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GLCCI1 and Glucocorticoid Receptor Genetic Diversity and Response to Glucocorticoid-Based Treatment of Graft-versus-Host Disease

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A B S T R A C T

The genetic diversity of loci implicated in glucocorticoid (GC) response has been associated with interindividual variations in responsiveness to GC in various diseases, such as asthma and inflammatory bowel disorders. In acute graft-versus-host disease (aGVHD), similar differences of first-line therapy responsiveness are also observed, with approximately 40% of patients failing to respond to GC. Here, the distribution of functionally relevant single nucleotide polymorphisms (SNP) belonging to the GC-induced transcript 1 GLCCI1 (rs37972) and the glucocorticoid receptor (rs41423247, rs6195 and rs6198) gene loci were analyzed alongside clinical factors for their association with the response to corticosteroids in aGVHD. The frequencies of variant alleles did not differ significantly between corticoresistant patients, their donors, and their corticosensitive peers ($P = .10$ to 1.00). Severe and early onset of aGVHD, bone marrow as the stem cell source, and an HLA mismatch were associated with the failure to respond to GC in logistic regression. After including the single SNPs to the model, carriers of the rs41423247 polymorphism had a higher probability of responding to GC, whereas all other polymorphisms did not affect the likelihood of response.

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INTRODUCTION

The curative potential of allogeneic hematopoietic stem cell transplantation (HSCT) for many hematologic disorders is significantly curtailed by the complications it occasions [1]. In particular, graft-versus-host disease (GVHD) will affect at the most 70% of subjects, of which approximately one-half will not respond to first-line treatment with glucocorticoids (GC). Steroid-refractory GVHD (SR-GVHD) has a particularly poor prognosis, with reported long-term survival rates between 10% and 30% [2]. The question of why some patients respond and others do not can be approached from different angles. Not only clinical characteristics, such as previous and/or concurrent treatment regimen, donor/recipient sex constellation, the interaction between GVHD and infections, but also genetically driven vulnerability may predispose an individual to treatment refractoriness [3-5].

GC resistance can be observed in various pathological settings, presumably arising from pharmacokinetic or pharmacogenetic variants in patients [6]. Tantisira et al. identified a significant correlation between a functional single nucleotide polymorphism (SNP) in the GLCCI1 gene and the clinical phenotype of resistance to steroid treatment of asthma [7]. Further advances on determinants of GC response were the findings of GC sensitivity modulation through polymorphisms of the glucocorticoid receptor (GR) gene, eg, at the BclI restriction fragment length, at codon N363 of exon 2 or in exon 9β [8,9]. We sought to determine if a correlation between previously described GC response–modifying SNPs and the clinical phenotype of response to acute GVHD (aGVHD) treatment with GC can be found.

PATIENTS AND METHODS

Patient Selection

In a previous study involving patients with SR-GVHD [10], we reported similar results of second-line therapy using either mycophenolate mofetil, inolimomab, or etanercept. The patients enrolled at this time (n = 64) were

analyzed in the present study for SNP distribution after the study of pharmacogenetic variants associated with steroid resistance in patients demonstrating GC refractoriness in other diseases, such as asthma [7]. As a control, a cohort of 80 patients with steroid-sensitive GVHD was analyzed for the same polymorphisms.

Study Design and Statistical Analyses

Patients gave their consent to the evaluation of the data and received treatment on a local ethical committee—approved research protocol (reference BIOGVH 14650). Patients having received cord blood transplantation were not included in this analysis. aGVHD was suspected at the appearance of the following symptoms after transplantation: erythema/exanthema, diarrhea, nausea, emesis, abdominal pain, anorexia, or cholestatic hepatitis. Glucksberg and consensus criteria determined the diagnosis, staging, and grading of GVHD [11,12]. SR-GVHD was defined as either the absence of remission by 14 days, stable disease by 7 days, or progression within 3 days after the beginning of corticosteroid treatment at a dose of 1 to 2 mg/kg/day. Corticoresistant patients were treated in second-line in an interventional trial evaluating treatment of SR-GVHD with mycophenolate mofetil, inolimomab, or etanercept [10].

The choice of SNPs for the analysis was based on their established functional impact and their previously demonstrated association with GC-related treatment response. Genomic DNA was extracted from EDTA-treated peripheral blood samples using a standard salting-out method to be subjected to routine HLA typing and then stored frozen until the present study. All participants were genotyped for functional polymorphisms in GLCCI1 (rs37972 C–>T) and GR (in the BclI restriction fragment length: rs41423247 G–>C, as a point mutation in exon 2 NS363: rs6195 A–>G or in exon 9β: rs6198 A–>G). The genotyping was performed by a TaqMan 5'-nuclease assay (Applied Biosystems, Foster City, CA) with allele-specific fluorogenic oligonucleotide probes using pre-developed TaqMan assay genotyping kits (Applied Biosystems).

