The role of genetic polymorphism of transporter proteins in acut lymphoblastic leukemia and other diseases; pharmacogenetic studies

Ph.D thesis

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Introduction

The acute lymphoblastic leukemia (ALL) is the most frequent pediatric hematological malignancy in Hungary. There is still little knowledge on the development of this disease, but genetic and environmental factor both contribute to the occurrence of the disease. Several genes were already described that might influence the risk of ALL. The ABC-transporters (ABC: adenosine triphosphate binding cassette) have important role in the protection of the mammal tissues against xenobiotics. Thus in can be supposed that their malfunctioning, altered expression level due to genetic reasons presumably contribute to the development of ALL. In order to gain more understanding on the risk of ALL we studied genetic variations which alter the function of ABCB1 and ABCG2 ABC-transporters. These are the 3435C>T (rs1045642, synonim) and 2677 G>T/A (rs2032582, Ala893Ser) SNPs (single nucleotid polymorphisms) in *ABCB1* gene and 34G>A (rs2231137, Val12Met) and 421C>A (rs2231142, Gln141Lys) SNPs in *ABCG2* gene, respectively. The *ABCB1* 3435C>T determines a rare codon, which cause an altered folding of the mRNA and reduced protein level. The *ABCB1* 2677 G>T/A SNP cause reduced transporter activity. The *ABCG2* 34G>A polymorphism results in impared membrane localisation, the *ABCG2* 421C>A SNP reduces the ATPase activity of ABCG2 protein.

Genomic regions with unknown function in the development of the disease might contribute to the appearance of the ALL. Genome-wide association studies (GWAS) are applied to map these regions. The result of GWA studies must be replicated in independent patient cohorts to validate the obtained results. According to a GWAS performed by a research group from England the rs3731217 SNP located in the first intronic region of *CDKN2A* (cyclin-dependent kinase inhibitor 2A) influences the development of ALL. In order to validate the results they analysed this relationship in larger populations, in which the Hungarian population was also included.

Nowadays children with acute lymphoblastic leukemia are cured in great majority, in 80-90% of the patients. But the long survival after ALL does not mean that the former patient is perfectly healthy. Approximately 40% of the survivors will develop health problems within 30 years of their initial cancer diagnosis. Frequently, patients die because of the late side effects, among others because of heart problems. There is an 8-fold increase in mortality due to cardiac problems among the survivors of childhood cancer. The prevention of cardiac impairment is particularly important in children as they might live for decades after the treatment. The early recognition of the abnormal cardiac function is important to stop the damaging therapy and to start the early treatment. Therefore it is essential to continuously monitor the patients to detect subclinical alterations which are without clinical symptoms but are detectable with diagnostic methods. Genetic variations, e.g. SNPs might alter the expression and function of xenobiotic metabolising enzymes, transporters of the xenobiotics or drug-targets. The aim of the pharmacogenetic studies is to tailor-make the therapy with personally choosing the drugs and drug doses on the base of the genetic background of the patient to improve the efficacy and safety of the treatment. The anthracyclines are part of the chemotherapy protocol used in the treatment of chilhood ALL. These belong to the most effective anticancer agents, but their usage is limited because their considerable damage to the cardiomyocites probably through the production of reactive oxygen species. The anthacyclines pass through the plasma membrane with passive diffusion and are eliminated from the cell with the help of the ABC-transporters, thus the main determinant of the anthracycline level in the cell is the function of the ABC-transporters. The ABCC1 transporter is involved in the transport of anthracyclines and has also important role in the defense mechanism against oxidative stress. Genetic polymorphism resulting in altered protein function might influence the effective response against oxidative stress induced by the anthacyclines and the appropriate protection of the cardiomyocytes.

Aims

I had the following aims during my work:

- To study functional polymorphisms in the *ABCB1* and *ABCG2* ABC-transporter genes in the development of acute lymphoblastic leukemia which play role in the protection of the body against xenobiotics. To compare the allelic and haplotype frequencies and genotype combinations determined by 3435C>T, 2677G>T,A polymorphisms in *ABCB1* gene and 34G>A and 421C>A polymorphisms in *ABCG2* gene among the patient and control groups. To analyse the effect of the polymorphisms on the characteristics and outcome of the disease.

- To study the ALL risk within an international cooperation with IALLGC (International childhood Acute Lymphoblastic Leukaemia Genetics Consortium). To study the childhood ALL on adequate sample size and to validate the result obtained from a previous GWAS. To perform the genotyping of rs3731217 polymorphism in *CDKN2A* (cyclin-dependent kinase inhibitor 2A) gene on our patient population and to contribute to the confirmation of the validity of the result.

