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ORIGINAL ARTICLE

In interaction with gender a common *CYP3A4* polymorphism may influence the survival rate of chemotherapy for childhood acute lymphoblastic leukemia

A Gézsi[1], O Lautner-Csorba[1], DJ Erdélyi[2], G Hullám[3], P Antal[3], ÁF Semsei[1], N Kutszegi[1], M Hegyi[2], K Csordás[2], G Kovács[2] and C Szalai[1,4,5]

CYP3A4 has an important role in the metabolisms of many drugs used in acute lymphoblastic leukemia (ALL) therapy; still, there are practically no publications about the role of *CYP3A4* polymorphisms in ALL pharmacogenomics. We genotyped eight common single-nucleotide polymorphisms (SNPs) in the *CYP3A4* and *CYP3A5* genes in 511 children with ALL and investigated whether they influenced the survival of the patients. We involved additional 127 SNPs in 34 candidate genes and searched for interactions with respect to the survival rates. Significant association between the survival rates and the common rs2246709 SNP in the *CYP3A4* gene was observed. The gender of the patients and the rs1076991 in the *MTHFD1* gene strongly influenced this effect. We calculated new risk assessments involving the gender-rs2246709 interaction and showed that they significantly outperformed the earlier risk-group assessments at every time point. If this finding is confirmed in other populations, it can have a considerable prognostic significance.

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INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most frequent haematopoetic malignancy in childhood worldwide.[1] It is cured in ~80–90% of the patients,[2] but relapse and unresponsiveness to the therapy remain unacceptably high for some subgroups. Inherited genomic variations contribute to the risk of relapse and to the risk of failing to respond to the therapy.

CYP3A4 is the most abundant CYP450 enzyme in the liver and the gut, and the main drug-metabolizing protein in humans. It has an important role in the metabolisms of many drugs used in ALL therapy, for example, vincristine, cyclophosphamide, dexamethasone and doxorubicin. In a study of healthy volunteers, the heritability of the observed high enzyme variability of CYP3A4 has been estimated at 90%, suggesting an important role of genetic variations in the gene and in the regulation of the gene expression.[3] Still, there are practically no publications about the role of *CYP3A4* polymorphisms in ALL pharmacogenomics. One possible reason for this that the frequency of the functionally relevant variations in the gene are relatively low (maximum about 4 – 5%) and thus the studies were underpowered in the usually small ALL populations.

In the present study, we aimed to investigate whether more frequent polymorphisms in the *CYP3A4* gene influenced the survival of the patients in pediatric ALL. We selected and genotyped common single-nucleotide polymorphisms (SNPs; minor allele frequency $\geqslant$10% in Caucasian populations) in the *CYP3A4* gene in a relatively large pediatric ALL population. In addition, we also included rarer functional polymorphisms in *CYP3A4* and in *CYP3A5* genes, which have overlapping substrate specificities and which SNPs in some studies significantly influenced the pharmacokinetics of different drugs.[4,5]

It is well known that individual alleles do not act alone, but in interaction with other genetic and environmental factors. Unfortunately, these interactions are very difficult to detect, especially, when larger numbers of variables are studied. Recently, we have developed and tested a novel statistical method named Bayesian network based Bayesian multilevel analysis of relevance (BN-BMLA), which proved to be superior in the detection of interactions over other methods.[2,6–11] In the present study, we involved additional 127 SNPs in 34 genes that, according to the scientific literature and our own previous researches,[2,10] can have a role in the pathomechanism and pharmacogenomics of ALL and together with clinical data and with the help of the BN-BMLA we searched for interactions which influence the survival of the ALL patients.

SUBJECTS AND METHODS

Subjects

In a retrospective manner, DNA was obtained from 511 children who were diagnosed with ALL between 1990 and 2010. According to the data of the Hungarian Paediatric Cancer Registry this corresponds to >40% of all children diagnosed with ALL in Hungary in the given time period. There was no significant difference in the distribution of age groups, genders or ALL-immunophenotypes between the whole ALL-children population and our sample collection.[12]

Treatment of the patients and risk-group stratification were performed according to the ALL Berlin-Frankfurt-Münster 90, 95 or ALL IC-Berlin-Frankfurt-Münster 2002 chemotherapy protocols. Comparison of the different protocols can be found in the Supplementary information.

