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Glucocorticoid Receptor Gene Variants and Neonatal Outcome in Very-Low-Birth-Weight Preterm Infants

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Key Words
Glucocorticoid receptor · Polymorphism · Neonatal outcome · Preterm infants

Abstract
Background: Induction of lung maturation by prenatal steroid treatment has become the standard of care for pregnant women at risk for preterm birth. In addition to the beneficial effects on lung maturation, prenatal steroids have been shown to reduce the incidence of neonatal death, necrotizing enterocolitis, sepsis, and intraventricular hemorrhage. However, little is known about the role of interindividual differences in corticoid sensitivity arising from polymorphisms in the glucocorticoid receptor *(GR)* gene. ***Objectives:*** To assess the impact of *GR* polymorphisms *N363S* (rs56149945), *R23K* (rs6190), and *BclI* (rs41423247) on neonatal outcome. ***Methods:*** The *GR* polymorphisms *N363S, R23K,* and *BclI* were examined in 10,490 very-low-birth-weight (VLBW) preterm infants from 49 German tertiary level neonatal units (German Neonatal Network, GNN) with respect to neonatal outcome. ***Results:*** Infants carrying the *BclI* genotype were at higher risk to develop bronchopulmonary dysplasia (BPD) (OR 1.12 per *BclI* allele, 95% CI: 1.02–1.23, p = 0.013) in a logistic regression model adjusted for gestational age, mechanical ventilation, and small for gestational age status. A similar relative risk was seen in the children (89.4%) who received antenatal betamethasone treatment (OR 1.16, 95% CI: 1.05–1.27, p = 0.003), whereas no such effect was detectable in infants without antenatal steroids. *N363S* and *R23K* did not show any stable association with neonatal outcome parameters. ***Conclusion:*** Except for a slightly higher risk of BPD in carriers of the *GR BclI* variant, the *GR* gene polymorphisms *BclI, N363S,* and *R23K* did not affect neonatal outcome parameters in this large multicenter cohort of VLBW preterm infants.

Introduction

In 1969 Graham Liggins demonstrated accelerated fetal lung maturation after antenatal corticosteroid administration in premature lambs [1] and shortly thereafter in humans [2]. Antenatal corticosteroid treatment significantly reduces the risk of respiratory distress syndrome

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(RDS) and has become a standard of care for all pregnant women at risk of preterm birth less than 34 weeks of gestation. In addition to the beneficial effect on lung maturation, prenatal steroid treatment has been shown to reduce the incidence of neonatal death, necrotizing enterocolitis (NEC), sepsis, and intraventricular hemorrhage (IVH) [3].

We hypothesized that genetically determined differences in glucocorticoid sensitivity may modify the response to corticosteroids and thus represent a factor influencing the clinical outcome of preterm infants. Differences in the individual glucocorticoid sensitivity are mediated by glucocorticoid receptor (*GR*) gene polymorphisms, among which both variants with increased *(N363S*, *BclI)* and decreased *(R23K)* receptor sensitivity exist [4]. These variants may not only modify the response to exogenous synthetic steroids such as betamethasone, but could also modify the effects of endogenous corticosteroids during pre- and perinatal life.

So far, *GR* gene polymorphisms have been analyzed in mostly small cohorts of preterm infants with conflicting results [5–10]. However, considering the comparatively low allele frequencies for *N363S* and *R23K* and the need to stratify for antenatal corticosteroid treatment, verification of this compelling hypothesis may require substantially larger study samples. In this study, we aimed to determine the effects of *GR* gene polymorphisms *BclI*, *N363S*, and *R23K* on neonatal outcome parameters in a large multicenter cohort of more than 10,000 very-low-birth-weight (VLBW; birth weight <1,500 g) preterm infants.

