The association between multidrug resistance-1 gene polymorphisms and outcomes of allogeneic HLA-identical stem cell transplantation

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The impact of single nucleotide polymorphisms of two loci (C3435T and G2677T/A) of the multidrug resistance-1 gene (*MDR1*) was investigated in 82 patients undergoing allogeneic stem cell transplantation (SCT). The GG genotype on G2677T/A loci was associated with higher non-relapse mortality than was the non-GG genotype (67% vs. 32%, p=0.0073), but not the C3435T (p=0.2026) or *MDR1* haplotype (p=0.2238). Accordingly, overall survival was significantly correlated with the G2677T/A genotype (p=0.0048). Multivariate analysis also showed that the GG genotype at G2677T/A had an unfavorable prognosis in terms of overall survival (p=0.003) and non-relapse mortality (p=0.031). In conclusion, the G2677T/A genotype seems to be associated with transplantation outcomes, especially non-relapse mortality.

Key words: single nucleotide polymorphism, multidrug resistance-1 gene, allogeneic stem cell transplantation.

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P-glycoprotein (P-gp), which is produced by expression of the multidrug resistance-1 gene (*MDR1*), mediates one of the pathways involving the metabolism of diverse drugs. Its importance was first recognized because of its role in the development of multidrug resistance against anti-cancer drugs in cultured tumor cells.[1] Furthermore, it is well known that P-gp plays an important role in the disposition of a broad variety of drugs.[2] However, the mechanisms by which P-gp is upregulated are still under debate. Recently, various single nucleotide polymorphisms (SNP) of *MDR1* have been identified, some of which appear to be associated with altered transporter functions and expression, thereby affecting the metabolism and disposition of drugs. Previous research has identified 29 kinds of *MDR1* SNP,[2,3] including the G2677T SNP at exon 21, which has been found to be the most frequent polymorphism and leads to a change in the amino acid sequence from Ala to Ser (G2677T) or Thr (G2677A) in the second transmembrane domain of P-gp, thereby affecting the function of P-gp.[4,5] In addition, SNP on exon 26 position 3435 (C3435T)[6] has also been found to be associated with the expression and function of P-pg.[2,7] Recently, Illmer *et al.*[8] reported that *MDR1* SNP in acute myeloid leukemia (AML) can predict the achievement of complete remission but not long-term survival, suggesting the need for further investigations, including pharmacokinetic studies, to assess the impact of *MDR1* SNP on AML. However, studies on the role of P-gp in the drug disposition of calcineurin-inhibitors (i.e. cyclosporine; CSA) or corticosteroids in the setting of solid organ transplantation,[9-12,11,13,14] have not produced consistent results. Furthermore, there are no data available on the role of *MDR1* SNP on the outcomes of allogeneic hematopoietic stem cell transplants. On the basis that *MDR1* may influence the pharmacokinetics of CSA, we evaluated the association between *MDR1* SNP and outcomes of allogeneic SCT, especially with respect to overall survival (OS) and non-relapse mortality (NRM).

Design and Methods

Eighty-two consecutive patients who had received an allogeneic SCT were enrolled in the current study; there were 29 female (35%) and 53 male patients (65%) with a median age of 34 years (range16-58). Thirty-six had AML, 9 had acute lymphoblastic leukemia, 16 had chronic myeloid leukemia, 5 had myelodysplasia, 10 had aplastic anemia, 4 had lymphoma, 1 had paroxysmal nocturnal hemoglobinuria and 1 had metastatic colorectal cancer. The extraction of genomic DNA and genotyping of *MDR1* were performed as previously described.[5] The transplant procedures were conducted as previously described.[15] Briefly, the conditioning regimens for the allogeneic recipients were busulfan/cyclophosphamide (n=53) or cyclophosphamide/ATG-containing (n=10) myeloablative or fludarabine-based reduced-intensity conditioning (n=19). Apheresis procedures were performed on 61 donors, of whom 60 were siblings and one was an unrelated donor, while 21 patients (26%) received a marrow harvest from siblings (n=10) or unrelated donors (n=11). The infused cell doses of mononuclear cells, $CD34^+$ and $CD3^+$ cells were 7.24±0.55

$\times 10^8$/Kg, 7.67±0.63$\times 10^6$/Kg, and 2.36±0.22$\times 10^8$/Kg (mean±standard error), respectively. Prophylaxis against acute graft-versus-host disease (GVHD) consisted of methotrexate and CSA for all patients. A continuous infusion of CSA (5.0mg/Kg/day) was started on day-1, then tapered to 2.5 mg/Kg/day from day+5 and continued until oral intake was tolerable and engraftment confirmed. The blood CSA concentration was first measured 48 hours after starting the CSA infusion (day+1), then the CSA dose was adjusted to maintain a blood CSA concentration between 200 and 400 ng/mL which, according to the creatinine level, required decrements in the CSA dose of between 25 to 75%.

