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PHARMACOGENETICS

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Abstract
Purpose The aim of this pilot study was to assess the association between polymorphisms in genes that encode for proteins involved in the pharmacokinetics/pharmacodynamics of glucocorticoids and the occurrence of respiratory distress syndrome (RDS) in preterm infants born to mothers treated with a complete course of betamethasone.
Methods Sixty-two preterm infants were enrolled. The C1236T, G2677T, and C3435T polymorphisms in the *ABCB1* gene, *Bcl*I, N363S and ER22/23EK in the *NR3C1* gene, I105V in the *GST-P1* gene and *GST-M1* and *GST-T1* deletions were analyzed, and their association with the occurrence of RDS was assessed.
Results In univariate analysis, the heterozygous and homozygous presence of the I105V variant in the *GST-P1* gene seemed to confer protection against the occurrence of RDS (P=0.032), while no association for all other polymorphisms was observed. In multivariate analysis, selection from the reference model of independent variables based on AIC (Akaike information criteria) maintained three variables in the model: gestation, C3435T, and *GST-P1* genotype.
Conclusions Polymorphisms of the *GST-P1* gene may influence the effect of antenatal steroids on the newborn lung.

Keywords Glucocorticoids · Respiratory distress syndrome · ATP binding cassette, subfamily B, member 1 (*ABCB1*) · Nuclear receptor subfamily 3, group C, member 1 (*NR3C1*) · Glutathione-S-transferase (*GST*)

Introduction

In spite of considerable progress in fetal/maternal surveillance, the overall frequency of preterm birth remains high and actually seems to be increasing [1]. Preterm labor is responsible for 70–85% of fetal, neonatal, and infant deaths, due to the complications of prematurity. Respiratory distress syndrome (RDS) is the most frequent form of respiratory failure in preterm infants, and the main cause of neonatal death and disability [2]. This complication occurs in about 50% of infants with birth weight between 501 and 1,500 g [3]. The incidence is inversely proportional to gestational age and birth weight. Both incidence and severity of RDS have markedly decreased since the introduction of antenatal steroids. RDS is the clinical expression of a deficiency in pulmonary surfactant, which prevents the collapse of alveoli [4]. In the absence of surfactant, small air spaces collapse, and each expiration results in progressive atelectasis.

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Glucocorticoids (GC) promote the structural maturation of lungs by thinning pulmonary mesenchyma and inducing surfactant axis maturation through increased biosynthesis of phosphatidylcholine [5]. Antenatal GC treatment is associated with improved survival rates and decreased incidence of RDS and intraventricular hemorrhage [2]. However, a significant number of premature infants do not respond to this treatment [6]. Thus, the identification of factors associated with the response to GCs in preterm infants is of considerable importance.

Fetal GC exposure is determined largely by the placental barrier, which serves as a metabolic gatekeeper to limit access of drugs and xenobiotics to the placenta and the fetus [7]. The mechanisms of placental transport involve passive or facilitated diffusion, active transport, and pynocitosis [8]. In addition, the concentration of drugs in the fetal blood is also modulated by the action of metabolic placental enzymes, whose expression varies considerably during gestation [9].

An important component of the placental barrier is represented by active transporters, including P-glycoprotein (P-gp). P-gp is encoded by the *ABCB1* (ATP binding cassette, subfamily B, member 1) gene [10, 11], which, using the energy derived by ATP hydrolysis, allows the extrusion of substances from cells. This transporter was originally found to be associated with multidrug resistance in cancer cells and is known for its role in modulation of drug absorption, distribution, and metabolism [12]. GCs are good substrates for P-gp [13], and the protein may limit the access of these hormones to the placenta [7] and the fetus [11]. The *ABCB1* gene is highly polymorphic, and three frequently occurring single nucleotide polymorphisms (SNPs), *C1236T* in exon 12, *G2677T* in exon 21, and *C3435T* in exon 26, have been related to different protein expression levels and function [14, 15] and could therefore alter the transplacental passage of GCs.