Patients and Methods

The study cohort comprised a total of 10,490 VLBW infants. 10,413 infants were prospectively enrolled in a multicenter study of the German Neonatal Network (GNN; 48 German tertiary level neonatal units) between 2003 and 2013; 77 infants were initially recruited as part of a single-center study at the Department of Neonatology, University of Bonn, which became affiliated with the GNN network later on. Since inclusion of multiple siblings with identical or similar genetic backgrounds as well as shared intrauterine environment increases the risk of spurious genotype-phenotype associations, only one sibling of each group of multiples was selected on a random basis to be included into the study samples. Personal data were pseudonymized prior to genotyping and statistical analysis. The study was approved by the ethics committees of all participating hospitals. Written informed consent was obtained from all parents.

DNA for genotyping was extracted from buccal swabs or umbilical cord tissue using standard protocols (QIAamp 96 DNA Kit and Gentra Puregene Tissue Kit; Qiagen, Hilden, Germany). Genotypes for the *GR* gene variants *N363S* (rs56149945), *R23K* (rs6190), and *BclI* (rs41423247) were analyzed automatically on an Applied Biosystems 7900HT platform (GNN, Lübeck, Germany) or by restriction fragment length polymorphism (initial Bonn cohort; for primers and reaction conditions see Schreiner [11]). Out of a total of 10,490 samples, genotyping was successful in 10,201 *(BclI)*, 9,851 *(R23K)*, and 10,322 *(N363S)* samples. Because of the small number of samples homozygous for 363S or 23K alleles, those heterozygous and homozygous for the mutant allele were grouped for statistical analyses. In order to exclude potential confounding effects of compound heterozygosity or double mutants, all statistic tests were repeated comparing samples carrying only one *GR* variant (= pure carrier) against those carrying only wild-type alleles at all three polymorphic loci (= pure noncarrier).

Detailed information on clinical definitions is given in previous publications of the GNN group [12, 13]. Small for gestational age (SGA) was defined as birth weight below the 10th percentile according to Voigt et al. [14]. Ethnicity was assessed by the mother's report on her racial background. Bronchopulmonary dysplasia (BPD) was classified according to Walsh et al. [15]. Supplemental oxygen <30% with desaturation <90% during an oxygen reduction test, supplemental oxygen >30%, and/or requirement of CPAP/mechanical ventilation were classified as 'BPD' at 36 weeks' gestational age. Any kind of mechanical ventilation after endotracheal intubation during the hospital stay was classified as mechanical ventilation, whereas CPAP was documented as a separate variable. Any use of surfactant was expressed by the variable surfactant therapy. IVH was defined as any bleeding into the germinal matrix or ventricles. The lowest blood pressure on the first day of life was defined as the lowest documented mean arterial blood pressure during the first day of life. We separately assessed clinical sepsis, defined as any suspected sepsis with obvious clinical symptoms [16], and blood-culture confirmed sepsis. Blood-culture-confirmed sepsis was further separated into early-onset sepsis if the onset was within 72 h of life and late-onset sepsis. Treatment of retinopathy of prematurity (ROP) with laser coagulation or cryocoagulation was expressed as surgery for ROP. Any surgical treatment of NEC was expressed by the variable surgery for NEC. Indications for surgery of ROP, PDA, and NEC were based on the local treatment standard of each participating center.

Statistical analyses were performed using the SPSS software package, version 22 (SPSS Inc., Chicago, Ill., USA). Differences between genotype groups were analyzed by a χ^2 test, Fisher's exact test, or ANOVA. Logistic regression analysis was used to assess the influence of *GR* genotypes and other factors on binary variables such as BPD or IVH. Effect sizes for other variables were analyzed by linear regression analyses. Generally, statistical significance was assumed for p values <0.05.

Results

Clinical data stratified for *GR* polymorphisms *BclI*, *N363S*, and *R23K* are presented in tables 1–3. Genotypes were in Hardy-Weinberg equilibrium and allele frequencies were comparable with those reported previously from other Western European populations [4, 11].

