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Pharmacogenetics of Steroid Responsive Acute Graft-Versus Host Disease

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Abstract

Glucocorticoids are central to effective therapy of acute graft-versus-host disease (GVHD). However, only about half of the patients respond to steroids in initial therapy. Based on postulated mechanisms for anti-inflammatory effectiveness, we explored genetic-variations in glucocorticoid receptor, co-chaperone proteins, membrane transporters, inflammatory mediators, and variants in the T-cell receptor complex in hematopoietic cell transplant recipients with acute GVHD requiring treatment with steroids and their donors towards response at day 28 after initiation of therapy. 300 recipient and donor samples were analyzed. Twenty-three SNPs in 17 genes affecting glucocorticoid pathways were included in the analysis. In multiple regression analysis, donor SNP rs3192177 in the *ZAP70* gene (O.R. 2.8, 95% CI: 1.3–6.0, $p=0.008$), and donor SNP rs34471628 in the *DUSP1* gene (O.R. 0.3, 95% CI: 0.1–1.0, $p=0.048$) were significantly associated with complete or partial response. However, after adjustment for multiple testing, these SNPs did not remain statistically significant. Our results, on this small, exploratory, hypothesis generating analysis suggest that common genetic variation in glucocorticoid pathways may help identify subjects with differential response to glucocorticoids. This needs further assessment in larger datasets, and if validated, could help identify subjects for alternative treatments and design targeted treatments to overcome steroid resistance.

Keywords

Allogeneic; HCT; graft-versus-host disease; pharmacogenetics; SNPs

Introduction

Glucocorticoids are central to effective therapy of acute graft-versus-host disease (GVHD). However, only about half of the patients respond and only one third have a durable

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Author contributions

M.A. and B.T. were involved in the concept/design, data analysis/interpretation, drafting article and critical revision of article. D.W. reviewed the data analysis, and contributed to critical revision of the article. R.S. performed the data analysis and contributed to critical revision of the article. All authors approved the final article.

response¹. Several studies have evaluated clinical and biological parameters to predict response at day 28, however none has explored glucocorticoid pathways directly to identify steroid resistance in acute GVHD^{2–5}. Based on postulated mechanisms for anti-inflammatory effectiveness^{6,7}, we explored genetic variations in glucocorticoid receptor (GR), co-chaperone proteins, membrane transporters, inflammatory mediators, and variants in the T-cell receptor complex in hematopoietic cell transplant (HCT) recipients with acute GVHD requiring treatment with steroids and their donors towards response at day 28 after initiation of therapy.

Patients and Methods

175 HCT recipients with acute graft-versus-host disease⁸ requiring treatment with systemic steroids, who underwent a HCT between 2003–2008 at the University of MN or enrolled in the BMT CTN 0302 trial⁹, with either recipient or donor DNA available were included in this study (87 from University of MN and 88 from CTN 0302). 300 samples (149 recipients and 151 donors) were available for this preliminary study. Fifty- three SNPs were genotyped. SNPs with very low variation in our cohort (< 4% in both donor and recipient sample) were excluded from analysis. Twenty-three SNPs in 17 genes affecting glucocorticoid pathways were included in the analysis. Our endpoints were overall response (complete or partial response: CR/PR) and CR alone. Response was graded as described previously by MacMillan et al for both the University of MN and BMT CTN cohorts.² This study was approved by the Institutional Review Board (IRB) at the University of Minnesota.

