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Glucocorticoid receptor polymorphisms and outcomes in pediatric septic shock

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Abstract

Objective—Polymorphisms of the glucocorticoid receptor (GCR) gene are associated with outcome and corticosteroid responsiveness among patients with inflammatory disorders. We

conducted a candidate gene association study to test the hypothesis that these polymorphisms are associated with outcome and corticosteroid responsiveness among children with septic shock.

Design—We genotyped 482 children with septic shock for the presence of two GCR polymorphisms (rs56149945 and rs41423247) associated with increased sensitivity and one GCR polymorphism (rs6198) associated with decreased sensitivity to corticosteroids. The primary outcome variable was complicated course, defined as 28-day mortality or the persistence of two or more organ failures seven days after a septic shock diagnosis. We used logistic regression to test for an association between corticosteroid exposure and outcome, within genotype group, and adjusted for illness severity.

Setting—Multiple pediatric intensive care units in the United States.

Interventions—Standard care.

Measurements and Main Results—There were no differences in outcome when comparing the various genotype groups. Among patients homozygous for the wild type GCR allele, corticosteroids were independently associated with increased odds of complicated course (O.R. 2.30; 95% C.I. 1.01 to 5.21; $p = 0.047$).

Conclusions—Based on these GCR polymorphisms, we could not detect a beneficial effect of corticosteroids among any genotype group. Among children homozygous for the wild type allele, corticosteroids were independently associated with increased odds of poor outcome.

Keywords

sepsis; polymorphisms; outcome; corticosteroids; organ failure

INTRODUCTION

The role of corticosteroids for pediatric septic shock remains controversial. Corticosteroids are widely prescribed for children with septic shock, particularly those perceived to have refractory shock, based on guidelines and long standing anecdotal experiences [1-3]. Yet, there are no large, randomized trials demonstrating efficacy and several observational studies report either no benefit or potential harm [4-9]. This discrepancy between clinical practice and evidence reflects many factors, including the inability to reliably identify which patients are most likely to benefit from corticosteroid prescription [10].

Corticosteroid activity is, in part, dependent on the glucocorticoid receptor (GCR). There exist functional polymorphisms of the GCR gene, which are associated with either increased or decreased sensitivity to corticosteroids [11]. Among these, the minor alleles of the GCR polymorphisms N363 (rs56149945, formerly rs6195) and *BcI* (rs41423247) are associated with increased sensitivity to corticosteroids. The N363 polymorphism affects the GCR trans-activating domain, whereas the *BcI* polymorphism is an intronic restriction length polymorphism. Conversely, the minor allele of GCR polymorphism β (rs6198) is associated with decreased sensitivity to corticosteroids. This polymorphism increases the stability of the GCR β splice variant, which functions as a dominant negative receptor for corticosteroids, relative to GCR α .

Among patients with a variety of inflammatory disorders, these functional GCR polymorphisms have been associated with clinical outcomes and response to exogenous corticosteroids [12-15]. Here, we conducted a candidate gene association study to test the hypothesis that these polymorphisms are associated with outcome and corticosteroid responsiveness among children with septic shock.

METHODS

Study Subjects

The protocol for consenting and enrolling study subjects was approved by the Institutional Review Board of each participating institution and was previously detailed [16, 17]. Briefly, children 10 years of age admitted to the pediatric intensive care unit (PICU) and meeting pediatric-specific consensus criteria for septic shock were eligible for enrollment [18]. There were no exclusion criteria, other than the inability to obtain informed consent. After informed consent from the parents or legal guardians, blood samples for DNA isolation and clinical data were collected. Clinical and laboratory data were collected daily while in the PICU. The protocol for coding corticosteroid exposure was previously detailed [5]. Organ failure data were tracked up to day seven of septic shock using published criteria [18]. Mortality was tracked for 28 days after enrollment. Illness severity was estimated using the PRISM III score [19]. Control subjects consisted of critically ill children meeting criteria for systemic inflammatory response syndrome (PICU controls) and healthy children presenting to the ambulatory department for screening laboratory studies [17].

