**The role of matrix metalloproteinase gene polymorphism-1 (1607insG) in the bronchopulmonary dysplasia development in neonates**

**Abstract:** The environmental (0.54) and hereditary (0.46) factors contribute equally to development of BPD, which requires studying the polymorphism of genes and their regulatory functions for the prediction of chronic disease during pregnancy and in newborn. MMP-1 gene (1607insG) polymorphisms affects the individual tending to bronchopulmonary dysplasia (KW = H (n = 58) = 18.85, p = 0.0001). The prevalence of dominant homozygotes (AA) and heterozygotes (Aa) by insertion of guanine at position 1607 (p <0.001) is specific for children with BPD. This was probable basis for increased expression of MMP-1, which is peculiar to children with BPD.

Moderate violation of Hardy-Weinberg equation suggests high probability of non-mendelian polygenic inheritance of predisposition to bronchopulmonary dysplasia.

**Keywords:** children, bronchopulmonary dysplasia, hereditary factors, MMP-1 gene polymorphism

The issue of clinical variability high level of bronchopulmonary dysplasia (BPD) among individuals in a population who have the same gestational age and iatrogenic involvement during resuscitation still is not resolved [8]. This initiates a study of the genetic factors role and the influence of trigger genes polymorphisms in the etiology of this disease [1,2,3,4,5]. By physiological meaning, at the phase of BPD development and at the developed BPD feature of pulmonary and vascular remodeling is a sign of cellular and extracellular regulatory processes that are provided by molecular inducers, regulators and signaling systems that determine cardio-respiratory remodeling processes. Cellular and / or intracellular expression of cytokines, inductors, and the level of specific receptors presentation are determined by difficult genetic mechanisms [2,3,5]. The study of these mechanisms is important for prevention of growth inhibition and lung tissue fibrosis[7].

The development of bronchopulmonary dysplasia depends on the combined influence of several factors that can cause pneumofibrosis and inhibition of the pulmonary ontogenesis. We selected matrix metalloproteinase-1 gene as the trigger gene of pulmonary ontogenesis inhibition and pneumofibrosis [1,4,6]. Pathogenic role of MMP-1 in the development of BPD is demonstrated in early studies [9]. MMP-1 is now regarded as a special form of biological control at bronchopulmonary dysplasia, and takes main part in to the implementation of various biochemical processes and rapid physiological responses to changing conditions.

**Objective:** improvement of bronchopulmonary dysplasia developement diagnosis by determining the role of genetic and environmental factors in the formation of bronchopulmonary dysplasia in infants

**Materials and methods:** the study was performed at the Department of Pediatrics №1 and Neonatology of Kharkiv National Medical University (Head of Department - G. Senatorova) in the regional center for diagnosis and treatment of bronchopulmonary dysplasia in Kharkiv Regional Children's Clinical Hospital (head physician – G. Muratov). 60 twins /30 pairs (24,1 ± 2,7%) were under supervision: 54 patients (90,0±3,9%) with a diagnosis of bronchopulmonary dysplasia (study group) and 6 observed (10,0 ± 3,9 %) were born prematurely, had respiratory distress, but bronchopulmonary dysplasia didn’t develop (control group). The diagnosis of bronchopulmonary dysplasia was established by the 10th edition of International Classification of Diseases (code R27.0). All 6 babies of control group were from twins with child suffered from BPD. The twins method was used to assess the relative contributions of hereditary and environmental factors. We determined the concordance among pairs, evaluated the role of environmental factors. The fraction of heredity in the formation of BPD was calculated with Holzinger’s formula.

The matrix metalloproteinase-1 gene polymorphism was determined by PCR and allelic discrimination diagnostic kits «SNP-Express" produced by "Liteh Ltd.". DNA samples were obtained from buccal epithelium with reagent "DNA Express". Frequencies of alleles and allele frequency combinations and their accordance to Hardy - Weinberg equilibrium on the criterion χ2 were calculated.

Detection of polymorphism was performed by horizontal electrophoresis [12]. Statistical analysis of data was performed using the «Statistica-6".