Table 1. Clinical data with respect to the *BclI* genotype

	Genotype *BclI* (n = 10,201)			p value	
	–/– (4,427)	+/– (4,600)	+/+ (1,174)	–/–, +/–, +/+	–/– vs. carrier
Gestational age, weeks	28.28±2.74	28.32±2.70	28.29±2.71	0.760	0.506
Birth weight, g	1,058±302	1,063±305	1,067±294	0.578	0.299
Gender, males	50.0	51.4	52.1	0.289	0.131
Singletons	66.6	66.5	65.4	0.731	0.733
SGA <10th percentile	17.9	19.1	16.6	0.091	0.356
Apgar 5 min	7.72±1.50	7.78±1.82	7.81±1.47	0.139	0.077
Apgar 10 min	8.59±1.10	8.60±1.10	8.63±1.07	0.616	0.380
PDA /drug intervention	22.5	22.4	23.7	0.629	0.893
Lowest RR 1st dol, mm Hg	27.90±6.62	28.08±6.36	27.94±6.88	0.358	0.204
Inotropes	17.9	19.5	19.1	0.144	0.052
Antenatal steroids	89.2	89.7	87.4	0.076	0.916
Surfactant	56.4	56.2	57.5	0.737	0.986
CPAP	83.1	83.7	83.4	0.739	0.460
Mechanical ventilation	47.0	47.6	48.8	0.561	0.405
BPD	14.6	16.3	17.0	*0.045*	*0.015*
Dexamethasone (postnatal)	4.0	4.7	5.2	0.127	0.056
Steroids (postnatal)	11.2	11.9	13.7	0.064	0.112
IVH	16.7	17.1	18.3	0.402	0.370
PVL	2.9	3.8	3.6	0.073	*0.023*
PDA surgery	4.7	4.8	4.2	0.714	1.000
NEC surgery	2.5	2.6	3.1	0.506	0.464
ROP surgery	2.7	3.3	3.4	0.142	*0.048*
Sepsis (clinical)	31.8	31.5	32.7	0.716	0.877
Sepsis (culture-proven)	13.6	12.5	14.2	0.176	0.257
Sepsis (early-onset)	1.4	1.2	2.0	0.152	0.935
Sepsis (late-onset)	9.8	9.1	11.5	*0.041*	0.681
Death	3.0	3.5	2.7	0.234	0.285

Data are expressed as means ± SD or %. Italics represent significant values.

BclI

Carriers of the *BclI* variant were more likely to suffer from BPD (16.4 vs. 14.6%, p = 0.015), whereas birth parameters and frequencies of CPAP and/or mechanical ventilation requirement did not differ between *BclI* genotype groups (table 1). This genotype effect was reproducible in different subgroups of infants with a high BPD risk (table 4). Binary logistic regression analysis revealed a significant contribution of mechanical ventilation (OR 6.50, 95% CI: 5.43–7.85), gestational age (OR 0.74 per week, 95% CI: 0.72–0.76), SGA status (OR 2.82, 95% CI: 2.41–3.30), and *BclI* genotype (OR 1.12 per *BclI* allele, 95% CI: 1.02–1.23, p = 0.013) to the individual risk of BPD. A similar relative risk was seen in the subcohort of infants with antenatal steroid treatment (n = 9,065, OR 1.16, 95% CI: 1.05–1.27, p = 0.003). In contrast, no such effect was detectable in the subgroup without antenatal steroid exposure (n = 1,094, OR 0.86, 95% CI: 0.64–1.15). A subcohort analysis of 8,359 individuals being either *BclI* pure carriers (i.e. wild-type at positions p.363 and p.23) or pure noncarriers (wild-type at all three loci), however, slightly attenuated the observed association between *BclI* and BPD (CC 15.1% vs. CG 16.1% vs. GG 17.0%, p > 0.2; OR 1.09, 95% CI: 0.99–1.21, p = 0.08; infants with antenatal steroids: OR 1.12, 95% CI: 1.01–1.24, p = 0.030). Inclusion of ethnicity into analysis also did not further strengthen the observed association between BPD and *BclI* genotype (Caucasians, total n = 8,465: 16.4 vs. 14.4%, p = 0.051; OR 1.14, 95% CI: 1.02–1.27, p = 0.025; Middle East and Turkey, total n = 895: 16.5 vs. 15.2%, p > 0.2; OR 1.14, 95% CI: 0.78–1.66, p > 0.2).