Genotyping

The three polymorphisms of interest were detected using polymerase chain reaction (PCR) technology and a QuantStudio™ 6 instrument (ThermoFisher Scientific, Waltham, MA). Taqman PCR primers and Taqman genotyping master mix were purchased from Applied Biosystems, ThermoFisher Scientific. Genotype calls were made using Applied Biosystems TaqMan Genotyper Software.

Data Analysis

Initially, data are described using medians, interquartile ranges, frequencies, and percentages. Comparisons between groups used ANOVA on Ranks or the Chi-square test as appropriate. Descriptive statistics and comparisons used SigmaStat Software (Systat Software, Inc., San Jose, CA).

For testing the association between genotype and outcome we grouped the patients with septic shock into four genotype groups: 1) those with at least one allele associated with increased sensitivity to corticosteroids (rs56149945 or rs41423247); 2) those with at least one allele associated with decreased sensitivity to corticosteroids (rs6198); 3) those with a mixture of increased and decreased sensitivity alleles; and 4) those homozygous for the wild type allele. The primary outcome variable was complicated course, defined as the persistence of two or more organ failures at day seven of septic shock or 28-day mortality [6]. We used logistic regression to test for an association between corticosteroid exposure and complicated course, within genotype group, and adjusted for PRISM III score.

RESULTS

The three polymorphisms were in Hardy-Weinberg equilibrium. The allele frequencies were not different when comparing subjects with septic shock (n = 482) to healthy controls (n = 49) and PICU controls (n = 103) (*data not shown*). Table 1 shows the demographic and clinical data for the subjects with septic shock, grouped according to the GCR alleles. There was no difference in the rate of complicated course among the four groups ($p = 0.267$, Chi-square, 3 degrees of freedom). The proportion of subjects with gram-negative bacteria was different across the four groups, as was the proportion of Caucasian and African-American subjects. No other differences were noted.

Table 2 shows the results of logistic regression testing for an association between corticosteroid exposure and complicated course, within each allele group. Among all subjects, corticosteroids were not associated with complicated course. Among subjects who were homozygous for the wild type GCR allele, exposure to corticosteroids was independently associated with increased odds of complicated course. This association remained when also accounting for age and the presence of co-morbidity (O.R. 2.32; 95% C.I. 1.01 to 5.34; $p = 0.047$). Corticosteroids exposure was not associated with outcome among the other GCR allele groups.

DISCUSSION

In 2002, Annane et al. published a landmark study indicating that a corticotropin stimulation test and the corresponding blood cortisol concentrations could identify a subgroup of patients with septic shock most likely to benefit from corticosteroids [20]. The results were not replicated in a subsequent study [21], suggesting that the information provided by corticotropin stimulation test is not sufficient to consistently select patients who are more likely to benefit from corticosteroids and that other factors affect corticosteroid responsiveness among patient with septic shock. Indeed, critically ill patients demonstrate significant variability with regard to cortisol metabolism and GCR expression, and among critically ill children, a polymorphism in the corticotropin receptor gene was associated with a blunted cortisol response [22-25].

In the current study, we explored three functional GCR polymorphisms and tested their association with outcome and corticosteroid responsiveness. None of the polymorphisms was associated with outcome or corticosteroid responsiveness, as measured by the rate of complicated course. However, among patients who were homozygous for the wild type allele, corticosteroid exposure was associated with increased risk of complicated course, after adjusting for illness severity.

Collectively, the data suggest that corticosteroids might be harmful for children with septic shock who are homozygous for the GCR wild type allele, relative to the polymorphisms examined in the current study. This assertion must be interpreted with caution because we conducted a candidate association study, and consequently did not test all of the existing GCR polymorphisms. Further, the number of patients in the decreased and mixed sensitivity allele groups was relatively small. We did not conduct *a priori* power calculations, which is a

characteristic of a well conducted gene association study [26]. This reflects a lack of prior data linking these polymorphisms to pediatric sepsis outcomes on which to base power calculations. It is therefore possible that we were underpowered to detect an association between the gene variants and complicated course. We note, however, that the number of patients in the current study is greater than that of prior studies with positive findings, albeit in adults and conditions other than sepsis [13, 14].

