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Research Article

Polymorphism 129 C/T of Glutamate Cysteine Ligase Catalytic Subunit Gene Could be Associated with Bronchopulmonary Dysplasia Development: Prospective Cohort Pilot Study

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Abstract

Introduction: Recently, determining some single-nucleotide replacements in the antioxidant enzymes genes is one of the promising trends in the study of bronchopulmonary dysplasia (BPD). The goal of the research is to investigate the genetic polymorphism frequency analysis of glutamate cysteine ligase catalytic subunit (GCLC) enzyme at preterm infants from the BPD development high-risk group to clarify their contribution to the formation and course of the disease. Study design: prospective cohort pilot study.

Materials and Methods: 59 premature newborns with the high risk of BPD development were under observation. The patients to be examined underwent molecular-genetic studying of polymorphic variants of the GCLC gene. The identification of allele variables determined by the localized nucleotide replacements was performed with the allele-specific polymerase chain reaction. 2 cohorts were formed: children with "wild" type of the gene (control group, n = 36) and children who had GCLC mutations (main group, n = 23). All patients received treatment according to protocols for the management of the infant respiratory distress syndrome, regardless of cohort. The diagnosis of BPD was established according to the criteria of Jobe and Bancalary. Statistical processing was performed using the licensed software "Statistica 6.0".

Results: BPD was diagnosed in 21 children of the main group (21/23, 91.3%) and in 19 children of the control group (19/36, 52.7%). BPD developed more frequently in the main group (two-tailed Fisher's test, p = 0.004), the relative risk was 1.7 (95% confidence interval 1.2; 2.4). The predictive value of features promoting BPD development was significantly higher in preterm infants of the risk group who had heterozygous and homozygous carriership of GCLC gene minor alleles. The presence of the minor allele -129T of GCLC increased the relative chance of the disease developing. In a half (50.0%) of children to have developed BPD had heterozygous genotype 129 CT GCLC versus 10.5% in patients who did not develop the disease.

Conclusion: The genetic polymorphism of GCLC could be supposed to take pathogenetic part in the BPD development.

Keywords: *Bronchopulmonary Dysplasia; GCLC Gene; Genetic Polymorphism*

Abbreviations

129 C/T: Polymorphism of the GCLC gene, where Cytosine at position 129 is replaced by Thymine; BPD: Bronchopulmonary Dysplasia; CI: Confidence Interval; DNA: Deoxyribonucleic Acid; GCLC: Glutamate Cysteine Ligase Catalytic Subunit; OR: Odds Ratio; PCR: Polymerase Chain Reaction; ROS: Reactive Oxygen Species; RR: Relative Risk; Se: Sensitivity; Sp: Specificity

Introduction

Bronchopulmonary dysplasia (BPD) is considered to be a chronic obstructive pulmonary disease of young children to gain features of the medico-social problem because such patients have the much higher susceptibility to morbidity and mortality during all their life [1,2]. Introduction of the neonatal care modern methods, implementing surfactant, high-tech, and as much as possible sparing mechanical ventilation support have resulted in increasing preterm infants survival that in turn has affected on BPD occurrence.

The role of oxidative stress (OS) in BPD pathogenesis has been proved. The oxidative stress peculiarity at BPD is that the reactive oxygen species (ROS) result in significant damaging lung parenchyma at the expense of different enzymes activity malfunction, inactivation of protease inhibitors, suppression of DNA and surfactant synthesis, initiation of fibrogenesis due to airway morphofunctional immaturity and antioxidant protection insufficiency in premature infants [3]. Specialized systems of antioxidant protection such as superoxide dismutase catalyzing the dismutation of superoxide anion radical into hydrogen peroxide (H2O2), catalase decomposing H2O2, glutathione-dependent peroxidases and transferases removing organic peroxides have developed in cells for protection from ROS during the evolutionary process [4]. Researchers have demonstrated activity interruption of the principal enzymes destroying ROS at BPD [5,6]. The severity of pathological changes and the BPD development risk does not seem to depend on the level of immaturity of antioxidant protection and lung tissues in premature infants [7], but also on such fundamental component of endogenous risk as genetic predisposition.