Results and discussion: The distribution of the patients by gender and zygosity is presented in Table 1. The difference was observed only in monozygotic female twins (p <0.05), which in our view requires further investigation.

Table 1.

Twins distribution by gender and zygosity

(main group and control group)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Main group  n=54 | | Control group  n=6 | | р о.г.-г.п. |
| abs. | M±m% | abs. | M±m% |
| Monozygotic twins:   * males * females | 10  12 | 18,5±5,3%  22,2±5,7% | 1  0 | 16,6±16,6%  - | 0,823  0,039*1* |
| Total: | 22 | 30,1±6,7% | 1 | 16,6±16,6% | 0,317 |
| Dizygotic twins:   * males * females | 18  14 | 33,3±6,4%  25,9±6,0% | 1  4 | 16,6±16,6%  66,6±21,1% | 0,913  0,213 |
| Total: | 32 | 59,1±6,4% | 5 | 83,2±25,7% | 0,754 |

Note: significant difference (Х *1*– р<0,05).

A significant percentage of twins in the general population (24.1±2.7%) twins allowed us to use a method for evaluation of bronchopulmonary dysplasia heredity for, which includes estimation of possible BPD inheritance in pairs of mono- and dizygotic twins. The method is based on high concordance frequency of signs in monozygotic pairs. Comparison of concordance among pairs by each sign in genetically identical monozygotic and dizygotic twins makes it possible to make a conclusion about the role of genotype in the formation of bronchopulmonary dysplasia.

Pair concordance for BPD was 0.8, which indicates the big value of hereditary influence on the development of the disease (formula 1).

**С = с / (с+d) = 24 / (24+6) = 0,8 (1)**

Where, C-pair concordance; c and d - number of cocnocrdant and discordant couples.

24 pairs (80 ± 7,4%) were concordant by BPD, and 6 pairs (20 ± 7,4%) were discordant. The amount of concordant pairs by development of BPD was significantly higher (p <0.0001). That indicates on the possible influence of genetic factors on the formation of the disease.

It was found that from 16 pairs of monozygotic twins 9 pairs were concordant by BPD in the neonatal period (pair concordance - 0.562). Among 10 pairs of dizygotic twins 7 pairs were concordant by BPD (pair concordance - 0.7).

The role of heredity by the Holzinger’s formula is 0.46, which in the range from 0.3 to 0.7, and the part of environmental factors influence was 0.54. This indicates on the equal role of hereditary and environmental factors in the formation of BPD (formulas 2 and 3).

**Н = (СMz- СDz) / 1 – СDz = (0,625-0,7) / 1 – 0,7 = 0,46(2)**

**Н** – part of heredital influence of bronchopulmonaary dysplasia development;

**СMz, СDz –**pair concordance of monozygotic and dizygotic twins.

**Е = 1 – Н = 1 – 0,46 = 0,54 (3)**

**Е** – part of environmental factors influence; **Н** – part of heredital influence of bronchopulmonary dysplasia development.

Our study proved equal influence of environmental and genetic factors on the formation of BPD, which requires studying of gene polymorphism allelic variants to assess the possibility of this chronic disease prediction during pregnancy and in newborns.

60 twins were examined for the presence of matrix metalloproteinase-1 gene trigger polymorphism (insertion). Polymorphisms of MMP-1 gene (1607insG) affected on individual predisposity to bronchopulmonary dysplasia (KW = H (n = 58) = 18.85, p = 0.0001).

Allelic variants frequency of the MMP-1 gene polymorphism 1607insG in groups is shown in Table 2.

Table 2.

