$P2X_7$ receptor polymorphism and clinical outcomes in HLA-matched sibling allogeneic hematopoietic stem cell transplantation

Kyung-Hun Lee, Sung Sup Park, Inho Kim, Jin Hee Kim, Eun Kyung Ra, Sung-Soo Yoon, Yun-Chul Hong, Seonyang Park, Byoung Kook Kim

From the Department of Internal Medicine (K-HL, IK, S-SY, SP, BKK); Department of Laboratory Medicine (SSP, EKR); Department of Preventive Medicine, Seoul National University, College of Medicine, Seoul, Korea (JHK, Y-CH); Diagnostic DNA Chip Center, The Ilchun Molecular Medicine Institute, Medical Research Center, Seoul National University, College of Medicine, Seoul, Korea (IK, SP); Cancer Research Institute, Seoul National University, College of Medicine, Seoul, Korea (IK, S-SY, SP, BKK).

K-HL and SSP contributed equally to this work.

Funding: this study was supported by grant no 04-2006-013 from the SNUH Research Fund and by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (0405-BC02-0604-0004).

Manuscript received September 25, 2006.
Manuscript accepted January 26, 2007.

Correspondence:
Inho Kim, Professor Department of Internal Medicine, Seoul National University College of Medicine 28 Yongon-Dong, Chongno-Gu Seoul, 110-744, Korea.
E-mail: kim_dajung@hanmail.net

ABSTRACT

Background and Objectives

The $P2X_7$ receptor ($P2X_7$ R) is a key player in the processing and release of interleukin (IL)-1. To evaluate whether the A1513C polymorphism of the $P2X_7$ R gene is related to allogeneic stem cell transplantation outcome, we performed an association analysis between this polymorphism and clinical outcomes in patients treated with an HLA-matched sibling stem cell transplant.

Design and Methods

Patients (n=152) with a malignancy or aplastic anemia underwent allogeneic stem cell transplantation at a single institute. Peripheral blood DNA of these 152 patients and their 152 donors was genotyped. Genotypes of 145 recipients and 150 donors were obtained and analyzed for the polymorphism.

Results

The frequencies of the A and C alleles in all 295 study subjects were 72% and 28%, respectively. The genotypes in patients were AA in 75, AC in 58, and CC in 12; the genotypes in donors were AA in 74, AC in 70, and CC in 6. Overall survival was significantly shorter for recipients with the CC genotype than for those with the AA or AC genotype (92 days for 1513CC vs. 821 days for 1513AA or 1513AC, p=0.012), and for recipients from donors with the CC genotype than for recipients from donors with the AA or AC genotype (63 days for 1513CC vs. 702 days for 1513AA or 1513AC, p=0.024). Multivariate analyses, which included sex, age, transplant method (reduced intensity conditioning vs. conventional conditioning), stem cell source, risk group, and $P2X_7$R recipient and donor genotypes, as parameters, identified high-risk group (hazard ratio 3.25, 95% confidence interval 1.83~5.77) and a donor 1513CC genotype (hazard ratio 2.66, 95% confidence interval 1.02~6.91) as risk factors for a shorter survival. Microbiologically documented bacteremia occurred in 66.7% of recipients with the CC donor genotype and in 17.6% of recipients of transplants of AA or AC genotype (p=0.014).

Interpretation and Conclusions

We conclude that the A1513C polymorphism in the $P2X_7$R gene is related to the occurrence of infections and survival after allogeneic stem cell transplantation. Thus, the determination of this polymorphism may be helpful for the optimal selection of patients and donors.

Key words: $P2X_7$, polymorphism, hematopoietic stem cell transplantation, IL-1, IL-18.

Haematologica 2007; 92:651-657

Hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment for many hematologic malignancies and for aplastic anemia and inherited immune disorders. However, the incidence of transplant-related mortality (TRM) is substantial and it is important that risks be reduced by tailoring and individualizing therapies. Single nucleotide polymorphisms (SNP) are believed to play an important role in determining individual characteristics, and are also being actively investigated in the field of HSCT.[1-3] Several polymorphisms of cytokine genes, including those for interleukin (IL)-1, IL-2, IL-10, interferon (IFN)-γ, tumor necrosis factor (TNF)-α, and TNF-β, have been investigated in relation to HSCT outcomes, i.e., with respect to graft-versus-host disease (GVHD) and infections.[4-8] However, cytokine pathways are diverse and complex, and thus polymorphisms of cytokine receptors and their regulatory molecules must be considered in studies on the biological effects of cytokines.

