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Association of polymorphisms in *PATE1* gene with idiopathic asthenozoospermia in Sichuan, China

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ABSTRACT

Purpose: Idiopathic Asthenozoospermia (AZS) is a common symptom of male infertility described as reduced forward motility or absence of sperm motility. The *PATE1* is generally expressed in male genital tract and related to sperm development, maturation and fertilization. However, the single nucleotide polymorphisms (SNPs) of the PATE1 gene which contribute to AZS were still unknown. For this reason, the possible association between the single nucleotide polymorphisms of the *PATE1* gene and idiopathic asthenozoospermia was investigated in this research.
Methods: 108 idiopathic asthenozoospermia were screened by karyotype analysis, detection of Y microdeletions and mutations in 5 other genes from 140 clinical AZS. The sequence analyses of the PATE1 gene were conducted in 108 idiopathic asthenozoospermia and 106 fertile men with normospermic parameters in Sichuan, China.
Results: In this study, a total 108 patients without chromosomal abnormalities, Y microdeletions and selected genes mutation were confirmed. The 1423G (odds ratio [OR] 1.939, 95% confidence interval [CI] 1.320–2.848, P = 0.001) was found to be increased significantly in idiopathic asthenozoospermic patients compared with their fertile counterparts. This mutation substitutes a highly conserved glutamic to arginine at the position of the 47th amino acid which was shown to be located on the flank of the pleated sheet domain in *PATE1* protein by the 3D model given by the Protein Model Portal (PMP). Moreover, PolyPhen-2 analysis predicted that this variant was "probably damaging".
Conclusions: These results suggested that *PATE1* variant (A1423G) was probably one of the high risk genetic factors for idiopathic asthenozoospermia among males in Sichuan, China.

1. Introduction

Human infertility presents a prevalent as well as difficult problem which affects approximately 15% couples in the world. Male factors are responsible for an estimated half of human infertility cases (Zhang et al., 2016). Most infertile men showed a high incidence of dyszoospermia and sperm dysfunction with potential causes of environmental toxins or genetic variants (Song et al., 2015). The genetic abnormalities may be recognized in about 15% of male infertile cases (Ni et al., 2015). Azoospermia, oligozoospermia, asthenozoospermia (AZS) and teratospermia are the four major semen anomalies and are present in approximately 50% cases of couples with infertility and almost 90% of infertile males (Christova et al., 2002). Routine semen analysis is present to be the most important test in clinical diagnosis of infertility, including assessment of sperm motility, concentration and morphology. And the motility of sperm is considered as an important indicator of sperm function (Andrade-Rocha, 2003; Franken and Oehninger, 2012). Idiopathic AZS is a common symptom of male infertility described by reduced forward motility or absent sperm motility (A sperm <25%, or A + B sperm motility <50%, in fresh ejaculate, according to World Health Organization (2010) Guidelines) (Zuccarello et al., 2008a; Zuccarello et al., 2008b).

Idiopathic AZS might be due to complicated causes such as motility problems or increased level of sperm degradation.

