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RESEARCH LETTER

No association between glucocorticoid receptor polymorphisms and long-term respiratory outcome after very preterm birth

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In preterm infants in their first weeks of life, the hypothalamic-pituitary-adrenal (HPA) axis is unable to produce sufficient amounts of cortisol for the degree of illness [1, 2]. The causes for this phenomenon are prolonged central suppression, immature adrenocortical enzymes and a cortisol-cortisone shuttle that favors cortisone [1, 3]. Cortisol is known for its anti-inflammatory effect. It has been demonstrated among preterm infants that low-cortisol levels may predispose to disrupted lung development, manifested as bronchopulmonary dysplasia (BPD) in approximately 20% of all infants born <32 weeks of gestation [4, 5].

A factor that might contribute to lung development after preterm birth is sensitivity to glucocorticoids. In adults, single-nucleotide polymorphisms (SNPs) in the glucocorticoid receptor (GR) gene have previously been associated with alterations in glucocorticoid sensitivity [6, 7]. The R23K polymorphism has been associated with decreased sensitivity to glucocorticoids [8], while the N363S polymorphism has been associated with increased glucocorticoid sensitivity [9].

We hypothesized that GR polymorphisms, might influence respiratory morbidity after preterm birth. Previous studies among preterm infants showed that these GR polymorphisms were not associated with the risk of developing BPD [10, 11]. However, the consequences of GR polymorphisms on long-term respiratory outcome after very preterm birth remain to be explored. Therefore, we investigated associations between the GR polymorphisms R23K and N363S and long-term respiratory outcomes in a cohort of subjects born very preterm birth who were followed up until young adulthood.

Materials and methods

Study design and participants

For this study, data from the Project On Preterm and Small-for-gestational-age infants (POPS) study were used. The POPS study is a birth cohort study that had included 94% ($N = 1338$) of all liveborn very preterm (i.e., gestational age <32 weeks) and/or very-low-birth-weight (i.e., <1500 grams) infants throughout the Netherlands in 1983. Various perinatal and neonatal characteristics were collected from birth onwards, including the presence of BPD. BPD was defined as clinical signs of respiratory distress in conjunction with abnormal findings on a chest X-ray and supplemental oxygen at 28 days of age [5]. At the age of 19 years, all alive subjects were approached to participate in a follow-up study (POPS-19 study), in which, among other data, GR polymorphisms were collected. The study was approved by the medical ethics committee of all participating centers. All subjects gave written informed consent for participation in this study [8].

Data collection

Respiratory outcomes were determined by self-report using the European Community Respiratory Health Survey (ECRHS) questionnaire [12]. The ECRHS questionnaire

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Table 1 Respiratory outcomes at age 19 and neonatal respiratory outcomes by genotype

	Genotype			OR + 95% CI		*P* value	
	R23K *N* = 21	N363S *N* = 15	Non-carriers *N* = 258	R23K vs non-carriers	N363S vs non-carriers	R23K vs non-carriers	N363S vs non-carriers
Respiratory outcomes at age 19							
Eczema—*n* (%)	5 (23.8%)	1 (6.7%)	24 (9.3%)	3.05 (1.03, 9.05)	0.70 (0.09, 5.53)	0.045	0.73
Hay fever—*n* (%)	1 (4.8%)	3 (20.0%)	18 (7.0%)	0.67 (0.09, 5.26)	3.33 (0.86,12.89)	0.70	0.08
Asthma—*n* (%)	1 (4.8%)	1 (6.7%)	19 (7.4%)	0.68 (0.09, 5.36)	1.02 (0.13, 8.25)	0.71	0.99
Asthma attack last 12 months—*n* (%)	1 (4.8%)	0 (0.0%)	5 (1.9%)	NA	NA	1.00	1.00
Chest wheezing last 12 months—*n* (%)	8 (38.1%)	3 (20.0%)	56 (21.7%)	2.19 (0.86, 5.54)	0.89 (0.24, 3.29)	0.10	0.86
SOB during exercise —*n* (%)	4 (19.0%)	0 (0.0%)	34 (13.2%)	1.28 (0.40, 4.06)	NA	0.68	1.00
SOB while walking with people same age—*n* (%)	1 (4.8%)	0 (0.0%)	12 (4.7%)	0.86 (0.11, 6.98)	NA	0.89	1.00
Need to stop for breath when walking at own pace on level ground—*n* (%)	0 (0.0%)	0 (0.0%)	8 (3.1%)	NA	NA	1.00	1.00
Neonatal respiratory outcomes							
BPD—*n* (%)	1 (4.8%)	2 (13.3%)	20 (7.8%)	0.60 (0.08, 4.67)	1.83 (0.39, 8.69)	0.62	0.45
CPAP or IPPV, days—median [IQR]	3 [0–11]	1 [0, 6]	2 [0, 8]	1.03 (0.98, 1.07)	1.02 (0.97, 1.07)	0.23	0.40

SOB shortness of breath, *BPD* bronchopulmonary dysplasia, *CPAP* continuous positive airway pressure, *IPPV* Intermittent positive pressure ventilation, *N* number, *IQR* interquartile range

was developed from pre-existing questionnaires that had already been used in multinational studies [12–14], and contains questions about the presence of respiratory problems or symptoms. Symptoms on the ECRHS questionnaire showed correlation with objective measures of lung function [15]. This questionnaire was sent to all subjects known to be alive. At the same time, subjects were seen for blood sampling at one of the participating centers [8].