The $P2X_7$ receptor ($P2X_7R$), a plasma membrane receptor for extracellular ATP, is known to be a key player in the processing and release of IL-1 cytokines.[9-11] The IL-1 family presently contains nine members, including IL-1α, IL-1β, IL-1 receptor antagonist (IL-1Ra) and IL-18. These cytokines are released from a variety of cells including activated monocytes, macrophages, and microglia, and are known to play an important role in immune responses. The loss-of-function SNP at nucleotide position 1513 of the human $P2X_7R$ gene (1513A→C) changes a glutamic acid to alanine at amino acid 496. The presence of this polymorphism impairs ATP-induced $Ca2^+$ and ethidium$^+$ influx, ATP-induced Rb^+ efflux, ATP-induced release of IL-1β and IL-18, and the killing of intracellular bacteria.[12-15]

Several studies have concluded that IL-1 is related to HSCT outcome, i.e., GVHD and mortality, and that polymorphisms of the gene for IL-1Ra affect the incidence and severity of acute GVHD.[8] Moreover, because IL-1 expression is regulated by $P2X_7R$, it is possible that $P2X_7R$ status may also affect HSCT outcome. Therefore, we investigated the role of the $P2X_7R$ polymorphism in HSCT. Association analysis was performed to determine the relations between this polymorphism and the clinical outcomes of patients treated with HLA matched sibling stem cell transplants at a single institute.

Design and Methods

Patient population and data collection

Patients who underwent allogeneic HSCT from human leukocyte antigen (HLA)-matched sibling donors at a single institution (Seoul National University Hospital) between 1998 and 2005 for malignancies or aplastic anemia were included in this study. All the patients were 16 years or older at transplantation. Finally, 152 patients were included.

Table 1. Patients' characteristics (n=152).

Age (median, range), years	40 (16-70)
Gender, n (%)	
Male	86 (56.6%)
Female	66 (43.4%)
Transplant method, n (%)	
Reduced intensity conditioning	70 (46.1%)
Conventional conditioning	82 (53.9%)
Stem cell source	
Bone marrow	64 (42.1%)
Peripheral blood stem cells	80 (52.6%)
Bone marrow and peripheral blood stem cells	8 (5.3%)
Diagnosis	
Acute myeloid leukemia	51
Acute lymphoblastic leukemia	21
Chronic myeloid leukemia	21
Severe aplastic anemia	19
Myelodysplastic syndrome	12
Non-Hodgkin's lymphoma	10
Multiple myeloma	5
Renal cell carcinoma	5
Others	8
High risk disease*	79/152 (46%)
Granulocyte recovery	
Median days to reach 500/μL	15 (4-38)
Platelet recovery	
Median days to reach 20,000/μL	16 (5-73)

**Includes all other states than acute leukemia in first complete remission, chronic myeloid leukemia in first chronic phase and severe aplastic anemia.*

Clinical data were obtained from thorough review of medical records before $P2X_7R$ genotyping. The data collected were: patient demographics, transplantation procedure details including the conditioning regimens, time to recovery of platelet and granulocyte counts, liver and renal function as reflected by total bilirubin, aspartic transaminase (AST), alanine transaminase (ALT), and creatinine levels, transplantation complications such as acute GVHD and hepatic veno-occlusive disease (VOD), infections documented by blood culture and febrile episodes not confirmed microbiologically, survival status and cause of death. Times to platelet and granulocyte recovery were defined as the time to the first of 3 consecutive days of counts exceeding 500/μL, and to the first of 7 consecutive days of counts exceeding 20,000/μL without transfusion, respectively. Acute GVHD was graded from 0 to IV using conventional criteria.[16] VOD of the liver was defined as an increase in bilirubin of more than 2 mg/dL with at least two of the following; hepatomegaly, ascites, and a body weight gain of greater than 5%.[17]

The patients' characteristics, including underlying diseases and conditioning regimens are shown in Table 1. Acute myeloid leukemia (AML) was the most common underlying diagnosis (51 acute myeloid leukemia; 21 chronic myeloid leukemia; 21 acute lymphocytic leukemia; 21 severe aplastic anemia; 12 myelodysplastic syndrome; 10 non-Hodgkin's lymphoma). Low-risk disease was defined as follows: acute leukemia in first remission, chronic myeloid leukemia in first chronic phase, and severe aplastic anemia. Other diseases were

defined as high-risk diseases. Seventy-nine patients (46%) were transplanted in a high-risk disease status. The median age at the time of HSCT was 40 years (range, 16-70 years). The median follow-up of survivors was 935 days. Seventy patients received conventional myeloablative conditioning regimens and the other 82 underwent stem cell transplantation after reduced intensity conditioning. Myeloablative conditioning regimens were busulfan and cyclophosphamide in 69 patients, and total body irradiation and cyclophosphamide in one. The reduced intensity conditioning regimens used were fludarabine and melphalan in 35 patients, fludarabine and cyclophosphamide in 23, antithymocyte globulin and cyclophosphamide in 6, total lymphoid irradiation and cyclophosphamide in 6, fludarabine and cytarabine in 4, and others in 8. Sixty-four patients (42.1%) received bone marrow as the source of stem cells and 80 (52.6%) received peripheral blood stem cells. Eight patients (5.3%) received both bone marrow and peripheral blood stem cells. Eighty-six (56.6%) of the 152 patients were male.

