THE ROLE OF MOLECULAR-GENETIC INVESTIGATIONS IN PERIVENTRICULAR LEUKOMALACIA IN PREMATURE INFANTS OF UZBEK POPULATION

Sayfutdinova S.R.
Tashkent Medical Institute of Postgraduate Education, Chair of Pediatric Neurology named after Shamansurov Sh.Sh., Tashkent, Republic of Uzbekistan

The insufficient reliable antepartum prognosis of unfavourable primary results, for example, development of cerebral intraventricular hemorrhage (CIVH), periventricular leukomalacia (PVL) is one of the current significant problem in premature low birth weight infants (4). We suggest that hemostasis gene variants may play a role in development of some diseases in low birth weight infants.

One of the major directions is molecular-genetic investigation for identification of so called genes candidates. However, study of the results of the measurement of genetic fund remains to be at the early age.

For confirmation of the preliminary investigations of genetic relations with cerebral-vascular disorders we studied effect of genetic variants biochemically connected with hemostasis (factor V Leiden, prothrombin G20210A, factor VII -232 del/ins and factor XII – Val 34 Leu) on the grate number of infants who was born with low birth weight.

Material and Methods

In 200 newborns with very low birth weight who was born in the Scientific Research Institute of Obstetric and Gynecology of the Ministry of Health of the Republic of Uzbekistan as well as in the OPN department of Hospital N 5 of Mirobad District there were studied effect of gene variants participating in hemostasis during the period from 2007 to 2009 (including such criteria as age of gestation from 28 to 36 weeks, birth weight 1500 g and excluding such criteria as lethal malformation, chromosome inherited diseases: trisomia 13 and trisomia 18). The premature infants who were born at the gestational age 22-26 weeks were not included into our investigation because the mortality rate in this group is very high. In many homozygous infants from the parents of Uzbek nationality there was similar genetic phone from 50% to 100%. This significant genetic effect interfered clinical data, that is, genotype/phenotype relations were noted more often in these infants, than in children born from parents of different nationalities. However, these relations may occur due to other genetic factors which were not studied. For this purpose there was taken samples of DNA for studying of its distribution.

There was performed study of genetic associations in the great number of complex diseases for determination of the groups of increased risk. These trombophilic disorders include mutation of Leiden factor V, that results in resistance of factor V to decomposition of activated protein C and mutation of prothrombin G20210A connected with increased plasma prothrombin concentration.

For achievement of balanced hemostasis the structure of thrombus fibrin is considerably depended on factor XIII activity. The XIII factor catalyzes formation of the links between monomer fibrins and includes various adhesive and antifibrinolytic proteins into final thrombus fibrin, that increases mechanical strength. There were performed some studied of the functional effect of factor XIII gene polymorphisms on the structure of fibrin clot and risk of hemostatic disbalance (studies of Cobbervig and Williams). Polymorphism of factor XIII-Val34Leu is the best studied genetic variant because the transmission of the factor XIII-Leu 34 allele has biochemical connection with formation of fibrin mesh structure with thinner fibers, less pores and change of the characteristics of penetration in comparison with fibrin clots forming in homozygous factor XIII-Val34leu. The ef-

fect of prothrombin concentrations on the clot structure indicated that thinner clots were more resistant to fibrinolysis and accompanied increased risk of thrombosis development. In relation to coagulation factor VII there were described many polymorphisms connected with change of the factor VII levels. Two polymorphisms seem to be connected with increase in factor VII levels and risk of cerebral-vascular diseases (factor VII-C122T). Other genetic variants of factor VII including polymorphism of intron 7 and R353Q were connected with reduction of factor VII levels which may have different effect on the hemostatic balance (Mariani e.a.). The stimulator of factor VII-323 del/ins (323 A1/A2) results in 20% coefficient reduction of factor VII coagulant activity.

In relation to effect of factor XIII-Val34Leu polymorphism on the neurological short-term outcome in very low birth weight infants when in children there was noted increased risk of white substance damages (PVL stage 1-2). Besides, alleles of factor XII-Leu34 may be as protective factor in infants born at 23 week of gestation.

