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Fund “Nauka” Project № 18011 Resume – Competitive-based Session 2018:
“Study of molecular-genetic factors in CFTR – gene in males with primary infertility”
Project leader: Assoc. prof. Trifon Georgiev Chervenkov, MD, PhD

The aim of the research project is to study the role of molecular-genetic changes in the CFTR gene as an etiological factor for male primary infertility in order to improve the genetic prophylaxis in families with reproductive failure.

The tasks of the project are as follows:

1. To select patients who have sperm count in the ejaculate below 5×10^6/ml and are suitable for the study.
2. To define the prevalence of homo-and heterozygous genotype for the included genetic variants (mutations and polymorphic variants IVS8 poly-T, TG repeats, F508del and R117H) in the CFTR gene of the studied male participants.
3. To conduct a comparative analysis between the reported results from the molecular-genetic tests for mutations and polymorphisms in the CFTR gene and the concentration of the sperm cells in the ejaculate among the carriers of these genetic changes in order to define the genotype-phenotype correlation between the established genetic finding and impaired spermatogenesis.
4. To analyze the role and problems of medical-genetic counseling of families with primary infertility.
5. To summarize and analyze the results from the molecular-genetic tests as a part of the dissertation of Mariya Levkova, MD.

The methods include the use of Real-time PCR machine of Applied Biosystems QuantStudio Dx, gel electrophoresis, sequencing by using capillary electrophoresis of Beckman Coulter GeXP, analysis of the genotypes of the participants for the included variants in the CFTR gene - IVS8 poly-T, TG repeats, F508del, and R117H.

The expected results are proving the role of the CFTR gene as a reason for the observed deviations in the sperm count of the tested male subjects.

Achieved results:

DNA was isolated from men with primary idiopathic infertility, who had a sperm count in the ejaculate below 5×10^6/ml.

Molecular-genetic analysis was conducted for pathogenic and polymorphic variants, which could be related to impaired spermatogenesis, in the CFTR gene. The following genotypes for 5T variant were found: one participant (2.00%) from the case group was a homozygote for 5T/5T variant, and two participants (4.00%) were heterozygotes for 5T/7T variant. The genotypes of the other 47 participants (94.00%) with impaired spermatogenesis were wild type. One participant from the males with impaired spermatogenesis (2.00%) was a heterozygote carrier of the delF508 mutation in the CFTR gene. It was together with the 7T variant in the CFTR gene and this combination was not considered a pathogenic finding. None of the males with impaired spermatogenesis carried the R117H mutation in the CFTR gene in homo- or heterozygous state.

The genotypes of the patients with impaired spermatogenesis for the variant IVS8(n)T were compared to the genotypes of 20 fertile control male individuals. None of the participants from the control group had a delF508 or R117H mutation in the CFTR gene.

The non-parametric test One-Way ANOVA was applied in order to compare the distribution of the genotypes for the IVS8(n)T variant in the CFTR gene among the case subjects and the control group. According to the null hypothesis, there was no difference. However, the tested polymorphism in the CFTR gene showed a statistically significant difference ($p = 0.04$) and the null hypothesis was rejected.

The number of the TG repeats in the CFTR gene was also analyzed. The men with impaired spermatogenesis had the following distribution: 47 (94.00%) of them had 11 TG repeats, and three (6.00%) – 10 TG repeats. These results are considered normal findings and did not correlate with the impaired spermatogenesis.

The distribution of 5T variant in the CFTR gene, which has a potential role as an etiological factor for impaired spermatogenesis in its carriers was analyzed among Bulgarian males with infertility for the first time. The necessity of the molecular-genetic tests for mutations and polymorphic variants in the CFTR gene was confirmed in order to establish the cause of impaired spermatogenesis.