Published in final edited form as:

Int J Immunogenet. 2014 December ; 41(6): 521–527. doi:10.1111/iji.12158.

The influence of interleukin 7 receptor α chain haplotypes on outcome after allogeneic hematopoietic cell transplantation

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Summary

We investigated the influence of IL-7 receptor α chain (IL-7R α) gene haplotypes in donors on the outcome of haematopoietic cell transplantation (HCT). Unlike the association between single donor SNPs and HCT outcome found previously, only trends towards association were found here, due to “dilution” of SNPs into haplotypes.

Introduction

Interleukin-7 (IL-7) is essential for thymic T cell development (Hong *et al.*, 2012) and peripheral T cell maintenance (Carrette & Surh, 2012). The IL-7 receptor (IL-7R) is a heterodimer consisting of the α -chain (IL-7R α /CD127) and the common γ chain (CD132). IL-7R α also combines with the receptor of thymic stromal lymphopoietin (TSLP), a cytokine with diverse effects, including thymic development of Foxp3⁺ regulatory T cells (Tregs) (Watanabe *et al.*, 2005), peripheral differentiation of T helper (Th) 2 cells (Ito *et al.*, 2005) and stimulation of TNF production by dendritic cells (DCs) (Soumelis *et al.*, 2002). The IL-7R α gene is polymorphic and has been shown to be associated with risk of developing immune disorders such as multiple sclerosis (Broux *et al.*, 2010; Gregory *et al.*, 2007; Lundmark *et al.*, 2007) and sarcoid inflammation (Heron *et al.*, 2009), and with poor outcome after haematopoietic stem cell transplantation (HCT) (Shamim *et al.*, 2013).

HCT is a treatment for severe haematologic malignancies as well as a number of benign diseases including severe aplastic anemia and immunodeficiencies. However, recipients suffer from a prolonged post-transplant immune deficiency resulting in significant morbidity and mortality and a significant number of patients experience a relapse of leukemia after the transplant (Socie *et al.*, 1999). Previous studies indicated that certain IL-7R α single nucleotide polymorphisms (SNPs), when present in the HCT donor, are associated with a worse outcome after transplantation. More specifically, rs1494555GG and rs1494558TT donor genotypes are associated with acute and chronic graft versus host disease (GvHD) (Shamim *et al.*, 2006; Shamim *et al.*, 2013), while rs6897932T has been associated with relapse of leukaemia after HCT (Shamim *et al.*, 2013). These results indicate a role of the IL-7 pathway and IL-7R α polymorphisms in the outcome after HCT. These findings have suggested that selection of donors based on IL-7R α genotyping may lead to an improved survival. In addition to single SNPs, genotyping of IL-7R α can also be performed by analyzing the four common haplotypes, thus providing information about all the SNPs in these haplotypes in one assay (Teutsch *et al.*, 2003). Therefore, this study aims at validating the previous studies that were limited to single SNPs, by analyzing the complete IL-7R α haplotype of donors.

Materials and methods

Study population

A total of 591 donor/recipient pairs receiving a bone marrow (BM) or growth factor-mobilized peripheral blood stem cell (PBSC) transplant following a myeloablative conditioning regimen between 1988 and 2004 were included. All HCT were facilitated through the National Marrow Donor Program (NMDP) with clinical outcome data collected through the Center for International Blood and Marrow Transplant Research (CIBMTR). For this study, patients from 78 centers are included. The study population is described in detail by (Shamim *et al.*, 2013) and in Table 1.

Ethics statement

Observational studies conducted by the CIBMTR are performed in compliance with the privacy rule (HIPAA) as a Public Health Authority and in compliance with all applicable federal regulations pertaining to the protection of human research participants as determined by continuous review of the Institutional Review Boards of the NMDP and the Medical College of Wisconsin. Surviving patients who did not provide signed, informed consent to allow analysis of their clinical data or HLA typing of stored NMDP Research Repository samples were excluded. All surviving recipients included in this analysis were retrospectively contacted and provided informed consent for participation in the NMDP/CIBMTR research program. To adjust for the potential bias introduced by exclusion of non-consenting surviving patients, a modeling process randomly excluded the same percentage of deceased patients using a biased coin randomization with exclusion probabilities based on characteristics associated with not providing consent for use of the data in survivors. (Farag *et al.*, 2006) This procedure is standard for CIBMTR analyses to avoid bias from the retrospective consent process.