Detailed information about our ALL population in terms of the administered drugs, their dosage, their time periods and co-medications can be found in our previous study.[13]

[1]Department of Genetics, Cell- and Immunobiology, Semmelweis University, Budapest, Hungary; [2]2nd Department of Pediatrics, Semmelweis University, Budapest, Hungary; [3]Department of Measurement and Information Systems, University of Technology and Economics, Budapest, Hungary; [4]Heim Pal Children Hospital, Budapest, Hungary and [5]Csertex Research Laboratory, Budapest, Hungary . Correspondence: Professor C Szalai, Department of Genetics, Cell- and Immunobiology, Semmelweis University, Nagyvárad tér 4, Ulloi ut 86, Budapest H-1089, Hungary.
E-mail: szalaics@gmail.com
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Peripheral blood samples were taken in remission phase in order to analyze only normal leukocytes and thus investigate germline polymorphisms. Moreover in our ALL population in the case of stem cell transplanted patients, their peripheral blood samples were taken before the transplantation, thus we used only the patient's germinal samples for genotyping.

The study was approved through institutional human ethics review board (Hungarian Scientific and Research Ethics Committee of the Medical Research Council, ETT TUKEB; Case No.:8-374/2009-1018EKU 914/PI/08), and all patients or their next of kin, caretakers or guardians provided written informed consent in accordance with the Declaration of Helsinki.

Summary of the patients' characteristics is shown in Table 1.

SNP selection

We selected six SNPs in the *CYP3A4* gene and two SNPs in the *CYP3A5* gene based on their minor allele frequencies (⩾10%) in Caucasian populations (Table 2) or possible relevancy in ALL chemotherapy and genotyped them in a relatively large sample cohort.

Each of the selected SNPs met the requirements of the Hardy–Weinberg Equilibrium, hence all the chosen eight SNPs could be involved in the analyses (Table 2).

For the interaction studies, additional 127 SNPs in 34 candidate genes in ALL, which had been previously genotyped in this population,[2,10] were also involved.

Table 1. Basic characteristics of the investigated population

Features of the ALL cases	
Gender (%)	
Male	290 (56.8)
Female	221 (43.2)
Years of age at diagnosis	
Mean (± s.d.)	6.4 (±4.2)
Median (range)	5.1 (1-18)
Risk category (%)	
Low risk, LR	94 (21.1)
Medium risk, MR	299 (67.0)
High risk, HR	53 (11.9)
Morphological subgroup (%)	
pre-B, B-ALL	372 (82.9)
pre-T, T-ALL	77 (17.1)
Cytogenetics (%)	
Normal	127 (27.9)
Hyperdiploidy	79 (17.4)
Other	249 (54.7)

Genotyping

Genomic DNA was isolated with QIAmp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) from peripheral blood taken retrospectively. SNPs of *CYP3A4* gene (rs2404955, rs12333983, rs2246709, rs2242480, rs4646437, rs35599367) and *CYP3A5* gene (rs15524, rs776746) were genotyped by Sequenom iPLEX Gold MassARRAY technology at the McGill University and Génome Québec Innovation Centre, Montréal, Canada. Only SNPs with genotyping call rate over 90% were included in the analysis.

Statistical Analysis

For data analysis, R statistical software[14] (R Foundation for Statistical Computing, Vienna, Austria; version 3.0.3) was used. Cox proportional-hazards (PH) regression models were applied for uni- and multivariate analysis using the survival package[15,16] in R. The significance of the models was evaluated by the log-rank test, and results with two-sided *P*-values < 0.05 were considered significant. In cases where the Cox PH model did not reach convergence, the log-rank test was used to test the difference between survival curves.

Kaplan–Meier curves were plotted using the survMisc package[17] in R.

Power analysis was conducted by bootstrapping. We simulated 10 000 data replicates by sampling with replacement from the original data set. We applied the statistical test to each generated data set, and estimated power as the percentage of cases in which the null hypothesis was rejected.

To determine the discriminative power of different risk-group assessment variables, we calculated the C-index[18,19] (condordance index) using the pec package[20] in R. Confidence intervals were estimated by bootstrapping. First, we simulated 100 data replicates by sampling with replacement from the original data set. For each of the replicates we calculated the *C*-index in 75 time points (from 0.2 years to 15 years by 0.2 years). Finally, the 95% confidence intervals were calculated by determining the 2.5th and 97.5th percentiles at each time point. The differences between the risk-group assessment variables were evaluated by a two-sided *t*-test at each time point. *P*-values were adjusted using the Benjamini and Hochberg method.[21]

In addition, the BN-BMLA software was used to detect strongly relevant variables with respect to 5-year event-free survival (EFS) and overall survival (OS) as a target variable. For a summary of the method, see Supplementary information; for a detailed description, see ref 11.

