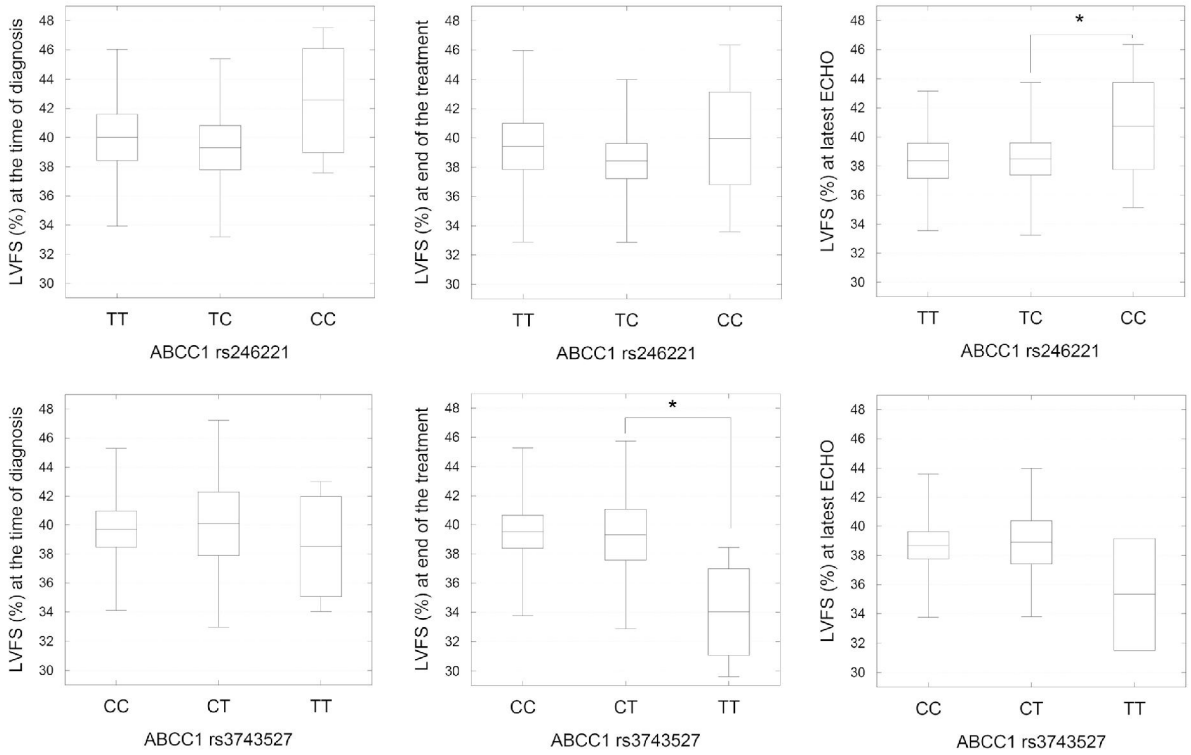

Figure 3.

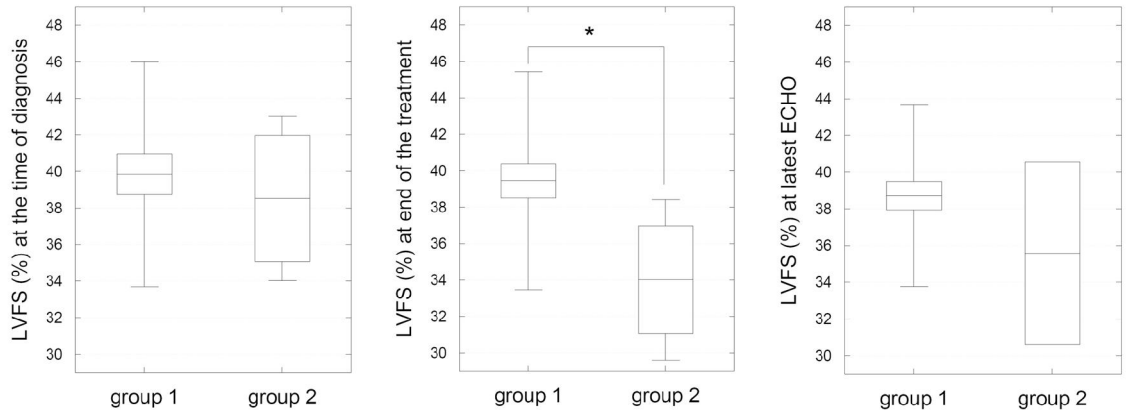

Table 1.
Characteristics of the study population and all cases diagnosed with ALL between 1990 and 2002 in Hungary in the hospitals included to the study from the Hungarian Paediatric Cancer Registry.

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 (mean ± SD, 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 (mean ± SD, %)			
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 echocardiography 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: ECHO, echocardiography; HR, high risk; LR, low risk; LVFS, left ventricular fractional shortening; MR, medium risk; n.d.a., no data available; SD, standard deviation

Table 2.
Genotyped SNPs and left ventricular fractional shortening in the three time points in different genotype groups.

SNP	Alleles on the forward strand	Position according to NCBI Genome Build 36.3	B [a]	Function	MAF	Deviation from the HWE (χ^2 test)	LVFS time	N all	LVFS 11 [b]	SD	N 11	LVFS 12 [b]	SD	N 12	LVFS 22 [b]	SD	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

[a] B: haplotype blocks determined by Haploview 4.1 using the HapMap data; [b] 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 with dashed line in column LVFS 22.; LVFS is indicated in mean %; HWE, Hardy-Weinberg equilibrium; LVFS, left ventricular fractional shortening; 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; SD, standard deviation

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

LVFS time	Patients involved	LVFS group 1 [a]	SD	Number of patients in group 1	LVFS group 2 [b]	SD	Number of patients in group 2	p value
LVFS dg.	118	39.8	6.2	122	38.5	4.5	9	0.4
LVFS e.t.	167	39.4	6	157	34.0	4.4	11	0.001
LVFS l.	166	38.7	4.9	156	35.6	4	5	0.1

[a] group 1: patients with genotype other than group 2 patients; [b] group 2: patients with rs3743527TT and rs246221TC or TT genotypes; LVFS is indicated in mean %; LVFS, left ventricular fractional shortening; 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; SD, standard deviation