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이학석사 학위논문

Effect of donor STAT4 polymorphism
rs7574865 on clinical outcomes of
pediatric acute leukemia patients after
hematopoietic stem cell transplant

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유전자다형성 rs7574865이
동종조혈모세포이식 후 임상결과에
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Effect of donor STAT4 polymorphism
rs7574865 on clinical outcomes of pediatric
acute leukemia patients after hematopoietic
stem cell transplant

by
Cheng Mun Wun

A Thesis Submitted to the Department of Medicine in Partial
Fulfillment of the Requirement for the Degree of Masters of
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Medicine

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Approved by Thesis Committee:

Professor _____, Chairman

Professor _____, Vice Chairman

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Abstract

Effect of donor STAT4 polymorphism rs7574865 on clinical outcomes of pediatric acute leukemia patients after hematopoietic stem cell transplant

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STAT4 polymorphism, rs7574865 is linked to various autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis. Its T minor allele is associated with higher STAT4 mRNA and protein expression, indicating a stronger skewed immune response than the norm. Although widely studied in autoimmune disease patients and the general population, its effect on immunocompromised subjects is still unknown. Especially in situations, i.e. post-hematopoietic stem cell transplantation (post-HSCT), where control of the immune response is crucial. Hence, this study investigates if the presence of the T minor allele in donors would affect immunological response and clinical outcomes post-HSCT. Samples from 161

pediatric patients who underwent allogeneic HSCT for acute leukemia and showed complete chimerism by donor cells were obtained. Six clinical outcomes were investigated; hepatic veno-occlusive disease, acute graft-vs-host disease, chronic graft-vs-host disease, cytomegalovirus (CMV) infection, relapse and overall survival. The TT genotype was found to be significant in the occurrence of CMV infection ($P=0.049$), showing higher incidence of CMV infection compared to the others. Multivariate analysis confirmed that association of the TT genotype is independent from other variables in CMV infection occurrence ($P=0.010$). This is the first study on STAT4 polymorphism rs7574865 in allogeneic HSCT as well as immunocompromised patients. As the TT genotype is associated with autoimmune diseases, our results seem at a paradox with current evidence hinting at a different role of STAT4 in normal circumstances versus immunocompromised patients. Further investigation is needed to elicit the reason behind this and discover novel applications for better post-transplant outcomes.

Keywords : HSCT, SNP, STAT4, rs7574865, Acute Leukemia, CMV infection

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List of Abbreviations

ABL	Acute Biphenotypic Leukemia
aGvHD	Acute Graft versus Host Disease
ALL	Acute Lymphoblastic Leukemia
AML	Acute Myeloid Leukemia
BM	Bone Marrow
Bu	Busulfan
CBT	Cord Blood Therapy
cGvHD	Chronic Graft versus Host Disease
CMV	Cytomegalovirus
HLA	Human Leukocyte Antigen
HSCT	Hematopoietic Stem Cell Transplantation
HVOD	Hepatic Veno-occlusive Disease
IFN- γ	Interferon gamma
IgG	Immunoglobulin G
OS	Overall Survival
PB	Peripheral Blood
PCR	Polymerase Chain Reaction
STAT4	Signal Transducer and Activator of Transcription 4
TBI	Total Body Irradiation
TRM	Transplant Related Mortality

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1. Introduction

Signal transducer and activator of transcription 4 (STAT4) is a member of the JAK-STAT pathway which is a vital signalling pathway in the immune response. The STAT protein is phosphorylated by Janus kinase (JAK) receptors on the cell surface after cytokine activation and translocates into the nucleus to initiate transcription of target genes [1]. It is the only tissue-specific STAT, expressed in myeloid and lymphoid tissues and has most commonly been associated with the regulation of interleukin-12 (IL-12) and its biological functions [2]. IL-12 binds specifically to two receptor subunits expressed on natural killer (NK) cells and activated B and T cells [3]. It is responsible for the production of IFN- γ , monocyte activation and T-helper type 1 (T_H1) cell differentiation [4]. Therefore it is a critical mediator of the immune response and it is highly plausible that any change in STAT4 activity or expression can alter the function and response of the immune system, leading to autoimmune disorders or immunosuppression.

As a matter of fact, many STAT4 polymorphisms have increasingly been associated with systemic autoimmune diseases such as systemic lupus erythematosus (SLE) [5], rheumatoid arthritis (RA) [6] etc. in various populations [7–10]. In particular, evidence has pointed to the presence of

polymorphism rs7574865 G/T as having the strongest associations. The presence of the T allele showed significant increase in odds ratio for SLE, RA, type 1 diabetes (T1D), Systemic Sclerosis (SSc) and Primary Sjogren's syndrome (pSS) [11] and possibly associated with juvenile idiopathic arthritis (JIA) and Primary antiphospholipid syndrome (APS) [12,13]. In addition, the TT genotype has been proven to increase susceptibility to autoimmune thyroid diseases (AITD) [14]. Its minor T allele has also been proven to be associated with acute renal allograft rejection [15], further implying a significant role in the immune response. Therefore we looked into the possibility of an association of the rs7574865 G/T polymorphism with other transplant outcomes as well, especially in hematopoietic stem cell transplant (HSCT).