P2X7R A1513C genotyping

Genomic DNA was prepared from peripheral blood samples using a Puregene® DNA purification kit (Gentra, Minneapolis, MN, USA). Peripheral blood DNA of the 152 patients and 152 donors was genotyped. Genotypes of 145 recipients and 150 donors were obtained and analyzed for the polymorphism. SNP genotyping was performed using SNP-IT™ assays and the SNPstream 25K® System (Orchid Biosciences, Princeton, NJ, USA). Briefly, the genomic DNA region spanning the polymorphic site was amplified using one phosphothiolated primer and one regular polymerase chain reaction (PCR) primer (sense: AAGCTGCCTCCCATCTCA, antisense: AACAGCTCTGAGGTGGTGAT). Amplified PCR products were then digested with exonuclease (Amersham Biosciences, Uppsala, Sweden). 5´ phosphothiolates were used in this study to protect one strand of the PCR-product from exonuclease digestion. The single-strand PCR template generated by exonuclease digestion was overlaid onto a 384-well plate precoated covalently with the extension primer (SNP-IT™ primer with the GAGAGCCACAGGTGCCTGGAGG sequence). These SNP-IT™ primers were designed to hybridize immediately adjacent to the polymorphic site. After hybridization of template strands, SNP-IT™ primers were extended by a single base using DNA polymerase at the polymorphic site of interest. Extension mixtures contained two labeled terminating nucleotides (one fluorescein isothiocyanate, one biotin) and two unlabeled terminating nucleotides. The final single base incorporated was identified by serial colorimetric reactions using anti-fluorescein-AP (Roche, Basel, Switzerland) and streptavidin-horse radish peroxidase (Pierce, Rockford, IL, USA), respectively. Respective blue and/or yellow color developments were analyzed using an enzyme linked immunosorbent assay (ELISA) reader and the final genotyping calls were made using the QC Review™ program.

Statistical data analysis

Statistical analyses of categorical variables were performed using the Pearson's χ^2 test or Fisher's exact test as appropriate. Continuous data were compared using linear regression. To determine whether the P2X7R SNP site is in Hardy-Weinberg equilibrium, the distributions of observed genotype frequencies and expected genotype frequencies were compared using the χ^2 test. Mean values of peak total bilirubin, AST, and ALT were calculated for individual genotypes. Engraftment rates and the incidences of complications, such as acute GVHD and hepatic VOD, were compared between genotypes. Treatment-related mortality (TRM) was defined as death from any cause, other than progression of the underlying disease, during the course of treatment. TRM and overall survival (OS) were calculated using the Kaplan-Meier method and comparisons between genotypes were made using the log-rank test. Cox regression analysis was used for multivariate analyses. The variables included in the models were age, gender, stem cell source, conditioning regimen used, and disease status at the time of transplantation (i.e., low risk vs. high risk). Two-sided p values <0.05 were considered statistically significant.

Ethics

The study protocol was reviewed and approved by the institutional review board of Seoul National University Hospital, and complied with the recommendations of the Declaration of Helsinki for biomedical research involving human subjects.

Results

Frequencies of P2X7R A1513C genotypes

The frequencies of the A and C alleles of the P2X7R A1513C polymorphism were 72% and 28%, respectively. The genotypes in patients were AA in 75 (51.7%), AC in 58 (40.0%), and CC in 12 (8.3%); the genotypes in donors were AA in 74 (49.3%), AC in 70 (46.7%), and CC in 6 (4.0%). The P2X7R SNP site was in Hardy-Weinberg equilibrium (p>0.05). The baseline characteristics of patients with different genotypes were comparable (Table 2).

Treatment-related mortality, disease-free survival, and overall survival analysis according to P2X7R A1513C genotype

Treatment-related mortality and survival analyses were performed in donors and recipients separately. Overall survival was significantly shorter for recipients with the CC genotype than for those with the AA or AC genotype (92 days for 1513CC vs. 821 days for 1513AA or 1513AC, p=0.0121), and for recipients from

Table 2. Comparison of patients' characteristics according to the P2X$_7$R genotypes of donors and patients.

