IMPACT OF CTLA-4 AND NOD2/CARD15 GENE VARIATIONS ON GRAFT- VERSUS-HOST DISEASE AFTER ALLOGENEIC HSCT: A STRUCTURED REVIEW

AL-Battawi S[1], Hameed S[1], Ng ESC[1], and Amini F[1].

[1] School of Healthy Aging, Medical Aesthetics and Regenerative Medicine, Faculty of Medicine and Health Science, UCSI University, Kuala Lumpur, Malaysia

Correspondence:
Farahnaz Amini
School of Healthy Aging
Medical Aesthetics and Regenerative Medicine
UCSI University KL Campus
No.1, Jalan Menara Gading, UCSI Heights (Taman Connaught)Cheras
56000 Kuala Lumpur, Malaysia
Tel: +603 9101 8880 (Extn: 2269)
Fax: +603 9131 7044
Email: farahnaz@ucsiuniversity.edu.my

Abstract

Graft-versus-host Disease (GVHD) is the main cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (alloHSCT). In spite of immune-suppressive prophylaxis, most survivors suffer from acute and chronic GVHD (aGVHD and cGVHD). The outcome of alloHSCT may be affected by the presence of single nucleotide polymorphism (SNP) in non-HLA genes including those involved in innate immune responses. This study aimed to evaluate the impact of cytotoxic T-lymphocyte antigen-4 (CTLA-4) and caspase recruitment domain 15 (NOD2/CARD15) gene polymorphisms on the incidence and severity of aGVHD and cGVHD following alloHSCT. A structured literature review was carried out using various keywords and MESH terms such as stem cell transplantation, allogenic haematopoietic stem cell transplantation, GVHD, and non-HLA gene polymorphism, in PubMed, Google Scholar and Cochrane Database. A total of 8 studies that met inclusion criteria (English publications from 2006 to 2017) were included. Ten SNPs in CTLA-4 gene and three SNPs in NOD2/CARD15 gene were tested in patients with underlying haematological malignancies. Four studies tested the SNPs of CTLA-4 gene and two were found to have an association with CTLA-4 SNPs (rs3087243, rs231775) and increased incidence of aGVHD. The other four studies tested the SNPs of NOD2/CARD15 gene and one found an association between SNP13 and increased incidence of aGVHD. None of these eight studies found any effect on severity of GVHD. In conclusion, two SNPs in CTLA-4 and one SNP in NOD2/CARD15 increased the incidence of aGVHD but not its severity. The higher incidence of aGVHD in studies with larger sample size could support the impact of SNPs in the outcome of alloHSCT. However, due to the heterogeneity of studies in regard to the age of patients and donor, and conditioning regimen, it is difficult to draw a definite conclusion.

Keywords: *Graft-versus-Host Disease, GVHD, Single Nucleotide Polymorphism, SNPs, CTLA-4, NOD 2, CARD 15*

Introduction

The Human Genome Project was completed in 2003. Shortly thereafter, great interest has been directed towards identifying genetic variations, including single nucleotide polymorphisms (SNPs), which may influence the susceptibility to different diseases as well as diverse responses to specific treatment in different ethnic groups (1-4).

Graft-versus-host disease (GVHD) is the main complication after allogeneic hematopoietic stem cell transplantation (alloHSCT) in patients with underlying haematological malignancies. Moreover, GVHD is the major cause of serious adverse side effects and mortality in these patients (5). GVHD has two forms: acute GVHD (aGVHD) that occurs within 100 days after alloHSCT and chronic GVHD (cGVHD), developing after day 100. For over 20 years, development of GVHD has been thought to be the result of the differences among the major and minor HLA (Human Leukocyte Antigen) genotypes of both donor and recipient, which provoke donor T cell activation. However, in the last decade, other genetic variations, including non-HLA gene

polymorphisms, have been tested to identify their crucial effects in the development and severity of GVHD (6).