2. Materials and Methods

2.1 Study Population

The study population consisted of pediatric patients with acute leukemia who underwent hematopoietic stem cell transplantation between November 2001 and April 2014 in the Seoul National University Children's Hospital. Out of 279 patients who underwent HSCT in this period, only 201 showed chimerism with donor cells. Out of this 201, only 161 produced viable DNA samples

from HLA-matched transplants, resulting in a final of 161 patient samples used in this study. Peripheral blood or bone marrow aspiration samples were collected after the transplantation procedure and stored in the human-derived sample storage facility. DNA was then isolated and analysed from each sample. Samples used met the following inclusion criteria: (i) pediatric patients who were diagnosed with acute leukemia and received allogeneic-HSCT (allo-HSCT); (ii) post-transplant samples showed complete chimerism of hematopoietic cells by donor cells (short tandem repeat (STR) test on patients' DNA < 0.8%).

This study was designed in accordance with the regulations for research involving human subjects and approved by the Institutional Review Board for Human Research (IRB number: 1512-061-728).

2.2 Transplantation Protocol

Before 2005, donors were selected based on human leukocyte antigen (HLA) serologic typing performed for class I antigens and HLA molecular typing for the DR loci. From 2006 onwards, typing for the DQ loci was included. HLA-A, -B, -C, -DR, -DQ were confirmed through a high-resolution molecular method for both recipients and donors.

Bu-based and TBI-based conditioning regimens were carried out to prepare patients for transplant. Graft-vs-host disease (GvHD) prophylaxis consisted of cyclosporine and prednisolone for related HSCT, cyclosporine and mycophenolate mofetil for cord blood therapy (CBT) or tacrolimus and methotrexate for unrelated bone marrow (BM) or peripheral blood (PB) stem cell transplantation. Hepatic veno-occlusive disease (HVOD) and infection prophylaxis were administered according to our center guidelines for HSCT. For HVOD prophylaxis, patients received lipo-PGE1 at a dose of 1 mcg/kg/day through continuous infusion with or without low molecular heparin (nadroparine calcium). For infection prophylaxis, patients received ciprofloxacin, itraconazole, or micafungin, and acyclovir. Intravenous immune globulin (0.5/g/kg/dose) was infused every two weeks until Day 100, then monthly until Day 180. Sulfamethotrexate/trimethoprim was discontinued three days before HSCT and restarted after engraftment.

2.3 Chimerism analysis

Serial analysis of STR in BM aspirates were performed to evaluate the hematopoietic chimerism in patients at 1, 3, 6 months and 1 year after the allo-HSCT, excluding patients who underwent CBT. Patients who underwent CBT had the chimerism analysis carried out more frequently beginning at 1,2

and 3 weeks from peripheral blood and 1, 3, 6 months and 1 year from BM aspirate. The chimerism analysis was based on the quantitative amplification of specific STR regions in the patient and donor by using an AmpFISTR profiler polymerase chain reaction (PCR) amplification kit (Applied Biosystems, Foster City, CA, USA).

2.4 SNP analysis

DNA was extracted from the samples by using the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA purity and concentration were determined by spectrophotometric measurements using absorbance levels at 260 nm and 280 nm. The extracted genomic DNA was stored at -20 °C before analysis for its genotype. The upstream primer sequence used was 5'-AAA GAA GTG GGA TAA AAA GAA GTT TG-3' and the downstream primer sequence 5'-CCA CTG AAA TAA GAT AAC CAC TGT-3' to generate a 147 base pair region of the STAT4 polymorphism, rs7574865 [15]. The polymerase chain reaction (PCR) was carried out on a Bio-Rad MyCycler™ Thermal Cycler. A total reaction volume of 30 µl containing 200 ng genomic DNA template, 20 pmol of each primer was added into a PCR pre-mix (Solg™ 2X Taq PCR Pre-Mix, SolGent, Daejeon, Korea). The amplification profile was as follows: 95 °C for

2 min, followed by 40 cycles of 95 °C for 20 s, 61 °C for 40 s and 72 °C for 60 s, with a final cycle of extension at 72 °C for 5 min. In addition, tubes containing all the same PCR components but with the DNA template replaced with distilled water served as negative controls to ensure that there was no contamination of DNA carried over from prior reactions.

2 µl of each PCR products was then ran on agarose gel to check for successful replication of the 147 bp product. The remaining 28 µl was purified with a PCR purification kit (QIAquick® PCR Purification Kit, QIAGEN, Hilden, Germany), according to the manufacturer's instructions, to a volume of 30 µl. 15 µl of the purified product was incubated with a three times excess in units of *Hpa* I restriction enzyme (RE) (Takara Bio Inc., Otsu, Japan) for 90 min at 37 °C. This RE specifically recognises and digests DNA amplified from the minor allele (T) into 122 bp and 25 bp products. Therefore GG homozygous individuals will show only 147bp bands, GT heterozygous individuals bands of both 147 bp and 122 bp while homozygous TT individuals show only the 122 bp band (Figure 1). Reaction mixtures using distilled water in place of the REs were used as negative controls while mixtures using a known TT genotype DNA sample was used as positive controls.

Both undigested and digested PCR products were visualised in 2.5% agarose

gel stained with StaySafe™ Nucleic Acid Gel Stain (Real Biotech Corporation, Taipei, Taiwan). Genotypes were scored blindly and all ambiguous samples repeated.

Figure 1. PCR-based RFLP analysis of STAT4 polymorphism, rs7574865. Lane 1 represents a 1kb DNA ladder. Lane 2 is a homozygous sample of the polymorphism with 2 T alleles while lane 3 is a heterozygous sample of 1 T allele and 1 G allele. Lane 4 represents a homozygous sample with 2 T alleles. The 25bp restriction fragments from lane 2 and 3 run off the gel during electrophoresis.