Nominally significant associations were also detected for *BclI* and the individual risk to develop PVL, ROP requiring surgical intervention, and late-onset sepsis, respec-

Table 2. Clinical data with respect to the *N363S* genotype

	Genotype *N363S* (n = 10,322)		p value
	–/– (9,593)	+/– or +/+ (718 + 11)	–/– vs. carrier
Gestational age, weeks	28.30±2.73	28.22±2.55	0.278
Birth weight, g	1,061±303	1,056±293	0.568
Gender, males	50.9	50.8	0.956
Singletons	66.4	64.8	0.371
SGA <10th percentile	18.4	16.6	0.224
Apgar 5 min	7.76±1.66	7.69±1.48	0.176
Apgar 10 min	8.60±1.10	8.59±1.07	0.915
PDA/drug intervention	22.7	22.8	0.931
Lowest RR 1st dol, mm Hg	28.03±6.55	27.26±6.31	*0.008*
Inotropes	18.7	21.0	0.132
Antenatal steroids	89.4	89.1	0.753
Surfactant	56.5	57.8	0.489
CPAP	83.3	83.7	0.817
Mechanical ventilation	47.3	51.4	*0.032*
BPD	15.7	16.4	0.608
Dexamethasone (postnatal)	4.4	4.8	0.621
Steroids (postnatal)	11.8	13.2	0.258
IVH	17.0	18.0	0.493
PVL	3.3	4.4	0.119
PDA surgery	4.7	4.2	0.528
NEC surgery	2.7	2.4	0.590
ROP surgery	3.0	2.8	0.736
Sepsis (clinical)	31.8	32.4	0.743
Sepsis (culture-proven)	13.1	13.1	0.969
Sepsis (early-onset)	1.4	2.0	0.192
Sepsis (late-onset)	9.7	9.3	0.757
Death	3.3	3.0	0.749

Data are expressed as means ± SD or %. Italics represent significant values.

Table 3. Clinical data with respect to the *R23K* genotype

	Genotype *R23K* (n = 9,851)		p value
	–/– (9,353)	+/– or +/+ (493+5)	–/– vs. carrier
Gestational age, weeks	28.27±2.70	28.48±2.74	0.105
Birth weight, g	1,058±303	1,080±294	0.142
Gender, males	51.1	49.4	0.449
Singletons	66.2	63.2	0.159
SGA <10th percentile	18.3	16.1	0.210
Apgar 5 min	7.74±1.50	7.96±3.49	0.424
Apgar 10 min	8.59±1.10	8.63±1.08	0.322
PDA/drug intervention	23.0	21.1	0.326
Lowest RR 1st dol, mm Hg	27.97±6.54	28.35±6.66	0.358
Inotropes	19.2	17.8	0.439
Antenatal steroids	89.5	87.7	0.196
Surfactant	57.0	53.5	0.131
CPAP	83.3	85.1	0.316
Mechanical ventilation	47.8	46.2	0.485
BPD	15.9	13.6	0.165
Dexamethasone (postnatal)	4.6	3.5	0.309
Steroids (postnatal)	12.0	10.3	0.256
IVH	17.1	16.4	0.695
PVL	3.4	4.1	0.461
PDA surgery	4.7	4.7	0.980
NEC surgery	2.7	2.9	0.839
ROP surgery	3.1	2.7	0.601
Sepsis (clinical)	31.8	31.9	0.997
Sepsis (culture-proven)	13.1	15.3	0.155
Sepsis (early-onset)	1.4	2.2	0.104
Sepsis (late-onset)	9.7	10.6	0.517
Death	3.3	4.5	0.154

Data are expressed as means ± SD or %.