Placental P-gp is also likely to restrict the access of GCs to their receptor (GR). Upon binding to GCs, the GR translocates into the nucleus and transcriptionally regulates various genes through its binding to GC response elements (GRE) in the promoter regions of target genes. Several functional polymorphisms of the GR gene (*NR3C1*, nuclear receptor subfamily 3, group C, member 1, glucocorticoid receptor) have been described; the N363S polymorphism in codon 363 and the *Bcl*I restriction site in intron 2 have been associated with hypersensitivity to GCs [16, 17], whereas the ER22/23EK polymorphism, also located in exon 2, seems implicated in GC resistance [18].

Glutathione-S-tranferase (GST) is an important metabolic pathway for the detoxification of a variety of hydrophobic and electrophilic compounds [19]. These enzymes have previously been shown to bind to GCs and to play a role in the metabolism and response to these hormones [20–23]. Phenotypically relevant genetic polymorphisms for some GST subclasses (*GST-M1*, *GST-T1*, and *GST-P1*) have been related to the outcome of several diseases, including acute lymphoblastic leukemia and solid tumors [24], and to response to GCs [25]. In addition, of particular interest, a polymorphism at codon 105 in the *GST-P1* gene has been recently associated with the occurrence of bronchopulmonary displasia in preterm infants [26].

As GCs decrease the rate of RDS by approximately 50% and fail to do so in the remaining 50% [2, 3], the aim of this pilot study was to assess the association between lack of response to these hormones and polymorphisms in the genes that encode for transporters, drug receptors, and metabolizing enzymes involved in the pharmacodynamics and pharmacokinetics of these drugs. We tested the hypothesis that sequence variants in these genes may contribute to the variation in GC response.

Methods

Subjects

From February 1, 2005, to August 30, 2006, blood samples were obtained for genotype analysis from 62 newborn Caucasian infants, born prior to 34 weeks of gestation at Burlo Garofolo Children's Hospital, Trieste, Italy, and Clinica Mangiagalli, Milan, Italy. All mothers received a complete course of antenatal betamethasone, i.e., two 12-mg doses given intramuscularly 24 h apart [27].

The study protocol was approved by the local ethics committee, and informed consent was obtained. Investigations were conducted according to the principles expressed in the Declaration of Helsinki. Clinical data on infants and their mothers were collected prospectively. The diagnosis of RDS was based on clinical and radiographic criteria: tachypnea, chest retractions, cyanosis in room air that persists or progresses over the first 48–96 h of life, and a typical chest X-ray (uniform reticulogranular pattern with air bronchograms). Infants were divided into two groups according to the occurrence of RDS: group 1: RDS (n=22), group 2: no RDS (n=40).

Genetic analysis

Genomic DNA was obtained from cord blood by a standard phenol/chloroform extraction procedure, and the polymorphisms were determined by sequence-specific primers and polymerase chain reaction-restriction fragment length polymorphism (PCR RFLP) assays or PCR allele specific oligonucleotide (ASO) assays.

For *ABCB1* gene, the PCR consisted of an initial denaturation step at 94°C for 3 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 1 cycle of 72°C for 7 min [28, 29]. To evaluate all *NR3C1* polymorphisms, DNA was amplified by a single PCR program, consisting on 95°C for 7 min, 40 cycles of 94°C for 1 min, 56°C for 1 min, 72°C for 1 min, and 1 cycle of 72°C for 7 min [30, 31]. For *GST-P1*, the PCR consisted of an initial denaturation step at 94°C for 5 min, 35 cycles of 94°C for 30 s, 60°C for 30 s, 72°C for 30 s, and 1 cycle of 72°C for 7 min [32]. DNA fragments, generated after digestion with restriction enzymes, were separated on agarose gel; primers, restriction enzymes, and conditions are shown in Table 1.

