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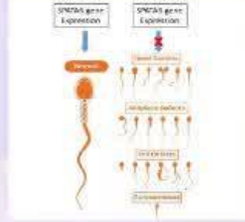
Investigation of the SPATA6 gene Expression in men with terato-asthenozoospermia

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Background

Infertility has emerged as a significant issue in contemporary society, with its prevalence on the rise globally. A proportion of men suffer from a condition referred to as idiopathic male infertility, which is marked by an ambiguous cause of the disorder. The SPATA (Spermatogenesis-Associated Protein) gene family is responsible for encoding proteins that are crucial for the processes of spermatogenesis and fertilization. Within this family, the SPATA6 gene is notably conserved throughout evolution and exhibits expression that is restricted to testicular tissue.

Objective

In the present study, aimed to examine the expression level of the SPATA6 in individuals with terato-asthenozoospermia (TAZ) and Normozoosperima (NZ) as a control group.

Conclusion

Overall, the results of this study suggested the role of the SPAT6A gene in sperm motility and morphology.

Materials and Methods

Semen sampling and preparation

We selected 30 men with terato-asthenozoospermia and 30 cases with Normozoospermia based on the clinical criteria.Sperm morphology was investigated using microscopy and Video test Image Analysis-Sperm 2.1 (Video test, Moscow, Russia). The concentration and motility of sperms were assessed by Computer-Aided Sperm Analysis. The semen samples were centrifuged at 4°C for 5 minutes at 2500 g and supernatants were removed, and then the spermatozoa separated from seminal plasma were washed with PBS and centrifuged again at 4°C for 5 minutes at 2500 g in duplicate.

RNA Extraction and cDNA synthesis

The total RNA was extracted using spermatozoa precipitation by RNX plusTM kit (Cat.no: RN7713c/EX6101; Sinaclon, Iran). The RNA quality and concentration were measured according to 260/230nm and260/280nm absorbance by Thermo Scientific™ Nano Drop 2000, and the RNA was stored at -80°C until cDNA synthesis. Random hexamer primers and Yekta Tajhiz cDNA synthesis kit (Cat. no: YTA4500; Yekta Tajhiz, Iran) were used to prepare cDNA from 2 µg total RNA.

Quantitative Real-Time PCR (qRT-PCR)

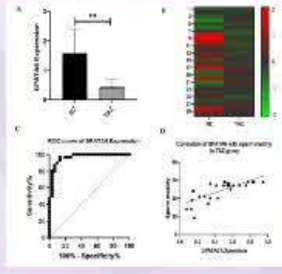
The qRT-PCR was performed using 1µl of cDNA in 12µl. reaction volume. The qPCR reaction solution included 6ul of SYBR Green Real-time PCR Master Mix Kit (Cat.no: YT2551; Yekta Tajhiz, Iran) and 0.25µl of 100nM of each forward and reverse primer for gene. Quantitative RT-PCR was performed using Rotor-Gene Q (Corbett Research, Sydney, AU).

Statistical analysis

The statistical analyses were performed using REST 2009 software (for qPCR data analysis) and the Graph Pad/Prism8.0.2 software (for comparison of the qPCR data between the studied groups). Data were presented as mean±SD. The comparison of unpaired data was done by the Kruskal-Wallis test after investigating the normality of data distribution by the Kolmogorov-Smirnov test. Correlations analysis was performed by the Spearman's correlation coefficient and $P<0.05$ was considered significance level.

Results

The findings of this study indicated that the expression level of SPATA6 was significantly ($p<0.0001$) lower in the TAZ group than in the control group. There were also significant ($p<0.0001$) positive associations between the expressions of SPATA6 with sperm motility and morphology.



References

Bedard, N., et al., Mice lacking the USP2 deubiquitinating enzyme have severe male subfertility associated with defects in fertilization and sperm motility. Biology of reproduction, 2011. 85(3): p. 594-604.