Endpoints

The outcomes analysed in this study are non-relapse mortality (NRM), relapse, acute and chronic GvHD, disease-free survival (DFS) and overall survival (OS). Relapse was defined as leukaemia recurrence, with death in continuous remission as a competing risk. NRM was death in the absence of relapse, with relapse as a competing risk. Acute GvHD was defined as development of grades 2–4 and grades 3–4 according to the Glucksberg criteria, with death as a competing risk (Glucksberg *et al.*, 1974). Chronic GvHD was diagnosed following standard definitions, with death as a competing risk (Shulman *et al.*, 1980). DFS was defined as survival in complete remission after HCT, with relapse as a competing risk. For OS, death from any cause was considered an event. All living patients were censored at last follow-up.

Detection of IL-7R α haplotypes

HCT donors were genotyped for the IL-7R α locus using a restriction fragment length polymorphism (RFLP) procedure, as previously described (Broux *et al.*, 2010; Teutsch *et al.*, 2003). Briefly, a fragment of the IL-7R α gene promoter region, including three tagging SNPs (rs7718919, rs11567685 and rs11567686), was PCR amplified from genomic DNA of donors. This fragment was then cut by the restriction enzymes HphI (Fermentas, Burlington, Ontario, Canada) and PstI (New England Biolabs, Ipswich, MA, USA) to obtain banding patterns specific for each of the four common haplotypes. To verify our genotyping assay, 4 to 6 samples per haplotype were sequenced using an ABI automated DNA sequencer (PerkinElmer, Waltham, MA, USA) to validate our gel-based genotyping technique. For 16 donors, no genotype could be determined due to technical issues (indicated as “not typed” in Table 1).

Statistics

Patient, donor, disease and transplant-related factors were compared between groups using the Chi-square test for categorical variables and the Wilcoxon sample test for continuous variables. Patient factors included age, sex, Karnofsky score and cytomegalovirus (CMV) status. Donor factors included age, sex, CMV status and number of pregnancies. Disease factors included disease and disease stage. Transplant-related factors included graft type, GvHD prophylaxis, use of anti-thymocyte globulin (ATG), donor/patient CMV match, donor/patient sex/match, graft type, time from diagnosis to transplant and year of transplant. Surviving patients were censored at the time of last contact.

Multivariate analysis used Cox's proportional hazard model. All clinical variables were tested for proportional hazards assumptions. Factors violating the proportional hazards assumption were adjusted through stratification. A stepwise model building procedure was then used to select clinical risk factors for each outcome with a threshold of $p = 0.05$ for entering into the model. The main variable of IL-7R α haplotypes was then tested by forcing it into the models. Due to multiple testing, $p < 0.01$ was used to determine statistical significance for the main effect. The transplantation outcome analyses were performed using SAS version 9.2 (SAS Institute, Inc). The Hardy-Weinberg equilibrium tests were performed using R Package Genetics.

Results and discussion

IL-7R α haplotype frequencies

For this study, 591 donors were genotyped for the four common IL-7R α haplotypes, as described in Table 1. There were no deviations from Hardy-Weinberg equilibrium, and the haplotype frequencies correspond to those previously reported (Broux et al., 2010; Gregory et al., 2007; Hafler *et al.*, 2007; Lundmark et al., 2007; Teutsch et al., 2003).

IL-7R α haplotypes and HCT outcome

In order to identify an effect of donor IL-7R α haplotypes on outcome after HCT, we performed both univariate and multivariate analyses. The outcome measures which were analyzed included survival, NRM, relapse, aGvHD and cGvHD. In contrast to single SNP associations found in previous studies using the same cohort of donors, we did not find an association of any haplotype combination or carriage of any haplotype with the outcome measures, either in a univariate analysis model (Table 2) or a multivariate model (Table 3) in this study. However, the trends that were observed, although they were not significant due to multiple testing and a low number of individuals or events in certain groups ($0.01 < p < 0.05$), were comparable to those found in the previous single SNP study (Shamim et al., 2013). Specifically, we found that Hap1 (containing rs1494555G and rs1494558T) was suggestive for association with acute and chronic GvHD, and that Hap2 (containing rs6897932T) was suggestive for association with relapse and aGvHD in the univariate analysis (Table 2). Hap2 was also suggestive for association with relapse in the multivariate analysis (Table 3). These results confirm our previous data, although significance was not reached in the present study.