The haplotypes were determined based on a Bayesian algorithm using the Phase program[16] (available at *http://www.stat.washington.edu/stephens/phase.html*). The univariate analyses according to *MDR1* SNP were performed using Fisher's exact test or Mann-Whitney's U-test. The blood CSA concentrations according to *MDR1* SNP were compared using Mann-Whitney's U-test.

OS was defined as the time from transplantation until death from any cause. NRM was defined as death from any cause except recurrence or disease progression. The OS estimates were calculated using the method of Kaplan and Meier, and were compared using log-rank tests. The cumulative incidences of NRM and relapse were estimated with the Cmprsk package in R software (The R Foundation, Vienna, Austria). Death from any cause was treated as a competing event.[17]

The multivariate survival analyses using Cox's proportional hazard model was performed by backward conditional procedures to define the prognostic factors for OS, NRM, and the probability of relapse. The hazard ratio (HR) and 95% confidence interval (CI) were also estimated. A cut-off *p*-value of 0.05 was adopted for all the statistical analyses, and the statistical data were analyzed using an SPSS software package (SPSS 11.5 Inc. Chicago, IL, USA).

Results and Discussion

The frequencies of the G-, T-, and A-alleles for G2677T/A were 42.1%, 34.1%, and 23.8%, while the frequencies of the C- and T-alleles for C3435T were 61.0% and 39.0%, respectively. The frequencies of genotypes were as follows: for G2677T/A at exon 21, the GG genotype was detected in 20 patients (24.4%), GT in 20 (24.4%), GA in 9 (11.0%), TT in 14 (17.0%), TA in 8 (9.8%), and AA in 11 (13.4%). For C3435T at exon 26, the CC genotype was detected in 30 patients (36.6%), CT in 40 (48.8%), and TT in 12 (14.6%). In this series of patients, three haplotypes (GC, TT and AC) out of six predominated, such that their frequencies were as follows: GC 45.1%, TT 29.3%, AC 12.2%, AT 6.7%, TC 3.7%, and GT 3.0%. When comparing the patients' characteristics according to the *MDR1* gene polymorphism, no differences except sex were noted between patients with the GG- or non-GG genotype for G2677T/A. The transplant outcomes are summarized in Table 1. Briefly, with a median follow-up of 347 days (range 15 to 2,181 days), 42 patients had died of causes other than relapse (n=29, 36%) or progression (n=13, 16%). The 2-year OS rate was estimated to be 46.5±5.7%, while the 2-year cumulative incidence of NRM and relapse was 38.9±5.8% and 42.9±6.6%, respectively. The patients with the GG-genotype for G2677T/A had a worse outcome than those without the GG-genotype in terms of OS (18.6±9.5% vs. 53.8±6.6%; *p*=0.0048, Figure 1A) and higher NRM (67.4±13.3 vs. 32.4±6.4%; p=0.0073, Figure 1B). However, the probability of relapse was not different according to G2677T/A genotype (*p*=0.0933). No differences in OS, NRM, or relapse were noted according to the C3435T genotype (Figure 1C) or *MDR1* haplotype (Figure 1D). In addition, no differences in other transplant outcomes, such as the development of acute or chronic GVHD, were noted according to the G2677T/A (Table 1) or C3435T genotypes (*data not shown*).

Table 1. Transplantation outcomes according to *MDR1* genotype or haplotype.