Clinical-laboratory methods of examinations included analysis of obstetric-gynecological and somatic medical history of mothers, clinical course of pregnancy, the chest roentgenography data, neurosonography and dopplerography of the brain as well as other investigations in combination with the traditional tactics of surveys.

Results and Discussion

Out of 200 infants with low birth weight 109 children of Uzbek nationality participated in the study as main group and the rest children from mixed marriages were control group. In this population the genotyping was successful in 73 cases (Leiden factor), in 76 cases the mutation of prothrombin G20210A was noted, in 74 cases – factor XIII-323 del/ins polymorphism, and in 78 cases – factor XIII-Val34leu; 98%-99%). The distribution of hemostasis gene polymorphism in low birth weight infants was defined by equation Hardy-Winberg.

During examination of children born with periventricular leukomalacia (PVM) there were observed increased echodense lesions which may transform into the porencephalitic cyst and periventricular hemorrhages which distributed accordingly to the stage: PVH 1(periventricular hemorrhage stage 1) was considered as blood presence in the areas of embryo matrix, stage II – as system with ≤50% of ventricular volume or ventricular extension, stage III – as blood in the ventricular system of >50% ventricular volume or ventricular extension, and stage IV – as blood presence in the ventricular system and parenchymatous lesion with consequent parenchymatous destruction.

Genotyping. All the polymorphisms were found with use of polymerase chain reaction and restriction analysis. Primers and DNA-sequences were selected. The polymerase chain reaction for factor V Leiden mutation and prothrombine G20210A were performed as described earlier. The primer pares for identification of factor XIII-Val34Leu polymorphism were 5’ CAT GCC TTT TCT GTT GTC TTC-3’ and 5’-Tac CTT GCA GGT TGA CGC CCC GGG GCA CTA-3’ (Ddel-digest) and 5’-GGC CTG GTC TGG AGG CTC TCT TC-3’ and 5’-GAG CGG ACG GTT TTG TTG CCA CCG-3’ (Ddel-digest) and 5’ GGC CTG GTC TGG AGG CTC TCT TC-3” and 5’-GAG GGG ACG GTT TTG TTG CCA GCG-3’ (*Hind*III digest) for mutation of factor VII-323del/ins.

On the basis of these data it will be enough for identification of the total difference of frequency of carriers between children with low birth weight or studied healthy children of 5% (prothrombin G20210A and factor XIII-Val34Leu) to 9% (factor VII ins/del).

The expected frequencies of the carriers for Uzbek population of homozygous or geterozygous factor V Leiden are 4,9%, prothrombin G20210A – 3,0%, and factor VII-121del/ins – 19,9% and factor Val34Leu homozygous polymorphism in 3% of children.

Conclusions

Out of 200 infants with low birth weight 109 children of Uzbek nationality participated in the study as main group and the rest children from mixed marriages were control group. In this population the genotyping was successful in 73 cases (Leiden factor), in 76 cases the mutation of prothrombin G20210A was noted, in 74 cases – factor XIII-323 del/ins polymorphism, and in 78 cases – factor XIII-Val34leu; 98%-99%). The distribution of hemostasis gene polymorphism in low birth weight infants was defined by equation Hardy-Winberg.

Finally it should be stressed the effects on the factors of thrombophilic risk such as factor V Leiden and mutation of prothrombine G20210A on the development of intracranial hemorrhages and PVL in premature infants. On the contrary, genetically based low levels of coagulation factors such as polymorphism of factor VII-232del/ins stimulator may both increase risk of intracranial hemorrhage (as showed studied on adults) and effect on the cerebral circulation disturbances in premature infants, for example, on the development of periventricular leukomalacia.

References

1. Fogel F., Motulsky A. Genetika cheloveka (transl from Engl.).-M.,1990.-P.142-149. (Human genetics).

2. Bianchi D.W. Prenatal diagnosis by analysis of fetal cells in maternal blood // J.Pediatr.-1995.-Vol.127, N 6.-P.847-856.

3. Roy J.C., Johnsen J., Breese K. Fragile X syndrome, which is the impact of diagnosis on families // Dev.Brain.D.-1995.

4. Hartel C., Konig I., Koster S., et al Genetic polymorphism of hemostasis genes and primary outcome of very low birth weight infants // Pediatrics.-2006.-Vol.118, N 2.-P.683-689.