The frequencies of GLCCI1 and GR polymorphisms were assessed in patients affected by aGVHD and in their donors and compared according to their response to GC. Variables were compiled and compared using tests for categorical or continuous data. Differences in genotype distribution in patients and donors were tested using contingency tables and compared using Pearson's chi-squared and Fischer's exact test, where appropriate. For the SNP data, in departure from Hardy-Weinberg equilibrium, statistical analyses involved Cochran-Armitage trend testing on the basis of the genotypes. Factors possibly influencing the response to GC, such as patient age, donor/host combinations for gender and HLA mismatch, stem cell source, grade and acuteness of aGVHD onset, as well as the SNPs, were first tested by means of univariate analysis. Adding each covariable subsequently to forward conditional logistic regression, we then examined the significance of clinical predictors of response to corticosteroid treatment as well as that of the studied polymorphisms. Overall survival was estimated by Kaplan-Meier analysis. All tests were 2-sided and P values $\leq$ .05 were considered significant. Statistical analyses were performed on SPSS version 19 (SPSS Inc, Chicago, IL), GraphPad Prism 5.0a (GraphPad Software, San Diego, CA), and Stata/SE 12.1 (StataCorp LP, College Station, TX).

RESULTS

Patient, Transplantation, and Disease Characteristics

One hundred forty-four patients (57 female, 87 male) ages 5 to 66 years (median, 44 years) presenting corticosensitive (CS) or corticorefractory aGVHD after allogeneic HSCT (between 1999 and 2013) were identified for this analysis. Stem cell source at first transplantation was bone marrow in 38 (26%) and mobilized peripheral blood stem cells in 106 (74%). Stem cell donors were identical siblings (n = 63, 44%), matched unrelated donors (n = 60, 42%), or mismatched unrelated donor (n = 21, 14%). Median follow-up from HSCT was 19.5 months (range, 1 to 141).

aGVHD arose at a median time of 20 days after HSCT. GVHD grades were 4 in 24 (17%), 3 in 42 (29%), 2 in 63 (44%), and 1 in 15 patients (10%). Sixty-four patients qualified as being SR, as defined above, and received second-line immunosuppression, consisting either of MMF in 27 patients who had not previously received this substance in prophylaxis (42% of the patients with SR-GVHD), inolimomab in 18 (28%), and etanercept in 19 patients (30%). Second-line treatment began at a median of 13 days after the diagnosis of GVHD. Eighty-three patients developed chronic
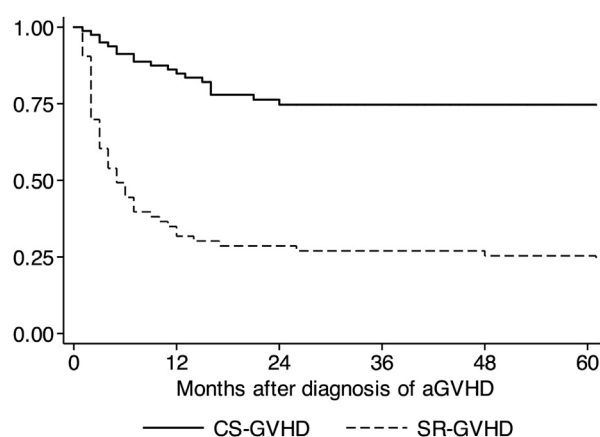

Figure 1. Overall survival after the time of diagnosis of aGVHD.

GVHD. Kaplan-Meier estimated overall survival at 5 years after the time of diagnosis of acute GVHD was significantly lower for patients affected by SR-GVHD than for the patients who responded to corticosteroids (23% $\pm$ 5% standard error versus 75% $\pm$ 5%; log-rank $P <$ .0001) (Figure 1).

As previously described by Westin et al. [5], severe (grade 3 or 4) GVHD occurring early in the course, ie, within 14 days of HSCT, resulted in the strongest risk of failure to respond to corticosteroids (odds ratio [OR], 18.1; 95% confidence interval [CI], 6.46 to 50.73; $P <$ .0001) on univariate analysis. Further predictors of the failure of aGVHD to respond to GC were, in decreasing order of importance, the following: bone marrow stem cell source (OR, 3.88; 95% CI, 1.76 to 8.55; $P =$ .001), HLA mismatch (OR, 5; 95% CI, 1.72 to 14.54; $P =$ .002), myeloablative conditioning (OR, 2.89; 95% CI, 1.47 to 5.72; $P =$ .002), and sex mismatch in the direction of a female donor to male recipient (OR, 2.4; 95% CI, 1.14 to 5.06; $P =$ .02). There was a trend for higher response rates to corticosteroids in patients receiving antithymoglobulin (ATG) before transplantation (OR, 0.54; 95% CI, .27 to 1.11; $P =$ .09), which was independent of the stem cell source (bone marrow versus peripheral blood stem cell source; $P =$ .13). Patient and transplantation characteristics are detailed in Table 1.