Evidence on the importance of non-HLA genes in the occurrence of GVHD and advanced knowledge about pathogenesis of GVHD have increased interest in the effect of these genes and the risk of complications following alloHSCT (7). Recently, many studies have proposed that polymorphisms in immune-related genes influence the risk and severity of GVHD as well as mortality following alloHSCT. Among these genes, those involved in the innate immune system that detects invading pathogens through several pattern-recognition receptors, are crucial. It is known that acute alloimmune responses are amplified by innate immunity.

The cytotoxic T-lymphocyte antigen-4 (CTLA-4), an inhibitory molecule, is expressed on T-cells upon activation and has important roles in the balance between pro-immune and anti-immune responses by downregulating T-cell signalling (8). Human CTLA-4 has two isoforms: a membrane-bound receptor isoform (mCTLA-4) and a secreted, soluble isoform (sCTLA-4). While mCTLA-4 consists of both extracellular and intracellular domains, sCTLA-4 has only the extracellular domain for ligand-binding (9). These 2 isoforms establish negative feedback loops to decrease T-cell activation both intrinsically and extrinsically, thereby preserving homeostasis and immune self-tolerance. However, a study that screened recipients and their HLA-matched donors for presence of a SNP in CTLA-4 reported a significant association between recipient genotype and lower disease-free survival, and overall survival following alloHSCT (10).

Furthermore, studies have showed a significant association between GVHD and three SNPs in the caspase recruitment domain 15 (CARD15) gene (which is also known as the nucleotide-binding oligomerisation domain 2 (NOD2) gene) (11). NOD2/CARD15 gene contains a highly conserved CARD linked to a nucleotide-binding domain, which is thought to regulate apoptosis and nuclear factor-kB (NFkB) activation. The C-terminal domain of NOD2/CARD15 comprises a leucine-rich repeat (LRR) region, which has sequence homology with a number of plant disease resistance genes.

In the current study, we performed a review to assess the impact of CTLA-4 and NOD2/CARD15 gene variations on the incidence and severity of GVHD.

Materials & Methods

A literature search was performed for retrieving articles which were published from 2006 to 2017 in PubMed, Google Scholar and Cochrane Database. The process of searching was carried out using keywords and MESH terms such as stem cell transplantation, allogenic haematopoietic stem cell transplantation, GVHD, non-HLA gene polymorphism, and single nucleotide polymorphism. Randomized clinical trials, retrospective and case studies from literature published in English were considered. The PICO framework (Table 1) and flow chart (Figure 1) indicate the method of extraction and selection of pertinent studies.

Table 1: PICO framework for selecting the studies for this review

Patients	Patients with haematological malignancies
Intervention	Allogenic HSCT
Comparison	With or without SNPs
Outcome	Impact of CTLA-4 and NOD2/CARD15 gene variations on the incidence and severity of GVHD

Quality assessment of the articles was done using a subjective scoring (12). The answer matrix was prepared by 3 authors separately and discussed. Studies with scores 0-3 were excluded and studies with scores 4-8 were considered to have good quality and included (Table 2).

Results

A total of 117 articles were found in different databases using keywords and two filters, i.e., English Language and year (2006-2017). Forty articles were viewed according to the inclusion and exclusion criteria (Figure 1). Finally, 8 articles with a total of 3264 patients were selected for this review (Table 2).