Taking into account the pathogenetic aspects of BPD development a degree of lung tissue damage by ROS and, the severity of disease clinical implications can be the result of the action of either one or several mutant antioxidant enzyme genes.

The goal of the study is to investigate the genetic polymorphism frequency analysis of glutamate cysteine ligase catalytic subunit (GCLC) enzyme at preterm infants from the BPD development high-risk group to clarify their contribution to the formation and course of the disease.

Material and Methods

Study design

At the first step of the study, the prognostic indices of the BPD development high-risk group had been determined by retrospective analysis of 2 preterm infants' case reports groups. The 1 group included children with verified BPD (n = 27), the 2 group included children without verified BPD (n = 34). The minimum sample size had been determined according to the Altman's nomogram (the input parameters: 95% test power, standardized quantitative difference 1.18 - based on the presence of prematurity less than 32 weeks, significance level with an error of 0.05) and had consisted of 25 people in each group. The obtained data were used to construct a table of prognostic factors by A Wald (Table 1).

At the second step, 59 premature infants with high risk of BPD development according to the prognostic model were included in the cohort prospective controlled study. There were children with 27 - 35 weeks gestational age to have admitted to the Neonatal Intensive Care Unit of the Hospital, Russian Federation (37 boys, 22 girls, 2 twins). All patients from this group had a respiratory distress syndrome and, mechanical ventilation support was required for 37 children. Exclusion criteria were premature infants with malformations of lungs or with congenital heart diseases (for excepting patent ductus arteriosus and patent foramen ovale); premature infants with generalized

intrauterine infection, sepsis, aspiration syndrome; premature infants to be on mechanical ventilation due to abnormalities or severe hypoxic damage of the central nervous system.

Sign	Options	DC	I
Prematurity*	32 - 38 weeks	-12	331.0
	Less than 32 weeks	+4	101.7
Pneumonia*	No	-5	78.5
	Yes, typical	+1	4.6
	Yes, atypical	+5	62.9
Severe anemia*	Yes	+5	78.2
	No	-2	25.0
Mechanical ventilation support immediately after birth*	Yes	+4	71.7
	No	-3	48.9
Surfactant Therapy*	Yes	+2	33.3
	No	-5	80.9
Presence of respiratory failure symptoms more than 3 days*	Yes	+3	62.5
	No	-4	77.0
Oxygen dependence more than 7 days*	Yes	+14	566.5
	No	-7	282.5

Table 1: *The diagnostic table to predict the occurrence of BPD in premature infants.*

Note:: Chi-squared test with Yates's correction, $p < 0.05$ when comparing characteristic frequencies in the groups*

DC: Diagnostic Coefficient

I: Informative Value

Molecular-genetic studying of the GCLC gene was performed to the patients. Based on the results, 2 cohorts were formed. In the control group (n = 36), children with a "wild" gene variant were included, children with GCLC mutations formed the main group (n = 23). All patients received treatment according to the protocols for the management of the infant respiratory distress syndrome, regardless of cohort. The diagnosis of BPD was established according to the criteria of Jobe and Bancalary [8]. BPD severity was determined at 36 weeks of post-conceptual age for children with gestational age less than 32 weeks and, at 56 days age for children with a gestational age of more than 32 weeks.

Methods of the molecular-genetic study

The patients to be examined underwent molecular-genetic studying. Genomic deoxyribonucleic acid (DNA) samples were isolated from the leukocyte fraction using the SNP-Express reagent complex (manufactured by Liteh, Moscow, Russian Federation). The allelic polymorphism of GCLC gene has been investigated, where Cytosine at position 129 is replaced by Thymine (129 C/T) to determine lower promoter activity [9].

Amplification of genomic DNA regions containing these polymorphisms was performed by the polymerase chain reaction (PCR). Identification of allelic variants conditioned by single-nucleotide replacements was carried out with the help of allele-specific PCR using "Liteh" kits (Moscow, Russian Federation). After having performed 37 cycles of PCR at thermocycler "Tercik" (Helicon, Russian Federation) the amplification products were analyzed by electrophoresis in 3% agarose gel. DNA fragments were visualized in transmitted ultraviolet light after having stained the gel with ethidium bromide in the concentration of 1 μg/ml.