GLCCI1 and GR variant frequencies in patients with SR-GVHD and CS-GVHD

The GLCCI1 rs37972 T variant allele did not occur more frequently either in patients with CS- or SR-GVHD (OR, 1.00; 95% CI, .62 to 1.62; $P =$ 1.00). The frequency of the T allele in donors to patients with CS-GVHD was higher than in the donors to patients who developed SR-GVHD, albeit not reaching the level of statistical significance (58 of 150 alleles in the donors of patients with CS-GVHD versus 41 of 118 alleles in the donors of patients with SR-GVHD; OR, .85; 95% CI, .51 to 1.40; $P =$ .51) (Table 2, Figure 2).

In terms of GR polymorphism, we found that the frequency of the BclI rs41423247 C allele was higher in patients with CS-GVHD than in those with SR-GVHD (63 of 158 [40%] versus 42 of 128 [33%] alleles; OR, .74; 95% CI, .45 to 1.20; $P =$ .22 in CS-GVHD and SR-GVHD, respectively). As for the potential influence of the donor BclI rs41423247 C allele, we observed 47 of 150 variant alleles in donors to patients who later developed CS-GVHD and 41 of 118 in donors to patients with SR-GVHD (OR, 1.17; 95% CI, .69 to 1.95; $P =$ .56) (Table 2, Figure 2). Concerning the NS363 rs6195, we found that the G allele was equally distributed in the 2 patient groups (OR,

Table 1
Patient, Transplantation, and GVHD Characteristics

Variables	CS-GVHD	SR-GVHD	P Value
n	80	64	
Age, median (range), yr	48 (15-66)	37.5 (5-64)	.01
Gender, male/female	47(59)/33(41)	40(63)/24(37)	.65
Diagnosis			
Acute lymphoblastic/ myelogenous leukemia	26 (32)	33 (52)	.10
Myelodysplastic/ myeloproliferative neoplasia	20 (25)	10 (16)	
Lymphoid neoplasia*	27 (34)	13 (20)	
Aplastic anemia	7 (9)	8 (13)	
Transplant cell source			
Bone marrow	12 (15)	26 (41)	.001
Peripheral blood	68 (85)	38 (59)	
Conditioning			
Reduced intensity	52 (65)	25 (39)	.002
Myeloablative	28 (35)	39 (61)	
ATG			
No	48 (60)	47 (73)	.09
Yes	32 (40)	17 (27)	
GVHD prophylaxis			
CsA/MTX	28 (35)	35 (55)	.16
CsA/MMF	49 (61)	20 (31)	
CsA	3 (4)	9 (14)	
Donor type			
Identical sibling	45 (56)	18 (28)	<.001
Matched unrelated	30 (38)	30 (47)	
Mismatched unrelated	5 (6)	16 (25)	

CsA indicates cyclosporine; MTX, methotrexate; MMF, mycophenolate mofetil.

Data presented are n (%), unless otherwise indicated.

* Lymphoid neoplasia includes Hodgkin and non-Hodgkin lymphoma, chronic lymphocytic leukemia, plasma cell neoplasia.

1.79; $P = .98$, Cochran-Armitage) (Table 2) and absent amongst donors. Finally, the GR exon 9β rs6198 G allele was found at a higher frequency in patients with CS-GVHD (OR, .74; 95% CI, .39 to 1.43; $P = .38$) and at a higher frequency in the donors to patients with CS-GVHD compared with the donors to patients with SR-GVHD, although the difference in

Table 2
GR and GLCCI1 Polymorphisms in Patients Affected by CS- and SR-GVHD and their Respective Donors

Variables	CS-GVHD Patients	SR-GVHD Patients	CS-GVHD Donors	SR-GVHD Donors
rs37972 Allele frequency				
C	100 (63)	81 (63)	92 (61)	77 (65)
T	58 (37)	47 (37)	58 (39)	41 (35)
P	1.00		.51	
rs41423247 Allele frequency				
G	95 (60)	86 (67)	103 (69)	77 (65)
C	63 (40)	42 (33)	47 (31)	41 (35)
P	.22		.56	
rs6195 Genotype frequency				
AA	74 (94)	61 (95)	75 (100)	59 (100)
AG	5 (6)	2 (3)	0	0
GG	0	1 (2)	0	0
P	.98		n.a.	
Rs6198 Allele frequency				
A	131 (83)	111 (87)	121 (81)	104 (88)
G	27 (17)	17 (13)	29 (19)	14 (12)
P	.38		.10	

