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

Alix O'Meara [1], Wahid Boukouaci [2], Marie Robin [1], Aliénor Xhaard [1], Catherine Fortier [3], François Marzais [3], Flore Sicre de Fontbrune [1], Régis Peffault de Latour [1], Dominique Charron [3], Gerard Socié [1,2], Ryad Tamouza [2,3,*]

[1] *Hematology Transplantation, Assistance Publique-Hôpitaux de Paris, Saint Louis Hospital, Paris, France*
[2] *Institut National de la Santé et de la Recherche Médicale, UMRS-1160, F-75010 Paris, France*
[3] *Laboratoire Jean Dausset and LabEx Transplantex, Hôpital Saint Louis, Paris, France*

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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* (*rs*37972) and the glucocorticoid receptor (*rs*41423247, *rs*6195 and *rs*6198) 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 *rs*41423247 polymorphism had a higher probability of responding to GC, whereas all other polymorphisms did not affect the likelihood of response.

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 *Bcl*I 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

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* Correspondence and reprint requests: Dr. Ryad Tamouza, Jean Dausset Laboratory, Saint Louis Hospital, 1 Ave Claude Vellefaux, 75475, Paris CEDEX 10, France.
E-mail address: tamouza.ryad@gmail.com (R. Tamouza).

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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 *Bcl*I 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 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% ± 5% standard error versus 75% ± 5%; log-rank $P < .0001$) (Figure 1).

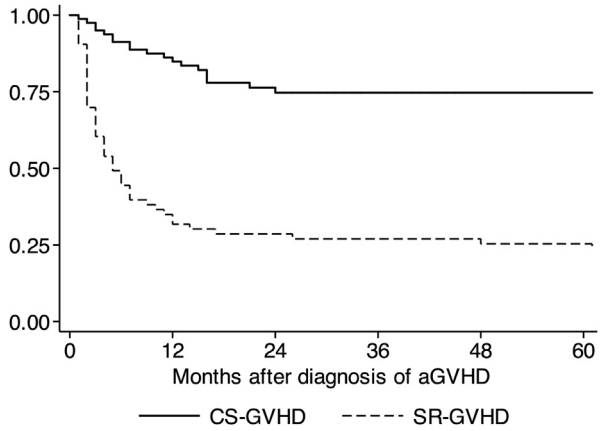

Figure 1. Overall survival after the time of diagnosis of aGVHD.

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 *rs*37972 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 *Bcl*I *rs*41423247 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 *Bcl*I *rs*41423247 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 *rs*6195, we found that the G allele was equally distributed in the 2 patient groups (OR,