2.5 Definitions

The diagnosis of HVOD was made based on the modified Seattle criteria [16], acute graft-versus-host disease (aGvHD) and chronic graft-versus-disease (cGvHD) according to their respective standard criteria [17,18]. Cytomegalovirus (CMV) infection was defined as the presence of CMV pp65 antigen positive cells at a frequency of $\geq 1/2 \times 10^5$ cells. CMV disease was diagnosed through histopathological examinations or ophthalmoscopy exam showing characteristic retinal changes.

2.6 Statistical Analysis

Statistical calculations were performed with SPSS statistical software version 23.0 for Mac (SPSS Inc., Chicago, IL, USA) and R statistical software version 3.2.3 for Mac. The Kaplan-Meier method and Log-rank test were used for univariate survival analysis. Cumulative incidences (CI) were analyzed with the Gray test. Competing events were defined as death without HVOD for HVOD; death without CMV infection for CMV infection; death without aGvHD for aGvHD and death without cGvHD for cGvHD. Factors that showed a statistically significant difference upon univariate analysis were included in the multivariate analysis. All tests were two tailed and a statistical value of $P < 0.05$ was considered to be significant.

3. Results

3.1 rs7574865 genotyping

161 patient samples were analysed for the rs7574865 polymorphism genotype. 78 (48.4%) patients were found to have the homozygous GG genotype, 69 (42.9%) had the heterozygous GT phenotype while 14 (8.7%) had the homozygous TT genotype. This is in consensus with the population average of about 10% for the TT genotype [14,19]. This along with other baseline and demographic clinical characteristics of patients involved in the study are listed in Table 1.

Table 1 Patient Characteristics

Characteristics	n=161
Gender, No. (%)	
Male	88 (54.7%)
Female	73 (45.3%)
Mean age, yr.(range)	8.96 (0.6-22.3)
Genotype, No.(%)	
GG	78 (48.4%)
GT	69 (42.9%)
TT	14 (8.7%)
Diagnosis, No.(%)	
ALL	72 (44.7%)
AML	70 (43.5%)
ABL	19 (11.8%)
Conditioning regimens, No. (%)	
BU-based	125 (77.6%)
TBI-based	36 (22.4%)
Type of donor, No.(%)	
Related donor	39 (24.2%)
Unrelated donor	122 (74.8%)
Type of stem cell source, No.(%)	
Bone marrow	41 (25.5%)
Peripheral blood	81 (50.3%)
Cord blood	39 (24.2%)
First transplantation, No.(%)	
Yes	146 (90.7%)
No	15 (9.3%)
CMV infection, No.(%)	105 (65.2%)
CMV disease, No.(%)	12 (7.5%)
Outcome, No.(%)	
HVD	30(18.6%)
Death	53 (32.9%)
TRM	16 (9.9%)
Relapse	36 (22.4%)

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia;
 ABL, acute biphenotypic leukemia; BU, busulfan; TBI, total body
 irradiation; CMV, cytomegalovirus; HVD, hepatic veno-occlusive
 disease; TRM, transplant-related mortality

3.2 Cumulative incidence

The influence of the genotypes was compared in six outcomes; HVOD, CMV infection, aGvHD, cGvHD, relapse and survival. When comparing the three genotypes (GG, GT and TT) individually, no statistically significant difference was observed (Figure 2). Then, the GG and GT genotypes were combined and compared against the TT genotype (Figure 3). In the case of CMV infection, the TT genotype was found to significantly affect disease occurrence compared to the GG and GT group ($P= 0.05$). Previous studies have also shown that the source of stem cells for transplantation may have an impact on certain outcomes [20,21]. Therefore, we investigated the significance of the target genotype for CMV infection in each of the three different stem cell sources (Figure 4). Results show that the TT genotype has a significant influence on CMV infection after transplantation from cord blood ($P= 0.01$) but does not affect those with bone marrow or peripheral blood as stem cell sources. No statistically significant results were observed for HVOD, aGvHD, cGvHD, relapse or survival in the patients but a general trend where the TT genotype shows a lower rate of disease occurrence and higher overall survival is observed.

Figure 2. Cumulative incidence of various outcomes after HSCT. The outcomes include HVOD, CMV infection, aGvHD, cGvHD, relapse and survival. In all six events, no statistical significance was observed between the three different genotypes. However, observing the general trends of the three curves, the GG and GT phenotypes seem to show close association with each other in most of the events.

Figure 3. Cumulative incidence of outcomes after HSCT compared among GG and GT genotype patients with those with homozygous TT genotype. Statistical evidence was not found for the above five events, HVOD, aGvHD, cGvHD, relapse and overall survival. Proportions denote the number of patients with the event/total number of patients in the group.

Figure 4. Cumulative incidence of CMV infection, according to the stem cell source, in patients with the G allele compared with homozygous T allele genotype from donors. A denotes the incidence rate from bone marrow as the source, B from peripheral blood and C from cord blood. A and B both do not show that the TT genotype has a statistical significance in the occurrence of CMV ($P = 0.722$ and $P = 0.175$ respectively). C however, shows statistical significance ($P = 0.008$) in the occurrence of CMV for TT genotypes compared to other genotypes.