N.A. indicates not available.

frequencies did not reach the level of statistical significance (OR, .56; 95% CI, .28 to 1.11; $P = .10$) (Table 2, Figure 2). rs37972, rs41423247 and rs6198 were in Hardy-Weinberg equilibrium, whereas the rs6195 was not.

Association between clinical characteristics, gene polymorphisms and the response to GC

The clinical factors that resulted in a significant effect on univariate analysis were retained for logistic regression, ie, hyperacute severe GVHD, stem cell source, conditioning regimen intensity, HLA mismatch, and sex mismatch, a model to which the individual SNPs were included. As in univariate analysis, patients who had a severe and hyper acute manifestation of GVHD had a significantly higher risk of not responding to GC than the patients who either developed early but mild aGVHD or severe aGVHD beyond the first 14 days after transplantation (n = 40, $P < .0001$). Further factors associated with nonresponse to steroid therapy were bone marrow as the cell source of HSCT (n = 38, $P = .001$) and an HLA mismatch (n = 21, $P = .019$). The clinical factors of a myeloablative conditioning (n = 67, $P = .61$) and sex mismatch in the direction of a female donor to a male patient (n = 40, $P = .33$) did not prove to be significant in logistic regression.

The effects of the individual SNPs in donors and patients in forward conditional logistic regression were nonsignificant for the following SNP in donors and recipients: rs6195 SNP-positive recipient (n = 8, $P = .97$), rs6198 SNP-positive donor (n = 36, $P = .15$), rs6198 SNP-positive recipient (n = 40, $P = .83$), rs37972 SNP-positive donor (n = 77, $P = .50$), and rs37972 SNP-positive recipient (n = 83, $P = .92$). The association between the presence of the SNP rs41423247 and a response to GC was significant in the recipient (n = 88, $P = .009$) but not in the donor (n = 73, $P = .12$) (results detailed in Table 3).

DISCUSSION

The development of GVHD seriously diminishes the curative potential of allogeneic HSCT. Around one half of allogeneic HSCT recipients will develop this complication, of which between 40% and 60% will respond to standard treatment with GC [13]. For the patients who do not respond, a second-line treatment is often initiated, however, at the price of even lower response rates and a higher infectious burden, and ultimately resulting in a very poor prognosis [14].

The mechanisms of action of GC are primarily that of inducing lymphocyte apoptosis and increasing the transcription of anti-inflammatory cytokines while decreasing the transcription of their proinflammatory counterparts [15,16]. In the context of aGVHD, GC has further been found to suppress CD8-positive T cells [17]. Our understanding of GC resistance mainly derives from research conducted in the field of chronic inflammatory diseases and, in particular, asthma. Aside from pharmacokinetic interferences, such as suboptimal drug absorption or distribution, various molecular mechanisms have been identified, such as an increased drug efflux, eg, under the control of multidrug resistance transporter pump SNPs [18], defective binding, or failure of the GR to translocate to the nucleus due to post-translational alterations or through competition [19,20]. In addition, interindividual differences in the response to corticosteroids have been ascribed to genetic variants of GLCCI1 and GR [7,8].

Previous studies have suggested lower response rates to GC in patients experiencing higher initial GVHD stages [21],
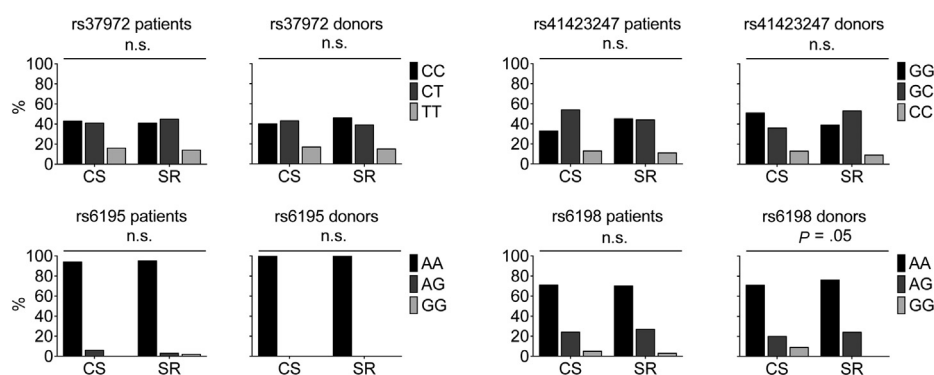
Figure 2. Allele frequencies in patients and donors.