Results

Recipients ranged in age from < 1 to 68 years, 63% underwent a HCT using a myeloablative conditioning, 29% underwent a HCT from an HLA-identical sibling donor, 26% from matched URD and 39% from an umbilical cord blood (Table 1). Acute GVHD grade at onset included grade I disease in 27 recipients (15%), grade II in 109 recipients (62%), grade III in 36 (21%) and grade IV in 3 (2%). All recipients received initial therapy with systemic steroids. Patients enrolled in BMT CTN 0302 were in addition treated with etanercept (n=21, 24%), mycophenolate mofetil (MMF) (N=21, 24%), Ontak (n=21, 24%) or Pentostatin (n=25, 28%). Initial multivariate logistic regression analysis was performed to determine clinical risk factors associated with CR or PR. Clinical variables included in the analysis were recipient age, conditioning regimen, donor type, acute GVHD grade and year of HCT. Only year of HCT was significantly associated with CR or CR+ PR (p=0.01). Each recipient and donor SNP was subsequently evaluated individually in logistic regression analysis after controlling for year of HCT.

Results of analysis are presented in Table 2. The reference category was the genotype consisting of two copies of the more common allele. The odds ratio represents the change in odds for each copy of the less common allele. The Holm-Sidak¹⁰ method was used to adjust p-values for multiple testing This method attempts to control the family wise error rate at no more than 5%. All SNPs were checked for Hardy-Weinberg equilibrium. SNPs rs864058 and rs179008 (both in *TLR7* gene) were identified not to be in Hardy-Weinberg equilibrium (p<0.02).

Outcomes evaluated include complete response (CR) and complete or partial response (CR/PR). After excluding SNPs that were not in Hardy-Weinberg equilibrium, donor SNP rs3192177 in the *ZAP70* gene, and donor SNP rs34471628 in the *DUSP1* gene were significantly associated with CR/PR. However, after adjustment for multiple testing, none of the SNPs remained statistically significant.

Discussion

T-cells have been shown to be critical cellular targets of GR signaling as T cell-specific GR knockout mice show significant mortality following immune activation.¹¹ It has been shown that T cells defective in Zap70 signaling show loss of retinoid receptor dependent transcription.¹² It has also been demonstrated that Zap70 signaling is also essential in dexamethasone inducible GR-mediated transactivation in T lymphocytes.¹³ Zap70 and its upstream kinase Lck have been identified to be essential enzymes in maintaining normal levels of ANXA1, a protein known to be induced and released by the glucocorticoid action. Zap70 and Lck were also found to regulate ANXA1 promoter function in part through the modulation of GR-dependent transcription¹³.

Another mechanism of action by which glucocorticoids inhibit inflammation is through induction of the dual specificity phosphatase-1 (DUSP-1) gene, which reduces the activation of mitogen activated protein kinases (MAPKs)¹⁴. MAPKs can enhance the transcriptional activity of NF- κ B and AP-1, and also cause mRNA stabilization and translational activation of inflammatory genes¹⁴.

Our results, on this small, exploratory, hypothesis generating analysis suggest that common genetic variation in glucocorticoid pathways may help identify subjects who may be less likely to respond to gluco-corticoids. This needs further assessment in larger datasets, and if validated, could help identify subjects for alternative treatments and also help design targeted treatments to overcome steroid resistance.

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References

1. MacMillan ML, Robin M, Harris AC, et al. A refined risk score for acute graft-versus-host disease that predicts response to initial therapy, survival, and transplant-related mortality. *Biol Blood Marrow Transplant.* 2015; 21:761–7. [PubMed: 25585275]