RESULTS

Effect of the *CYP3A4* and *CYP3A5* SNPs on the survival rates of the population

The survival rate of this population has already been described.[2] In summary, the OS rate was 85.5%, whereas the EFS rate was 81.0% in this pediatric ALL population.

The genotype and allele frequencies of the SNPs in the *CYP3A4* and *CYP3A5* genes in our study population are shown in Table 2. These values correspond to the frequency data in different databases and studies for Caucasian/European populations.[3–5,22–24]

Table 2. Information of the investigated SNPs in *CYP3A4* and *CYP3A5* genes

SNP (rs#)	*Gene*	*Alleles (1/2)*[a]	*Position in the genome*[b]	*Genotype Frequency*			*MAF*	*Position in the gene/alternative name*
				1/1[1]	*1/2*[1]	*2/2*[1]		
rs15524	*CYP3A5*	A/G	chr7:99245914	0.835	0.155	0.010	0.088	Exon, UTR
rs776746		G/A	chr7:99270539	0.844	0.149	0.007	0.082	Entron/ CYP3A5*3/*1
rs2404955	*CYP3A4*	G/A	chr7:99353279	0.768	0.214	0.018	0.124	Near-gene-3
rs12333983		T/A	chr7:99354114	0.771	0.211	0.018	0.123	Near-gene-3
rs2242480		C/T	chr7:99361466	0.773	0.217	0.010	0.119	Intron
rs4646437		G/A	chr7:99365083	0.747	0.237	0.016	0.134	Intron
rs2246709		A/G	chr7:99365719	0.538	0.395	0.067	0.264	Intron
rs35599367		G/A	chr7:99366316	0.914	0.084	0.002	0.044	Intron/ CYP3A4*22

Abbreviations: MAF, minor allele frequency; SNP, single-nucleotide polymorphism; UTR, untranslated region. [a]Alleles on the forward strand; 1, major allele; 2, risk allele. [b]Position according to GRCh37.p10 Genome Build 104.0.

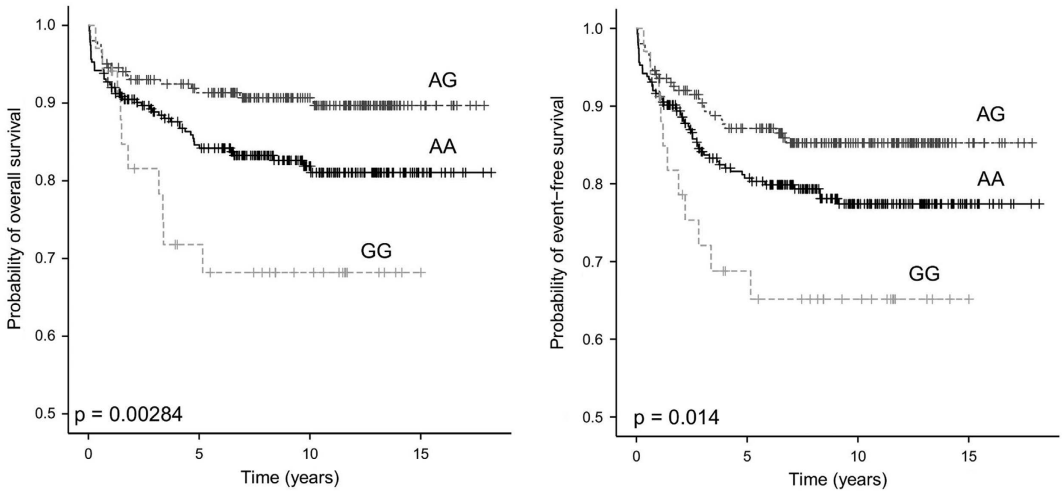

Figure 1. Kaplan–Meier survival curves. Overall survival (left) and event-free survival (right) according to rs2246709 of *CYP3A4*. *P*-value represents the statistical significance of the difference among the three genotypes.

Linkage disequilibrium between these SNPs was also calculated (Supplementary Figure S1, Supplementary Table S1).

The log-rank test was used to estimate whether the investigated SNPs influenced the overall or the EFS rates (Supplementary Table S2). An association between the OS rates and the rs2246709 SNP in the *CYP3A4* gene was observed ($P = 0.0028$). The association between rs2246709 and EFS rates was also observed ($P = 0.014$). These associations were also confirmed by the BN-BMLA method, which showed that the rs2246709 SNP influenced both the overall and the EFS rate with high posterior probabilities ($Pr > 0.85$ and $Pr > 0.55$, respectively). The survival rates in the different genotype groups are depicted in Figure 1.