	Patient P2X7R A1513 genotype				*Donor P2X7R A1513 genotype*			
	AA (n=75)	*AC (n=58)*	*CC (n=12)*	*p value*[a]	*AA (n=74)*	*AC (n=70)*	*CC (n=6)*	*p value*[a]
Sex (male), n (%)	45 (60%)	31 (53.4%)	8 (66.7%)	0.611	40 (54.1%)	41 (58.6%)	3 (50.0%)	0.823
Age [mean±SD(years)]	41.3±12.6	40.2±13.0	40.83±16.1	0.876	39.4±13.0	41.7±12.3	46.2±14.8	0.330
Transplant method (reduced intensity conditioning), n (%)	40 (53.3%)	32 (55.2%)	6 (50.0%)	0.942	39 (52.7%)	37 (52.9%)	5 (83.3%)	0.339
Stem cell source (BM), n(%)	33 (44.0%)	24 (41.4%)	4 (33.3%)	0.961	31 (41.9%)	31 (44.3%)	1 (16.7%)	0.436
High-risk disease, n(%)	43 (57.3%)	24 (41.4%)	9 (75%)	0.104	42 (56.8%)	31 (44.3%)	4 (66.7%)	0.534

Excluding patients whose laboratory tests or genotyping results were unavailable; p-values were calculated using the χ^2 test or ANOVA.

donors with the CC genotype than for recipients from donors with the AA or AC genotype (63 days for 1513CC vs. 702 days for 1513AA or 1513AC, p=0.0241; Figure 1). Multivariate analyses including sex, age, transplant method (reduced intensity conditioning vs. conventional conditioning), stem cell source, risk group, and P2X$_7$R genotype as variables identified high-risk group (hazard ratio 3.25, 95% confidence interval 1.83~5.77) and donor 1513CC genotype (hazard ratio 2.66, 95% confidence interval 1.02~6.91) as risk factors for shorter survival (Table 3).

Median disease-free survival was shorter for recipients with the CC genotype than for those with the AA or AC genotype (76 days for 1513CC vs. not reached for 1513AA or 1513AC, p=0.028), and for recipients from donors with the CC genotype (163 days for 1513CC vs. not reached for 1513AA or 1513AC, p=0.054). However, more high-risk patients were of the CC genotype, but the CC genotype in patients was not found to be an independent predictor of shorter disease-free survival (p=0.372) by Cox regression analysis using genotype and risk group as covariates. The 1-year TRM rate after transplantation appeared to be higher for recipients from donors with the CC genotype (33.3% for donors with 1513CC vs. 22.2% for 1513AA or 1513AC, p=0.3325), although this was not statistically significant.

Complications after HSCT and the A1513C polymorphism

Microbiologically documented infections with a positive blood culture were significantly more frequent in recipients of grafts from donors with a CC genotype (66.7% for donors with 1513CC genotype vs. 17.6% for those with a 1513AA or 1513AC donor, p=0.014). However, no difference was observed according to recipient genotype (27.3% for recipients with 1513CC vs. 22.2% for recipients with 1513AA or 1513AC, p=0.712). In addition, the occurrence of TRM was found to be related to a microbiologically documented infection (TRM rate, 71.4% in patients with a positive blood culture vs. 12.2% in patients without a positive blood culture, p<0.001). The identified pathogens were Gram-

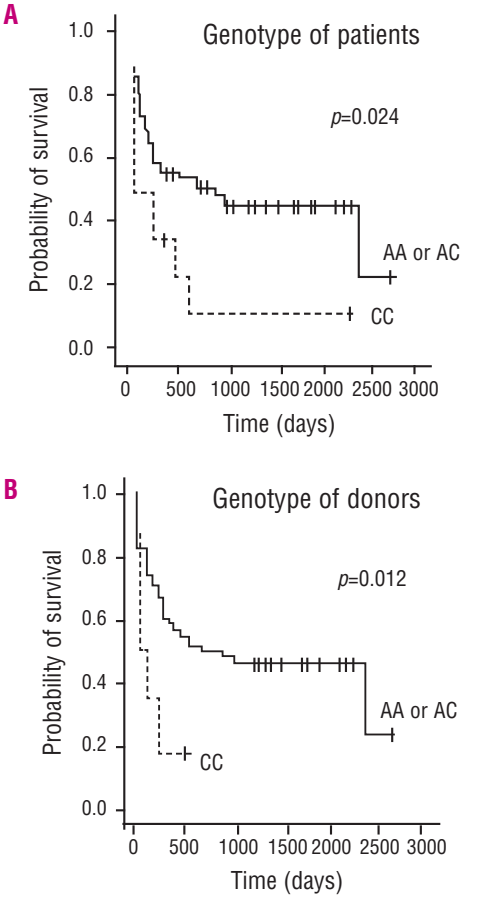

Figure 1. Kaplan-Meier plots of overall survival according to (A) patient and (B) donor P2X$_7$R A1513C genotype.

Table 3. Effects of individual characteristics on overall patient survival.