early onset of GVHD [5], in female to male donor-patient pairs [4], and in case of HLA disparities between donors and recipients [22]. Our findings replicate the negative effect of severe and early onset of GVHD, as well as the effect of a mismatched donor. Further, a detrimental effect of bone marrow as the stem cell source was observed in this study. With the addition of ATG to GVHD prophylaxis, acute and chronic GVHD incidences can be significantly decreased [23]. Interestingly, should aGVHD occur after an ATG-containing regimen, a trend to higher response rates to GC could be observed in our data.

The following most common SNPs involving a modified response to GC were screened for their association with the response to GC in aGVHD: rs37972, rs41423247, rs6195, and rs6198. Patients with asthma carrying mutant alleles at rs37972 and rs37973 of the GLCCI1 gene (both SNPs being in complete linkage disequilibrium) were found to be more likely to have an inferior response to GC treatment of asthma [7]. In our study, rs37972 SNP-positive donors and recipients were neither increased nor were more likely to have SR-GVHD.

Unlike previous reports of an association between rs6195 variants and GC resistance [24], an identical frequency of the G allele in the donors and patients of both groups was found in our study. The rs6198 G allele has been implicated in reduced responsiveness to GC in patients [8,25]. Although an increased number of mutant alleles was noted in the donors to patients with CS-GVHD, logistic regression did not support an association between this variant and a particular clinical phenotype of GC responsiveness. The rs6195 was not in Hardy-Weinberg equilibrium. After having excluded genotyping errors, departure from Hardy-Weinberg equilibrium can be explained either by chance, particularly in a small set of cases or by a genuine genetic association with the development of a disease [26]. The focus of our study on patients with aGVHD implied a patient selection within the collective of all HSCT recipients. Thus, departure from Hardy-Weinberg equilibrium could also indirectly indicate a genetic GC

homeostasis background to the development of GVHD. Finally, individuals carrying the Bcl1 C allele (rs41423247) have been reported to have a constitutionally determined higher sensitivity to GC [27,28]. Although the enhanced sensitivity to GC due to this SNP has been demonstrated as cited in autoimmune disorders, knowledge of the underlying mechanism of action is limited. Patients who responded to GC had higher frequencies of the C allele and an association between this allele and a lower risk of developing SR-GVHD was seen in logistic regression.

In a recent study by Theiss-Suennemann et al. in a murine model, when recipients received allogeneic T cells lacking GR expression, aGVHD had a far more severe course than in controls [17]. This finding suggests an important role of donor T cells in the response to GC, although we have detected an effect on the determination of response in the genetic background of the recipient. Several factors may explain this discrepancy; foremost, the different subjects studied. On account of the differences in donor, host, and transplantation characteristics in mice and humans (ie, different conditioning regimens, cell sources, genetic and immunological matches, host microbiome, and age), the results of murine models cannot be fully aligned with the results in humans [29]. Moreover, further cell sets, eg, host antigen-presenting cells, cytokine modulation, and also tissue response to GC play a role in the complex interplay of host and donor immunity in human aGVHD [30-32]. Our findings warrant further validation of the genetic determinants of response to GC in donor and recipient pairs both in functional assays and in a larger cohort.

In conclusion, the SNP rs41423247 can be linked to the response to treatment with GC in patients who have experienced aGVHD. Other SNPs may play response-modifying roles in the treatment of aGVHD with GC, by way of example other genetic variants either on the GR gene, possibly in linkage disequilibrium to the rs41423247, or encoded on other genes (eg, multiple drug resistance). Our data suggest that the severity of presentation of aGVHD, donor-recipient disparities, and the transplantation source can influence, at least partly, the response to GC. Our findings provide new insights on constitutional GC response–modulating variants in aGVHD and should they be further validated, could contribute to the management of immunosuppressive treatment of aGVHD. Wider investigations of the genome and functional assays would allow a more comprehensive understanding of the pharmacogenetics behind the treatment of aGVHD.

Table 3
Odds Ratios of Failure to Respond to GC Therapy of aGVHD in Logistic Regression

Predictor	n	OR	95% CI	P Value
Hyperacute severe aGVHD	40	34.50	10.15-117.22	<.0001
BM	38	5.76	2.07-16.03	.001
HLA mismatch	21	4.49	1.29-15.64	.019
Recipient rs41423247 carrier	88	0.28	.11-0.72	.009

BM indicates bone marrow.

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