3.3 Determination of rs7574865 as CMV risk factor through multivariate analysis

Table 2 shows a multivariate analysis to identify predictors of CMV infection through the Cox regression model. The analysis was used to determine the influence of rs7574865 and other clinical variables (gender, age, disease, pre-SCT status, donor type, conditioning regime and CMV prophylaxis) on its occurrence after significance was found in univariate analysis. It supports the results shown in the previous figures, identifying the rs7574865 TT genotype as a risk factor for CMV infection ($P= 0.03$), with carriers twice as likely as the GG and GT group to contract CMV infection. Current research has also shown that donor CMV seropositivity can be a risk factor for CMV infection [22,23], hence the donor CMV IgG status was also investigated in this experiment. However, only 9% of the donors was confirmed to be IgG-negative, with the majority of the donors showing seropositivity. Therefore, its influence on CMV infection does not undermine the significance of the influence from the TT genotype. The multivariate analysis shows that except for the rs7574865 polymorphism, other clinical variables did not influence CMV infection.

Table 2 Multivariate Analysis For Transplantation Outcomes

	n	CMV			TRM			OS		
		HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
rs7574865										
GG>	147	1			1			1		
TT	14	2.06	1.09-3.89	0.03	0.64	0.08-5.05	0.67	1.10	0.57-2.12	0.77
Gender										
male	88	1			1			1		
female	73	1.00	0.67-1.48	0.99	0.57	0.20-1.61	0.29	1.34	0.91-2.00	0.14
Age at HSCT	161	1.02	0.98-1.07	0.27	0.95	0.85-1.05	0.29	0.99	0.95-1.04	0.78
Diagnosis										
ALL	72	1			1			1		
ABL	19	0.90	0.48-1.68	0.74	0.45	0.55-3.71	0.46	0.45	0.23-0.88	0.45
AML	70	0.68	0.44-1.05	0.08	1.57	0.54-4.52	0.41	0.89	0.58-1.35	0.89
preSCT status										
1st CR	112	1			1			1		
2nd CR	32	1.12	0.67-1.86	0.68	1.60	0.44-5.78	0.48	1.11	0.66-1.87	0.70
advanced	17	1.61	0.84-3.10	0.15	2.37	0.61-9.20	0.21	2.25	0.86-5.89	0.10
Donor type										
Bone Marrow	41	1			1			1		
Peripheral Blood	81	0.95	0.58-1.55	0.82	1.77	0.47-6.76	0.40	7.47	4.04-13.82	0.00
Cord	39	1.41	0.82-2.42	0.22	1.42	0.30-6.68	0.66	1.57	0.91-2.71	0.10
Conditioning Regimen										
BU	127	1			1			1		
TBI	34	0.35	0.48-1.30	0.35	2.70	0.86-8.44	0.09	0.57	0.30-1.06	0.08

HSCT: hematopoietic stem cell transplant; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ABL, acute biphenotypic leukemia; BM, bone marrow; PB, peripheral blood; BU, busulfan; TBI, total body irradiation; CMV, cytomegalovirus; TRM, transplant-related mortality;

4. Discussion

HSCT is now the common practice in the treatment of congenital or acquired hematologic malignancies or immunological disorders [24]. However, the success of HSCT hinges on the careful balance of the donor immune response to elicit a Graft vs Tumor effect [25] while keeping the recipient's immune response carefully in check to prevent GvHD and rejection of the graft but minimise the risk of infections [26]. Hence research on changes that can occur in an individual's immune response stands to benefit increasing the success of HSCT. The study is focused specifically on the rs7574865 polymorphism due to its increasing popularity in recent immunology studies. As mentioned previously, the polymorphism has influence in various immunological diseases but as it is found in the third intron of the gene, its relation with protein function is yet unknown. A recent study on the polymorphism has shown higher sensitivity to IFN- γ , an inflammatory cytokine which is involved in signaling of the immune response [27]. The T allele has also been associated with higher STAT4 mRNA and protein levels [28]. STAT4 is crucial for the initiation and maintenance of the immune and inflammatory responses [29]. Our findings suggest that donor TT genotype from rs7574865 G/T polymorphism may affect the immune response of recipients and hence influence the success of HSCT.

To our knowledge, this is the first study of a STAT4 polymorphism in HSCT, in immunocompromised patients and in pediatrics. In our study, we assessed the potential influence of the presence of the rs7574865 minor T allele in donor cells on the clinical outcomes of patients post-aHSCT. Out of the six outcomes investigated, the T minor allele was found to be significantly associated with the occurrence of CMV infection. Although no statistical significance was noted, the increase in CMV infection and the lower occurrence of unfavourable outcomes (lower incidences of aGvHD, cGvHD and relapse with higher incidence of overall survival) in the TT genotype seem to indicate a possible weaker immune response in this genotype compared to the others. This seemingly appears as a contradiction to current knowledge of this polymorphism yet reports showing similar paradoxes have also been reported. Rs7574865 has been reported to be linked to a predisposition to SLE as well as increased STAT4 mRNA and protein expression which theoretically leads to a stronger immune and/or inflammation response. Yet a report by *Singh et.al* shows accelerated nephritis in STAT4-deficient lupus mice models [30]. *Chaim et al.* showed that STAT4 deficiency accelerated kidney disease and led to higher mortality for SLE but lower levels of autoantibodies were observed compared to the wildtype [31]. Additionally, a study by Shin et.al found that CMV infection and aGvHD occurrence showed differential expression in suppressors of

cytokine signaling (SOCS) proteins [32]. As SOCS proteins function via the Jak/STAT pathway, the difference in expression of immune regulatory molecules gives support to the results found in our study. Hence, these reports suggest that more detailed research is needed to ascertain the role of STAT4 in the progression of various diseases as well as to fully determine its capacity and its mechanism of action in an immune response. The lower incidences of negative outcomes (i.e. occurrence of GvHD and relapse) also brings to question the graft vs leukemia effect of this polymorphism and is an area of interest for future research.