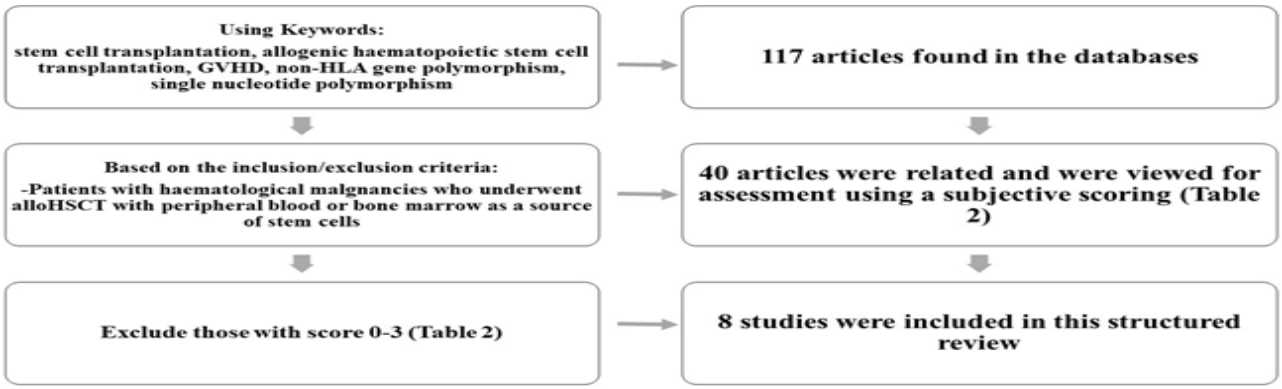

Figure 1: Flow chart illustrating the method of extraction and selection of studies

Table 2: Scoring of selected studies based on 8 questions for assessment

	Pe´rez Garcı´a *et al.* (13)	Bosch Vizcaya *et al.* (14)	Jagasia *et al.* (15)	Sengsayadeth *et al.* (16)	Mayor *et al.* (17)	Sairafi *et al.* (18)	Holler *et al.* (19)	Nguyen *et al.* (20)
Did the study clearly focus on the issue?	√	√	√	√	√	√	√	√
Did the study clearly mention the treatment plan?	√	√	√	√	√	√	√	√
Did the study mention a measurement system for the outcomes?	√	√	√	√	√	√	√	√
Were the outcomes accurately measured to minimize the bias?	√	√	√	√	√	√	√	√
Did the studies have accurate follow-up measures?	√	√	√	√	√	√	√	√
Was there any detail of sample size calculation?	-	-	-	-	-	-	-	-
Was the proper statistical analysis employed?	√	√	√	√	√	√	√	√
Was the study conducted multicentre?	√	√	-	√	√	-	√	√
Total score	7	7	6	7	7	6	7	7

All of these articles were cohort studies with patients who suffered from haematological malignancies and were treated with alloHSCT. These studies looked at 10 SNPs of CTLA-4 gene and three SNPs of NOD2/CARD15 gene. The characteristics of patients such as their age, gender, and ethnicity, as well as other variables, e.g., study design, sample size, disease under treatment, median time of follow up, single- or multi-centre study, are all summarized in Table 3. In addition, the gene, SNP, derived allele and its frequency, as well as the conditioning regimen are all listed in Table 4. Also, the latter includes the clinical outcomes of these studies, such as overall survival, incidence of aGVHD and cGVHD.

Table 3: Characteristics of selected studies

Author	Study design	Disease under treatment	Sample size	Ethnicity	Median time of follow up (months)	Patients' characteristics	Single/ Multi-Centre Study
Pe´rez Garcı´a *et al.* (13)	Retrospective cohort study	Hematologic malignancies	536	Caucasian	60	Median age: 34 years (male: female) 311:225	Multi-centre
Bosch Vizcaya *et al.* (14)	Retrospective cohort study	Hematologic malignancies	136	Caucasian	132	Median age: 40 years (male: female) 75 :61	Multi-centre
Jagasia *et al.* (15)	Cohort study	Hematologic malignancies	164	Caucasian	17	Median age: 47years (male: female) 80 :84	Single centre
Sengsayadeth *et al.* (16)	Cohort study	Hematologic malignancies	780 patients	Caucasian - African American - Hispanic - Others	63	Median age of recipients: 50 years Median age of donors: 34 years	Multi-Centre