Variables	Univariate			Multivariate		
	Hazard ratio†	95% CI‡	p value	Hazard ratio†	95% CI‡	p value
Age	1.02	1.00, 1.04	0.066	1.02	0.99, 1.04	0.175
Gender	1.15	0.73, 1.80	0.556	0.99	0.61, 1.59	0.949
Stem cell source (bone marrow)	0.70	0.45, 1.09	0.117	1.20	0.60, 2.38	0.611
Transplant method (reduced intensity conditioning)	1.13	0.73, 1.75	0.593	0.81	0.43, 1.51	0.506
Disease status (high risk)	3.08	1.90, 4.98	<0.001	3.25	1.83, 5.77	0.000
Patient P2X7R 1513CC	2.30	1.18, 4.48	0.015	1.67	0.83, 3.37	0.150
Donor P2X7R 1513CC	2.74	1.10, 6.85	0.031	2.66	1.02, 6.91	0.045

†‡*Hazard ratios and 95% confidence intervals were determined using the Cox proportional hazard model.*

Table 4. Comparison of outcomes according to P2X7R genotype.*

	Patient P2X7R A1513 genotype				Donor P2X7R A1513 genotype			
	AA (n=74)	AC (n=56)	CC (n=11)	p value†	AA (n=74)	AC (n=70)	CC (n=6)	p value†
Peak total birilubin (mg/dL)	7.1±1.3*	4.2±0.7	8.7±4.5	0.161	6.2±1.21	5.6±1.1	3.6±1.0	0.793
Peak ALT (IU/L)	160.4±40.3	173.0±27.2	184.1±57.8	0.950	156.8±33.8	156.4±24.1	123.0±39.3	0.947
Peak AST (mg/dL)	197.9±76.5	127.7± 22.1	219.1±120.3	0.694	183.4±69.1	128.6±26.1	91.8±18.4	0.720
Peak creatinine (mg/dL)	1.8± 0.1	1.5±0.1	2.4±0.5	0.010	1.7±0.1	1.66±0.1	1.48±0.58	0.804
Acute GVHD (>Gr II, %)	21.3	31.0	16.7	0.347	23.0	22.9	50.0	0.314
Hepatic VOD (%)	13.3	10.3	16.7	0.784	13.5	12.9	0.0	0.63

**Excluding patients whose laboratory tests or genotype were unavailable; values are means±SE; †p-values were determined using the χ^2 test or ANOVA; ALT: alanine transaminase; AST: aspartic transaminase; GVHD: graft-versus-host disease; VOD: veno-occlusive disease.*

positive bacteria in 21 patients, Gram-negative bacteria in nine patients, and *Candida* species in three patients (four patients were infected by more than one pathogen). Peak bilirubin, AST, ALT, and creatinine levels during the initial 30-day post-transplantation period were not associated with the A1513C polymorphism in either donors or recipients, and neither was platelet or granulocyte recovery.

Thirty-seven patients (24.3%) developed grade II-IV acute GVHD; 16 patients grade II acute GVHD, 10 patients grade III, and 11 patients grade IV. The occurrence of acute GVHD was not related to the P2X7R A1513C polymorphisms. Hepatic VOD developed in 19 patients (12.5%), but showed no association with A1513C genotype (Table 4).

Discussion

The present study describes the importance of the A1513C P2X7R gene polymorphism in HSCT, with regard to infections and survival after transplantation. Patients who received stem cells from matched sibling donors and who were homozygous for the minor C allele were found to be vulnerable to infections (as proven by blood culture) and had a shorter survival. However, no association was found between the polymorphism and acute GVHD.

IL-1α, IL-1β and IL-18 belong to the IL-1 family, and mediate inflammation and a wide range of other biological effects associated with infection, inflammation, and autoimmune processes. IL-1 is pyrogenic, induces hepatic acute-phase proteins, activates lymphocytes, and promotes prostanoid synthesis.[18] It also augments antimicrobial defenses and enhances immunologic responses; on the other hand it causes hypotension and has a deleterious effect in the pathogenesis of sepsis syndrome.[19-21] With regard to tumors and chemotherapy, IL-1 has a direct antiproliferative effect on human tumors and can accelerate hematopoietic recovery after myelosuppressive therapies.[22-23] Moreover, IL-1Ra binds competitively to IL-1 receptor and militates against the biological effects of IL-1α and IL-1β.[24]