2. MacMillan ML, Weisdorf DJ, Wagner JE, et al. Response of 443 patients to steroids as primary therapy for acute graft-versus-host disease: comparison of grading systems. *Biol Blood Marrow Transplant.* 2002; 8:387–94. [PubMed: 12171485]
3. Levine JE, Logan BR, Wu J, et al. Acute graft-versus-host disease biomarkers measured during therapy can predict treatment outcomes: a Blood and Marrow Transplant Clinical Trials Network study. *Blood.* 2012; 119:3854–60. [PubMed: 22383800]
4. Levine JE, Huber E, Hammer ST, et al. Low Paneth cell numbers at onset of gastrointestinal graft-versus-host disease identify patients at high risk for nonrelapse mortality. *Blood.* 2013; 122:1505–9. [PubMed: 23760615]
5. Vander Lugt MT, Braun TM, Hanash S, et al. ST2 as a marker for risk of therapy-resistant graft-versus-host disease and death. *N Engl J Med.* 2013; 369:529–39. [PubMed: 23924003]
6. Trevor JL, Deshane JS. Refractory asthma: mechanisms, targets, and therapy. *Allergy.* 2014; 69:817–27. [PubMed: 24773466]
7. Rhen T, Cidlowski JA. Antiinflammatory action of glucocorticoids—new mechanisms for old drugs. *N Engl J Med.* 2005; 353:1711–23. [PubMed: 16236742]
8. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995; 15:825–8. [PubMed: 7581076]
9. Alousi AM, Weisdorf DJ, Logan BR, et al. Etanercept, mycophenolate, denileukin, or pentostatin plus corticosteroids for acute graft-versus-host disease: a randomized phase 2 trial from the Blood and Marrow Transplant Clinical Trials Network. *Blood.* 2009; 114:511–7. [PubMed: 19443659]
10. Holm S. A Simple Sequentially Rejective Multiple Test Procedure. *Scand J Stat.* 1979; 6:65–70.
11. Wintermantel TM, Berger S, Greiner EF, Schutz G. Genetic dissection of corticosteroid receptor function in mice. *Horm Metab Res.* 2004; 36:387–91. [PubMed: 15241729]
12. Ishaq M, DeGray G, Natarajan V. Evidence for the involvement of tyrosine kinase ZAP 70 in nuclear retinoid receptor-dependent transactivation in T lymphocytes. *J Biol Chem.* 2005; 280:34152–8. [PubMed: 16096284]
13. Ishaq M, DeGray G, Mou K, et al. Zap70 signaling pathway mediates glucocorticoid receptor-dependent transcriptional activation: role in the regulation of annexin 1 expression in T cells. *J Immunol.* 2007; 179:3851–8. [PubMed: 17785822]
14. Newton R. Anti-inflammatory glucocorticoids: changing concepts. *Eur J Pharmacol.* 2014; 724:231–6. [PubMed: 23747654]

Table 1

Demographic Characteristics

	N	N with CR (%)	N with CR/PR (%)
N	175	99 (57%)	136 (78%)
Conditioning regimen			
Myeloablative	111	60 (54%)	82 (74%)
Reduced intensity conditioning	64	39 (61%)	54 (84%)
Gender			
Male	114	61 (54%)	86 (75%)
Female	61	38 (62%)	50 (82%)
Recipient age (years)			
Age 0–20	32	22 (69%)	27 (84%)
Age 21–40	39	17 (44%)	30 (77%)
Age 41–68	104	60 (58%)	79 (76%)
Source			
Clinical Trials Network (CTN 0302)	88	42 (48%)	63 (72%)
University of Minnesota	87	57 (65%)	73 (84%)
Donor type			
Matched sibling	51	31 (61%)	42 (82%)
Other related	7	3 (43%)	5 (71%)
UCB	68	42 (62%)	57 (84%)
Matched URD	46	23 (50%)	31 (67%)
Mismatched URD	3	0 (0%)	1 (33%)
Diagnosis			
ALL	47	30 (64%)	37 (79%)
AML	33	12 (36%)	21 (64%)
CML	12	8 (67%)	9 (75%)
Other leukemia	12	8 (67%)	11 (92%)
MDS	20	11 (55%)	13 (65%)
NHL	30	17 (57%)	25 (83%)
Multiple Myeloma	4	2 (50%)	4 (100%)
Other	17	11 (65%)	16 (94%)
AGVHD grade at onset			
1	27	17 (63%)	21 (78%)
2	109	63 (58%)	89 (82%)
3	36	17 (47%)	24 (67%)
4	3	2 (67%)	2 (67%)
AGVHD treatment (CTN 0302)			
Etanercept	21	5 (24%)	13 (62%)