Besides, associations between both the overall and the EFS rates and rs15524 in *CYP3A5* were also observed ($P = 0.031$ and $P = 0.00058$, respectively). Following further analyses of these effects, we found, that they occurred owing to the unexpectedly high relative proportion, but altogether low number of rare homozygous patients in the groups of died or relapsed patients (two and three patients in OS and EFS, respectively). These associations might be clinically important, but, because of the low number of patients, their validation is needed in a larger population.

No significant association was found with any analyses between the survival rates and the two functional polymorphisms in the two genes (rs776746 in *CYP3A5* and rs35599367 in *CYP3A4*), although a high LD value ($D' = 0.88$) was found between the rs2246709 and the rs35599367 (Supplementary Figure S1). But as the allele frequency of the rs35599367 (4.4%) was significantly lower than that of the rs2246709 (26.4%) this linkage proved to be only one-sided, which can also be deduced from the low r^2 (0.1) value between the two SNPs (see Supplementary Table S1). Nevertheless, the power of the statistical analyses was low in case of these functional SNPs (Supplementary Table S2), because of the low minor allele frequencies of these SNPs in the study population (Table 2).

Next, we calculated the hazard ratios for OS and EFS with univariate (unadjusted) and multivariate (adjusted) Cox's proportional regression analysis, including clinical parameters (risk-group assessment, lineage of ALL, cytogenetic abnormalities, treatment protocol and gender) and the rs2246709 SNP. As can be seen in Table 3 the effect of the rs2246709 on the OS was confirmed by this analysis. The most favorable genotype was the AG heterozygote, which showed a 0.52 (0.28–0.98) hazard ratio (HR) ($P = 0.04$, adjusted for all clinical parameters) (Figure 1).

There was, however, no significant association in this analysis between EFS and rs2246709 (see Supplementary Table S3).

We analyzed the association between the genotypes of the SNPs in the study and the different causes of death as well (Supplementary Table S4). As the number of patients was low or moderate in each category, the power of the analyses was generally low to detect significant associations. However, three SNPs in *CYP3A4* gene (rs2404955, rs12333983 and rs2246709) were significantly associated (*P*-value < 0.003) with survival when the cause of death was the progression of the disease (Supplementary Table S4).

Interaction between clinical parameters and genetic variations with rs2246709 in respect of survival

Then, with the help of BN-BMLA we searched for interactions that could influence the survival of the patients. To extend our analysis, we involved additional 127 SNPs in 34 genes which, according to the scientific literature and our own previous researches,[2,10] can have a role in the pathomechanism and pharmacogenomics of ALL. To be able to detect statistically significant interactions in this population we selected SNPs with minor allele frequency of $> 10\%$. As can be seen in Supplementary Table S5, according to BN-BMLA, among all these SNPs, only the *CYP3A4* rs2246709 showed high posterior probabilities in respect of both OS and EFS. In addition, the BN-BMLA revealed several potential interactions between these variables (Figures 2a and b). In the followings, we focus on interactions involving *CYP3A4*.

We observed an interaction between rs2246709 (*CYP3A4*) and the gender of the patients on OS (interaction ratio = 0.22, Figure 2a). We investigated this interaction with Cox PH regression analysis as well by including an interaction term in the model. As can be seen from the results the gender strongly influenced the effect of rs2246709 on both OS (Table 4) and EFS (Table 5). The influence of the gender on OS and EFS was especially strong in heterozygous and wild type homozygous patients. In case of patients with AG genotype the gender reversed the effect of this genotype relative to the AA females (HR = 1.53 in females, whereas 0.39 in males; HR = 0.26 (0.09–0.77); $P = 0.0158$ for AG males relative to AG females). AA homozygous males had a HR = 2.39 relative to AA females ($P = 0.012$). These correlations were similar in EFS.