Table 3 ***Continued***

Author	Study design	Disease under treatment	Sample size	Ethnicity	Median time of follow up (months)	Patients' characteristics	Single/ Multi-Centre
Mayor *et al.* (17)	Cohort study	Acute leukaemia	196 patients	European descent	26	Median age of recipients: 20 years Median age of donors: 35 years	Multi-Centre
Sairafi *et al.* (18)	Cohort study	Hematologic malignancies	198 patients	European descent	78	Median age of recipients: 37 years Median age of donors: 37 years (male: female) 114:84	Single centre
Holler *et al.* (19)	Cohort studies	Acute leukaemia	Cohort 1 (n = 169) and Cohort 2 (n =531)	European descent	30	Median age at cohort 1: 44 years Median age at cohort 2: 36 years	Multi-Centre
Nguyen *et al.* (20)	Retrospective cohort study	Hematologic malignancies	390 patients	-European descent -American - Others	77	Median age: 37 years (male: female) 211:179	Multi-Centre

Table 4: Associations between SNPs of CTLA-4 and NOD2/CARD15 and clinical outcomes

Study	Gene	SNP and Derived Allele	Frequency of Allele (%)	Overall Survival (p value)	Incidence of aGVHD (p value)	Incidence of cGVHD **(p value)**	**Conditioning Regimen**
Pe´rez Garcı´a *et al.* (13)	CTLA-4	1. rs733618 T allele C allele	 91.7 8.3	1. No effect (.454)	1. No effect (.112)	-	-CP + TBI -Busulfan + CP -Other
		2. rs4553808 A allele G allele	 87.9 12.1	2. No effect (.538)	2. No effect (.679)	-	
		3. rs5742909 T allele C allele	 10.2 89.8	3. No effect (.393)	3. No effect (.882)	-	
		4. rs231775 A allele G allele	 67.8 32.2	4**. Increase (.019)**	**4. Increase (.072)**	-	
		5. rs3087243 A allele G allele	 52.5 47.5	5. **Decrease (.016)**	**5. Increase (.108)**	-	
Bosch Vizcaya *et al.* (14)	CTLA-4	rs3087243 A allele G allele	 22 78	Increase (.008)	No effect	No effect	Myeloablation with T-cell depletion

aGVHD= Acute Graft-versus-Host Disease, cGVHD= Chronic Graft-versus-Host Disease, CP= Cyclophosphamide, TBI=Total body irradiation

Table 4 *Continued*

Study	Gene	SNP and Derived Allele	Frequency of Allele (%)	**Overall Survival (p value)**	**Incidence of aGVHD (p value)**	**Incidence of cGVHD (p value)**	**Conditioning Regimen**
Jagasia *et al.* (15)	CTLA-4	1. rs11571315 T allele C allele	 41 59	1. No effect (.69)	1. No effect	1. No effect	Myeloablation
		2. rs4553808 A allele G allele	 88 12	2. **Increase (.02)**	2. No effect	2. No effect	
		3. rs11571316 A allele G allele	 46 54	3. No effect (.29)	3. No effect	3. No effect	
		4. rs16840252 T allele C allele	 14 86	4. No effect (.06)	4. No effect	4. No effect	
		5. rs231775 A allele G allele	 60 40	5. No effect (.59)	5. No effect	5. No effect	
		6. rs231777 T allele C allele	 13 87	6. No effect (.24)	6. No effect	6. No effect	
		7. rs231779 T allele C allele	 40 60	7. No effect (.61)	7. No effect	7. No effect	
		8. rs3087243 A allele G allele	 46 54	8. No effect (.41)	8. No effect	8. No effect	
		9. rs1019701 C allele A allele	 15 85	9. No effect (.92)	9. No effect	9. No effect	
		10. rs231725 A allele G allele	 33 67	10. No effect (.52)	10. No effect	10. No effect	
Mayor *et al.* (17)	NOD/ CARD15	1.rs2066844 (SNP 8) 2.rs2066845 (SNP 12) 3. rs2066847 (SNP 13)	Not mentioned	Decrease (.01)	No effect	No effect	Myeloablation with T-cell depletion
Sairafi *et al.* (18)	NOD/ CARD15	1.rs2066844 (SNP 8) 2. rs2066845 (SNP 12) 3. rs2066847 (SNP 13)	Not mentioned	No effect	No effect	-	Myeloablation with T-cell depletion
Holler *et al.* (19)	NOD/ CARD15	1. rs2066844 (SNP 8) 2. rs2066845 (SNP 12) 3. rs2066847 (SNP 13)	Not mentioned	**Decrease** (.007) (only in MRD)	**Increase** (.004) (only in MRD)	-	Myeloablation with T-cell depletion
Nguyen *et al.* (20)	NOD/ CARD15	1. rs2066844 (SNP 8) 2. rs2066845 (SNP 12) 3. rs2066847 (SNP 13)	Not mentioned	No effect	No effect	No effect	Myeloablation