Table 4. BPD and *BclI* genotypes in BPD high-risk subgroups

High-risk subgroup	Infants with BPD according to *BclI* genotype			p value	
	–/–	+/–	+/+	–/–, +/–, +/+	–/– vs. carrier
SGA (n = 1,858)	19.4 (153/787)	23.5 (206/877)	24.7 (48/65)	0.082	*0.028*
Pneumothorax (n = 517)	25.5 (51/200)	32.5 (82/252)	36.9 (24/65)	0.127	0.056
Treatment with surfactant (n = 5,705)	22.2 (549/2,475)	25.4 (653/2,566)	25.3 (168/664)	*0.018*	*0.005*

Data are expressed as % (n/n). Italics represent significant values.

tively. Whereas *BclI* and PVL also exhibited a significant relation in binary regression analysis including gestational age and antenatal steroids as covariables (OR 1.18, 95% CI: 1.01–1.39, p = 0.038), outcome variables ROP surgery and late-onset sepsis did not reveal significant associations in regression analyses. Similarly, pure carrier analyses did not show significant associations between any of the variables PVL, ROP, and sepsis to the *BclI* genotype.

N363S

Infants carrying the *N363S* variant had lower blood pressure at the first day of life and higher rates of mechanical ventilation (table 2). The genotype effect on blood pressure remained significant after adjustment for gestational age ($\beta = -0.023$, $p = 0.024$). Analysis of only the children born to Caucasian mothers (n = 8,761) revealed similar effect sizes (group comparison 28.10 ± 6.51 vs. 27.35 ± 6.35, $p = 0.012$; regression $\beta = -0.021$, $p = 0.052$) compared to the entire cohort. On the other hand, in children of Middle East or Turkish ethnic origin (n = 915), none of the analyses revealed a significant relation between *N363S* and blood pressure (all $p > 0.2$). Similarly, selective analyses of pure *N363S* carriers against pure noncarriers (403 vs. 3,407 infants) did not confirm the effects observed in the entire cohort (all $p > 0.2$).

Regarding mechanical ventilation, logistic regression analysis with adjustment for gestational age only revealed a trend for the impact of *N363S* ($p = 0.060$). Again, exclusion of compound-heterozygous and double mutant individuals abolished the effects seen in the entire cohort (47.0 vs. 48.3%, $p > 0.2$).

R23K

The *R23K* variant did not influence any of the neonatal outcome parameters, neither in the entire cohort nor in subgroup analyses comparing only pure carriers against pure noncarriers (all $p > 0.1$).

The infants included in our study were born over a considerably long period (2003–2013) during which assistance of premature infants was developed further. In order to identify potential confounders related to changes of neonatal care during this period, we compared the core parameters gestational age, BPD, and death according to each birth year, but did not find significant differences in our cohort (online suppl. table S1; see www.karger.com/doi/10.1159/000446908).

Discussion

In the present study we have assessed the impact of polymorphisms in the *GR* gene on neonatal outcome parameters in a cohort of 10,490 VLBW infants, which is the largest pediatric study on this topic so far. Although some previous studies found significant relations between *GR* gene polymorphisms and several aspects of neonatal outcome in preterm infants such as RDS, BPD [9, 10], and birth weight [7], the only significant association observed in our cohort was a slightly increased risk of developing BPD (OR 1.12–1.16 per allele) in carriers of the *BclI* variant. Further associations observed in the group comparisons for *BclI* (PVL, ROP, late-onset sepsis) and *N363S* (RDS/mechanical ventilation, blood pressure) did not withstand adjustment for cross-genotype effects (e.g. mechanical ventilation and *N363S* vs. BPD and *BclI*).