According to the results the heterozygote genotype in males associated with the most favorable OS and EFS (Supplementary

Table 3. Univariate (unadjusted) and multivariate (adjusted) analysis of covariates in overall survival

	Univariate analysis				*Multivariate analysis* (N = *373, NE = 59,* P = *$9.3*10^{-6}$*)			
Covariate	N, *NE*	*HR*	*95% CI*	P-*value*	N, *NE*	*HR*	*95% CI*	P-*value*
Risk group	LR = 94, 7 MR = 299, 39 HR = 53, 20	2.87	1.87–4.40	**$1.4*10^{-6}$**	LR = 90, 7 MR = 244, 36 HR = 39, 16	2.53	1.63–3.94	**$3.8*10^{-5}$**
Lineage (Pre-B, B)	Pre-T, *T* = 77, 15 Pre-B, *B* = 372, 54	0.72	0.40-1.27	0.249	Pre-T, *T* = 64, 13 Pre-B, *B* = 309, 46	1.25	0.65–2.40	0.511
Cytogenetic								
Normal	127, 20	(1.00)			104, 20	(1.00)		
Hyperdiploid	79, 8	0.63	0.28–1.43	0.268	66, 5	0.46	0.17–1.23	0.122
Other	249, 44	1.14	0.67–1.93	0.638	203, 34	0.89	0.50–1.58	0.688
Protocol								
88–90	123, 18	(1.00)			98, 15	(1.00)		
95	244, 45	1.34	0.78–2.33	0.289	235, 43	1.60	0.87–2.95	0.129
2002	117, 10	0.76	0.35–1.66	0.493	40, 1	0.22	0.03–1.70	0.148
Gender (male)	Female = 221, 29 Male = 290, 46	1.26	0.79–2.01	0.326	Female = 166, 22 Male = 207, 37	1.15	0.66–2.00	0.629
rs2246709								
AA	275, 46	(1.00)			207, 39	(1.00)		
AG	202, 19	0.53	0.31-0.90	**0.020**	144, 14	0.52	0.28–0.98	**0.042**
GG	34, 10	1.85	0.93-3.66	0.079	22, 6	1.22	0.51–2.96	0.653

Abbreviations: CI, confidence interval; HR, hazard ratio; N, number of observations; NE, number of events. Note that during univariate analyses different numbers of observations were used in case of each covariate owing to data missingness. Results with *P*-value less than 0.05 are in bold.

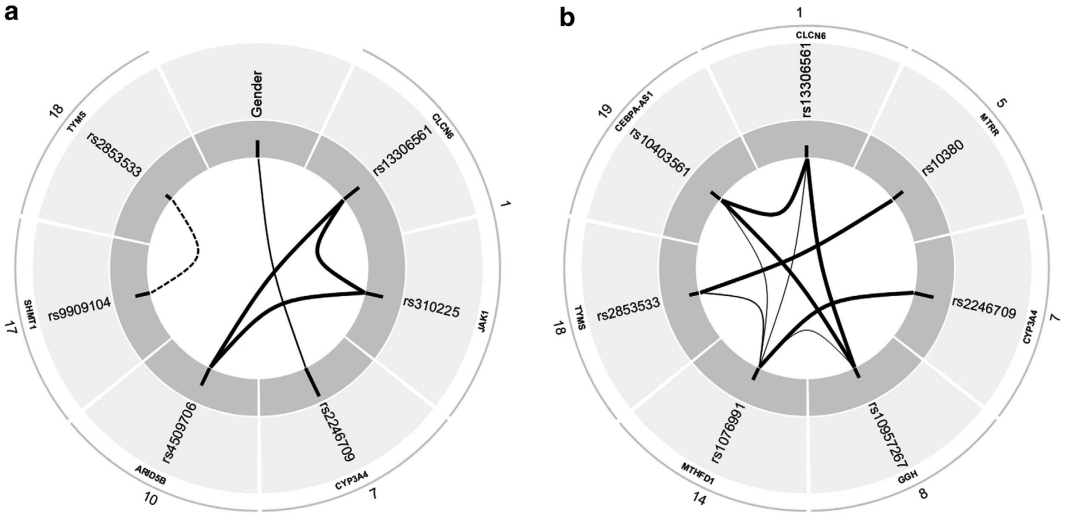

Figure 2. Redundancies and interactions according to the BN-BMLA method. The figure shows the magnitude of redundancies (dashed curved lines) and interactions (solid curved lines) between the variables in 5-year overall survival (**a**) and in 5-year event-free survival (**b**) according to the BN-BMLA method. See Methods for the computation of interaction and redundancy. The width of the curved lines is proportional to the strength of the effect. The a posteriori probability of the strong relevance of the variables is proportional to the length of the black columns next to the variable in the inner gray colored ring. The corresponding genes and chromosomes of the SNPs are shown on the outer ring.

Figure S2). The results remained significant after adjusting for risk-group assessment (Table 4 for OS and Table 5 for EFS) or for all clinical covariates (Supplementary Table S6).