G2677T/A genotype	*Total (n=82, 100%)*	*GG (n=20; 24%)*	*Non-GG (n=62, 76%)*	*p value*
Follow-up duration (days)	347 (15~2181)	232 (17~1290)	479 (15~2181)	
Acute GVHD				
Overall	64 (78)	15 (75)	49 (79)	0.759
≥ grade 2	55 (67)	14 (70)	41 (66)	1.000
≥ grade 3	20 (24)	6 (30)	14 (23)	0.554
Chronic GVHD	N=65			
Limited + extensive	49 (75)	10 (77)	39 (75)	1.000
Extensive	31 (48)	9 (69)	22 (42)	0.121
Survival				
Relapse	26 (32)	8 (40)	18 (29)	0.412
Death	42 (52)	15 (75)	27 (44)	0.014*
Cause of death				
Non-relapse mortality	29 (36)	11 (55)	18 (29)	0.058
Infection	19 (23)	5 (25)	14 (22)	1.000
GVHD+Infection	5 (6)	3 (15)	2 (3)	0.091
Other	5 (6)	3 (15)°	2 (3)#	0.091
Progression	13 (16)	4 (20)	9 (15)	0.725

*GVHD: graft-versus-host disease; *p=0.020 by Fisher's exact test; °others denote veno-occlusive disease (n=1), intracranial hemorrhage (n=1) and hemorrhagic uremic syndrome/thrombotic thrombocytopenic purpura (n=1); #other denotes veno-occlusive disease (n=2).*

In a multivariate survival analysis, the GG genotype for G2677T/A was found to have an unfavorable prognostic impact on OS (*p*=0.003, HR 2.651 [1.386~5.070]) and NRM (*p*=0.031, HR 2.388 [1.084~5.264]), but not on relapse (Table 2). As regards a possible pharmacokinetic impact of *MDR1* SNP on CSA metabolism, we failed to detect significant associations between CSA concentration and G2677T/A or C3435T genotypes or with the *MDR1* haplotype (*data not shown*). This study is the first investigation about the impact of *MDR1* SNP on the outcomes after allogeneic hematopoietic SCT. In the current study, the association of the G2677T/A genotype with transplant outcomes, especially NRM, revealed that the impact of P-gp needs to be interpreted with caution since it may depend on the clinical situation, e.g. a chemotherapy or transplantation setting. In contrast to its beneficial effect on preventing chemoresistance in AML,[5] the low *MDR1*-producing allele (GG

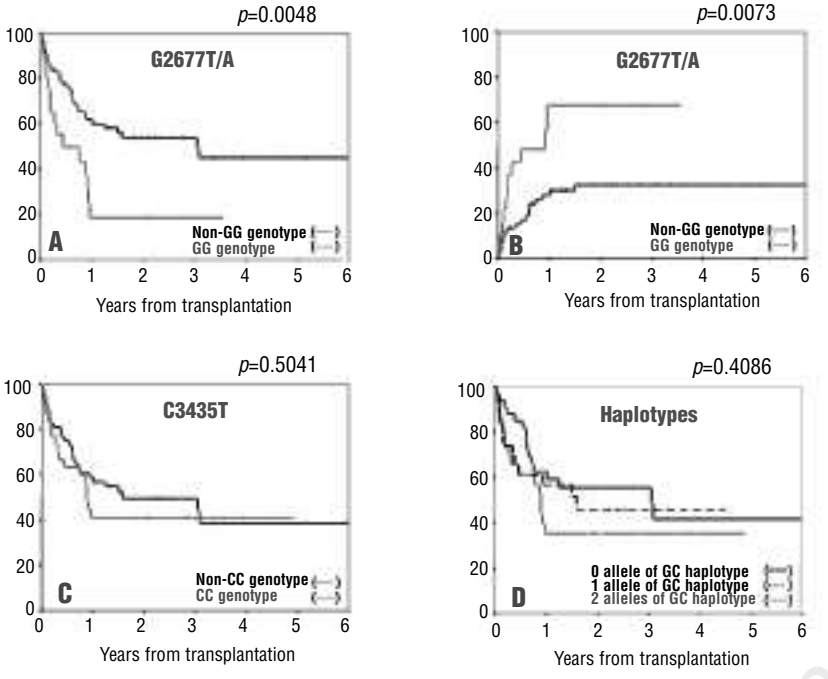

Figure 1. **Overall survival and cumulative incidence of non-relapse mortality according to *MDR1* genotype (A-C) or haplotype (D).**

Table 2. **Multivariate survival analysis of prognostic factors for overall survival and cumulative incidence of non-relapse mortality or relapse for all patients (n=82).**