	N	N with CR (%)	N with CR/PR (%)
MMF	21	10 (48%)	16 (76%)
Ontak	21	17 (81%)	18 (86%)
Pentostatin	25	10 (40%)	16 (64%)
Transplant year			
2003	4	3 (75%)	4 (100%)
2004	29	22 (76%)	27 (93%)
2005	33	21 (64%)	25 (76%)
2006	50	28 (56%)	37 (74%)
2007	53	21 (40%)	37 (70%)
2008	6	4 (67%)	6 (100%)

Abbreviations: CR: complete response; CR/PR: complete/partial response; UCB: umbilical cord blood; URD: unrelated donor; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; CML: chronic myeloid leukemia; MDS: myelodysplastic syndrome; NHL: non hodgkin's lymphoma; AGVHD: acute graft-versus-host disease

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Table 2

Multivariate analysis of SNPs as predictors of response (CR; CR or PR)

Gene	SNP (r= recipient d=donor)	Minor allele frequency	Outcome = CR					Outcome = CR or PR				
			Odds Ratio	Lower 95% CL	Upper 95% CL	p-value	Adjusted p-value	Odds Ratio	Lower 95% CL	Upper 95% CL	p-value	Adjusted p-value
ABCB1 (MDR1)	r_rs1045642	0.47	0.9	0.6	1.5	0.814	1.000	1.1	0.7	1.9	0.665	1.000
	d_rs1045642	0.46	1.1	0.7	1.7	0.808	1.000	1.0	0.6	1.7	0.880	1.000
ANXA-1 (annexin-1)	r_rs1050305	0.08	0.5	0.2	1.3	0.136	1.000	0.6	0.2	1.7	0.370	1.000
	d_rs1050305	0.07	0.6	0.2	1.7	0.353	1.000	0.6	0.2	1.7	0.331	1.000
NFKB1A (IκBa)	r_rs1050851	0.24	1.3	0.6	2.6	0.505	1.000	1.2	0.5	2.8	0.608	1.000
	d_rs1050851	0.24	0.6	0.3	1.3	0.206	1.000	0.8	0.4	1.8	0.654	1.000
HSP90AA1 (Hsp90)	r_rs10873531	0.18	0.8	0.4	1.6	0.466	1.000	1.0	0.4	2.2	0.921	1.000
	d_rs10873531	0.10	1.6	0.6	3.8	0.321	1.000	1.9	0.6	5.9	0.293	1.000
DNAJA1 (Hsp40)	r_rs20583	0.48	0.9	0.6	1.6	0.836	1.000	1.4	0.7	2.6	0.335	1.000
	d_rs20583	0.41	1.2	0.7	2.0	0.519	1.000	1.1	0.6	1.9	0.868	1.000
STIP1 (HOP)	r_rs2070232	0.07	1.7	0.6	4.6	0.312	1.000	1.2	0.4	4.1	0.720	1.000
	d_rs2070232	0.07	1.3	0.5	3.6	0.645	1.000	1.5	0.4	5.8	0.519	1.000
ABCB1 (MDR1)	r_rs2229109	0.05	1.7	0.5	6.0	0.440	1.000	1.5	0.3	7.3	0.624	1.000
	d_rs2229109	0.02	0.5	0.1	2.4	0.364	1.000	1.6	0.2	15	0.662	1.000
PPID	r_rs2230221	0.03	0.7	0.2	3.0	0.612	1.000	0.4	0.1	1.9	0.247	1.000
	d_rs2230221	0.07	1.1	0.4	2.9	0.876	1.000	0.7	0.2	2.1	0.556	1.000
DUSP1 (MKP-1)	r_rs2431663	0.08	0.7	0.3	1.7	0.379	1.000	0.4	0.2	1.2	0.094	1.000
	d_rs2431663	0.04	0.4	0.1	1.6	0.221	1.000	0.3	0.1	1.2	0.089	1.000
ZAP70	r_rs3192177	0.33	1.2	0.7	1.9	0.576	1.000	1.4	0.8	2.6	0.287	1.000
	d_rs3192177	0.28	1.6	0.9	2.8	0.108	1.000	2.8	1.3	6.0	0.008	0.405
CD247 (Zeta)	r_rs33937946	0.05	2.1	0.6	7.1	0.249	1.000	1.7	0.4	8.1	0.514	1.000
	d_rs33937946	0.04	0.9	0.3	3.1	0.875	1.000	0.5	0.1	1.8	0.286	1.000
DUSP1 (MKP-1)	r_rs34471628	0.05	0.7	0.2	2.2	0.531	1.000	0.5	0.1	1.6	0.241	1.000
	d_rs34471628	0.03	0.5	0.1	1.9	0.301	1.000	0.3	0.1	1.0	0.048	1.000
FKBP5 (FKBP51)	r_rs34866878	0.02	1.1	0.2	5.2	0.951	1.000	0.8	0.1	4.2	0.744	1.000
	d_rs34866878	0.04	1.4	0.4	5.1	0.630	1.000	0.5	0.1	2.0	0.335	1.000
TLR 9	r_rs352140	0.48	1.0	0.6	1.7	0.935	1.000	0.8	0.5	1.5	0.534	1.000