Statistical Methods

Statistical processing was performed using the licensed software "Statistica 6.0" (StatSoft, USA). The frequency of alleles and genotypes was determined by direct counting. The data were presented as a Mean ± standard deviation (minimum-maximum), and categorical variables as the frequency and percentage. Fisher's two-tailed test was used to compare genotype frequencies in observed groups. Also, the odds ratio (OR) and the relative risk (RR) with the lower limit and upper limit of 95% confidence interval (CI) were calculated for the frequencies of alleles and genotypes, sensitivity (Se, %) and specificity (Sp, %) were evaluated too. P values < 0.05 were considered significant.

Ethical expertise

This study was conducted in accordance with the Declaration of Helsinki. This study was approved by the Ethics committee of the Omsk State Medical University, Omsk, Russian Federation (IRB number: 31). Informed consents of patients' parents for participating in the study were obtained.

Results and Discussion

Results

The gestational age of the children with high risk of BPD development was 30.15 ± 2.68 weeks, the average birth weight was 1484.29 ± 395.32 grams (900.0 - 2290.0). All children had the severe or very severe condition at birth; the condition severity was caused by the presence of respiratory failure and neurologic symptoms (first of all central nervous system depression syndrome) at the background of prematurity. On physical examining lungs of all children with respiratory distress syndrome weakened breathing, scattered crepitation, less often wheezing and crackles were noted. The diagnosis of pneumonia had to be confirmed by chest X-ray examination.

The carriership of polymorphic variants of GCLC gene was detected in 23 children (main group). Most of them (21/23, 91.3%) formed BPD later and 2 (2/23, 8.7%) children did not have lung lesion. In the control group (n = 36), the favorable outcome and the development of the disease were met with the same frequency (17/36, 47.2%, and 19/36, 52.8% respectively). It is worth noting that the gestation age of children from the main group was 29.15±2.68 weeks, the average birth weight was 1484.29 ± 395.32 grams (900.0 - 2290.0); in the children of the control group, gestational age was 30.15 ± 1.59 weeks, birth weight was 1550.81 ± 237.56 grams (970.0 - 2490.0). Thus, the main group did not differ in the body weight and the gestational age from the control group, however in the main group BPD was formed significantly more often (two-tailed Fisher's test, p = 0.004), RR of BPD development was 1.7 (95% CI 1.2; 2.4).

We have analyzed the prognostic significance of phenotypic signs, to be favorable for BPD developing in the risk group children depending on the presence of mutant genotype. For this purpose, the following criteria such as using mechanical ventilation, the duration of mechanical ventilation more than 3 days, oxygen dependence at 28 days of life, as well as the presence of pneumonia were compared in children with polymorphic variants of GCLC. The premature infants with minor alleles of the glutamate cysteine ligase gene were established to be significantly more often on mechanical ventilation, to be performed for more than 3 days and also to need protractedly additional oxygen supply (Table 2).

Sign	Presence of the GCLC mutation n = 23		Absence of the GCLC mutation n = 36		p	OR	Lower limit 95% CI	Upper limit 95% CI	Se [%]	Sp [%]
	Yes	No	Yes	No						
The use of mechanical ventilation*	20	3	17	19	0.002	7.45	1.88	29.6	86.95	52.77
The duration of mechanical ventilation more than 3 days*	18	5	10	26	0.0002	9.36	2.74	32.03	78.26	72.22
Oxygen dependence at 28 days of life*	6	17	2	34	0.046	6.0	1.09	32.94	24.0	94.40
Presence of pneumonia	3	20	5	31	1.000	0.93	0.20	4.33	13.04	86.11

Table 2: *Prognostic value of clinical features in premature infants from the risk group of BPD development with polymorphic variants of the GCLC gene.*

Note:

**: Differences were significant*

p*: Two-tailed Fisher's test*

OR: Odds Ratio

CI: Confidence Interval

In total, 40 people were diagnosed with BPD in the main and control groups (Table 3). Slightly more than half of the children who developed BPD (21/40, 52.5%) were carriers of GCLC mutant genotypes, and in children without lung lesions, they were practically not detected (2/19, 10.5%).