OS	*Risk factor*	*2-year rate(%)*	*HR [95% CI]*	*p value*
G2677T/A	Non-GG genotype	53.8±6.6	1.0	0.003
	GG genotype	18.6±9.5	2.651[1.386~5.070]	
Chronic GVHD	Yes	58.2±7.2	1.0	<0.001
	No	21.4±9.1	3.395[1.842~6.256]	
Disease status	Standard risk	56.8±7.3	1.0	0.045
	Advanced risk	30.3±8.6	1.842[1.014~3.345]	
NRM°				
Risk factor	2-Yr rate(%)	HR [95% CI]	p-value	
G2677T/A	Non-GG genotype	32.4±6.4	1.0	0.031
	GG genotype	67.4±13.3	2.388 [1.084~5.264]	
Chronic GVHD	Yes	29.6±7.0	1.0	0.001
	No	51.5±9.1	3.799 [1.745~8.270]	
Acute GVHD	Grade 0~2	34.0±6.5	1.0	0.019
	Grade 3,4	59.2±11.6	2.613 [1.171~5.830]	
Relapse	Risk factor	2-Yr rate(%)	HR [95% CI]	p-value
Chronic GVHD	Yes	33.9±7.2	1.0	0.021
	No	70.6±14.8	2.683 [1.162~6.194]	
Acute GVHD	Grade 2~4	35.7±7.7	1.0	0.050
	Grade 0,1	59.7±12.1	2.324 [1.001~5.395]	
Disease status	Standard risk	31.6±8.1	1.0	0.003
	Advanced risk	61.7±10.5	3.345 [1.506~7.407]	

**For the multivariate analyses, the MDR1 SNP (GG genotype vs. non-GG genotype for G2677T/A genotype), disease status (standard vs. advanced risk), transplanted dose of CD34$^+$ cells (less than or more than 6×10^6/Kg), source of stem cells, peripheral blood vs. bone marrow, donor (sibling vs. unrelated), and GVHD (acute grade 0-2 vs. 3,4 for OS/NRM or grade 0,1 vs. 2-4 for the probability of relapse and development of chronic GVHD) were included in the final model. °When chronic GVHD was excluded as a covariate in a multivariate survival analysis for NRM, the following factors were identified as independent prognostic factors for NRM: 1) GG genotype, p=0.010, HR 2.734, 95% C.I. 1.276-5.860, 2) acute GVHD, grade 3,4, p=0.032, HR 2.298, 95% C.I. 1.075-4.901, and 3) lower transplant CD34$^+$ cell dose (less than 6×10^6/Kg), p=0.056, HR 2.092, 95% C.I. 0.983-4.454.*

genotype of G2677T/A) was not found to be correlated with a superior survival or lower risk of relapse (Table 1). Indeed, the GG genotype was strikingly associated with worse survival and NRM. A possible explanation for these findings might be the pharmacokinetic effects of *MDR1* SNP, of altering drug disposition, which could have influenced the transplant outcomes. However, the current study failed to provide clear evidence of the impact of *MDR1* SNP on the pharmacokinetics of CSA. The results of previous research on the association between the pharmacokinetics of calcineurin inhibitors and *MDR1* SNP in the setting of solid organ transplants are still under debate.[9-12,14,18-20] Further study on the association of *MDR1* SNP with CSA toxicity should provide some elucidation.

Another possible mechanism may be through the impact on the metabolism of other drugs such as antibiotics. Although the use of multiple drugs in a transplant setting hinders a precise interpretation of the effect of *MDR1* SNP on transplant outcomes, the fact that 40% of the patients with the GG-genotype died of infection-related causes implies that other mechanisms could be involved in *MDR1* SNP related to infection and/or GVHD. For example, patients with the lower *MDR1*-producing allele, who absorb less xenobiotics from the gut lumen, may have a higher risk of treatment failure against opportunistic infections.

In conclusion, the present findings suggest that *MDR1* SNP, especially the GG-genotype on G2677T/A, have an adverse impact on survival and NRM after allogeneic SCT. Further study is warranted with larger numbers of patients to clarify how *MDR1* SNP affect transplant outcomes.

D-H K, J-Y P and S-K S contributed equally to the work and assume primary responsibility for it. D-H K was responsible for the design of the study, supervision of data collection, data analysis, and writing the manuscript. J-Y P and S-K S were responsible for the supervision of data interpretation, data analysis, and critical revision of the manuscript. N-Y L and J-S S were involved in the laboratory investigations, interpretation of the data and critical revision of the manuscript. K-B L was involved in critical revision of the manuscript.

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