We also observed an interaction between rs1076991 in the *MTHFD1* gene and rs2246709 (*CYP3A4*) on EFS (interaction ratio = 0.9, Figure 2b). The Cox regression analysis could confirm this interaction as well, which also remained significant after adjusting for all clinical covariates both in overall and in EFS (see Supplementary Table S7 and Supplementary Table S8, respectively). As can be seen in these tables, the different genotype combinations were associated with significantly different HR values (Supplementary Figure S3).

Modifying risk-group assessment based on gender and rs2246709 genotype information

In the following, we investigated whether the information on gender and rs2246709 genotype could be used to modify the

Table 4. Interaction between gender and *CYP3A4* (rs2246709) genotype, unadjusted and adjusted (with risk group) results in overall survival

	Unadjusted (N = *511*, *NE* = *75*, P = $2.2*10^{-4}$)				*Adjusted* (N = *446*, *NE* = *66*, P = $4.1*10^{-7}$)			
Covariate	N, *NE*	*HR*	*95% CI*	P-*value*	N, *NE*	*HR*	*95% CI*	P-*value*
Risk Group	–	–	–	–	LR = 94, 7 MR = 299, 39 HR = 53, 20	2.48	1.61–3.82	**$3.4*10^{-5}$**
rs2246709, gender								
AA, female	111, 11	(1.00)			100, 10	(1.00)		
AA, male	164, 35	2.39	1.21–4.70	**0.012**	149, 33	2.10	1.03–4.28	**0.041**
AG, female	101, 15	1.53	0.70–3.32	0.287	87, 12	1.31	0.57–3.04	0.526
AG, male	101, 4	0.39	0.12–1.23	0.109	83, 3	0.36	0.10–1.31	0.121
GG, female	9, 3	3.93	1.10–14.12	**0.036**	5, 2	4.16	0.91–19.05	0.066
GG, male	25, 7	3.10	1.20–8.00	**0.019**	22, 6	2.31	0.83–6.40	0.108

Abbreviations: CI, confidence interval; HR, hazard ratio; N, number of observations; NE, number of events. Note that owing to data missingness different numbers of observations were used in unadjusted and adjusted analyses. Results with *P*-value less than 0.05 are in bold.

Table 5. Interaction between gender and *CYP3A4* (rs2246709) genotype, unadjusted and adjusted (with risk group) results in event-free survival

	Unadjusted (N = *511*, *NE* = *95*, P = $8.3*10^{-4}$)				*Adjusted* (N = *446*, *NE* = *82*, P = $2.6*10^{-6}$)			
Covariate	N, *NE*	*HR*	*95% CI*	P-*value*	N, *NE*	*HR*	*95% CI*	P-*value*
Risk Group	–	–	–	–	LR = 94, 9 MR = 299, 52 HR = 53, 21	2.24	1.52–3.31	**$4.7*10^{-5}$**
rs2246709, Gender								
AA, female	111, 13	(1.00)			100, 12	(1.00)		
AA, male	164, 43	2.55	1.37–4.74	**0.0031**	149, 39	2.18	1.13–4.17	**0.019**
AG, female	101, 18	1.57	0.77–3.21	0.214	87, 14	1.31	0.61–2.84	0.489
AG, male	101, 10	0.84	0.37–1.91	0.673	83, 8	0.81	0.33–1.99	0.649
GG, female	9, 3	3.31	0.94–11.63	0.062	5, 2	3.73	0.83–16.71	0.085
GG, male	25, 8	3.13	1.30–7.54	**0.011**	22, 7	2.44	0.96–6.24	0.062

Abbreviations: CI, confidence interval; HR, hazard ratio; N, number of observations; NE, number of events. Note that owing to data missingness different numbers of observations were used in unadjusted and adjusted analyses. Results with *P*-value less than 0.05 are in bold.

risk-group assessment so that it would better predict the risk of the patients.

First, based on the Cox PH model including risk group, gender, rs2246709 genotype and the interaction of the latter two factors (see Tables 4 and 5), we calculated the predicted relative risk of death for all individuals. In practice, it means to calculate the predicted relative risk of all possible configurations of the values of the covariates. We derived a new risk assessment covariate (named RiskGroupCoxModel) by categorizing the possible predicted relative risk values into three groups according to the following rule: risk below 0.85 was assigned to low-risk group; risk between 0.85 and 3.0 was assigned to medium-risk group; and risk above 3.0 was assigned to high-risk group.