In addition, some factors of this study should be noted when reviewing the results. For example, Figure 3 shows statistical significance of the TT genotype for CMV infection in cord blood but not in peripheral blood or bone marrow. To our knowledge, the congenital cord blood infection rate in Korea is only 1.5% [33] and hence is unlikely that the results were due to cord blood cells carrying CMV infection prior to transplant. However, CMV infection has been found to be highly prevalent in Korea (>90%) [34]. Hence most donors would have been exposed to and developed an immunity to CMV. When performing transplant with bone marrow or peripheral blood, the recipient inherits this immunity from the donor's immune cells and is less likely to develop CMV infection [35]. In cord blood transplants however, the

donor cord blood has not been exposed to the CMV virus and hence has lower immunity and higher susceptibility to CMV infection [36].

Another factor that should be accounted for is the setting in which this experiment was carried out. Previous research has been focused on the role of the polymorphism in patients with autoimmune diseases while comparing to the general population. In our experiment, we looked at the effects of this polymorphism on HSCT patients who are medically immunocompromised. Studies have shown that many commonly used drugs in cancer treatment such as glucocorticoids, i.e dexamethasone [37], and chemotherapy [38] inhibit STAT4 phosphorylation and result in acquired STAT4 deficiency respectively. This does not affect the results of our experiment as patients undergo similar regimens and hence does not pose as a problem when comparing within the sample. However, these discrepancies with the general population indicate that any comparison or translation of the results to other studies must be handled with caution. As the mechanism of the polymorphism is yet unknown, the possibility that the minor T allele has a higher susceptibility to these drugs and treatment is also another factor that must be considered. In addition, this is the first study of STAT4 polymorphism in children, more research has to be done to see if similar results can be found in adults.

In conclusion, our research sheds light on the importance and impact of donor STAT4 polymorphisms in transplant recipients and its correlation on transplantation outcomes. It highlights the significance of understanding the role of STAT4 in the immune response. Medication that affects STAT4 expression is already attainable (i.e. dexamethasone which inhibits STAT4 phosphorylation therefore inhibiting TH1 response [37]), but its role is still very limited. This study therefore also hopes to introduce novel drug development targets for STAT4-related diseases.

References

- [1] a L. Wurster, T. Tanaka, M.J. Grusby, The biology of Stat4 and Stat6., *Oncogene*. 19 (2000) 2577–2584. doi:10.1038/sj.onc.1203485.
- [2] M.H. Kaplan, STAT4, *Immunol. Res.* 31 (2005) 231–241. doi:10.1385/IR:31:3:231.
- [3] W.E. Thierfelder, J.M. van Deursen, K. Yamamoto, R.A. Tripp, S.R. Sarawar, R.T. Carson, M.Y. Sangster, D.A. Vignali, P.C. Doherty, G.C. Grosveld, J.N. Ihle, Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells., *Nature*. 382 (1996) 171–4. doi:10.1038/382171a0.
- [4] B.D. Korman, D.L. Kastner, P.K. Gregersen, E.F. Remmers, STAT4: genetics, mechanisms, and implications for autoimmunity., *Curr. Allergy Asthma Rep.* 8 (2008) 398–403. doi:10.1007/s11882-008-0077-8.
- [5] A. Kawasaki, I. Ito, K. Hikami, J. Ohashi, T. Hayashi, D. Goto, I. Matsumoto, S. Ito, A. Tsutsumi, M. Koga, T. Arinami, R.R. Graham, G. Hom, Y. Takasaki, H. Hashimoto, T.W. Behrens, T. Sumida, N. Tsuchiya, Role of STAT4 polymorphisms in systemic lupus erythematosus in a Japanese population: a case-control association study of the STAT1-STAT4 region., *Arthritis Res. Ther.* 10 (2008)

R113. doi:10.1186/ar2516.

- [6] S. Kobayashi, K. Ikari, H. Kaneko, Y. Kochi, K. Yamamoto, K. Shimane, Y. Nakamura, Y. Toyama, T. Mochizuki, S. Tsukahara, Y. Kawaguchi, C. Terai, M. Hara, T. Tomatsu, H. Yamanaka, T. Horiuchi, K. Tao, K. Yasutomo, D. Hamada, N. Yasui, H. Inoue, M. Itakura, H. Okamoto, N. Kamatani, S. Momohara, Association of STAT4 with susceptibility to rheumatoid arthritis and systemic lupus erythematosus in the Japanese population, *Arthritis Rheum.* 58 (2008) 1940–1946. doi:10.1002/art.23494.
- [7] B. Namjou, A.L. Sestak, D.L. Armstrong, R. Zidovetzki, J.A. Kelly, N.J.V. Ciobanu, K.M. Kaufman, J.O. Ojwang, J. Ziegler, F.P. Quismorio, A. Reiff, B.L. Myones, J.M. Guthridge, S.K. Nath, G.R. Bruner, R. Mehrian-Shai, E. Silverman, M. Klein-Gitelman, D. Mccurdy, L. Wagner-Weiner, J.J. Nocton, C. Putterman, S.C. Bae, Y.J. Kim, M. Petri, J.D. Reveille, T.J. Vyse, G.S. Gilkeson, D.L. Kamen, M.E. Alarc??n-Riquelme, P.M. Gaffney, K.L. Moser, J.T. Merrill, R.H. Scofield, J.A. James, C.D. Langefeld, J.B. Harley, C.O. Jacob, High-density genotyping of STAT4 reveals multiple haplotypic associations with Systemic lupus erythematosus in different racial groups, *Arthritis Rheum.* 60 (2009) 1085–1095. doi:10.1002/art.24387.
- [8] E.F. Remmers, R.M. Plenge, A.T. Lee, R.R. Graham, G. Hom, T.W.

