Investigation of piwi-interacting RNA pathway genes role in idiopathic non-obstructive azoospermia

Zeeba Kamaliyan1, Sara Pouriamanesh1, Mohsen Soosanabadi2, Milad Gholami1 & Reza Mirfakhraie1,3

Genes involved in piwi-interacting RNAs (piRNAs) pathway have an essential role in spermatogenesis. HIWI and TDRD proteins are critical for piRNA biogenesis and function. Therefore, Mutations and polymorphisms in HIWI and TDRD genes may play role in male infertility. The aim of the present study was to investigate the role of HIWI rs508485 (T>C) and HIWI3 rs11703686 (C>T) polymorphisms and mutational analysis of TDRD5 gene in idiopathic non-obstructive azoospermia in a case-control study including 226 non-obstructive azoospermia patients and 200 fertile males. Genotyping for both polymorphisms was performed using Tetra-Primer ARMS PCR. Mutation analysis of TDRD5 gene was done using multi-temperature single strand conformation polymorphism technique (MSSCP). The frequency of rs508485TC genotype was significantly different in the studied groups (P = 0.0032; OR = 2.12; 95% CI, 1.29–3.48). In addition, the genotype frequencies showed a significant difference under dominant model (P = 0.005; OR = 2.79; 95% CI, 1.22–3.13). No mutation was detected in the Tudor domain of the TDRD5 in the studied patients. In conclusion, we provide evidence for association between genetic variation in the HIWI gene and idiopathic non-obstructive azoospermia in Iranian patients. Therefore, piRNA pathway genes variants can be considered as risk factors for male infertility.

Infertility is defined as the failure to make a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. According to the worldwide statistics, infertility affects 10–15% of couples and almost in the half of the cases, men are responsible1. Azoospermia is the most common reason for male infertility with a prevalence of 10–15% in infertile men and 1% of all men2. Although several diverse reasons are mentioned for azoospermia, the main reason is still unknown. The most of our knowledge about the possible link between a gene and male infertility is the result of the gene knockout studies in animal models and the only way to confirm this relationship is to study the candidate genes structure and function in human diseases1.

Recent studies have revealed piRNA pathway as a new essential pathway for spermatogenesis. The genes involved in this pathway are expressed abundantly and solely in germline cells. This class of non-coding RNAs forms a retrotransposon silencing complex in germline via binding to a different subtype of Argonaute proteins3-5. Many studies showed that piRNAs are crucial for the differentiation and specificity of male germ line. In addition to cutting and degradation, they can repress transposons by histone modifications and DNA methylation6-7. Although piRNAs are expressed both in testis and ovaries, only mutant male mice for these genes become sterile probably due to the overexpression of transposons in the germine6-9.

The pathway function depends on P-element Induced Wimpy testis proteins (PIWIIs) and Tudor domain-containing proteins (TDRDs). PIWIIs are the most important proteins in this pathway that play an important role in piRNA biogenesis and function. In humans, this subclass of Argonaute protein family includes HIWI, HIWT2, HIWI3 and HILL. Knockout studies in mice have revealed that silencing of these genes results in meiotic arrest and male sterility10-11. In addition, it has been suggested that single nucleotide polymorphisms

1Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2Faculty of Medicine, Semnan University of Medical Sciences, Tehran, Iran.
3Genomic Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Zeeba Kamaliyan and Sara Pouriamanesh contributed equally to this work. Correspondence and requests for materials should be addressed to R.M. (email: reza_mirfakhraie@yahoo.com)