The Impact of Drug Metabolism Gene Polymorphisms on Therapeutic Response and Survival in Diffuse Large B-Cell Lymphoma Patients

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ABSTRACT: **Background:** Diffuse large B-cell lymphoma (DLBCL) accounts for 30% of all non-Hodgkin lymphomas (NHL) and 80% of agressive lymphomas. Besides the traditional International Prognostic Index (IPI), some other factors may also influence the prognosis of DLBCL patients.

Objectives: To study how the genetic polymorphisms in the metabolic pathway influence the event-free and overall survivals and therapeutic responses in DLBCL.

Methods: The study was comprised of 51 patients (32 men, 19 women). The average age was 53.1 years. DLBCL was diagnosed between 2011 and 2016 and the average follow-up time was 3.78 years. These patients received 1–8 cycles (an average of 6.2 cycles) of rituximab, cyclophosphamide, doxorubicin, vincristin, prednisolon (R-CHOP) immunochemotherapy. Real-time polymerase chain reaction was used to determine the genetic polymorphisms of *CYP2E1*, *GSTP1*, *NAT1*, and *NAT2* genes.

Results: Our results showed that the polymorphisms of *CYP2E1*, *GSTP1*, and *NAT1* genes did not influence the prognosis of DLBCL patients significantly. In terms of the *NAT2* gene, GG homozygous patients showed slightly better therapeutic response and survival results compared to those bearing an A allele; however, the differences were not statistically significant.

Conclusions: Our results could not confirm that genetic polymorphism in metabolic pathways has any predictive role in DLBCL.

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KEY WORDS: genetic polymorphism, non-Hodgkin lymphoma (NHL), event-free survival, therapeutic efficacy, diffuse large B-cell lymphoma (DLBCL)

Diffuse large B-cell lymphoma (DLBCL) accounts for 30% of all non-Hodgkin lymphoma cases and involves 80% of agressive lymphomas [1]. The prognosis is determined by using International Prognostic Index (IPI) scores that considers the patient's age, disease stage, serum lactate dehydrogenase (LDH) level, Eastern Cooperative Oncology Group (ECOG) performance state, and extranodal involvement [2]. Several other factors have been investigated to determine whether they have any prognostic significance in DLBCL. These factors include the result of interim positron emission tomography-computed tomography (PET-CT) scan, which may point to a favorable outcome if negative. The gene expression profile may also influence the therapeutic response and survival data of DLBCL patients [3,4]. The pathogenetic factors in the development of agressive lymphomas include genetic changes,infections, and immunological disorders, as well as several toxins and chemicals such as insecticides, pesticides, hair dyes, and tobacco smoke. Various enzymes eliminate these toxic agents after they enter the body [5]. Metabolic enzymes like cytochrome P450 (CYP), glutathione-S-transferase (GST), and N-acetyl-transferase (NAT) are involved in the elimination of carcinogenes and drug metabolism. Consequently, these metabolic enzymes may play a role not just in the lymphomagenesis, but may also influence the therapeutic response and survival of lymphoma patients. CYP enzymes initiate various oxydative pathways in the first phase of detoxification, while GST conjugates toxic agents with glutathione in the second phase. The polymorphisms of genes decoding these metabolic pathway enzymes may influence the development and remission of lymphomas [6-9]. Four single-nucleotide polymorphisms (SNPs) are derived from four genes involved in metabolic pathways. These genes include *CYP2E1* (rs2070673), *GSTP1* (rs1695), *NAT1* (rs4986782), and *NAT2* (rs1208), which we chose for genotyping [Table 1]. Our aim was to investigate whether drug metabolism gene polymorphisms have any effect on the treatment responses and survival data in DLBCL.

PATIENTS AND METHODS

CLINICAL DATA

The clinical files of DLBCL patients were reviewed with particular reference to age, gender, IPI, response to treatment, and survival. Examining the survival rates, overall survival was determined by consideration of death events due to any reason, while event-free survival was determined by consideration of death events, relapses, or disease progression that indicated further treatment. Descriptive statistical analysis was used to characterize the patient populations. Normality of the parameters

Table 1. The examined alleles, genotypes, and SNPs of metabolic genes

Gene	Number of SNP	Alleles	Genotypes
CYP2E1	rs2070673	A/T	TT, AT, or AA
GSTP1	rs1695	G/A	AA, AG, or GG
NAT1	rs4986782	A/G	GG, AG, or AA
NAT2	rs1208	A/G	AA, AG, or GG

SNP = single-nucleotide polymorphism

were examined by applying the Wilk–Saphiro test. Comparing two groups, the *F*-test and *t*-test were administered by normal distribution of the parameters, otherwise the non-parametrical Mann–Whitney test was applied. Differences were significant if the probability level was less than 5% ($P < 0.05$). Survival rates were calculated using the Kaplan–Meier method, while the survival data were compared using the log-rank test.