Alleles and allelic combinations frequencies of MMP-1 gene polymorphism (1607insG) in twins with BPD (main group) and twins who were born prematurely, had respiratory distress in the early neonatal period, but BPD didn’t develop (control group)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Alleles and allelic combinations | Main group  n=54 | | Control group  n=6 | | χ2  о.г-г.п. |
| abs. | Allele and allelic combinations frequencies | abs. | Allele and allelic combinations frequencies |
| Alleles:  *allele А*  *allele а* | 64  44 | 0,592  0,407 | 1  11 | 0,083  0,916 | 0,297*2*  0,120*2* |
| Total: | 108 | 1,000 | 12 | 1,000 |  |
| Allelic combinations:  *АА*  *Аа*  *аа* | 25  14  15 | 0,462  0,259  0,277 | 0  1  5 | -  0,166  0,833 | 0,388*2*  0,0161  0,1852 |
| Total: | 54 | 1,000 | 6 | 1,000 |  |

Note: significant difference (Х *1*– р<0,01; Х *1*– р<0,001).

Data in the table shows that allele A is more common (59,2±4,7%; p<0.05) in the main study group, while allele a was in 40,7±4,7%. The prevalence of dominant homozygotes (AA) in twins of main group were significant (p <0.001). Recessive homozygotes (aa) in 1607insG gene MMP-1 were observed more frequently in the control group (p <0.001).

Comparison of allelic variants frequencies (observed and predicted) by Hardy-Weinberg equation performed for assessment of data compliance of the epidemiology of zygotes in the population of children with bronchopulmonary dysplasia (tab. 3).

Table 3.

The allelic combinations frequencies of MMP-1 gene polymorphism (1607insG) in twins with BPD (main group; n = 54)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| allelic combinations | Observed frequency | | Predicted frequency | χ2  observed/predicted |
|  | abs. | frequency |
| АА | 25 | 0,462 | 0,321 | 0,018*1* |
| Аа | 14 | 0,259 | 0,424 | 0,029*2* |
| аа | 15 | 0,277 | 0,255 | 0,007*1* |
| Total | 54 | 1,000 | 1,000 |  |

Note: no difference Х *1*– р>0,05; significant difference Х *2*– р<0,01)

In the group of patients with BPD allele frequencies corresponded to Hardy-Weinberg equation mostly. No significant differences between the observed and the expected frequencies for the dominant (AA) and recessive homozygotes (aa) were found (χ2 0,007-0,018; p> 0.05). However, frequencies observed and expected of heterozygotic alleles (Aa) were different (χ2 0,029; p <0.01). Heterozygous variant alleles Aa met 2 times less than expected (0.259 / 0.424), which violated the Hardy-Weinberg equation.

Moderate violations of Hardy-Weinberg equation due to the rarity of heterozygous alleles may be due to polygenic inheritance of predisposition to bronchopulmonary dysplasia, reduced viability of heterozygous carriers, the presence of comorbid disease, trigger the influence of the MMP-1 gene and population differences that require further research.

Consequently, the study found the prevalence of dominant homozygotes (AA) and heterozygotes (Aa) by insertion of guanine at position 1607 in children with BPD (p <0.001) and demonstrated the influence of MMP-1 gene polymorphisms (1607insG) on the bronchopulmonary dysplasia development (KW =H(n=58)=18.85, p=0.0001). These features probably appeared due to intracellular, interstitial pneumofibrosis activation and increased expression of MMP-1. However, we identified a high probability non-mendelian polygenic inheritance of predisposition to bronchopulmonary dysplasia evidenced by a moderate violation of Hardy-Weinberg equation.

Conclusions:

1. The development of BPD is caused by environmental (0.54) and hereditary (0.46) equally which necessitates the study of genes polymorphism and their regulatory functions with regard to the predictability of chronic disease during pregnancy and in newborns.

2. MMP-1 gene polymorphism (1607insG) influences on individual predisposition of bronchopulmonary dysplasia development.

3. The predominance of dominant homozygotes (AA) and heterozygotes (Aa) by insertion of guanine at position 1607 (p <0.001) is specific for children with bronchopulmonary dysplasia, which probably appeared due to increased expression of MMP-1, which is peculiar to children with BPD.

4. Identified high probability of non-mendelian polygenic inheritance of predisposition to bronchopulmonary dysplasia, evidenced by a moderate violation of Hardy-Weinberg equation.