Laboratory analysis

GR SNPs were determined by performing polymerase chain reactions using 2.5 ng of genomic DNA and standard reagents. Subsequently, they were genotyped by mass spectrometry using standard conditions [8]. The genotype distribution for both the R23K and the N363S polymorphism was in agreement with the distribution predicted by the Hardy–Weinberg equilibrium ($P = 0.54$ for the R23K polymorphism, and $P = 0.66$ for the N363S polymorphism), and are mutual exclusives.

Statistical analysis

Statistical analyses were performed with SPSS Statistics version 26. Patient characteristics were compared between R23K carriers and non-carriers, and between N363S carriers and non-carriers, using the Chi square-test for dichotomous data and the independent samples *t*-test or their nonparametric equivalents for continuous data. Outcomes were compared between R23K carriers and non-carriers, and between N363S carriers and non-carriers, using logistic regression analysis.

Results

Of the 1012 very preterm born infants 676 (67%) were still alive at the age of 19. Of these, 498 (74%) consented to participate in the POPS-19 study. In 318 (64%) of them genomic data and ECHRS symptoms were known. After the exclusion of subjects with any congenital malformation, 294 were available for analysis, including 21 (7%) R23K carriers, 15 (5%) N363S carriers and 258 (88%) non-carriers.

Perinatal characteristics did not differ between the groups (data not shown).

Respiratory outcomes at age 19 are shown by GR genotype in Table 1. No differences in scores of asthma, chest wheezing or shortness of breath were found between R23K carriers and non-carriers, or between N363S carriers and non-carriers. Eczema was positively associated with R23K carriage (OR 3.05, 95% CI 1.03, 9.05, *p* value 0.045).

Furthermore, no differences in the duration of respiratory support with continuous positive airway pressure (CPAP), intermittent positive pressure ventilation (IPPV) or the numbers developing BPD were seen between R23K carriers and non-carriers and N363S carriers and non-carriers.

Discussion

In this study we found no association between the GR polymorphisms R23K and N363S and respiratory outcomes 19 years after very preterm birth.

Respiratory outcome after preterm birth is determined by a complex interplay of lung immaturity, need for and duration of assisted ventilation, oxygen supplies, and recurrent or enduring infections [16], in addition to other factors that are not specifically related to prematurity, such as socioeconomic status, (maternal) smoking, BMI, and physical activity [17]. Furthermore, our sample was born in the pre-surfactant era [18]. Increasing degrees of prematurity and BPD were associated with more airflow impairment later in life [17], possibly due to the presence of smaller airways [19, 20]. Although GR SNPs might influence some of these processes, due to the multitude of factors that determine respiratory outcome after preterm birth their overall contribution is probably negligible.

We found that carriers of the R23K polymorphism had a higher odds of eczema. Although this association might reflect a biologically plausible mechanism, it could also be a chance finding. However, contrary to expectation, we found that carriers of the N363S polymorphism had a higher odds of hay fever. Clearly, our results should be balanced against the small numbers of variant allele carriers.

We found GR polymorphisms to be unrelated to the odds of BPD, consistent with findings by Schreiner et al. [10]. The lack of association can be explained by the complex multifactorial pathophysiology of BPD or by selective survival of certain genotypes [21]. However, the genotype frequencies in this study did not differ from the genotype frequencies in the Dutch norm population, which argues against a major survivor effect [8].

Our study has several strengths, including a large sample size and long-term follow-up. Our study also has its limitations. Even though this study had a relatively large sample size, the number of variant allele carriers ($n = 36$) was small. Nonetheless, the genotype distribution for both polymorphisms was in agreement with the distribution predicted by the Hardy–Weinberg equilibrium. Second, the POPS-19 study was not designed specifically for our research question. GR SNPs were determined at age 19. In the early 1980s, many very preterm infants did not survive, which inevitably may give rise to selection bias. In addition, participants were not representative for the current generation of very preterm infants, which are more immature. Insufficient cortisol production and BPD are more frequent at lower gestational ages [2]. Third, patient-reported respiratory data are not as objective as data derived from spirometry. Since respiratory problems may develop many years after preterm birth, serial follow-up of spirometry would allow to determine the course of pulmonary development after very preterm birth. Fourth, our study lacked an assessment of adrenocortical output and inflammatory markers during the neonatal course.

In summary, our study did not show associations between the GR polymorphisms R23K and N363S and long-term respiratory outcome 19 years after very preterm birth. Future studies on this topic should include serial measurements of adrenocortical output along with inflammatory markers during the neonatal course, and should include an objective assessment of lung function.

Authors' contributions E.M.B., M.R., S.M.P., E.J.L.E.V., J.R., M.J.J.F: made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; drafted the work or revised it critically for important intellectual content; approved the version to be published; agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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