For *GST-M1* and *GST-T1* polymorphisms, PCR ASO assay consisted of an initial denaturation step at 94°C for 12 min, 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, and 1 cycle of 72°C for 7 min. This assay places individuals in two categories for *GST-T1* or *GST-M1*, one being homozygous or heterozygous for T or M and the other having a homozygous deletion [33]. Primers for this assay are shown in Table 1.

Data analysis

We used a sample of convenience, as we enrolled infants born on weekdays (Monday to Friday) during usual working hours (9:00 a.m. to 5:00 pm). Patients' characteristics in the two groups (RDS and no RDS) were compared using Mann-Whitney test. Any possible association between RDS occurrence and polymorphisms in each gene was investigated calculating odds ratio (OR) and 95% confidence intervals (CI) from contingency tables and using two-sided Fisher's exact test. To control for confounding variables, multivariate logistic regression was performed with a model considering RDS as the dependent variable and gender, birth weight, gestation, Apgar scores at 1 and 5 min, and genotypes as the independent variables. The model was reduced using a backward elimination approach; the Akaike information criteria (AIC) was used to select the covariates to build the most balanced regression model. To validate the AIC stepwise selection, the reduced model obtained by AIC was compared using an ANOVA test to the original model with all covariates and to a model with no covariate. Values below 0.05 were considered statistically significant. Statistical analysis was performed using the software R (version 2.6.1).

Results

Sixty-two newborns born to mothers given a complete course of betamethasone in the two hospitals of Trieste and Milan represented the study population. All infants were Caucasian. Clinical and demographic data are shown in

Table 1 Primers used for amplification of PCR fragments and restriction enzymes and conditions

Gene	SNP	Primers	Restriction	
			Enzyme	Conditions
ABCB1	C3435T [28]	5′-TGTTTTCAGCTGCTTGATGG-3′ 5′-AAGGCATGTATGTTGGCCTC-3′	*Dpn*II	37°C 90 min 2% gel
	G2677T [28]	5′-TGCAGGCTATAGGTTCCAGG-3′ 5′-TTTAGTTTGACTCACCTTCCCG-3′	*Ban*I	37°C 90 min 3% gel
	C1236T [29]	5′-TATCCTGTGTCTGTGAATTGCC -3′ 5′-CCTGACTCACCACACCAATG -3′	*Hae*III	37°C 90 min 2% gel
NR3C1	N363S [31]	5′-AGTACCTCTGGAGGACAGAT-3′ 5′-GTCCATTCTTAAGAAACAGG-3′	*Tsp509*I	65°C 90 min 3% gel
	*Bcl*I [30]	5′-TGCTGCCTTATTTGTAAATTCGT-3′ 5′-AAGCTTAACAATTTTGGCCATC-3′	*Bcl*I	50°C 90 min 2% gel
	ER22/23EK [31]	5′-GATTCGGAGTTAACTAAAAG-3′ 5′-ATCCCAGGTCATTTCCCATC-3′	*Mnl*I	37°C 90 min 3% gel
GST	GST-P1 [32]	5′-ACCCCAGGGCTCTATGGGAA-3′ 5′-AGGGGCTTCTTGTGCCCTCA-3′	*Bsma*I	55°C 90 min 2% gel
	GST-M1 [33]	5′-GAACTCCCTGAAAAGCTAAAGC -3′ 5′-GTTGGGCTCAAATATACGGTGC -3′	___	
	GST-T1 [33]	5′-TTCCTTACTGGTCCTCACATCTC -3′ 5′-TCACCGGATCATGGCCAGCA -3′		2% gel
	β-globin	5′-GAAGAGCCAAGGACAGGT -3′ 5′-CAACTTCATCCACGTTCACC-3′		

Tables 2 and 3. Twenty-two newborns developed RDS; 40 did not. Gestational age was lower in the RDS group. Apgar scores at 1 min ($P<0.01$) and 5 min ($P<0.001$) were also significantly lower in the RDS group. There were no differences in gender, mode of delivery, and parity. Additionally, occurrence of RDS was not associated with differences in rates of chorioamnionitis, premature rupture of membranes (PROM), or in the interval between PROM and birth. As expected, there were significant differences in postnatal features, such as use of surfactant and need for mechanical ventilation, continuous positive airway pressure, and supplemental oxygen. In the RDS group, five infants developed bronchopulmonary dysplasia, and three had intraventricular hemorrhage. In the no-RDS group, one infant had stage one necrotizing enterocolitis.