Next, we derived another risk assessment covariate (named RiskGroupCTree) by training a classification tree on the newly derived RiskGroupCoxModel covariate. Our aim was to derive simple rules that capture the essence of the Cox PH model. From the classification tree, the following assignment rules could be determined: (1) males with genotype AG of rs2246709 should be assigned to low-risk group regardless of the previous risk-group assessment. (2) Patients with genotype GG who belonged to the low-risk group should be assigned to medium-risk group regardless of the gender of the patient.

The difference between the number of patients according to the original and the two newly derived risk-group assessment variables can be seen in Supplementary Table S9.

We determined the discriminative power of the three risk-group assessments (the original and the two derivatives) by calculating the so-called *C*-index (concordance index; see Methods). This measure is the frequency of concordant pairs among all pairs of patients, that is the probability that a patient belongs to a higher risk group than another patient if he/she experiences an event at an earlier time point than the other patient. Figure 3 shows the results of our calculations. Both new risk-group assessment variables significantly outperformed the earlier risk-group assessment at every time point in case of OS (maximum of Benjamini and Hochberg-adjusted *P*-values < 0.001) with an average increase of 3.5 (RiskGroupCTree vs Risk Group) and 4.21 (RiskGroupCoxModel vs Risk Group) percentage points in *C*-index. The difference between RiskGroupCoxModel and RiskGroupCTree was not significant (minimum of Benjamini and Hochberg-adjusted *P*-values > 0.08).

DISCUSSION

In the present study, we found that a common SNP (rs2246709) in the *CYP3A4* gene significantly influenced the survival rates of the ALL patients after chemotherapy, which was strongly affected by the gender of the patients. Difference between genders was especially large in the AG heterozygotes where male gender was associated with a significantly higher survival rate compared with that of females.

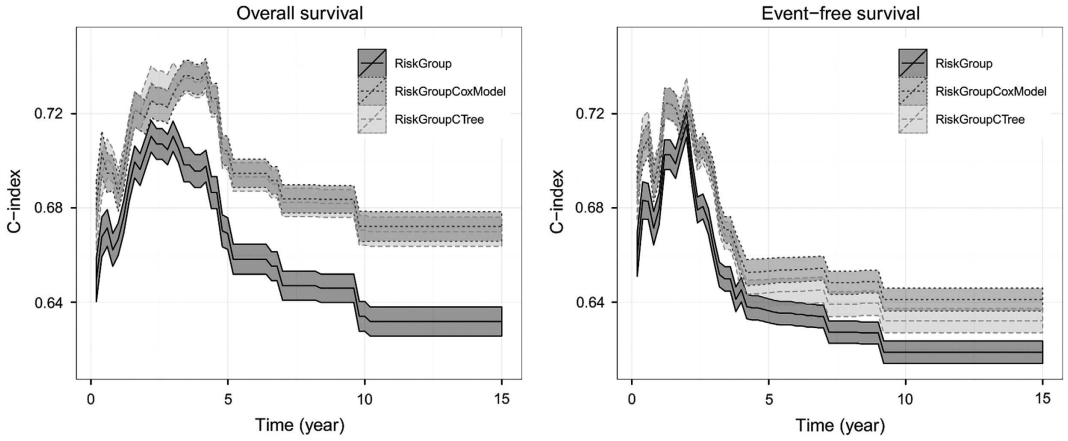

Figure 3. Comparing the discriminative power of risk-group assessments. The graphs show the estimated Concordance-index of different risk-group assessment variables during the full period of the study. The left panel refers to overall survival, and the right panel refers to event-free survival. Risk group is the original risk group assessment variable (solid black line). The other risk-group assessment variables are based on different covariates and interaction terms. RiskGroupCoxModel (gray dotted line) is based on the original risk group, gender, genotype of rs2246709 and the interaction of the latter two. RiskGroupCTree (light gray dashed line) is based on a classification tree learned on RiskGroupCoxModel variable. 95% confidence intervals of the estimates are shown as bands.