- Behrens, P.I.W. de Bakker, J.M. Le, H.-S. Lee, F. Batliwalla, W. Li, S.L. Masters, M.G. Booty, J.P. Carulli, L. Padyukov, L. Alfredsson, L. Klareskog, W. V. Chen, C.I. Amos, L.A. Criswell, M.F. Seldin, D.L. Kastner, P.K. Gregersen, STAT4 and the Risk of Rheumatoid Arthritis and Systemic Lupus Erythematosus, <http://dx.doi.org/10.1056/NEJMoa073003>. (2009).
- [9] O. Beltrán Ramírez, J.F. Mendoza Rincón, R.E. Barbosa Cobos, I. Alemán Ávila, J. Ramírez Bello, STAT4 confers risk for rheumatoid arthritis and systemic lupus erythematosus in Mexican patients, *Immunol. Lett.* 175 (2016) 40–43. doi:10.1016/j.imlet.2016.05.003.
- [10] H.M. El-Saadany, W.H. Amer, H.S. Khalil, R.A. Gaber, S.A. Elshweikh, Association of STAT4 polymorphism with susceptibility and severity of rheumatoid arthritis and systemic lupus erythematosus in Egyptian patients, Egypt. *Rheumatol.* 38 (2016) 21–27. doi:10.1016/j.ejr.2015.04.003.
- [11] J. Zheng, J. Yin, R. Huang, F. Petersen, X. Yu, Meta-analysis reveals an association of STAT4 polymorphisms with systemic autoimmune disorders and anti-dsDNA antibody, *Hum. Immunol.* 74 (2013) 986–992. doi:10.1016/j.humimm.2013.04.034.
- [12] Y.L. Liang, H. Wu, X. Shen, P.Q. Li, X.Q. Yang, L. Liang, W.H. Tian, L.F. Zhang, X.D. Xie, Association of STAT4 rs7574865 polymorphism with autoimmune diseases: A meta-analysis, *Mol. Biol.*

- Rep. 39 (2012) 8873–8882. doi:10.1007/s11033-012-1754-1.
- [13] A. Martínez, J. Varadé, A. Márquez, M.C. Cénit, L. Espino, N. Perdignes, J.L. Santiago, M. Fernández-Arquero, H. de la Calle, R. Arroyo, J.L. Mendoza, B. Fernández-Gutiérrez, E.G. de la Concha, E. Urcelay, Association of the STAT4 gene with increased susceptibility for some immune-mediated diseases, *Arthritis Rheum.* 58 (2008) 2598–2602. doi:10.1002/art.23792.
- [14] Y. Park, H.-S. Lee, Y. Park, D. Min, S. Yang, D. Kim, B. Cho, Evidence for the role of STAT4 as a general autoimmunity locus in the Korean population, *Diabetes. Metab. Res. Rev.* 27 (2011) 867–871. doi:10.1002/dmrr.1263.
- [15] H. Yang, Q. Zhou, Z.M. Chen, W.Q. Chen, M.M. Wang, J.H. Chen, Polymorphisms in STAT4 increase the risk of acute renal allograft rejection in the Chinese population, *Transpl. Immunol.* 24 (2011) 216–219. doi:10.1016/j.trim.2011.01.001.
- [16] G.B. McDonald, M.S. Hinds, L.D. Fisher, H.G. Schoch, J.L. Wolford, M. Banaji, B.J. Hardin, H.M. Shulman, R.A. Clift, Veno-occlusive disease of the liver and multiorgan failure after bone marrow transplantation: a cohort study of 355 patients., *Ann. Intern. Med.* 118 (1993) 255–67.
- [17] D. Przepiorka, D. Weisdorf, P. Martin, H.G. Klingemann, P. Beatty, J. Hows, E.D. Thomas, 1994 Consensus Conference on Acute GVHD

- Grading., Bone Marrow Transplant. 15 (1995) 825–8.
- [18] A.H. Filipovich, D. Weisdorf, S. Pavletic, G. Socie, J.R. Wingard, S.J. Lee, P. Martin, J. Chien, D. Przepiorka, D. Couriel, E.W. Cowen, P. Dinndorf, A. Farrell, R. Hartzman, J. Henslee-Downey, D. Jacobsohn, G. McDonald, B. Mittleman, J.D. Rizzo, M. Robinson, M. Schubert, K. Schultz, H. Shulman, M. Turner, G. Vogelsang, M.E.D. Flowers, National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Group Report, Biol. Blood Marrow Transplant. 11 (2005) 945–956. doi:10.1016/j.bbmt.2005.09.004.
- [19] E.S. Kim, S.W. Kim, C.M. Moon, J.J. Park, T. Il Kim, W.H. Kim, J.H. Cheon, Interactions between IL17A, IL23R, and STAT4 polymorphisms confer susceptibility to intestinal Behcet’s disease in Korean population, Life Sci. 90 (2012) 740–746. doi:10.1016/j.lfs.2012.03.017.
- [20] M. Eapen, J. Le Rademacher, J.H. Antin, R.E. Champlin, J. Carreras, J. Fay, J.R. Passweg, J. Tolar, M.M. Horowitz, J.C.W. Marsh, H.J. Deeg, N. Young, R. Calado, P. Scheinburg, S. Rosenfeld, D. Follman, O. Nunez, N. Young, J. Marsh, S. Ball, J. Cavenagh, W.M.D.A.D.R.W. Group, H. Schrezenmeier, J. Passweg, J. Marsh, A. Bacigalupo, G. Socie, H. Schrezenmeier, D. Przepiorka, D. Weisdorf, P. Martin, M. Flowers, E. Kansu, K. Sullivan, J. Klein, M.