Reconstitution of the T cell population after HCT is dependent on a combination of thymus-dependent de novo T cell production and extrathymic expansion of donor-derived T cells. Since IL-7 can be involved in both processes (Carrette and Surh, 2012; Hong et al., 2012), we hypothesized that genetic variations in the IL-7R α gene could influence T cell reconstitution and the risk of GvHD and NRM. Indeed, previous studies of single SNPs suggested that genetic variations in the donor is associated with the outcome after HCT. Specifically, rs1494555GG and rs1494558TT donor genotypes were associated with acute and chronic GvHD in a univariate analysis (Shamim et al., 2006; Shamim et al., 2013). The T allele of rs6897932, which was shown to be associated with the risk of developing multiple sclerosis (MS) (Gregory et al., 2007; Hafler et al., 2007; Lundmark et al., 2007) and inhalation allergy (Shamim *et al.*, 2007), was suggestive of an association with increased frequency of relapse by univariate analysis and multivariate analysis (Shamim et al., 2013).

In this study, validation of these results using haplotypes instead of single SNPs was performed in the same cohort of donors. SNPs in the IL-7R α gene combine into 4 common haplotypes, which can be determined using 3 tagging SNPs in the promoter region. This approach has been used successfully in the past in the context of MS (Booth *et al.*, 2005; Broux et al., 2010) and human immunodeficiency virus (HIV) (Rajasuriar *et al.*, 2010; Rajasuriar *et al.*, 2012), validating the use of this technique for identification of multiple SNPs in one assay, resulting in an increase of information per assay. We observed a trend

for increased risk of relapse when donors carried the Hap2/Hap2 genotype (containing the rs6897932T SNP), in line with the previous findings in a univariate analysis in the single SNP study (Shamim et al., 2013). Due to the limited size of this group, the statistic outcome was not considered to be conclusive. The SNPs rs1494555G and rs1494558T are in strong linkage disequilibrium with each other (Shamim et al., 2013) and are located in Hap1. Although these SNPs have previously been associated with aGvHD, we were not able to identify any statistically significant association for Hap1, indicating that other SNPs present in this haplotype do not add to or even decrease the risk previously identified at the single SNP level.

The apparent discrepancy between this study and the previous study of single SNPs in the same cohort may also be related to a lower power in the present study, due to “dilution” of SNPs into haplotypes. For example, when analyzing the rs6897932 SNP, only three combinations are found (TT, CT, CC). However, when analyzing the four haplotypes, 10 combinations are possible, of which 4 contain Hap2 (the haplotype carrying rs6897932T). This dramatically reduces the number of individuals and events in each group, decreasing the power of the study. Here, we genotyped 591 donor/recipient pairs, which might not be sufficient to detect any significant effects using this approach. Future studies using a larger population of donor/recipient pairs will provide a decisive validation of these results and a conclusive statement on whether IL-7R α genotyping can contribute to better clinical outcome after HCT.

Acknowledgments

This project was funded by Hasselt University, Fonds voor Wetenschappelijk Onderzoek, Belgian Charcot Foundation, University Hospital Rigshospitalet Copenhagen, the Dagmar Marshall Foundation and the Danish Cancer Society.