With regard to the polymorphisms studied, there were no significant deviations from Hardy-Weinberg equilibrium in any group (data not shown). The genotype frequencies in the two groups of premature infants are listed in Table 4. In univariate analysis, a significant difference ($P=0.032$) was observed for the I105V polymorphism in the *GST-P1* gene between newborns who developed RDS and those who did not. There were no other significant differences in any other polymorphism studied. The genotypes of premature children were also compared with those of 100 consecutive healthy blood donors from the same geographic area, but no significant difference was observed (data not shown). The results of the logistic regression analysis are reported in Table 5. As there was an obvious correlation between RDS and week of gestation, differences were corrected for gestational age. After correction, the difference in *GST-P1* was no longer significant (adjusted OR=6.47, 95% CI=0.87–48.10, $P=0.068$). However, selection from the reference model of independent variables based on AIC scores maintained three variables in the model: gestation, C3435T, and *GST-P1* genotypes (Table 6). The reduced model obtained by AIC was compared using ANOVA test to the original model with all covariates and to a model with no covariate. Results obtained showed no significant difference ($P=0.80$) between the reduced and the complete model, confirming that the simplified model performed as well as the complete one in predicting the dependent variable (i.e., incidence of SDR), while a significant difference with the model with no covariate was found ($P=1.73\times10^{-6}$), confirming that the simplified model was significantly more informative that the simplest possible model.

Discussion

RDS is a common complication of prematurity and represents a significant cause of early neonatal death and disability [2]. Pulmonary surfactant deficiency appears to be a major factor in its pathophysiology [4]. GCs, administered to mothers before birth, accelerate the appearance of pulmonary surfactant in fetal lungs [5], resulting in a significant reduction in both RDS and neonatal deaths [34]. Betamethasone is the preferred steroid because of its ability to cross the placenta, its weak immunosuppressive and mineralocorticoid activities, and its long duration of action. In addition, although data may not be conclusive, this steroid is more consistently associated with reduction

Table 2 Demographic and perinatal data of neonates with or without respiratory distress syndrome (RDS)

Neonatal data	No RDS (n=40)	RDS (n=22)
Gender		
Female (n)	19 (47.5%)	10 (45.5%)
Male (n)	21 (52.5%)	12 (54.5%)
Birth weight (g, mean ± SD)	1,552±313	1,147±451***
Gestation (weeks, mean ± SD)	31.9±1.8	28.8±2.8***
Mode of delivery		
Vaginal (n)	2 (5%)	1 (4.55%)
Cesarean (n)	38 (95%)	21 (95.45%)
Parity		
Singletons (n)	21 (52.5%)	9 (40.9%)
Twins (n)	14 (35.0%)	12 (54.5%)
Triplets (n)	5 (12.5%)	1 (4.5%)
Premature rupture of membranes (n)	13 (32.5%)	5 (22.7%)
Chorioamnionitis (n)	1 (2.5%)	1 (4.5%)
Apgar (median and range)		
1 min	7 (3–9)	6 (4–8)**
5 min	9 (7–10)	8 (5–9)***

**$P<0.01$, ***$P<0.001$, Mann-Whitney test

Table 3 Postnatal clinical data of neonates with or without respiratory distress syndrome (RDS)