Outcome	FO of the genotype [absolute amount (%)]			FO of an allele [absolute amount (%)]	
	129 CC	129 C/T	129 T/T	129 C	129 T
BPD (n = 40)	19 (47.5%)	20 (50.0%)	1 (2.5%)	58 (72.5%)	22 (27.5%)
without BPD (n = 19)	17 (89.5%)	2 (10.5%)	0	36 (94.7%)	2 (5.3%)
p, two-tailed Fisher's test	0.003		-	0.006	

Table 3: *Distribution of the GCLC polymorphisms in the children who developed BPD and the children who did not develop the disease.*

Note: FO: Frequency of Occurrence

The frequency of occurrence of the minor allele -129T GCLC was 5 times higher in the group of children with BPD. So, it could be possible to conclude that children with the pathological genotype of GCLC are more likely to form BPD than children with the risk of the disease developing but not to have any mutations. To sum up, premature infants with heterozygous and homozygous carriage of GCLC mutant alleles had the greatest risk of the BPD development.

Discussion

At present time the oxidative stress is considered as one of the pulmonary pathology development causes and in particular the cause of BPD [6]. Pulmonary adaptation of small premature infant is difficult due to low morphological, functional and especially biochemical parameters of the lungs. Oxidative stress with negligible antioxidant protection promotes the expression of cytokines and the initiation of the inflammatory process, which leads to damage to the epithelium of the respiratory tract and the deactivation of the surfactant [6,10]. Deficiency of antioxidants in premature infants is due to a lack of their endogenous production and the termination of their transfer from the mother through the placenta. It is logical that this category of children constitutes a special group of newborns to be threatened with the oxidative stress [5,11].

Glutathione cysteine ligase is considered to be the most important component of antioxidant protection in humans [12]. Glutathione cysteine ligase is a heterodimeric enzyme to be composed of two domains: the catalytic (GCLC) and modifier subunit (GCLM) [13]. GCLC is responsible for the catalytic activity of glutathione cysteine ligase, GCLM provides increasing catalytic efficiency of the enzyme [14]. They are coded by different genes on various chromosomes: GCLC is located at locus 6p12 and GCLM is located at locus 1p22-p21 [15]. Recently, more and more data are accumulating that the polymorphism of single nucleotides, due to the formation of specific alleles of genes, makes an important contribution to the development of protective reactions. Genetic predisposition can include specific polymorphisms that determine the level of the antioxidant protection [16-18].

Our study has been devoted to the investigation of the 129 C/T GCLC polymorphism contribution to the formation of BPD. As a result of the search, it was found the presence of single nucleotide replacements in GCLC gene increased the risk of BPD in premature infants with respiratory distress syndrome by 1.7 times, despite adequately conducted therapeutic measures. It has been supposed that the 129 C/T GCLC nucleotide polymorphism has been associated with susceptibility to oxidative stress [19]. Moreover, a lower level of glutathione has been registered in patients with BPD who had mutations in the GCLS gene [20], which could increase the damaging effect of ROS. The resulting defeat of the respiratory tract epithelium and the deactivation of the surfactant have led to a necessity of longer and more intensive respiratory support of infants with 129 C/T GCLC mutation.

Literary data about the role of genetic polymorphisms of GCLC in the formation of BPD are extremely scarce. One of the most significant works on this problem is the work of V Sampath., *et al* [1]. The authors have not found a connection between the 129 C/T GCLC polymorphism and the development of the disease in a survey of 659 newborns, of which 284 had BPD.

The limitation of this study is a limited number of observations. A continuation of the research about the contribution of 129 C/T GCLC polymorphism to the development of BPD will allow it to be considered as a possible risk factor for the development of the disease in the future.

Conclusion

BPD was 1.7 times more likely to develop in children with the 129 C/T GCLC mutation. The predictive value of features promoting BPD development was significantly higher in preterm infants of the risk group who had heterozygous and homozygous carriership of GCLC gene minor alleles. The presence of the minor allele -129T of GCLC increased the relative chance of the disease developing by reducing antioxidant protection and increasing oxidative stress. In a half (50.0%) of children to have developed BPD had heterozygous genotype 129 C/T GCLC versus 10.5% in patients who did not develop the disease. So, the genetic polymorphism of GCLC gene could be supposed to take pathogenic part in the BPD development.

Acknowledgments

None.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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