DETERMINATION OF DRUG METABOLISM GENE POLYMORPHISMS

High molecular weight DNA for genotyping was extracted from peripheral blood samples according to the manufacturer's recommendation using a QiaAmp DNA Blood Mini Kit (Qiagen GmbH, Germany). DNA was quantitated by UV absorption at 260 and 280 nm.

Genotyping of the single nucleotide polymorphisms (SNPs) was conducted by real-time polymerase chain reaction (PCR). We used a real-time PCR method that proved to be faster than the conventional methods based on restriction enzyme digestion. Genotyping of the single nucleotide substitutions were carried out using the allelic discrimination assay. PCR primers and TaqMan probe specific for the polymorphisms (C_3237198_20, C_572769_C, C_2431871_30, C_1204334_50) were purchased from Applied Biosystems (Foster City, CA, USA). The assay enables scoring of both alleles in a single well. Real-time PCR was performed using Corbett Rotor-Gene RG-3000 (Qiagen, Hilden, Germany) equipment, which was set to detect FAM and VIC reporter dyes simultaneously. The PCR reaction was carried out in a 20 µl reaction volume containing TaqMan Universal Master Mix (2X, 4331182, Applied Biosystems), TaqMan genotyping Assay (20X), and optimized quantities of genomic DNA. The Universal Master Mix contained AmpliTaq Gold DNA Polymerase, AmpErase UNG, dNTPs with dUTP, passive reference, and optimized buffer components. Reactions were set up in duplicate. Thermal cycling was initiated by incubation at 95°C for 10 minutes for optimal AmpErase UNG activity and activation of AmpliTaq Gold DNA polymerase. After this initial step, 40 cycles of PCR were performed. Each PCR cycle consisted of heating to 92°C for 15 seconds for melting, and to 60°C for 1 minute for annealing and extension.

RESULTS

Altogether 51 patients, 32 males, 19 females, were involved in the study. Their mean age was 53.1 years. They were all diagnosed with DLBCL between February 2011 and November 2016 and received 1–8 cycles (6.2 cycles as average) of R-CHOP-14 or R-CHOP-21 chemotherapies. The data collection was finished in May 2016, the mean follow-up time was 3.78 years. Of the patients, 42% had activated B-cell type, 24% had germinal center type disorder, and the rest were not classified. Therapeutic response and survival data in terms of the four drug metabolism gene polymorphisms (*CYP2E1*, *GSTP1*, *NAT1*, and *NAT2*) resulted in the following outcomes.

CYP2E1 GENE

In terms of the *CYP2E1* gene, encoding an important member of cytochrome P450 enzyme network, TT genotype was found to be dominant. There was no significant difference in clinical features and therapeutic response data between patients bearing AA, AT, or TT genotypes [Table 2]. Patients with AA genotype were found to have a bit more favorable overall and event-free survival results than those with either AT heterozigosity or TT homozigosity [Figure 1A and Figure 1B].

GSTP1 GENE

With respect to the *GSTP1* gene, the homozygous AA genotype was found to be most common when examining *GSTP1* gene polymorphisms and only five patients bore two G alleles. Clinical features and therapeutic responses were quite similar in all three groups. There were no significant differences found in the overall and event-free survival results [Table 2].

NAT1 GENE

In terms of the *NAT1* gene, no AA homozygous patients were found. Overall response rate and complete remission data were more favorable in AG heterozygous than in GG homozygous patients; however, the differences were not significant [Table 2]. Survival results were very similar in the two groups [Figure 2A and FIgure 2B].

NAT2 GENE

The distribution of A and G alleles was quite steady when examining the *NAT2* gene. GG homozygosity seemed to be more favorable in terms of therapeutic response and survival results; however, the differences were not statistically significant [Table 2].

