

## **A Single Nucleotide Polymorphism within the Novel Sex-Linked Testis-Specific Retrotransposed *PGAM4* Gene Influences Human Male Fertility**

**Introduction and Objective:** Retrotransposition is a critical phenomenon associated with the racial revolution. In testis, this has created many functional genes and pseudogenes. Recently, some glycolytic enzymes in testis has been reported to be created by retrotransposition and associated with function of spermatozoa. PGAM4 (Phosphoglycerate mutase4) remain to be elucidated, although created by retrotrans position from PGAM1, glycolytic enzyme expressed in somatic tissues. Thus, we examine the expression of the PGAM4 and its association with male infertility.

**Materials and Methods:** RT-PCR and PCR were performed using total RNA of Human testis and human testis specific cDNA library to prove the transcription of PGAM4. The expression and localization of PGAM4 protein were analysed by immunoblotting and immunofluorescence. The direct sequencing of PCR-amplified PGAM4 from semen or blood of male infertile patients were performed to detect the mutations or SNPs associated with male infertility. Furthermore, the enzymatic assay of PGAM4 with the SNP was measured *in vivo* and *in vitro* using frozen semen of the patients carrying the SNP and tranfected cells with PGAM4 inserted expression vector.

**Results:** Using western blot analyses, we identified that PGAM4 is a functional retrogene that is expressed predominantly in the testes and is associated with male infertility. PGAM4 is expressed in post-meiotic stages, including spermatids and spermatozoa in the testes, and the principal piece of the axoneme and acrosome in ejaculated spermatozoa. We carried out the prevalence of SNPs in the coding region of phosphoglycerate mutase 4 (PGAM4) on the X chromosome. A case-control study revealed that 4.5% of infertile patients carry the G75C polymorphism, which causes an amino acid substitution in the encoded protein. Furthermore, an assay for enzymatic activity demonstrated that this polymorphism decreases the enzyme's activity both *in vitro* and *in vivo*, significantly.

**Conclusions:** These results suggest that PGAM4, an X-linked retrogene, is a fundamental gene in human male reproduction and the G75C polymorphism is associated with male infertility.