The CIBMTR is supported by Public Health Service Grant/Cooperative Agreement U24-CA076518 from the National Cancer Institute (NCI), the National Heart, Lung and Blood Institute (NHLBI) and the National Institute of Allergy and Infectious Diseases (NIAID); a Grant/Cooperative Agreement 5U10HL069294 from NHLBI and NCI; a contract HHS250201200016C with Health Resources and Services Administration (HRSA/DHHS); two Grants N00014-14-1-0028 and N00014-13-1-0039 from the Office of Naval Research; and grants from Actinium Pharmaceuticals; Allos Therapeutics, Inc.; Amgen, Inc.; Anonymous donation to the Medical College of Wisconsin; Ariad; Be the Match Foundation; Blue Cross and Blue Shield Association; Celgene Corporation; Chimerix, Inc.; Fred Hutchinson Cancer Research Center; Fresenius-Biotech North America, Inc.; Gamida Cell Teva Joint Venture Ltd.; Genentech, Inc.; Gentium SpA; Genzyme Corporation; GlaxoSmithKline; Health Research, Inc. Roswell Park Cancer Institute; HistoGenetics, Inc.; Incyte Corporation; Jeff Gordon Children's Foundation; Kiadis Pharma; The Leukemia & Lymphoma Society; Medac GmbH; The Medical College of Wisconsin; Merck & Co, Inc.; Millennium: The Takeda Oncology Co.; Milliman USA, Inc.; Miltenyi Biotec, Inc.; National Marrow Donor Program; Onyx Pharmaceuticals; Optum Healthcare Solutions, Inc.; Osiris Therapeutics, Inc.; Otsuka America Pharmaceutical, Inc.; Perkin Elmer, Inc.; Remedy Informatics; Sanofi US; Seattle Genetics; Sigma-Tau Pharmaceuticals; Soligenix, Inc.; St. Baldrick's Foundation; StemCyte, A Global Cord Blood Therapeutics Co.; Stemsoft Software, Inc.; Swedish Orphan Biovitrum; Tarix Pharmaceuticals; TerumoBCT; *Teva Neuroscience, Inc.; THERAKOS, Inc.; University of Minnesota; University of Utah; and Wellpoint, Inc. The views expressed in this article do not reflect the official policy or position of the National Institute of Health, the Department of the Navy, the Department of Defense, Health Resources and Services Administration (HRSA) or any other agency of the U.S. Government.

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Table 1**Study population**

Variable	n (%)
Recipient age, median (range), years	38 (18–62)
Donor age, median (range), years	36 (18–59)
Recipient age at transplant	
18–19 years	18 (3)
20–29 years	126 (21)
30–39 years	176 (30)
40–49 years	202 (34)
50 and older	69 (12)
Males amongst recipients	344 (58)
Disease at transplant	
AML	111 (19)
ALL	76 (13)
CML	374 (63)
MDS	30 (5)
Disease status at transplant	
Early	427 (72)
Intermediate	164 (28)
GVHD prophylaxis	
FK506±MTX±MMF±Steroids±other	147 (25)
CsA + MTX ± other	415 (70)
CsA ± other (No MTX)	25 (4)
MTX ± other (No CsA)	4 (1)
Graft type	
Marrow	519 (88)
Peripheral blood	72 (12)
Donor/Recipient sex match	
Male/Male	239 (40)
Male/Female	143 (24)
Female/Male	105 (18)
Female/Female	104 (18)
Donor/Recipient CMV match	
Negative/Negative	233 (39)
Negative/Positive	159 (27)
Positive/Negative	85 (14)
Positive/Positive	94 (16)
Unknown	20 (3)
Donor Haplotype GTG (Hap1)	
GTG/GTG	58 (10)
one GTG	242 (41)

Variable	n (%)
no GTG	275 (47)
not typed	16 (3)
Donor Haplotype GTA (Hap2)	
GTA/GTA	45 (8)
one GTA	225 (38)
no GTA	305 (52)
not typed	16 (3)
Donor Haplotype TTA (Hap3)	
TTA/TTA	6 (1)
one TTA	135 (23)
no TTA	434 (73)
not typed	16 (3)
Donor Haplotype GCA (Hap4)	
GCA/GCA	47 (8)
one GCA	236 (40)
no GCA	292 (49)
not typed	16 (3)
Donor full haplotype distribution	
GTG/GTG	58 (10)
GTG/GTA	95 (16)
GTG/TTA	48 (8)
GTG/GCA	99 (17)
GTA/GTA	45 (8)
GTA/TTA	40 (7)
GTA/GCA	90 (15)
TTA/TTA	6 (1)
TTA/GCA	47 (8)
GCA/GCA	47 (8)
Not typed	16 (3)
Median follow-up of survivors, mo (range)	93 (10–207)