MRD= Matched related donor, MUD= Matched unrelated donor

Discussion

Allogeneic hematopoietic stem cell transplantation (alloHSCT) has been used to treat patients with different types of haematological malignancies. Although the preference is to have related HLA-identical donors, reports show a comparable outcome from unrelated donor transplantations by using different conditioning regimens (21-23). On the other hand, the role of non-HLA genes on successful outcome following alloHSCT has also been reported.

Acute GVHD is the most common complication which affects safety of allogeneic hematopoietic stem cell transplantation, resulting in significant morbidity and mortality. This review collected evidence from cohort studies focusing on the association between SNPs of CTLA-4 and NOD2/CARD15 genes and the incidence and severity of GVHD. There was some heterogeneity between these studies in the aspects of patients' and donors' age, using stem cells from matched related or matched unrelated, ethnicity of patients, different underlying haematological malignancies, stage of disease and the use of T-cell depletion (TCD) as conditioning regimen for the recipients. Similarly, the outcomes of these studies showed some variations regarding the effect of these non-HLA gene polymorphisms on the incidence and severity of GVHD.

Mutations in CTLA-4 gene have been associated with the onset and clinical presentation of some diseases, such as insulin-dependent diabetes mellitus, sporadic breast cancer clinical features and risks, systemic lupus erythematosus, Graves' disease, Hashimoto thyroiditis, celiac disease, thyroid-associated orbitopathy, and other autoimmune diseases in addition to some malignancies (24-29). The impact of CTLA-4 gene polymorphisms on the immune response following allo-HSCT, as well as on the immune surveillance that reduces the incidence of relapse after obtaining complete remission in acute leukemia, have been reported but the potential role of this gene on modulation of GVHD after allo-HSCT has shown mixed results. Pe´rez-Garcı´a *et al.* (13) showed that the presence of A allele in CTLA-4 SNP rs3087243 in donors elevated the incidence of aGVHD in all selected patients. While the A allele of this SNP was pathologic and increased the occurrence of aGVHD with an incidence of 46% among recipients, G allele was protective and lowered the incidence of aGVHD to 37%. They concluded that AA genotype of this SNP could be an independent risk factor for aGVHD of grades II-IV. On the other hand, Azarian *et al.* (26) reported that the recipients from donors with G allele in this SNP had a worse outcome on cGVHD. Similar findings were found in a Tunisian cohort study (27). However, Bosch-Vizcaya *et al.* (14), who studied both recipients' and donors' genotype, reported no significant differences between the carriers of any allele of this SNP (AA, AG/GG) and the incidence of aGVHD as well as cGVHD. Despite that, Jagasia *et al.* (15) and Sengsayadeth *et al.* (16) revealed that this SNP had no association with GVHD. The lack of association in these three studies (13-15) might be due to the age of the recipients, who were older compared to recipients in the study by Pe´rez-Garcı´a *et al.* (13).