Global and conditional gene-targeted respiratory mouse models of either glucocorticoid deficiency or glucocorticoid receptor ablation have highlighted the crucial role of glucocorticoids and the glucocorticoid receptor for fetal lung development in utero [17]. Therefore, functional relevant polymorphisms in the *GR* gene might modify lung maturation and/or neonatal respiratory outcome parameters in preterm infants who are at risk for RDS. In a cohort of 62 preterm infants, Oretti et al. [8] did not detect associations between RDS and any of the three *GR* gene polymorphisms. More recently, RDS risk was found to be associated with the maternal *GR* genotype by Haas et al. [9], who analyzed the neonatal outcome of 117 infants delivered by 109 mothers. The authors observed a significantly lower RDS rate in infants of mothers carrying the *BclI* variant than in those born to wild-type mothers. Further analysis of this cohort suggested an association of *BclI* with BPD (OR 2.56, 95% CI: 1.11–5.95, $p = 0.02$), although multivariable analysis did not confirm this association in a subsequent publication of this group [10]. The cohort appeared too small to exclude potential confounding effects of compound heterozygosity or double mutants. Furthermore, it was not possible to estimate the effect of antenatal steroid administration because all mothers had received prenatal betamethasone treatment in this study cohort.

In our study, analysis of the whole cohort revealed slightly higher rates of mechanical ventilation in *N363S* carriers and BPD in infants carrying the *BclI* variant, respectively. The effect on BPD remained statistically significant after adjustment for gestational age, mechanical ventilation, and SGA status. It was of similar effect size in the subgroup of infants who received antenatal steroid treatment (n = 9,011, OR 1.15), whereas no significant genotype effect was seen in the subgroup of infants without antenatal steroid treatment (n = 1,982, OR 0.96, n.s.). Of note, infants who received antenatal steroid treatment had a slightly higher BPD rate compared to those without prenatally given steroids (15.9 vs. 14.8%). These two groups, however, are not comparable in terms that those infants without prenatal steroids were more likely to be born either spontaneously (17.9 vs. 8.9%, $p < 0.001$) or by emergency Caesarean section (20.2 vs. 6.2%, $p < 0.001$),

and had higher birth weight (1,113 vs. 1,054 g, $p < 0.001$) as well as gestational age (28.87 vs. 28.23 weeks, $p < 0.001$). We further corrected for potential confounding effects of compound heterozygosity or double mutant individuals (e.g. rate of mechanical ventilation in *N363S* carriers vs. BPD risk in *BclI* carriers) and recalculated all analyses with only those infants being either *BclI* pure carriers (i.e. wild-type at positions p.23 and p.363) or pure noncarriers (wild-type at all three polymorphic positions). This step, however, did not further strengthen the association between *BclI* and BPD.

Considering the increased receptor sensitivity of the *BclI* variant, as reported from previous association studies [18], one may speculate a priori that the BPD rate should be lower in carriers of the *BclI* variant and not vice versa as observed in our cohort. However, our results do not allow any speculation of whether differences in the BPD rate may result from differing levels of circulating endogenous glucocorticoid levels during pregnancy or after birth, or are indicative of a modified glucocorticoid receptor response to exogenous steroids. The fact that we did not see any relation of the *BclI* genotype to preceding mechanical ventilation in our study sample and, furthermore, no clear genotype-related differences in circulating glucocorticoid levels can be detected at least in term neonates during newborn screening [11] favors the assumption of a tissue-specific effect linked to a modification in the *GR* action. Of note, although a greater response in a dexamethasone suppression test is generally indicative of *GR* hypersensitivity, converse tissue-specific effects regarding the *GR* response to synthetic corticoids have been discussed previously [4, 19].