DISCUSSION

In our study, therapeutic response and survival data of 51 diffuse large B-cell lymphoma patients were investigated in terms of the polymorphisms of four drug metabolism genes (*CYP2E1*, *GSTP1*, *NAT1*, and *NAT2*). *CYP2E1*, a member of the CYP enzyme family, is expressed mainly in the liver and participates in the metabolism of nitrose amins and several other small-sized toxic molecules entering the body. The polymorphism of the *CYP2E1* gene may play a role in the development of

Table 2. *CYP2A1*, *GSTP1*, *NAT1*, and *NAT2* polymorphisms and their relationships

CYP2E1				
CYP2E1 genotype	AA	AT	TT	*P*
Number of patients (%)	6 (12)	9 (18)	36 (70)	–
Female/male ratio	2/4	3/6	14/22	NS
Average age, years (range)	62.5 (37-70)	49.82 (19-83)	53.5 (25-86)	NS
Average IPI score	1.8	2.1	1.7	NS
Complete remission (%)	4 (66)	6 (66)	28 (77)	NS
Partial remission (%)	1 (17)	2 (22)	2 (6)	NS
Non-responders (%)	1 (17)	1 (12)	6 (17)	NS
Overall response (complete remission + partial remission) (%)	5 (83)	8 (88)	30 (83)	NS

NAT1				
NAT1 genotype	AA	AG	GG	*P*
Number of patients (%)	0	7 (14)	44 (86)	–
Female/male ratio	–	2/5	17/27	NS
Average age, years (range)	–	47.82 (19–83)	52.4 (28–86)	NS
Average IPI score	–	1.9	2.2	NS
Complete remission (%)	–	5 (72)	30 (68)	NS
Partial remission (%)	–	1 (14)	5 (11)	NS
Non-responders (%)	–	1 (14)	9 (21)	NS
Overall response (complete remission + partial remission) (%)	–	6 (86)	35 (79)	NS

GSTP1				
GSTP1 genotype	AA	AG	GG	*P*
Number of patients (%)	24 (47)	22 (43)	5 (10)	–
Female/male ratio	8/16	9/13	2/3	NS
Average age, years (range)	61.4 (39–83)	43.5 (19–63)	56.5 (25–66)	NS
Average IPI score	1.9	2	1.7	NS
Complete remission (%)	19 (80)	16 (72)	4 (80)	NS
Partial remission (%)	1 (4)	2 (9)	0	NS
Non-responders (%)	4 (16)	4 (19)	1 (20)	NS
Overall response (complete remission + partial remission) (%)	20 (84)	18 (81)	4 (80)	NS

NAT2				
NAT2 genotype	AA	AG	GG	*P*
Number of patients (%)	19 (37)	22 (43)	10 (20)	–
Female/male ratio	7/12	7/15	5/5	NS
Average age, years (range)	57.5 (23–70)	49.82 (19–83)	53.5 (25–86)	NS
Average IPI score	1.9	2	1.7	NS
Complete remission (%)	13 (68)	15 (68)	7 (70)	NS
Partial remission (%)	3 (16)	2 (9)	2 (20)	NS
Non-responders (%)	3 (16)	5 (23)	1 (10)	NS
Overall response (complete remission + partial remission) (%)	16 (84)	17 (77)	9 (90)	NS

NS = not significant

NHL. Moreover, the efficacy of R-CHOP treatment may vary in patients having different genotypes, as *CYP2E1* contributes to the metabolic process of both cyclophosphamide and rituximab [10]. In our experience, complete remission rates and event-free survival data were slightly more favorable in patients being homozygous for the T allele; however, the overall survival results were similar in all groups. Interestingly, Cho et al. [1] found that individuals bearing the T allele had an elevated risk for the development of DLBCL.

Glutathione-S-transferase has an important role in the metabolization of several drugs, such as prednisolon and vincristin, and consequently it influences the efficacy of treatment

Figure 1. [A] Overall survival results in case of *CYP2E1* polymorphism **[B]** Event-free survival results in case of *CYP2E1* polymorphism