Table 2

Univariate analysis

Univariate Probability of Outcomes for GTG (Hap1) distribution

Outcome	n	no GTG		one GTG		GTG/GTG	
		n	Prob (95% CI)	n	Prob (95% CI)	n	Prob (95% CI)
Survival	275			242		58	
@ 5 years			45 (40–51)		48 (41–54)		49 (36–62)
NRM	270			239		57	
@ 5 years			41 (35–47)		40 (34–46)		39 (27–52)
Relapse	270			239		57	
@ 5 years			16 (12–21)		16 (12–21)		14 (6–24)
AGVHD II-IV	272			239		58	
@ 100 days			51 (45–57)		55 (49–61)		69 (57–80)
AGVHD III-IV	264			233		54	
@ 100 days			25 (20–30)		23 (18–28)		22 (12–34)
CGVHD II-IV	273			241		56	
@ 1 year			50 (44–56)		48 (42–55)		68 (55–79)
							0.31 ^a
							0.82
							0.96
							0.90
							0.03
							0.86
							0.02

Univariate Probability of Outcomes for GTA (Hap2) distribution

Outcome	n	no GTA		one GTA		GTA/GTA	
		n	Prob (95% CI)	n	Prob (95% CI)	n	Prob (95% CI)
Survival	305			225		45	
@ 5 years			47 (42–53)		46 (40–53)		44 (30–59)
NRM	300			223		43	
@ 5 years			42 (36–48)		39 (33–45)		38 (24–53)
Relapse	300			223		43	
@ 5 years			12 (9–16)		19 (15–25)		24 (12–38)
AGVHD II-IV	302			222		45	
@ 100 days			56 (50–61)		55 (48–61)		44 (30–59)
AGVHD III-IV	292			215		44	
@ 100 days			25 (20–30)		24 (19–30)		14 (5–25)
CGVHD II-IV	303			223		44	
							0.47 ^a
							0.93
							0.74
							0.03
							0.36
							0.14

Univariate Probability of Outcomes for GTA (Hap2) distribution					
Outcome	no GTA		one GTA		GTA/GTA
	n	Prob (95% CI)	n	Prob (95% CI)	Pointwise p-value
@ 1 year		51 (45–57)		52 (45–59)	49 (34–64) 0.92

Univariate Probability of Outcomes for TTA (Hap3) distribution					
Outcome	no TTA		one TTA		TTA/TTA
	n	Prob (95% CI)	n	Prob (95% CI)	Pointwise p-value
Survival	434		135		6 0.39 ^a
@ 5 years		46 (41–50)		51 (43–59)	33 (5–72) 0.43
NRM	429		131		6 0.37
@ 5 years		41 (36–45)		39 (31–47)	67 (28–95)
Relapse	429		131		6 N/A
@ 5 years		17 (14–21)		13 (8–20)	0 (–) 0.24
AGVHD II-IV	429		134		6 0.83
@ 100 days		56 (51–61)		49 (40–57)	67 (28–95)
AGVHD III-IV	415		130		6 0.58
@ 100 days		23 (19–27)		25 (18–32)	33 (5–72)
CGVHD II-IV	430		134		6 0.58
@ 1 year		52 (47–57)		50 (41–58)	33 (5–72)

Univariate Probability of Outcomes for GCA (Hap4) distribution					
Outcome	no GCA		one GCA		GCA/GCA
	n	Prob (95% CI)	n	Prob (95% CI)	Pointwise p-value
Survival	292		236		47 0.62 ^a
@ 5 years		48 (42–54)		47 (41–53)	39 (25–54) 0.53
NRM	286		233		47 0.56
@ 5 years		39 (33–45)		41 (34–47)	47 (33–62)
Relapse	286		233		47 0.38
@ 5 years		18 (14–23)		14 (10–19)	13 (5–25)
AGVHD II-IV	290		233		46 0.87
@ 100 days		56 (50–61)		53 (47–60)	54 (40–68)
AGVHD III-IV	280		226		45