- Moeschberger, D. Cox, D. Margolis, J. Casper, G. Socie, M. Henry-Amar, A. Bacigalupo, H. Deeg, G. Socie, G. Schoch, H. Deeg, M. O'Donnell, J. Tolar, Effect of stem cell source on outcomes after unrelated donor transplantation in severe aplastic anemia., *Blood*. 118 (2011) 2618–21. doi:10.1182/blood-2011-05-354001.
- [21] C. Peters, J.M. Cornish, S.H. Parikh, J. Kurtzberg, Stem Cell Source and Outcome After Hematopoietic Stem Cell Transplantation (HSCT) in Children and Adolescents with Acute Leukemia, *Pediatr. Clin. NA*. 57 (2010) 27–46. doi:10.1016/j.pcl.2010.01.004.
- [22] M. Boeckh, W.G. Nichols, The impact of cytomegalovirus serostatus of donor and recipient before hematopoietic stem cell transplantation in the era of antiviral prophylaxis and preemptive therapy, 103 (2004) 2003–2008. doi:10.1182/blood.
- [23] J.D. Meyers, N. Flournoy, E. Donnall Thomas, Risk Factors for Cytomegalovirus Infection after Human Marrow Transplantation, *J. Infect. Dis*. 153 (1986) 478–488. doi:10.1093/infdis/153.3.478.
- [24] A. Gratwohl, H. Baldomero, M. Aljurf, et al, Hematopoietic stem cell transplantation: A global perspective, *JAMA*. 303 (2010) 1617–1624.
- [25] F.R. Appelbaum, Haematopoietic cell transplantation as immunotherapy, *Nature*. 411 (2001) 385–389. doi:10.1038/35077251.
- [26] J.L. Ferrara, J.E. Levine, P. Reddy, E. Holler, Graft-versus-host

- disease, *Lancet*. 373 (2009) 1550–1561. doi:10.1016/S0140-6736(09)60237-3.
- [27] S.S. Cho, C.M. Bacon, C. Sudarshan, R.C. Rees, D. Finbloom, R. Pine, J.J. O'Shea, Activation of STAT4 by IL-12 and IFN- α : evidence for the involvement of ligand-induced tyrosine and serine phosphorylation., *J. Immunol*. 157 (1996) 4781–9.
- [28] A. Lamana, M. López-Santalla, R. Castillo-González, A.M. Ortiz, J. Martín, R. García-Vicuña, I. González-Álvaro, N. Jacobson, S. Szabo, R. Weber-Nordt, Z. Zhong, R. Schreiber, J.D. Jr., B. Korman, D. Kastner, P. Gregersen, E. Remmers, B. Rueda, J. Broen, C. Simeon, R. Hesselstrand, B. Diaz, H. Suarez, A. Abelson, A. Delgado-Vega, S. Kozyrev, E. Sanchez, R. Velazquez-Cruz, N. Eriksson, S. Kariuki, K. Kirou, E. MacDermott, L. Barillas-Arias, M. Crow, T. Niewold, D. Jiang, J. Sun, G. Cao, Y. Liu, D. Lin, Y. Gao, M. Prevoo, M. van 't Hof, H. Kuper, M. van Leeuwen, L. van de Putte, P. van Riel, J. Esteve-Vives, E. Batlle-Gualda, A. Reig, K. Livak, T. Schmittgen, D. Aletaha, T. Neogi, A. Silman, J. Funovits, D. Felson, C.B. 3rd, A. Lamana, A. Balsa, B. Rueda, A. Ortiz, L. Nuno, M. Miranda-Carus, M. Remoli, J. Ragimbeau, E. Giacomini, V. Gafa, M. Severa, R. Lande, A. Rose, A. Nott, S. Meislin, M. Moore, The Minor Allele of rs7574865 in the STAT4 Gene Is Associated with Increased mRNA and Protein Expression, *PLoS One*. 10 (2015) e0142683.

doi:10.1371/journal.pone.0142683.