We are aware that we have analyzed only three selected *GR* gene polymorphisms. However, considering the heterogenous conclusions drawn from mostly small association studies published on this topic so far, we favored analyzing a few *GR* variants in a large multicenter cohort. *N363S* and *R23K* were selected because of their relevant functional effects shown in in vitro studies [20, 21]. For the more frequent *BclI* variant, which has been linked to increased *GR* sensitivity in several association studies, no in vitro transfection studies are available because this variant is not located within the coding region of the *GR* gene. Due to the vicinity to a known splicing site, linkage to other functional polymorphisms has been speculated. Indeed, in the study of Rautanen et al. [22] who investigated the relationship of six *GR* haplotypes to basal cortisol secretion, the *BclI* variant was part of two haplotypes, but only one of these was associated with higher basal cortisol levels. Interestingly, a recent case-control study on 675 preterm infants born with a gestational age less than 31 weeks did not find any association of 23 SNPs in the *GR* gene with BPD [23]; *BclI*, *N363S,* and *R23K*, however, were not analyzed in this study.

Given the almost ubiquitous *GR* expression and the broad spectrum of clinical challenges in neonatal medicine, other organ systems might also be affected by genetic variation of the *GR* gene. Both endogenous and exogenous glucocorticoids are well known to raise blood pressure. In our cohort, we detected an association between *N363S* and blood pressure; however, it disappeared in the pure carrier analysis after excluding any compound heterozygotes and carriers of *BclI* and *R23K*. This is in line with findings of other pediatric and adult studies, which did not detect an association of *N363S* and blood pressure later in life [6, 24, 25].

Referring to the concept of early developmental origins of adult disease as initially proposed by Barker et al. [26], low birth weight itself has been associated with the predisposition to metabolic-related disorders in numerous clinical studies [27].

In line with findings from a study comprising more than 2,400 term and late preterm infants [28], we did not detect any association between *GR* polymorphisms and either birth weight or being born SGA in our cohort. Interestingly, Finken et al. [5] reported carriers of the *GR R23K* variant to be protected from postnatal growth failure after preterm birth. Considering the assumed close relationship between the individual pattern of postnatal catch-up growth and metabolic sequelae of low birth weight, this finding certainly warrants further investigation.

Our study has some limitations. First, the study participants were enrolled over a relatively long period of time, namely 10 years, and assistance to premature infants, survival, and outcome of VLBW infants has changed considerably in the last two decades. Although there were no significant differences with respect to the core parameters gestational age, BPD, and death in our cohort, other variables may have been affected by medical and technical progress and could thus have masked further associations between *GR* variants and neonatal outcome parameters. Second, some of the analyzed neonatal outcome parameters are susceptive to inaccuracy arising from center-specific treatment algorithms in our cohort which comprises infants from a large number of neonatal units (e.g. initial ventilation support, indication and timing of surgery for ROP, PDA, and NEC). Third, the statistical power of our large sample of over 10,000 neonates is still debatable, especially in view of a variety of tested neonatal

outcome parameters with low prevalence in the total VLBW cohort and comparatively low genotype frequencies for polymorphisms *N363S* and *R23K*. Even for the observed association between BPD and the frequent *BclI* variant (1,584 BPD cases, OR 0.12–0.15), a power calculation yielded BPD case numbers between 1,423 and 1,805 (dominant 1 df/allelic 1 df test) necessary to reach 80% power ($p = 0.05$).

In summary, except for a slightly higher risk of BPD (OR 1.12–1.16 per allele) in carriers of the *GR BclI* variant, *GR* gene polymorphisms *BclI*, *N363S*, and *R23K* did not affect early neonatal outcome parameters in our large multicenter cohort of >10,000 VLBW preterm infants. In the near future, increasingly available genome-wide association studies together with large-scaled multicenter approaches will help to clarify whether and to what extent single genomic variants (alone or enriched in polygenetic clusters connecting biological pathways [29]) can predict individual risks during the challenging clinical course after preterm birth.

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Disclosure Statement

The authors have nothing to disclose.

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