- To study the genetic background of the development of cardiotoxicity as side effect of anthracyclines the very potent anticancer agents in the treatment of ALL. To characterize the heart function with fractional shortening calculated from the echocardiographic measurements performed regularly during the treatment. To search for association between the heart function and polymorphism of *ABCC1* ABC-transporter responsible the transport of anthracyclines out of cardiomyocites. To study the associations between the decrease of fractional shortening and 9 single nucleotide polymorphisms in the *ABCC1* gene.

Methods

Our patient cohort consisted of children with acute lymphoblastic leukemia diagnosed between 1990 and 2002 in Hungary. They were treated with the ALL Berlin-Frankfurt-Münster (BFM) 90 or 95 chemotherapy protocols. We analysed the all available clinical data in each study from our DNA and clinical databank. We used DNA collected from healthy blood donors as controls.

We studied the role of *ABCB1* and *ABCG2* genes in the susceptibility of development of ALL in population consisting of 396 patients and 192 controls. We selected the polymorphisms from the literature. We performed the genotyping of 3435C>T and 2677 G>T/A *ABCB1* polymorphisms with multiplex minisequencing with SNaPshot method. We genotyped the 34G>A and 421C>A polymorphisms of *ABCG2* gene with LightCycler PCR method. We estimated haplotypes and the frequency of the haplotypes by Haploview 4.1 software. We performed χ^2-test and logistic regression to statistically analyse the data.

In the international cooperation we genotyped 550 patients and 450 controls with KasPar^{TM} technics. In the IALLGC data from a previous genome wide association study was reanalysed. The rs3731217 SNP in *CDKN2A* associated with the susceptibility of ALL and we genotyped this SNP in this study. We analyzed the clinical data with χ^2-test and logistic regression.

To study the cardiotoxic effect of antracyclines we analysed the data collected from the patients medical records retrospectively. We calculated left ventricular fractional shortening from the echocardiographic measurements. Altogether the fractional shortening data were available from 235 patients. We selected 1-2 polymorphisms from every haplotype block of the *ABCC1* gene. We genotyped the selected 9 *ABCC1* SNPs with SNPstream method. We used multi-adjusted GLM (general linear model) to statistically analyze the data.

Results

We studied the role of *ABCB1* 3435C>T and 2677 G>T/A and *ABCG2* 34G>A and 421C>A polymorphisms in the development of childhood ALL. There were no significant difference between the patient and control group in the allelic frequencies and genotype distribution. By comparing the estimated haplotype frequencies we found that there were no significant differences in the prevalence of the two predominant *ABCB1* haplotypes (TT and GC), while the rare GT haplotype was more frequent in cases than in controls (9.4% vs. 3.9%; p=0.002; OR=2.5 (CI 95%: 1.4–4.4)). The TC haplotype were more prevalent in controls (6.7%) vs. patients (3.0%), p=0.006; OR = 0.4 (Cl 95%: 0.2–0.8). The haplotype distribution did not differ between the two groups in the *ABCG2* gene.

The genotype combinations 2677GT/3435TT and 2677GG/3435CT of the positions 2667 and 3435 in the *ABCB1* gene occurred more frequently in ALL patients. A frequencies were 8.7% in the patients, 3.2% in controls (p=0.02; OR=2.88 (1.19–7.11)) and 7.4% in the patients 2.7% in controls (p=0.04; OR=2.97 (1.12–7.77)) respectively. The 2677TT/3435CT genotype combination was more frequent in controls (7.9%) than in patients (2.1%) (p=0.002; OR=0.26 (0.11–0.61). The distribution of the genotype combinations in the *ABCG2* gene did not differ significantly between cases and controls.

Subsequently we investigated whether the alleles, haplotypes and genotype combinations influence the clinical characteristics of ALL. None of these genetic variants showed association with age at diagnosis, sex, immunophenotype, hyperdiploidy, risk group, relapse and leukaemia related death.

In the international cooperation the result of a genome association study performed on Caucasians by a research group from England was validated on larger population, in which the Hungarian patient population with acute lymphoblastic leukemia was also included. The IALLGC reanalysed the genotype data of a former genome wide association study performed on 907 patients and 2398 controls with the genotyping of 291371 SNPs. Altogether 34 statistically significant ($p<0.0001$) SNPs were genotyped on a larger, independent German population consisting of 1428 patients with ALL and 1516 controls, in which only one SNP (rs3731217) remained significant ($p=1.15x10^{-7}$). This polymorphism is located in the chromosome region 9p21.3 in the first intron of *CDKN2A* (cyclin-dependent kinase inhibitor 2A) gene.

The obtained result was validated also on Hispanic (148 patients and 187 controls), Hungarian (550 patients and 450 controls) and Canadian (260 patients and 266 controls) populations. The Hungarian population belongs to our sample-, and databank, the genotyping was performed by our group, thus I discuss this result in my PhD thesis.

