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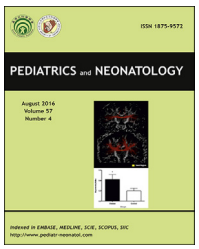

Original Article

A polymorphism in the glucocorticoid receptor gene is associated with refractory hypotension in premature infants

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Key Words
*Bcl*I polymorphism;
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Background: Glucocorticoids play an important role in endocrine control. The association of glucocorticoid receptor (GR) gene polymorphisms with altered sensitivity to glucocorticoid therapy has been reported in adults. However, there are few such reports in infants. The present study analyzed the prevalence of four GR polymorphisms in preterm infants born before 30 weeks of gestation and determined the associations between these polymorphisms and clinical outcomes in the infants.
Methods: Totally, 41 preterm infants born at two hospitals in Fukushima were retrospectively screened for the presence of four GR gene polymorphisms, using a TaqMan single-nucleotide polymorphism genotyping assay. The effect of GR gene polymorphisms on clinical outcomes during hospitalization was evaluated. The following primary clinical outcomes were assessed: refractory hypotension in the acute phase and/or severe bronchopulmonary dysplasia, maximum dopamine and dobutamine doses administered, and total hydrocortisone dose administered in the first 48 h of life. Multivariate analysis with logistic regression was used to assess the association between clinical factors and refractory hypotension.
Results: Of the four GR polymorphisms, only the *Bcl*I polymorphism was detected. The genotype distribution was as follows: C/C, 33; C/G, 8; and G/G, 0 infants. Significant differences were observed between the C/C and C/G genotypes with respect to the following variables: refractory hypotension (6% *vs.* 50%), dopamine dose [3.0 (2.0–4.0) *vs.* 4.8 (4.0–7.5) μg/kg/min], dobutamine dose [2.4 (0.0–3.6) *vs.* 4.0 (0–10.0) μg/kg/min], and total hydrocortisone

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dose administered in the first 48 h of life [2.0 (0—10.0) *vs.* 6.0 (0—12.0) mg/kg]. Multivariate analysis showed that the *Bcl*I genotype (C/C) was significantly less associated with refractory hypotension in the acute phase (odds ratio, 0.008; 95% confidence interval, 0.000—0.371; p = 0.013).

Conclusion: The incidence of refractory hypotension in infants with the C/C genotype was initially expected to be higher than that in infants with the C/G genotype. However, the results of this study were rather different from what we originally expected. The suppressive effect of antenatal steroid use on the HPA axis of the preterm infants with the *Bcl*I variant may be associated with refractory hypotension in the acute phase.

1. Introduction

Glucocorticoids (GCs) play an important role in endocrine control with regard to homeostasis, immune function, and cell growth and differentiation.[1] They are used to treat hypotension, bronchopulmonary dysplasia (BPD), and sepsis in premature infants. At the cellular level, the action of GCs is mediated by the intracellular glucocorticoid receptor (GR).[2] The GR, a member of the steroid hormone receptor family and the ligand-regulated nuclear receptor superfamily, is involved in the positive or negative regulation of the expression of GC-responsive genes.

The sensitivity of GRs to GCs can vary significantly among individuals,[3] and variability in the sensitivity to hydrocortisone therapy has been linked to several GR gene polymorphisms.[4] The following polymorphisms have been detected in the human GR-encoding gene: *N363S*, *Bcl*I, *R23K*, and *GR-9β*. The *N363S* polymorphism is associated with a high sensitivity to GCs *in vivo*, as demonstrated by the increased cortisol suppression observed using a 0.25-mg dexamethasone suppression test.[5] *Bcl*I polymorphism is associated with an increased sensitivity to GCs.[6] Conversely, *R23K* and *GR-9β* polymorphisms are associated with a relative resistance to GCs. Notably, *R23K* harbors two linked single-nucleotide polymorphisms (SNPs) in codons 22 and 23 of exon 2 in the human GR gene.

Several reports have described the effects of GR polymorphisms on clinical outcomes in adults; however, there are few such reports in infants.[7—9] We hypothesized that GR polymorphisms could affect clinical outcomes in preterm infants.

The present study analyzed the prevalence of four GR polymorphisms in preterm infants born before 30 weeks of gestation and determined the associations between these polymorphisms and clinical outcomes.

2. Methods

2.1. Study subjects

We enrolled infants born at 23—29 weeks of gestation to Japanese parents, between October 2010 and July 2013. Infants with major congenital defects were excluded from the study. The Ethics Committees of Fukushima Medical University School of Medicine and National Hospital Organization Fukushima National Hospital approved the study. All mothers provided written informed consent for the inclusion of their infants.

2.2. Definitions

Refractory hypotension in the acute phase was defined as a mean arterial pressure (mmHg) that was lower than the numerical value of the gestational age (weeks) of an infant[10] despite optimal management, including volume expansion, inotrope administration, and the administration of a single dose of hydrocortisone (2 mg/kg),[11,12] in the first 48 h of life. Until the resolution of hypotension, infants were sequentially treated with the following therapies: (1) volume replacement (10 mL/kg of 0.9% saline), (2) dopamine administration (dose increased by 2—3 μg/kg/min every 30 min up to a maximum dose of 5—10 μg/kg/min), (3) dobutamine administration when the infant did not achieve the target blood pressure with only dopamine use, and (4) administration of a single dose of hydrocortisone (2 mg/kg).

BPD was diagnosed if oxygen therapy was administered for ≥28 days.[13] Severe BPD was diagnosed if an infant required positive airway pressure ventilation at 36 weeks' postmenstrual age or at discharge, whichever was earlier. A physiological test developed by Walsh et al. was used to confirm oxygen requirement at the time of assessment.[14]

2.3. Analysis of gene polymorphisms

The following previously investigated GR gene polymorphisms were selected because of their reported association with an altered sensitivity to GCs: *N363S* (rs56149945), *Bcl*I (rs41423247), *R23K* (rs6189), and *GR-9β* (rs6198). Blood samples were drawn immediately after birth from either the umbilical cord, a vein, or an artery. Genomic DNA was extracted from blood samples using the QIAamp® DNA Blood kit (Qiagen, Courtaboeuf, France). Genotypes were determined using a Custom TaqMan® SNP assay (Applied Biosystems, Foster City, CA, USA), which allowed custom allelic discrimination and included specific primers and corresponding probes for each SNP. Assay design was performed using the Custom TaqMan® SNP assay design tool (Applied Biosystems). *N363S*, *Bcl*I, and *R23K*

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