CYP3A4 is the major drug-metabolizing enzyme in humans but its role in ALL chemotherapy is quite complex. It has an important role in the clearance of vincristine, dexamethasone and doxorubicin, but vincristine and doxorubicin are also inhibitors, whereas dexamethasone is an inducer of CYP3A4. In addition, CYP3A4 not only catalyzes the activation of cyclophosphamide, but also catalyzes the formation of the neurotoxic chloroacetaldehyde leading to serious side effects.[25,26] Owing to these contradictory roles, it is impossible to predict the influence of the interindividual differences in CYP3A4 levels on the overall effects of chemotherapy in ALL. Although CYP3A4 activity varies widely, with 10- to 100-fold variation between individuals with 90% heritability, the genetic background of this has not yet been revealed. Recently, a functional SNP in intron 6 (rs35599367; *CYP3A4*22*) was identified that was associated with decreased CYP3A4 production and activity in liver cells and was correlated with statin dose requirement and tacrolimus pharmacokinetics.[4,5] In the same study the rs2246709 SNP was also found to be associated with an allelic mRNA imbalance, although the association was less significant and it was attributed by the authors to the LD with the rs35599367 SNP.

In our study the rs35599367 SNP did not influence the survival of the patients but the rs2246709 had a considerable effect on it. We also detected partial LD between the two SNPs, but because of the large differences in the allelic frequencies this was only one-sided, and thus the effect of the less frequent rs35599367 could not contribute significantly to that of the rs2246709.

There were large gender differences in the effect of the rs2246709 on survival rates. There are several data about the significantly higher CYP3A4 activity in females relative to males.[27–29] Interestingly, male heterozygosity was associated with the highest survival rate. When, according to our results we recalculated the risk-groups assessment, we found that males with genotype AG of rs2246709 should be assigned to low-risk group regardless of the previous risk-group assessment. In contrast, AA homozygosity was associated with a poorer survival rate in males relative to the females. Presently these findings cannot be explained. Theoretically it can be hypothesized that somehow the rs2246709, or a still unknown variation in linkage, influences the gender dependent regulation of the expression of the enzyme. Furthermore, perhaps because of the complex roles of the CYP3A4 in the chemotherapy, the two types of homozygosity seem to be less favorable in males in respect of responding to the therapy.

Involving additional 127 SNPs in 34 genes and applying our BN-BMLA method we also detected that the effect of the *CYP3A4* rs2246709 on survival was significantly influenced by a SNP in the *MTHFD1* gene. This gene is part of the folate pathway, which is the target of methotrexate. Earlier we found that the GG genotype of this SNP (rs1076991) increased the risk of B-cell ALL, but did not influence the survival rate.[10] CYP3A4 does not metabolize the methotrexate, and thus the effects of the two SNPs on the different pathways seem to be superimposing each other. But these results also show the difficulties of the studies of gene–gene interactions, especially when the different genotype combinations have different effects (Supplementary Figure S3). Here is known that the gender also influences the effects of rs2246709 on survival rates. But if gender were also involved in this analysis, it would create altogether 18 subgroups, which cannot be realistically analyzed in this number of patients. This shows the immense difficulties of the task of creating a decision support system involving all the genetic (for example, results of genome-wide association studies), clinical and environmental factors, which can influence the target variables (for example, response to a therapy).

In contrast, relative simple rules could be drawn from the results of the rs2246709 (*CYP3A4*) gender interactions (see Results). We calculated new risk assessments and showed that these, in respect of survival rates, significantly outperformed the earlier risk-group assessment at every time point in our population.

It must be noted that these results were obtained by a newly developed statistical method (BN-BMLA), which offers an automated and normative solution for the multiple hypothesis testing problem, which is one of the main limitations of the traditional (frequentist) statistical methods.[6–11] In addition, in contrast to the most similar studies, where the number of ALL patients is around or below 100,[30–32] in this study significantly more patients have been involved. There are, however, some weak points in the study. Although our sample set contains similar rate of relapsed patients to what was observed in the whole population,[12] patients who died during the chemotherapy due to therapy resistant progressive disease or due to infections or toxicities of therapy are underrepresented in our sample. Furthermore, in some patients certain data points were missing and thus different numbers of observations were used in some calculations.

It is also important to note, however, that the rs2246709 is a very frequent SNP with a heterozygous frequency of around 40% in different populations[5,33,34] (Table 2). This means, that in contrast to the much rarer functional SNPs (for example, rs35599367 with a MAF of 4.4%) even if it has only a weak effect it can have a large population impact and if our findings are confirmed in other populations, the possibility of its involvement in future risk assessment or personalized therapies should be investigated.

In summary, the most significant result of this study is that the interaction between the rs2246709 SNP in the *CYP3A4* gene and gender significantly influences the survival rate of chemotherapy of childhood ALL and this can change the assignment of patients to risk groups. If this finding is confirmed in other populations, it can have a considerable prognostic significance and therapeutic consequences.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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OTKA: http://www.otka.hu/
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