Postnatal data	No RDS (n=40)	RDS (n=22)
Surfactant use (n)	0 (0%)	20 (90.9%)*
Mechanical ventilation (n)	4 (10.0%)	21 (95.5%)***
Continuous positive airway pressure (n)	27 (67.5%)	20 (90.9%)*
Supplemental oxygen (n)	20 (50.0%)	19 (86.4%)**
Bronchopulmonary dysplasia (n)	–	5 (22.7%)**
Necrotizing enterocolitis (n)	1 (2.5%)[a]	–
Intraventricular hemorrhage (all grades) (n)	–	3 (13.6%)*

*P<0.05, **P<0.01, ***P<0.001, Mann-Whitney test
[a] Stage 1 necrotizing enterocolitis

in neonatal death and in periventricular leukomalacia [34–36]. However, a significant number of infants do not respond to this treatment and develop RDS. In agreement with previous research [2], in our study, 22 out of 62 preterm infants showed this complication. Univariate analysis revealed an association between the occurrence of RDS and body weight, gestational age, and Apgar scores. As in previous studies, only gestational age appeared to have an independent association with RDS in multivariate analysis [37, 38].

GCs are small hydrophobic molecules that bind to a receptor that is present in the cytoplasm as a multimeric complex with several chaperon proteins. Upon binding to the steroid, the receptor dissociates from the proteins, translocates to the nucleus, dimerizes, and binds to specific DNA sequences located in the promoter region of GC

Table 4 *ABCB1*, *NR3C1*, *GST-M1*, *GST-T1*, and *GST-P1* genotypes in premature infants and occurrence of respiratory distress syndrome (RDS)

Gene and polymorphism	No RDS (n=40)	RDS (n=22)	P value	OR (95% CI)
ABCB1				
C1236T				
WT	10 (25.0%)	4 (18.1%)	0.752	0.667 (0.182–2.443)
HET + MUT	30 (75.0%)	18 (81.8%)		
G2677T				
WT	10 (25.0%)	5 (22.7%)	1.000	0.882 (0.258–3.012)
HET + MUT	30 (75.0%)	17 (77.3%)		
C3435T				
WT	9 (22.5%)	6 (27.3%)	0.760	1.292 (0.390–4.274)
HET + MUT	31 (77.5%)	16 (72.7%)		
NR3C1				
BCLI				
WT	22 (55.0%)	10 (45.5%)	0.597	0.682 (0.240–1.939)
HET + MUT	18 (45.0%)	12 (54.5%)		
ER/EK				
WT	40 (100%)	21 (95.5%)	0.355	0.177 (0.007–4.536)
HET + MUT	0 (0%)	1 (4.5%)		
N363S				
WT	39 (97.5%)	21 (95.5%)	1.000	0.538 (0.032–9.059)
HET + MUT	1 (2.5%)	1 (4.5%)		
GST-M1				
Present	21 (52.5%)	9 (40.9%)	0.434	0.626 (0.219–1.795)
Deletion	19 (47.5%)	13 (59.1%)		
GST-T1				
Present	35 (87.5%)	20 (90.9%)	1.000	1.429 (0.253–8.057)
Deletion	5 (12.5%)	2 (9.1%)		
GST-P1				
WT	19 (47.5%)	17 (77.3%)	0.0319	3.758 (1.161–12.17)
HET + MUT	21 (52.5%)	5 (22.7%)		

WT Wild type, *HET* heterozygous, *MUT* mutated

Table 5 Odds ratios with 95% confidence intervals and *P* values for the independent variables in the logistic regression model for the occurrence of respiratory distress syndrome (RDS)