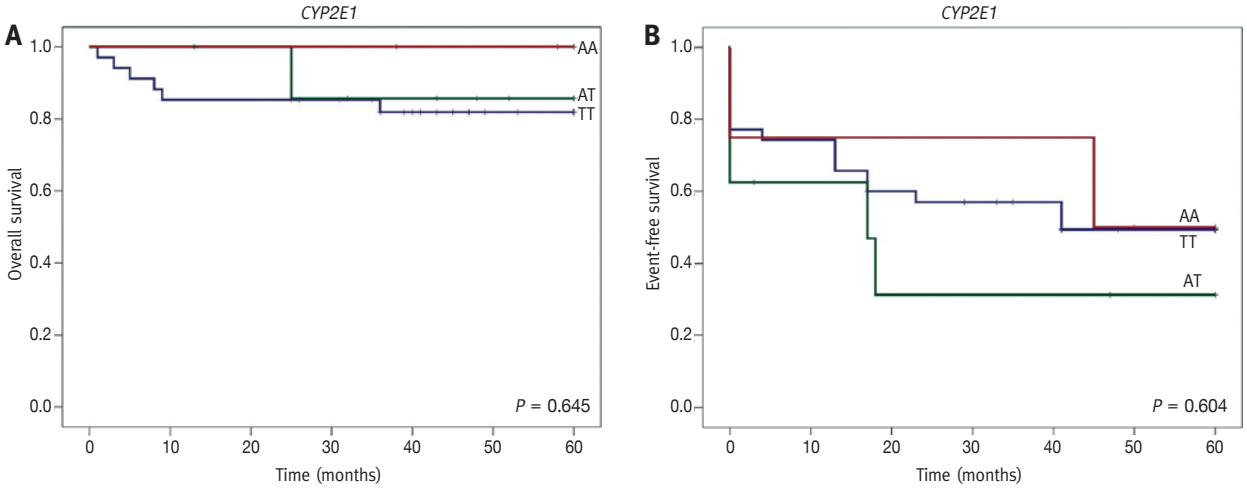
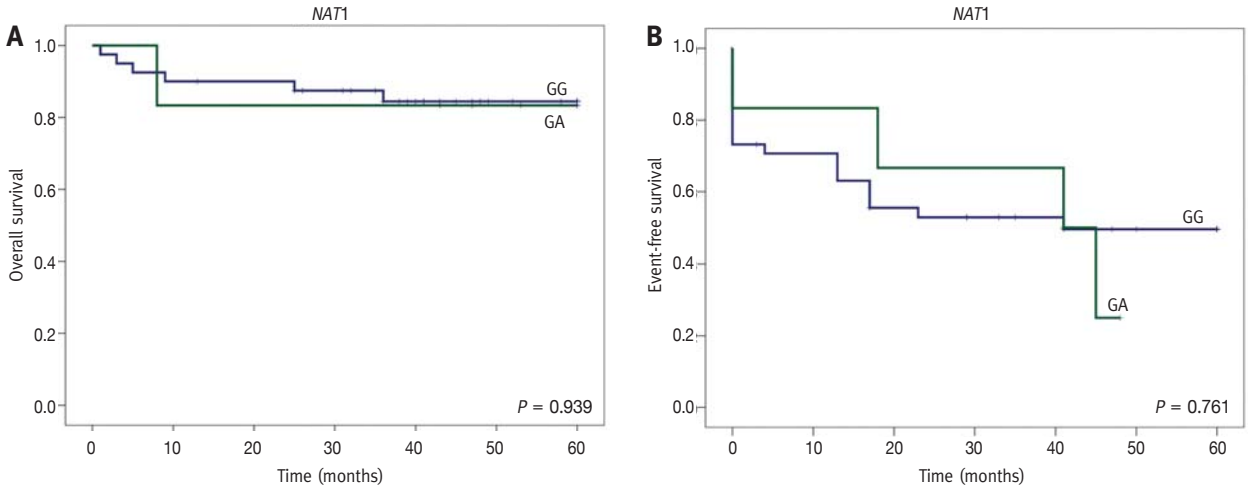


Figure 2. [A] Overall survival results in case of NAT1 polymorphism **[B]** Event-free survival results in case of NAT1 polymorphism



and the development of any toxicities [11-14]. Kim et al. [15] found that the AG genotype of *GSTP1* AG is associated with a lower risk for NHL development. Ibrahim and colleagues [16] reported similar results, as the presence of the G allele was associated with a lower prevalence of NHL in a test population. They also investigated the risk for lymphoma development in terms of both smoking and genetic alterations, but found no significant associations. Han and co-authors [10] declared that markedly favorable survival results can be expected in those NHL patients in which the deletion of *GSTT1* isoenzymes were detected; however Cho et al. [1] found that this deletion was associated with a higher toxicity of R-CHOP treatment. In the presence of the G allele of *GSTP1* isoenzyme, markedly higher myelo- and gastrointestinal toxicities can be observed [17].