Gene	SNP (r= recipient d=donor)	Minor allele frequency	Outcome = CR					Outcome = CR or PR				
			Odds Ratio	Lower 95% CL	Upper 95% CL	p-value	Adjusted p-value	Odds Ratio	Lower 95% CL	Upper 95% CL	p-value	Adjusted p-value
	d_rs352140	0.49	1.4	0.9	2.3	0.156	1.000	1.3	0.7	2.2	0.410	1.000
CD3G (Gamma)	r_rs3753058	0.17	0.8	0.4	1.7	0.625	1.000	1.0	0.4	2.3	0.959	1.000
	d_rs3753058	0.17	0.5	0.2	1.0	0.060	1.000	0.5	0.2	1.1	0.079	1.000
CD3E (Epsilon)	r_rs4606515	0.33	1.3	0.8	2.2	0.325	1.000	1.1	0.6	1.9	0.810	1.000
	d_rs4606515	0.34	1.4	0.8	2.5	0.206	1.000	1.0	0.5	1.9	0.939	1.000
HSD11B2 (11B-HSD T2)	r_rs5479	0.05	1.0	0.3	3.2	0.955	1.000	1.1	0.3	4.1	0.920	1.000
	d_rs5479	0.06	1.3	0.5	3.6	0.638	1.000	1.7	0.5	6.5	0.413	1.000
NR3C1 (GR)	r_rs6196	0.16	0.9	0.4	1.9	0.806	1.000	0.5	0.2	1.2	0.126	1.000
	d_rs6196	0.13	0.7	0.3	1.6	0.433	1.000	0.7	0.3	1.6	0.357	1.000
HSP90AA1 (Hsp90)	r_rs8005905	0.14	0.8	0.4	1.6	0.490	1.000	1.1	0.4	2.7	0.897	1.000
	d_rs8005905	0.07	1.9	0.7	5.5	0.226	1.000	1.3	0.4	4.2	0.705	1.000
ABCB1 (MDR1)	r_rs9282564	0.09	0.9	0.3	2.1	0.733	1.000	1.5	0.5	4.9	0.467	1.000
	d_rs9282564	0.11	1.5	0.6	3.6	0.362	1.000	2.3	0.7	7.2	0.167	1.000
PP1D (peptidylpropyl isomerase D)	r_rs9410	0.26	0.9	0.5	1.5	0.603	1.000	0.9	0.5	1.8	0.787	1.000
	d_rs9410	0.31	1.0	0.6	1.7	0.899	1.000	1.3	0.7	2.4	0.379	1.000

Abbreviation: CR: complete response, CR/PR: complete or partial response