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THE IMPACT OF SKEWED X-INACTIVATION IN SPINAL MUSCULAR ATROPHY PHENOTYPE MODIFICATION

Yurchenko A.A.[1], Hryshchenko N.V.[2]
1. Educational and Scientific Center "Institute of Biology and Medicine" of Taras Shevchenko National University of Kyiv, Ukraine
2. Institute of Molecular Biology and Genetics National Academy of Sciences of Ukraine, Ukraine

Proximal spinal muscular atrophy (SMA) is an autosomal recessive neurodegenerative disease caused by mutations in *SMN1* gene. In the great majority of SMA patients homozygous deletions in *SMN1* gene exon 7 have been detected [1]. Despite the fact that SMA is a classic example of a monogenic disease with a major mutation in the determinant gene, the clinical phenotype of patients varies in a wide range - from the most acute infantile type (SMA0-1) to a relatively mild chronic juvenile type (SMA3) [2, 3]. Some genetic factors, which modify SMA phenotype, are already known, such as deletion type of *SMN1* and *SMN2* copy number [4-5]. However, the variability of the SMA phenotypes cannot be completely determined by the known modifier genes. It was shown by us and in some similar studies that the female SMA phenotype was significantly differ from the phenotype of the male patients. One of the possible factors which may explain the gender dependent phenotype modifications is skewed X-inactivation [6, 7]. Due to this a female may have unequal expression of heterozygote alleles of the X-linked genes involved in the neurological pathways.The aimof our investigation is to study an association of SMA phenotype with X chromosome inactivation levels of skewingin female patients with *SMN1* deletions.

Materials and Methods. The analysis of X-inactivation patterns was carried out by genotyping of CAG repeats site allelic polymorphism in AR gene exon 1 in the group of female SMA patients (n = 46) with determined *SMN1* and *SMN2* genotypes.The PCR efficiency of the *AR* alleles from the DNA samples hydrolyzed by methyl-specific endonuclease Hin6I was compared with that one in non-hydrolyzed DNA. Genotyping of samples and semiquantitative analysis of AR gene alleles was carried out on an automatic fluorometer. As a comparison group, we used male SMA patients (n = 71) with determined *SMN1* and *SMN2* genotypes. To validate the results Spearman's rank correlation test, linear regression with breakpointand Mann-Whitney test were used in genotype-phenotype correlation and comparative analysis. Statistical processing was carried out using Statistica 10.0 software package.

Results. X-inactivation patterns in female patients with SMA have been analyzed. Skewed X-inactivation was detected in 56.5% of patients. The correlation analysis of the patients' genotypes (type of *SMN1* deletion and the number of *SMN2* copies) and SMA phenotype in male (n=71) and females (n=46) groups were performed. The correlation is stronger in the male group ($r = -0,31$; $p = 0,009$ in male group and $r = -0,28$; $p = 0,005$ in female). Expected phenotypes for females were calculated using the coefficients of linear regression calculated for the male group. It was demonstrated that SMA-females with skewed X-inactivation significantly more often ($p < 0.05$) had differ phenotype than expected.

Conclusions. The obtained data have confirmed the hypothesis that a level of skewing may influence on SMA phenotype. However, the phenotype modifications in patients with skewed X-inactivation lead both to a milder and more severe type of disease. Our results indicate that not X-inactivation level of skewing by itself, but nonrandom inactivated X-linked genes may participate in SMA phenotype modification.

Prospects for further research.Taking into account our current results we plan to study an association of X-linked genes, such as *PLS3* and *UBA1* with SMA phenotype modifications.

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SPECIFIC SURFACTANT PROTEINS AS CLINICAL MARKERS FOR LUNG MATURITY IN RISK NEWBORNS

V. Stoyanova[1], A. Tsanova[1], E. Stoimenova[1], A. Jordanova[1], E. Christova[2], Z. Lalchev[3]
[1] Faculty of Medicine, St. Kliment Ohridsky University of Sofia, Bulgaria
[2] Faculty of Public Health, Medical University of Sofia, Bulgaria
[3] Faculty of Biology, St. Kliment Ohridsky University of Sofia, Bulgaria

Alveolar surfactant (AS) provides stability during the dynamic process of inhalation/exhalation by maintaining low surface tension. AS consists mainly of phospholipids (80%), carbohydrates, and about 10% proteins, four of which, SP-A, SP-B, SP-C, and SP-D, are specific for AS. They play a crucial role in stabilization of alveoli at exhalation, as well as in the immune defense. The specific surfactant proteins are synthesized after 24th week of gestation. Therefore, in risk children born before 32nd week of gestation different respiratory pathologies, some of them of lethal outcome, can be observed. In the present study we tested the presence of specific surfactant proteins in gastric aspirates (GA) from newborns by Western blot with the aim to consider the advantage of GA as an adequate sample for assessment of surfactant maturity at birth.

Materials and Methods. In this study we analyzed 9 clinical samples GA, 2 of which are after application of betamethasone to the mothers, for detection of SP-A, SP-B, and SP-C. The separation of the proteins were carried out by 12% SDS-PAGE under reducing conditions followed by wet Western blotting. The blots were probed with specific polyclonal antibodies against human SP-A (28-36 кD), promature (40 kD) and mature SP-B (8 kD) and promature SP-C (21 kD) surfactant proteins in GA.

Results. The obtained results showed that GA from newborns with Neonatal Respiratory Distress Syndrome (NRDS) had the lowest concentrations of all analyzed surfactant proteins in contrast to GA from full term children (Fig. 1). In addition, the applied corticosteroid therapy did not show enhanced protein biosynthesis.

Fig. 1. Western blot SP-A: 1 - GA from infant with NRDS; 3, 4, 6, 8, 9 - GAs from full term infants; 5 - amniotic fluid of full term baby; 2, 7 - GAs after corticosteroid therapy; Western blot SP-B: 9 - GA from infant with NRDS; 1, 2, 6, 7 - GAs from full term infants; 5 - amniotic fluid of full term baby; 3, 8 - GAs after corticosteroid therapy; 4 - human serum albumin; Western blot SP-C: 2 - GA from infant with NRDS; 4, 5, 7, 8 - GAs from full