P2X7R plays an important role in secreting IL-1 family cytokines in response to bacterial lipopolysaccharides. Pro-IL-1β and pro-IL-1α, which accumulate in the cytosol in the presence of lipopolysaccharides, are released after stimulation of the P2X7R by extracellular ATP.[10,11] IL-1Ra is also released by blood cells such as monocytes, but not by endothelial cells after P2X7R activation.[24] P2X7R consists of 13 exons, of which exons 12 and 13 code for the C-terminus. The P2X7R gene is highly polymorphic and is known to harbor more than 260 SNP.[9] The A to C polymorphism at position 1513 of the coding region of the P2X7R gene causes the substitution of Glu-496 by Ala in the intracellular C-terminal tail. This polymorphism was

found not to affect the surface expression of P2X7R, though the C allele was non-functional when expressed at low density but regained normal function at high density in terms of monocyte differentiation to macrophages and the apoptosis of lymphocytes.[12] In addition, the A1513C polymorphism has been linked to reduced release of IL-1β and IL-18 from human monocytes.[13,14] With regard to infections, the Glu-496 to Ala polymorphism has been shown to impair ATP-mediated immune responses, such as the killing of mycobacteria by human macrophages,[15] and it is also believed to contribute to the pathogenesis of chronic lymphocytic leukemia. Moreover, because *P2X7R* -dependent apoptosis is impaired in the presence of the C allele, its expression could result in the accumulation of neoplastic B cells.[25]

The shorter survival of recipients whose donors were homozygous for the C allele could be explained by a higher incidence of bacteremia. IL-1 family cytokines are secreted mainly by immune cells (e.g. monocytes, macrophages, and dendritic cells), and recipients of hematopoietic stem cells of the *P2X7R* CC genotype might show impaired IL-1 cytokine release, which would contribute to the observed high incidence of bacteremia. In addition, evidence associates a reduced IL-1 level and increased susceptibility to infection. Serious infections tended to increase after the administration of anakinra, a recombinant human IL-1Ra, in patients with rheumatoid arthritis.[26] Moreover, a low dose of recombinant IL-1 protected granulocytopenic mice from lethal Gram-negative infection.[27]

In the present study, patients with the CC genotype also showed shorter survival. Recurrences of pre-transplant disease were more frequent in these patients, mainly because patients with the CC genotypes had more progressed disease which is reflected by the higher percentages of high-risk patients in the CC genotypes. In addition, the relation between IL-1 family cytokines and tumors should also be considered. IL-1 is thought to be a growth factor for acute and chronic leukemia and to act as a stimulator of colony-stimulating growth factors and other cytokines, such as IL-1[6,19,28-30] whereas IL-1 and IL-18 are known to have antitumor effects in experimental models.[31,32] The direct injection of IL-18 recombinant adenoviral vector into a murine fibrosarcoma model completely eradicated tumors in all animals and concomitantly induced protective systemic immunity.[33] The intratumoral co-administration of IL-18-expressing adenoviral vector and dendritic cells resulted in the complete regression of injected tumors.[34] IL-1 has also been tested in phase I and II clinical trials.[35] The above findings collectively suggest the possibility of a relation between tumor recurrence and *P2X7R* polymorphisms, but unfortunately this could not be examined during the present retrospective study. Further prospective studies on patients with a homogenous disease status are needed to investigate this issue.

Previous research on the effects of IL-1 and IL-18 in HSCT have focused on the occurrence of acute and chronic GVHD;[8,36] it has been reported that increased serum levels of IL-18 after engraftment correlate with acute GVHD in allogeneic HSCT.[37] However, we found no correlation between the *P2X7R* polymorphism and GVHD. IL-1 and IL-1Ra might be affected by *P2X7R* status, but the *P2X7R* polymorphism alone was not found to be associated with the occurrence of GVHD in the present study. Heterogeneity of study populations and treatment regimens should be considered and prospective studies on IL-1 family cytokines and GVHD are warranted in a homogenous group of patients.

The effects of IL-1 cytokines on hematopoiesis have been previously described. IL-1 was found to be a myeloprotective agent when administered before myelotoxic chemotherapy.[22,23] However, we did not find any differences between the recovery times of peripheral blood leukocytes or platelets and *P2X7R* genotype. One possible explanation for this is that previous studies used exogenous IL-1 species, whereas we examined the effects of endogenous IL-1. Moreover, impaired cytokine secretion, despite similar neutrophil counts, might have influenced leukocyte inflammatory and immunological responses against micro-organisms. As far as we are aware, the present study is the first to find that the A1513C *P2X7R* gene polymorphism is associated with clinical outcome in HSCT.[9] Several researchers have shown associations between SNP and infections after HSCT, but no data regarding the *P2X7R* gene have been reported.[36,38-40]

Future prospective research on the A1513C *P2X7R* polymorphism in a larger group of patients should aid our understanding of the role of this polymorphism in HSCT. Moreover, other SNP in this gene, including gain-of-function polymorphisms, should also be investigated to comprehensively define their roles.[41] Ethnicity should also be considered, because the allelic frequency of A1513C determined in the present Korean cohort appears to be higher than frequencies previously reported for Caucasians, which ranged from 8% to 24%.[25,26] We conclude that the A1513C polymorphism of the *P2X7R* gene is related to the occurrence of infections and survival following allogeneic stem cell transplantation, and that the determination of this polymorphism may be helpful for the optimal selection of patients and donors.