The enzymes NAT1 and NAT2 also play an important role in the elimination of toxic agents. Earlier studies reported that the polymorphism of *NAT1* influences cell growth and may contribute to the development of etoposide resistance [18]. Moreover, the survival of children suffering from neuroblastoma may be associated with N-acetyl-transferase gene polymorphism [19]. Han and co-authors [10] concluded that *NAT1* polymorphism plays an important role in tumor progression and the prognosis of cancer patients. We found GG genotype in most of our DLBCL patients; however, the presence of the A allele was associated with a markedly favorable survival result. Our results also highlighted that the *NAT1* gene polymorphism may have a role in the therapeutic response to R-CHOP treatment, as complete remission was more commonly achieved in GA heterozygous patients. In terms of the *NAT2* gene, the results were quite similar, as bearing the G allele was associated with a markedly favorable prognosis and therapeutic response.

Our results underline that the polymorphisms of some particular drug metabolism genes (*GSTP1*, *CYP2E1*, *NAT1*, *NAT2*) do not have any statistically significant effects on the prognosis and survival of diffuse large B-cell lymphoma patients; however, relatively few patients were involved in the study. Nevertheless, we predict that genomic tests will help us to determine the prognosis and to choose a personalized treatment for each patient.

CONCLUSIONS

Our results could not confirm that genetic polymorphism in metabolic pathways has any predictive role in diffuse large B-cell lymphoma but other studies reported on the importance of genetic polymorhism in metabolic pathways in DLBCL and these studies showed the role of genomic tests in lymphoma treatment.

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Capsule

Overcoming a barrier to inflammatory bowel disease

Inflammatory bowel disease (IBD) is a group of disorders linked to inflammation of the gastrointestinal tract. Colitis is a type of IBD that affects the inner lining of the colon and has been linked to a gene known as *C1orf106*. **Mohanan** and co-authors found that *C1orf106* encodes a protein that stabilizes the integrity of epithelial junctions and enhances barrier defense. IBD-associated mutations in *C1orf106* lead to greater cytohesin-1 protein levels, changes in E-cadherin localization and enhanced susceptibility to intestinal pathogens. Modulation of *C1orf106* may thus hold promise for treating colitis and other IBDs.

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Capsule

Association between estimated cumulative vaccine antigen exposure through the first 23 months of life and non-vaccine-targeted infections from 24–47 months of age

Some parents are concerned that multiple vaccines in early childhood could weaken their child's immune system. Biological data suggest that increased vaccine antigen exposure could increase the risk for infections not targeted by vaccines. **Glanz** and colleagues examined estimated cumulative vaccine antigen exposure through the first 23 months of life in children with and without non-vaccine-targeted infections from 24–47 months of age. A nested case-control study was conducted in six U.S. health care organizations participating in the Vaccine Safety Datalink. Cases were identified by International Classification of Diseases codes for infectious diseases in the emergency department and inpatient medical settings and then validated by medical record review. Cases of non-vaccine-targeted infection were matched to controls by age, gender, health care organization site, and chronic disease status. Participants were children ages 24 through 47 months, born between 1 January 2003 and 31 September 2013 and followed until 31 December 2015. Among the 944 patients (193 cases and 751 controls), the mean age was 32.5 ± 6.3 months, 422 were female (45%), and 61 (7%) had a complex chronic condition. Through the first 23 months, the estimated mean cumulative vaccine antigen exposure was 240.6 ± 48.3 months for cases and 242.9 ± 51.1 months for controls. The between-group difference for estimated cumulative antigen exposure was -2.3 (95% confidence interval [95%CI], -10.1 to 5.4, $P = 0.55$). Among children 24–47 months of age with non-vaccine-targeted infections vs. those without, the matched odds ratio for estimated cumulative antigen exposure through age 23 months was not significant (matched odds ratio, 0.94, 95%CI 0.84 to 1.07). The authors concluded that among children 24–47 months of age with emergency department and inpatient visits for infectious diseases not targeted by vaccines, compared with children without such visits, there was no significant difference in estimated cumulative vaccine antigen exposure through the first 23 months of life.

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