According to our results the *CDKN2A* rs373121G allele did not influence the risk of ALL in Hungarian population: p=0.1, OR=0.83 (CI 95%: 0.58–1.17). When we analysed the B-, and T-cell group of ALL separately, there was no significant association in the B-cell ALL in the Hungarian population: p=0.5, OR=0.91 (CI 95%: 0.68–1.22). In the Hungarian population with T-cell ALL the G allele influenced the risk of ALL significantly: p=0.046, OR=0.60 (CI 95%: 0.38–0.93).

When pooled genotype data from all of the populations was analysed the obtained result showed that the G allele significantly influence the risk of development of ALL ($p= 3.01\times10^{-11}$, OR:0.71, CI 95%: 0.64–0.78). When we analysed the data of all of the populations in the B-, and T-cell ALL, the allele also influenced the risk: $p=5,29x10^{-10}$, OR=0.72, (0.64–0.80), and $p=1,88x10^{-7}$, OR=0.68, (0.58–0.79), respectively.

To understand better the genetic background of anthracycline cardiotoxicity we studied whether nine polymorphisms of the *ABCC1* gene influence the function of the heart in children with ALL after treatment with anthracyclines. We characterized the heart function with left ventricular fractional shortening (LVFS). We analyzed the LVFS data in the time of diagnosis, at the end of the treatment and at the time of the latest follow up.

The LVFS data calculated from the echocardiographic measurements performed at the time of diagnosis did not differ in the three genotype group in the case of the polymorphisms. After the chemotherapy, at the end of the treatment patients with rs3743527TT genotype had significantly reduced LFVS data (34.0%) compared to patients with CC (39.5%) or CT (39.3 %) genotype (p=0.001). We could observe similar tendency in the LVFS at the time of the latest echocardiography. Patients with rs3743527TT genotype had the latest LVFS value 35.3%, the heterozygotes 38.9% and the CC homozygotes 38.7%. There was an association of harboring the rs246221 T allele and LVFS at the time of the latest echocardiography. Patients with TC and TT genotype had reduced LVFS (38.4% and 38.5%) compared to patients with CC genotype (40.7%, p=0.027).

We analyzed the effect of genotype combinations determined by the two significant SNPs. We compared the LVFS data of patients with rs3743527TT and rs246221TC or rs246221TT genotype (TT-TC/TT) with patients with all other genotype combination. The LVFS at the time of diagnosis was similar in the two group. However, after the chemotherapy the LVFS was significantly lower in patients with the TT-TC/TT genotype combination (34.0%) compared to the other patients (39.4%) (p=0.001).

Among the clinical factors included in the analysis of LVFS, the age at the time of diagnosis, treating hospital, chemotherapy protocol were found to be significant cofactors. While the gender, total anthracycline dose, dexrazoxane usage were not significant. In the case of the two significant SNPs, none of these parameters overcame the effect of the genotypes.

Conclusions

The studied polymorphisms of *ABCB1* and *ABCG2* genes and the haplotypes of these do not influence the development of childhood acute lymphoblastic leukemia on the examined population. The rare GT haplotype of *ABCB1* associated with increased, the TC haplotype with decreased risk of ALL. The rare genotype combinations GT/TT and GG/CT (2677G>T/A-3435C>T) generated from the two SNPs in *ABCB1* gene were more frequent in the patients, while the TT/CT was more frequent in controls. We can conclude that polymorphisms in *ABCB1* important in the defense against xenobiotics might influence the risk of ALL.

The *CDKN2A* rs3731217 polymorphism studied in the international cooperation did not confer susceptibility to ALL in our population, neither to B-cell ALL. But the development of T-cell ALL was significantly influenced by the rs3731217 T allele. According to the data of all of the six studied populations this polymorphism influenced the risk of ALL when all of the patients were analyzed together and also in subpopulations (B-, and T-cell ALL) separately. Thus the polymorphism of the *CDKN2A* gene might influence the development of ALL.

We found correlation with the reduction of left ventricular fractional shortening (LVFS) expressing the cardiotoxic effect of anthracyclines and polymorphisms of *ABCC1* gene. Patients with *ABCC1* rs3743527TT genotype had significantly reduced fractional shortening calculated from the echocardiography performed at the end of the treatment compared to patients with CC or CT genotype. Patients harboring the rs246221 T allele had reduced LVFS compared to patients with CC genotype at the time of the latest echocardiography. Patients with rs3743527TT-rs246221TC/TT genotype combination determined by the rs3743527 and rs246221 SNPs had reduced LVFS at the end of the treatment compared to the other patients. Variants of *ABCC1* might influence the defense of the body against anthracyclines and might cause the reduction of heart function.

List of own publications

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