Furthermore, SNP rs231775 (+49) was examined in donors in three studies. Pe´rez-Garcı´a *et al.* (13) reported a nonsignificant association with incidence of grades II to IV aGVHD in AA genotype but no effect on severity of GVHD. But, Jagasia *et al.* (15) and Sengsayadeth *et al.* (16) did not find any association. However, these two studies had some limitations, including the recruitment of only patients with acute myeloid leukaemia (AML) and myelodysplastic syndrome (MDS) in the unrelated donor group; their conclusion might be restricted to such patients.

Additionally, the other eight SNPs (rs11571315, rs4553808, rs11571316, rs16840252, rs231777, rs231779, rs1019701, rs231725) lacked any association with the incidence of aGVHD and cGVHD. Therefore, only two out of the ten SNPs of CTLA-4 gene showed an association with aGVHD grades II-IV. These two SNPs were rs3087243 and rs231775 in donors.

Mutations in NOD2 gene have been associated with Crohn's disease and Blau syndrome (30). Also, NOD2/CARD15 gene is known to modulate the response of innate immunity. Three polymorphisms of NOD2/CARD15 gene, i.e., rs2066844 (SNP 8), rs2066845 (SNP 12) and rs2066847 (SNP13) were studied to evaluate their association with GVHD (17-20). According to Mayor *et al.* (17), there was no significant difference in the incidence of aGVHD due to the presence of any SNPs. This observation could be due to the use of T-cell depletion (TCD) during the conditioning regimens in a large number of recipients. This explanation was based on a study by Granell *et al.* (31) who found no association between polymorphisms of NOD2/CARD15 and aGVHD in recipients who were given conditioning regimens with TCD. The same findings were reported by Nugyen *et al.* (20) for incidence of both aGVHD and cGVHD, as this study also used TCD in most recipients.

Sairafi *et al.* (18) showed that the donor or recipient NOD2/CARD15 SNPs were not associated with aGVHD. This study concluded that the lack of association could be due to low frequency of SNPs in their patients and/or a lower overall incidence of severe GVHD. This hypothesis is in agreement with findings by Hampe *et al.* (32) who revealed that the prevalence of SNPs in the Scandinavian population was less than in other populations. Additionally, the immunosuppressive treatment of TCD was used in a large number of patients (61%) and this could be another explanation to the lack of observed association.

In a study by Holler E *et al.* (19), these three SNPs were examined in both matched related donor (MRD) and matched unrelated donor (MUD) cohorts. The outcome showed the presence of an association between the NOD2/CARD15 SNPs and the incidence of severe aGVHD, particularly the donors rs2066847 (SNP13) of the MUD cohort group (42%). Similar observations regarding this SNP was seen by Brenmoehl *et al.* (33) in which this SNP was related to double sepsis-related mortality in non-

haematological patients. Moreover, Holler *et al.* (11) reported that these SNPs of NOD2/CARD15 gene in either the recipient or donor raised the incidence of aGVHD. Then, a follow-up study by Holler *et al.* (34) expanded the original study with an extra 225 donor/recipient pairs from four extra European centres, and the outcomes were again confirmed.

Overall, these three SNPs of NOD2/CARD15 showed mixed results regarding their association with GVHD according to these selected studies. This observation could be due to some limitations in these selected studies, such as the usage of TCD conditioning regimens and the low frequency of SNPs in some ethnic races.

Conclusion

In conclusion, there was no association between any SNPs in these two genes and incidence and severity of cGVHD but two SNPs in CTLA-4 and one SNP in NOD2/CARD15 increased the incidence of aGVHD but not its severity. The higher incidence of aGVHD in 3 studies with larger sample size, could support the impact of SNPs in the outcome of alloHSCT. However, due to the heterogeneity of studies, with regard to the age of patients and donors, as well as conditioning regimens, it is difficult to draw a definite conclusion. Therefore, studies with larger sample sizes are required to validate these findings in order to provide a risk-stratification tool that can predict the outcomes of transplantation. This goal will not only reduce the incidence and severity of GVHD, but also improve the chance of survival for patients who are undergoing alloHSCT.

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