Authors' Contributions

K-HL and SSP contributed equally to this work. K-HL collected clinical data and wrote this paper. SSP designed experiments and did genotypings. IK was the principal investigator of this study and was responsible for the conception and supervision of the study and of the paper. JHK and Y-CH performed statistical analysis. EKR collected and prepared samples. IK, SSY, SP and BKK was involved in diagnosis and management of patients.

Conflict of Interest

The authors reported no potential conflicts of interest.

References

1. Kallianpur AR. Genomic screening and complications of hematopoietic stem cell transplantation: has the time come? Bone Marrow Transplant 2005;35:1-16.
2. Dickinson AM, Middleton PG, Rocha V, Gluckman E, Holler E; Eurobank members. Genetic polymorphisms predicting the outcome of bone marrow transplants. Br J Haematol 2004; 127:479-90.
3. Dickinson AM, Middleton PG. Beyond the HLA typing age: genetic polymorphisms predicting transplant outcome. Blood Rev 2005; 19:333-40.
4. Middleton PG, Taylor PR, Jackson G, Proctor SJ, Dickinson AM. Cytokine gene polymorphisms associating with severe acute graft-versus host disease in HLA-identical sibling transplants. Blood 1998; 92:3943-8.
5. Cavet J, Middleton PG, Segall M, Noreen H, Davies SM, Dickinson AM. Recipient tumor necrosis factor- and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants. Blood 1999;94: 3941-6.
6. Cavet J, Dickinson AM, Norden J, Taylor PR, Jackson GH, Middleton PG. Interferon- and interleukin-6 gene polymorphisms associate with graft-versus-host disease in HLA-matched sibling bone marrow transplantation. Blood 2001;98:1594-600.
7. Socié G, Loiseau P, Tamouza R, Janin A, Busson M, Gluckman E, et al. Both genetic and clinical factors predict the development of graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. Transplantation 2001; 72:699–705.
8. Cullup H, Dickinson AM, Jackson GH, Taylor PRA, Cavet J, Middleton PG. Donor interleukin-1 receptor antagonist genotype associated with acute graft-versus-host disease in human leukocyte antigen-matched sibling allogeneic transplants. Br J Haematol 2001;113:807–13.
9. Ferrari D, Pizzirani C, Adinolfi E, Lemoli RM, Curti A, Idzko M, et al. The P2X7 receptor: a key player in IL-1 processing and release. J Immunol 2006;176:3877-83.
10. Solle M, Labasi J, Perregaux DG, Stam E, Petrushova N, Koller BH, et al. Altered cytokine production in mice lacking P2X(7) receptors. J Biol Chem 2001;276:125-32.
11. Labasi JM, Petrushova N, Donovan C, McCurdy S, Lira P, Payette MM, et al. Absence of the P2X7 receptor alters leukocyte function and attenuates an inflammatory response. J Immunol 2002;168:6436-45.
12. Gu BJ, Zhang W, Worthington RA, Sluyter R, Dao-Ung P, Petrou S, et al. A Glu-496 to Ala polymorphism leads to loss of function of the human P2X7 receptor. J Biol Chem 2001; 276: 11135-42.
13. Sluyter R, Shemon AN, Wiley JS. Glu496 to Ala polymorphism in the P2X7 receptor impairs ATP-induced IL-1 β release from human monocytes. J Immunol 2004;172:3399-405.
14. Sluyter R, Dalitz JG, Wiley JS. P2X7 receptor polymorphism impairs extracellular adenosine 5'-triphosphate-induced interleukin-18 release from human monocytes. Genes Immun 2004;5:588-91.
15. Saunders BM, Fernando SL, Sluyter R, Britton WJ, Wiley JS. A loss-of-function polymorphism in the human P2X7 receptor abolishes ATP-mediated killing of mycobacteria. J Immunol 2003;171:5442-6.
16. Thomas ED, Storb R, Clift RA, Fefer A, Johnson L, Neiman PE, et al. Bone-marrow transplantation. N Engl J Med 1975;292:895-902.
17. McDonald GB, Hinds MS, Fisher LD, Schoch HG, Wolford JL, Banaji M, et al. Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients. Ann Intern Med 1993;118:255-67.
18. Dinarello CA, Cannon JG, Mier JW, Bernheim HA, LoPreste G, Lynn DL, et al. Multiple biological activities of human recombinant interleukin 1. J Clin Invest 1986;77:1734-9.
19. Dinarello CA, Wolff SM. The role of interleukin-1 in disease. N Engl J Med 1993;328:106-13.
20. Castelli MP, Black PL, Schneider M, Pennington R, Abe F, Talmadge JE. Protective, restorative, and therapeutic properties of recombinant human IL-1 in rodent models. J Immunol 1988;140:3830-7.