Univariate Probability of Outcomes for GCA (Hap4) distribution

Outcome	no GCA		one GCA		GCA/GCA	
	n	Prob (95% CI)	n	Prob (95% CI)	n	Pointwise p-value
@ 100 days	287	22 (17–27)	236	23 (18–29)	33 (20–48)	0.32
CGVHD II-IV						
@ 1 year		53 (47–58)		51 (44–57)	45 (31–59)	0.59

Abbreviations: Prob = Probability (%); CI = Confidence interval

^aLog-rank p-value

Table 3

Multivariate analysis

Haplotype 1: GTG	no GTG	one GTG	GTG/GTG	Overall p-value
	Ref	HR (95% CI)	HR (95% CI)	
Overall survival ^a	1	0.91 (0.73–1.15)	0.72 (0.49–1.06)	0.24
NRM^b	1	0.94 (0.73–1.23)	0.79 (0.51–1.24)	0.59
Relapse ^c	1	0.84 (0.54–1.30)	0.61 (0.28–1.36)	0.42
aGVHD II-IV ^c	1	1.07 (0.84–1.35)	1.24 (0.87–1.76)	0.48
aGVHD III-IV^e	1	0.86 (0.61–1.23)	0.74 (0.40–1.37)	0.53
cGVHD ^f	1	0.88 (0.69–1.12)	1.20 (0.85–1.70)	0.21

Haplotype 2: G TA	no GTA	one GTA	GTA/GTA	Overall p-value
	Ref	HR (95% CI)	HR (95% CI)	
Overall survival ^a	1	1.04 (0.82–1.31)	1.26 (0.84–1.87)	0.53
NRM^b	1	1.01 (0.78–1.32)	1.17 (0.71–1.91)	0.83
Relapse ^c	1	1.53 (0.98–2.39)	2.51 (1.27–4.97)	0.02
aGVHD II-IV ^d	1	0.98 (0.78–1.24)	0.72 (0.46–1.15)	0.39
aGVHD III-IV^e	1	0.94 (0.67–1.34)	0.48 (0.21–1.11)	0.23
cGVHD ^f	1	1.09 (0.87–1.38)	0.88 (0.57–1.36)	0.57

Haplotype 3: TTA	no TTA	one TTA	TTA/TTA	Overall p-value
	Ref	HR (95% CI)	HR (95% CI)	
Overall survival ^a	1	0.95 (0.73–1.24)	NA	0.69
NRM^b	1	0.88 (0.65–1.19)	NA	0.42
Relapse ^c	1	1.06 (0.62–1.79)	NA	0.85
aGVHD II-IV ^d	1	0.79 (0.59–1.03)	NA	0.08
aGVHD III-IV^e	1	0.98 (0.66–1.46)	NA	0.92
cGVHD ^f	1	0.99 (0.77–1.29)	NA	0.99

Haplotype 4: GCA	no GCA	one GCA	GCA/GCA	Overall p-value
	Ref	HR (95% CI)	HR (95% CI)	
Overall survival ^a	1	1.04 (0.83–1.31)	1.19 (0.80–1.77)	0.64
NRM^b	1	1.10 (0.85–1.44)	1.28 (0.82–2.01)	0.48
Relapse ^c	1	0.67 (0.42–1.05)	0.61 (0.26–1.44)	0.26
aGVHD II-IV ^d	1	0.96 (0.76–1.21)	1.02 (0.67–1.55)	0.94
aGVHD III-IV^e	1	1.10 (0.77–1.57)	1.75 (0.98–3.12)	0.17
cGVHD ^f	1	0.96 (0.76–1.20)	0.84 (0.54–1.31)	0.74

^a Adjusted for patient age, year of transplant, disease and stratified by disease stage.

^b Adjusted for use of ATG, year of transplant and stratified by patient age.

^c Adjusted for disease, Karnofsky score, time from diagnosis to transplant and number of donor pregnancies.

^d Adjusted for disease stage, disease, graft type and GVHD prophylaxis.

^e Adjusted for disease, graft type, year of transplant and number of donor pregnancies.

^f Adjusted for disease, graft type, ATG GVHD prophylaxis, donor sex and number of donor pregnancies.