- [29] M.H. Kaplan, A.L. Wurster, M.J. Grusby, A Signal Transducer and Activator of Transcription (Stat)4-independent Pathway for the Development of T Helper Type 1 Cells, *J. Exp. Med.* 188 (1998) 1191–1196. doi:10.1084/jem.188.6.1191.
- [30] R.R. Singh, V. Saxena, S. Zang, L. Li, F.D. Finkelman, D.P. Witte, C.O. Jacob, Differential Contribution of IL-4 and STAT6 vs STAT4 to the Development of Lupus Nephritis, *J. Immunol.* 170 (2003) 4818–4825. doi:10.4049/jimmunol.170.9.4818.
- [31] C.O. Jacob, S. Zang, L. Li, V. Ciobanu, F. Quismorio, A. Mizutani, M. Satoh, M. Koss, Pivotal Role of Stat4 and Stat6 in the Pathogenesis of the Lupus-Like Disease in the New Zealand Mixed 2328 Mice, *J. Immunol.* 171 (2003) 1564–1571. doi:10.4049/jimmunol.171.3.1564.
- [32] S. Shin, J.Y. Lee, T.H. Lee, S. Park, S. Yahng, J. Yoon, S. Lee, B. Cho, D. Lee, Y. Kim, S. Lee, C. Min, S. Cho, D. Kim, J. Lee, W. Min, C. Park, H. Kim, SOCS 1 and SOCS3 are expressed in mononuclear cells in human cytomegalovirus viremia after allogeneic hematopoietic stem cell transplantation, 50 (2015).
- [33] Y.M. Sohn, K.I. Park, C. Lee, D.G. Han, W.Y. Lee, Congenital cytomegalovirus infection in Korean population with very high prevalence of maternal Immunity, *J Korean Med Sci.* 7 (1992) 47–51.
- [34] J.D. Park, E.H. Choi, H.J. Lee, H.S.A. Half-dose, Half-dose

- ganciclovir preemptive treatment of cytomegalovirus infection after pediatric allogeneic hematopoietic stem cell transplantation, (2016) 396–404. doi:10.1111/tid.12539.
- [35] F.E. Chen, G. Aubert, P. Travers, I.A. Dodi, J.A. Madrigal, HLA tetramers and anti-CMV immune responses: from epitope to immunotherapy, *Cytotherapy*. 4 (2002) 41–48. doi:10.1080/146532402317251518.
- [36] H.J. Kang, K.H. Yoo, J.W. Lee, H. Kim, S.H. Lee, Double umbilical cord blood transplantation for children and adolescents, (2010) 1035–1044. doi:10.1007/s00277-010-0985-2.
- [37] D. Franchimont, J. Galon, M. Gadina, R. Visconti, Y.-J. Zhou, M. Aringer, D.M. Frucht, G.P. Chrousos, J.J. O’Shea, Inhibition of Th1 Immune Response by Glucocorticoids: Dexamethasone Selectively Inhibits IL-12-Induced Stat4 Phosphorylation in T Lymphocytes, *J. Immunol.* 164 (2000) 1768–1774. doi:10.4049/jimmunol.164.4.1768.
- [38] I.P. Lupov, L. Voiles, L. Han, A. Schwartz, M. De, L. Rosa, K. Oza, D. Pelloso, R.P. Sahu, J.B. Travers, M.J. Robertson, H.-C. Chang, Acquired STAT4 deficiency as a consequence of cancer chemotherapy, *Blood*. 118 (2011) 6097–6016. doi:10.1182/blood-2011.

국문초록

STAT4 다형성 rs7574865는 전신 홍반성 루프스, 류마티스 관절염과 같은 다양한 자가면역질환과 관련된 유전자이다. STAT4 rs7574865 T 대립유전자는 STAT4 mRNA와 단백질 발현을 증가시키는 것으로 알려져 있고, 이는 표준보다 왜곡된 면역반응을 일으킬 가능성을 시사한다. 자가면역질환 환자 또는 일반인을 대상으로 STAT4에 대해 많은 연구가 이루어졌으나 면역억제 환자를 대상으로 하는 연구는 많이 부족한 실정이다. 특히 조혈모세포이식 후와 같이 면역반응의 조절이 매우 중요한 특수 상황에서 STAT4의 역할을 발견하는 것은 큰 의미를 가진다. 이에 저자들은 공여자의 rs7574865 T 대립유전자의 존재가 동종조혈모세포이식 후의 면역반응과 임상결과에 미치는 영향에 대하여 연구하였다.

2001년 11월부터 2014년 4월까지 서울대학교 어린이병원에서 급성백혈병으로 조혈모세포이식을 시행 받았으며, 이식 후 공여자의

조혈세포로 완전히 치환된 결과를 보이는 161명의 환자를 대상으로 하였다. 유전형질분석을 위해 중합효소 연쇄반응(PCR) 및 제한단편길이다형성 분석을 시행하였다.

TT 유전자형을 가진 경우 자가면역질환에 취약하다는 것이 알려진 바 있어, TT 유전자형을 가진 그룹과 나머지 GG와 GT 유전자형을 가진 환자의 두 그룹으로 나누어 합병증, 재발, 전체 생존율에 대해 비교분석을 시행하였다. 비교분석에 포함된 조혈모세포이식의 합병증은 간정맥폐쇄질환, 급성 및 만성 이식편대숙주병, 사이토메갈로바이러스 (CMV) 감염이었다. 분석 결과, TT 유전자형을 가지는 환자군에서 CMV감염 발생률이 다른 유전자형을 가진 그룹에 비하여 높았다. 다변량 분석을 통하여 TT 유전자형은 CMV 감염의 발생과 독립적으로 유의한 관계가 있음을 밝혀내었다.

이는 조혈모세포이식을 시행 받는 면역 저하 환자에서 rs7574865 에 대해 시행된 첫 번째 연구이다. 기존 연구들에서 TT 유전자형이

자가면역질환과의 연관되어 있음이 밝혀진 것과는 달리, 본 연구의 결과는 면역 억제자들에서는 역설적으로 STAT4 가 다른 역할을 할 가능성이 있음을 시사한다. 상기 결과를 보여준 근거에 대해 밝혀내고, 조혈모세포이식의 성적을 향상시킬 수 있도록 추가적인 연구가 필요할 것으로 사료된다.