Variable	*P* value	OR (95% CI)
Gender (M vs F)	0.50	1.77 (0.34–9.26)
APGAR 1 min	0.81	0.91 (0.43–1.94)
APGAR 5 min	0.16	0.36 (0.089–1.47)
Birth weight	0.18	1.00 (1.00–1.01)
Gestational age	0.013	0.37 (0.17–0.81)
C3435T (WT vs non WT)	0.091	19.79 (0.62–632.11)
G2677T (WT vs non WT)	0.70	0.44 (0.007–29.05)
C1236T (WT vs non WT)	0.74	1.85 (0.049–69.63)
Bcl (WT vs non WT)	0.41	2.12 (0.36–12.66)
GST T1 (normal vs deletion)	0.75	0.67 (0.067–7.55)
GST M1 (normal vs deletion)	0.23	0.33 (0.05–2.03)
GST P1 (WT vs non WT)	0.068	6.47 (0.87–48.10)

WT Wild type

responsive genes, triggering their transactivation or repression. There is increasing evidence that the entrance of steroids inside the cells, and hence their intracellular concentrations, can be modulated by the activity of multidrug transporters such as P-gp as well as by the activity of several metabolic enzymes. Theoretically, an altered expression of proteins involved in the pharmacodynamics (NR3C1) or pharmacokinetics (ABCB1, GST) of steroids could play a role in the reduced efficacy of GCs in preventing RDS. As polymorphisms in these proteins have been identified, we hypothesized that sequence variants in these genes may contribute to the variation in GC response.

In this study we evaluated the presence of common SNPs in the GC receptor gene (*NR3C1*), the *Bcl*I and N363S polymorphisms that have been related to increased corticosensitivity, and a linked polymorphism in codons 22 and 23 in exon 2 that seems, on the contrary, to be related to a relative GC resistance. The common *Bcl*I SNP is a G/C transversion in intron 2, 647 bp downstream from the exon2/intron2 junction. In patients mutated for this polymorphism, changes in metabolism, including hyperinsulinemia, higher abdominal fat, higher body mass index, higher leptin levels, and larger increases in body weight following experimentally induced overfeeding, have been described [17, 39]. The N363S SNP is an AAT to AGT transition in codon 363 in exon 2 and has been related to metabolic changes, such as higher BMI, higher waist-to-hip ratio, and a higher insulin response following 0.25 mg dexamethasone [39, 40]. The ER22/23EK polymorphism has been associated with a favorable metabolic profile, lower fasting insulin, and lower LDL cholesterol concentrations [18]. In our study population, we did not find any difference in the genotype distribution for all studied *NR3C1* polymorphisms in two groups of preterm infants.

Table 6 Multivariate analysis: reduction in the number of independent variables through stepwise selection based on AIC (Akaike information criteria) index. Odds ratios with confidence intervals and *P* values for the independent variables in the logistic regression model for the occurrence of RDS

Variable	*P* value	OR (95% CI)
Gestational age	0.00019	0.52 (0.37–0.74)
C3435T (WT vs non WT)	0.10	4.21 (0.76–23.46)
GST-P1 (WT vs non WT)	0.10	4.59 (0.98–21.39)

WT Wild type

Another potential mechanism responsible for the low sensitivity to GCs could be decreased cellular GC concentrations, secondary to increased P-gp-mediated efflux from target cells, or increased activity in the placental tissue, due to overexpression of the *ABCB1* gene. P-gp has the capacity to bind and transport different steroids [13, 41, 42] and is highly expressed in the apical brush-border membrane of the syncytiotrophoblast at different stages of pregnancy [43–46]. This protein could therefore play a role in the protection of the fetus both against xenobiotics [10, 11] and steroids [7]. Previous studies have suggested that its overexpression could lead to clinical resistance to GCs in inflammatory bowel disease [47] and nephrotic syndrome [48]. A number of polymorphisms have been identified in the *ABCB1* gene. Among these, a G2677T/A missense polymorphism in exon 21 has been shown to be significantly related to a higher level of expression and function of P-gp in healthy individuals [15]. On the contrary, the C3435T SNP in exon 26 has been associated with a reduced expression and activity of the pump. Another *ABCB1* polymorphism is the C1236T synonymous SNP, located in exon 12, whose functional significance is not yet clear. In our study, multivariate analysis demonstrated a borderline significance for the C3435T polymorphism (Table 5), and selection of independent variables, based on AIC score, maintained this variable into the model. The mutated genotype has been correlated with lower P-gp expression and function [15], hence lower placental expression should allow GCs to better cross the placenta, reaching fetal circulation. As this is a synonymous SNP, it should not affect protein expression. However, it has been suggested that it might have an indirect effect on RNA stability [49]. Alternatively, the presence of a rare codon, marked by the synonymous SNP, could affect the timing of cotranslational folding and insertion of the protein in the membrane [49].