21. McConkey DJ, Hartzell P, Chow SC, Orrenius S, Jondal M. Interleukin 1 inhibits T cell receptor-mediated apoptosis in immature thymocytes. J Biol Chem 1990;265:3009-11.
22. Johnson CS. Interleukin-1: therapeutic potential for solid tumors. Cancer Invest. 1993;11(5):600-8.
23. Smith JW 2nd, Longo DL, Alvord WG, Janik JE, Sharfman WH, Gause BL, et al. The effects of treatment with interleukin-1 alpha on platelet recovery after high-dose carboplatin. N Engl J Med 1993;328:756-61.
24. Wilson HL, Francis SE, Dower SK, Crossman DC. Secretion of intracellular IL-1 receptor antagonist (type 1) is dependent on P2X7 receptor activation. J Immunol 2004; 173:1202-8.
25. Wiley JS, Dao-Ung LP, Gu BJ, Sluyter R, Shemon AN, Li C, et al. A loss-of-function polymorphic mutation in the cytolytic P2X7 receptor gene and chronic lymphocytic leukaemia: a molecular study. Lancet 2002; 359: 1114-9.
26. Giles JT, Bathon JM. Serious infections associated with anticytokine therapies in the rheumatic diseases. J Intensive Care Med 2004;19:320-34.
27. van der Meer JW, Barza M, Wolff SM, Dinarello CA. A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. Proc Natl Acad Sci USA 1988;85:1620-3.
28. Zhang B, Ma XT, Zheng GG, Li G, Rao Q, Wu KF. Expression of IL-18 and its receptor in human leukemia cells. Leuk Res 2003;27:813-22.
29. Griffin JD, Rambaldi A, Vellenga E, Young DC, Ostapovicz D, Cannistra SA. Secretion of interleukin-1 by acute myeloblastic leukemia cells in vitro induces endothelial cells to secrete colony stimulating factors. Blood 1987;70:1218-21.
30. Lebel-Binay S, Thiounn N, De Pinieux G, Vieillefond A, Debre B, Bonnefoy JY, et al. IL-18 is produced by prostate cancer cells and secreted in response to interferons. Int J Cancer 2003;106:827-35.
31. Apte RN, Voronov E. Interleukin-1: a major pleiotropic cytokine in tumor-host interactions. Semin Cancer Biol 2002;12:277-90.
32. Osaki T, Peron JM, Cai Q, Okamura H, Robbins PD, Kurimoto M, et al. IFN-γ-inducing factor/IL-18 administration mediates IFN-γ- and IL-12-independent antitumor effects. J Immunol. 1998;160:1742-9.
33. Osaki T, Hashimoto W, Gambotto A, Okamura H, Robbins PD, Kurimoto M, et al. Potent antitumor effects mediated by local expression of the mature form of the interferon-gamma inducing factor, interleukin-18 (IL-18). Gene Ther 1999;6:808-15.
34. Tanaka F, Hashimoto W, Robbins PD, Lotze MT, Tahara H. Therapeutic and specific antitumor immunity induced by co-administration of immature dendritic cells and adenoviral vector expressing biologically active IL-18. Gene Ther 2002;9:1480-6.
35. Veltri S, Smith JW 2nd. Interleukin 1 trials in cancer patients: a review of the toxicity, antitumor and hematopoietic effects. Stem Cells 1996;14:164-76.
36. Rocha V, Franco RF, Porcher R, Bittencourt H, Silva WA Jr, Latouche A, et al. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. Blood 2002;100:3908-18.
37. Scholl S, Sayer HG, Mugge LO, Kasper C, Pietraszczyk M, Kliche KO, et al. Increase of interleukin-18 serum levels after engraftment correlates with acute graft-versus-host disease in allogeneic peripheral blood stem cell transplantation. J Cancer Res Clin Oncol 2004; 130:704-10.
38. Mullighan CG, Heatley S, Doherty K, Szabo F, Grigg A, Hughes TP, et al. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. Blood 2002;99:3524-9.
39. Holler E, Rogler G, Brenmoehl J, Hahn J, Herfarth H, Greinix H, et al. Prognostic significance of NOD2/CARD15 variants in HLA-identical sibling hematopoietic stem cell transplantation: effect on long-term outcome is confirmed in 2 independent cohorts and may be modulated by the type of gastrointestinal decontamination. Blood 2006;107: 4189-93.
40. Granell M, Urbano-Ispizua A, Suarez B, Rovira M, Fernandez-Aviles F, Martinez C, et al. Mannan-binding lectin pathway deficiencies and invasive fungal infections following allogeneic stem cell transplantation. Exp Hematol 2006; 34:1435-41.
41. Cabrini G, Falzoni S, Forchap SL, Pellegatti P, Balboni A, Agostini P, et al. A His-155 to Tyr polymorphism confers gain-of-function to the human P2X7 receptor of human leukemic lymphocytes. J Immunol 2005;175:82-9.