Corticosteroid prescription was not protocolized, thus raising the possibility of confounding by variability in patient care, inconsistent indications for corticosteroid prescription, and illness severity. We note, however, that the association between outcomes, corticosteroid prescription, and homozygosity for the wild type allele persisted after adjustments for illness severity, age, and the presence of co-morbidity. Nonetheless, our study also did not detect any benefit of corticosteroids in this cohort, thus strengthening the appeal for equipoise on the subject pending a randomized controlled trial of corticosteroids in pediatric septic shock.

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Table 1

Demographic and clinical data for the patients with septic shock, based on allele groups.

Variable	Increased Sensitivity Allele	Decreased Sensitivity Allele	Mixed Increased and Decreased Sensitivity Alleles	Homozygous for the Wild Type Allele
N	227	50	60	145
Complicated Course, n (%)	64 (28)	8 (16)	19 (32)	38 (26)
Mortality, n (%)	23 (10)	2 (4)	7 (12)	16 (11)
Maximum number of organ failures, median (IQR)	2 (2 – 3)	2 (1 – 2)	2 (2 – 3)	2 (2 – 3)
PICU length of stay, median days (IQR) ^a	8 (3 – 14)	6 (4 – 9)	7 (3 – 12)	9 (4 – 16)
PICU free days, median (IQR)	19 (9 – 24)	22 (18 – 24)	21 (9 – 25)	18 (8 – 24)
PRISM, median (IQR)	12 (7 – 19)	11 (9 – 17)	11 (8 – 15)	12 (7 – 18)
Age, median years (IQR)	3 (1 – 7)	3 (2 – 8)	5 (1 – 8)	3 (1 – 7)
Males, n (%)	135 (59)	25 (50)	36 (60)	84 (56)
Co-morbidity, n (%)	93 (41)	22 (44)	28 (47)	65 (45)
Malignancy, n (%)	21 (9)	7 (14)	6 (10)	13 (9)
Immunesuppression, n (%)	23 (10)	7 (14)	6 (10)	18 (12)
Bone marrow transplant, n (%)	10 (4)	1 (2)	3 (5)	5 (3)
Corticosteroids, n (%)	101 (44)	32 (64)	31 (52)	75 (52)
Gram negative bacteria, n (%) ^b	46 (20)	8 (16)	8 (13)	49 (34)
Gram positive bacteria, n (%)	62 (27)	14 (28)	13 (22)	34 (23)
Other organism, n (%)	26 (11)	5 (10)	9 (15)	14 (10)
Culture negative, n (%)	93 (41)	23 (46)	30 (50)	48 (33)
Race, n (%)				
<i>Caucasian</i> ^b	172 (76)	45 (90)	55 (92)	79 (54)
<i>African American</i> ^b	28 (12)	1 (2)	1 (2)	37 (26)
<i>Asian</i>	3 (1)	2 (4)	0 (0)	7 (5)
<i>Other</i>	11 (5)	0 (0)	1 (2)	14 (10)
<i>Unreported</i>	13 (6)	2 (4)	3 (5)	8 (6)

^aIncludes survivors only.^bp < 0.05, Chi-square, 3 degrees of freedom.

Table 2

Logistic regression testing the association between corticosteroid exposure and complicated course within each GCR allele group.

Genotype Group	Variable	O.R.	95% CI	P value
All subjects	Corticosteroids	1.33	0.87 – 2.05	0.190
	PRISM	1.09	1.06 – 1.12	<0.001
Increased sensitivity allele	Corticosteroids	1.34	0.72 – 2.49	0.356
	PRISM	1.09	1.05 – 1.13	<0.001
Decreased sensitivity allele	Corticosteroids	0.78	0.14 – 4.29	0.778
	PRISM	1.14	1.02 – 1.28	0.027
Mixed sensitivity alleles	Corticosteroids	0.75	0.26 – 2.30	0.614
	PRISM	1.07	0.99 – 1.16	0.101
Homozygous, wild type allele	Corticosteroids	2.30	1.01 – 5.21	0.047
	PRISM	1.09	1.04 – 1.14	<0.001