Several metabolic and detoxifying enzymes are also expressed in the placental trophoblast at different developmental stages [50] and further contribute to the physical and metabolic barrier between the maternal and fetal blood circulation. The GST family of enzymes, and in particular the GST-P1 isoform, is localized mainly in the cytotrophoblast at all stages of pregnancy, and its expression increases

with development [51]. These enzymes bind various ligands such as steroids [20–22] and may play a role in the clinical response to GCs in acute lymphoblastic leukemia [25, 52]. Phenotypically relevant genetic polymorphisms have been identified also in some human cytosolic GST isoenzymes, and in particular, in families M1, P1, and T1. Three different polymorphisms have been described at the GST-M1 locus, the most important of which is characterized by a partial deletion resulting in complete absence of enzyme activity [53]. One polymorphism has been described at the GST-T1 locus, encoding for a partial gene deletion that causes the loss of functional activity [53]. At least four different polymorphisms have been described at the GST-P1 locus, encoding for the 1a, 1b, 1c, and 1d forms. The 1c and 1d are extremely rare, while the 1b polymorphism is more common and is characterized by a single functional nucleotide substitution (A-G transition at base 1578), resulting in the amino-acid change I105V in the substrate binding site [54].

While no association was observed in our study between RDS occurrence and GST-M1 and T1 polymorphisms, an association between the GST-P1 codon 105 genotype was observed, and a decreased risk of developing RDS was evident in children with the heterozygous or mutated genotypes. GST-P1 is the main isoform in normal placenta and represents 36% of GST activity in this tissue [55]. This nonsynonymous SNP has been associated with reduced enzyme activity [56], hence the reduction in placental steroid biotransformation could permit higher GC concentrations to reach the fetus.

Our data are in accordance with those obtained by Manar et al. [26]. These authors have indeed demonstrated that the less efficient ile/ile isoform is more common in preterm infants who developed bronchopulmonary displasia than in controls; however, prenatal treatment with GCs was not considered by these authors. In childhood acute lymphoblastic leukemia, Stanulla et al. [57] demonstrated an association between central nervous system relapse and the presence of *GST-P1* and *ABCB1* polymorphisms, suggesting a role for these proteins in limiting drug transport also across the blood brain barrier.

This study has obvious limitations. First, this being a pilot study, we did not have data on which to calculate sample size. Underpowered studies carry the risk of yielding false negative results. Based on our preliminary data, 90 infants should have been enrolled to detect a significant difference in *GST-P1* genotype. Second, even with a significant difference, association by no means implies cause and effect. However, a plausible biological explanation for this association may exist. A reduced steroid metabolism in the placental tissue, mediated by the P1 isoform of GST, may enhance the effects of steroids on the fetal lung. Third, given their variability, studies on genetic polymorphisms—even when they give significant results—should be replicated prior to drawing any general conclusion. Only a limited number of polymorphisms potentially involved in GC pharmacokinetics/pharmacodynamics have been considered in this paper; the role of other SNPs in these or in different genes can not be excluded. In addition, the study has considered a Caucasian population, and hence further work is needed to evaluate the effect of these polymorphisms across different ethnicities.

In conclusion, genetic variations in *GST-P1* may influence the risk of developing RDS in preterm infants born after a complete course of antenatal corticosteroids. This pilot study was underpowered to detect a statistically significant difference, but may offer data on which further studies with larger sample sizes may be planned.

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