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Decreased motility may be secondary to sperm dysfunction, cryptorchidism, orchitis, epididymitis, varicocele, obstruction of the vas deferens, endocrine hypogonadism, infectious diseases or genetic factors (chromosomal abnormalities, Y microdeletions and genes variation) (Zuccarello et al., 2008b; Navarro-Costa et al., 2010; Mcauliffe et al., 2012). To date, only a few genes have been found to be associated with idiopathic AZS in humans, such as Methylenetetrahydrofolate reductase (MTHFR), human β-defensin 1/2(DEFB 1/2), Heat shock transcription factor, Y-linked 1/2 (HSFY1/2) (Navarro-Costa et al., 2010; Gong et al., 2015), while most causes for idiopathic AZS are still unknown. However, emerging data suggested that environmental factors played important roles in male infertility caused by decreased motility of sperm, especially the environmental of male genital tract (Li et al., 2010). Recently, more than 6000 different proteins which are expressed in male genital system have been identified by proteomics studies, and a large proportion of them were detected to be expressed in the prostate and testis, which could because of what the spermatozoa produced by the testis are immature which a special protein-rich microenvironment provided by epididymis is necessary for their maturation and storage before ejaculation, in which proteins expressed were assigned to various function pathways, including apoptosis, cell cycle, meiosis, membrane trafficking and the process of capacitation (Martinez-Heredia et al., 2006; Oliva et al., 2008; Li et al., 2010). Among others, the protein of prostate and testis 1(*PATE1*) is an important member in the male genital tract proteins family, that *PATE1* is generally expressed in male genital tract, including testis, prostate, epididymis and seminal vesicle (Soler-Garcia et al., 2005). *PATE1* is a novel sperm-associated protein, with an important role in mammalian sperm maturation, which bounds to different sperm domains and currently thought to regulate the process of capacitation and was localized to a band-like pattern in the sperm head (Bera et al., 2002; Levitin et al., 2008). *PATE1* could exhibit phospholipase activity, which is important because phospholipases have been shown to influence a wide range of cellular activities such as inflammation, proliferation, apoptosis, carcinogenesis, and protection against microbial infection (Soler-Garcia et al., 2005). The phospholipase activity can be an important property of the *PATE1* protein given that the membranes of the mammalian spermatozoa undergo extensive plasma membrane remodeling during maturation in the epididymal duct (Anon, Soler-Garcia et al., 2005). *PATE1* is a highly expressed gene in the male genital tract that encodes a novel secreted sperm associated protein that may play crucial roles during sperm development, maturation and fertilization (Bera et al., 2002; Soler-Garcia et al., 2005; Levitin et al., 2008). In consideration the important biological function of *PATE1* and the molecular mechanism of the association between PATE1 and AZS has been reported recently, which makes it a good candidate gene for screening in AZS.

In this research, a screening in 108 idiopathic AZS patients [After screening of Karyotype analysis, detection of Y microdeletions and a comprehensive AZS related genes mutation screening, including MTHFR, DEFB1, DEFB2, HSFY1, HSFY2] and 106 fertile men as a control group, have been performed, in order to study the association polymorphisms of the PATE1 gene with idiopathic AZS infertile patients in Sichuan, China.

2. Materials and methods

2.1. Patients of AZS and the controls

In this study, 140 AZS patients and 106 controls were investigated from the Chinese population. Patients with AZS were collected from the Affiliate Reproductive Hospital Genitalia Hygiene Research Center (Sichuan, China) between March 2015 and Jan 2016. To screen the idiopathic AZS, patients with known diseases such as cryptorchidism, orchitis, epididymitis, varicocele, obstruction of the vas deferens, endocrine hypogonadism were excluded from the study. Patients with drug, alcohol, substance abuse, and tobacco use were also excluded. The controls were all fertility men who had fathered one or more healthy children without assisted reproduction. All control donors were enrolled from the same hospital where the patients were recruited. All participants were informed about the study according to a protocol that was approved by the Institutional Ethical Review boards of Sichuan University (Chengdu, China), and all gave their written consent.

2.2. Semen analysis

For AZS patients, at least three independent semen examinations were conducted and results were within the following range; the semen analyses for sperm concentration, motility and morphology were performed following the fifth edition of the World Health Organization (WHO) manuals. These isolated asthenozoospermic infertility patients had sperm concentration more than 20×10^6/ml; sperm motility: rapid forward progressive motile sperm (grade A <25%) and total progressive motile sperm (grades A + B < 50%) in fresh ejaculation according to the WHO (2010) criteria.

2.3. Karyotype analysis

Karyotyping was performed using standard G-banding. Briefly, peripheral blood lymphocytes were cultured for 72 h in RPMI-1640 with phytohemagglutinin and fetal bovine serum. Two hours before the completion of culturing, colcemid was added to the medium. G-banding of metaphase chromosomes was performed using Giemsa staining. At least 20 metaphase spreads were analyzed for each patient, and at least 50 metaphase spreads were analyzed to confirm abnormalities. The normalities were reserved for next study.

2.4. Extraction of genomic DNA

Total DNA of human spermatozoa was extracted using the EasyPure Blood Genomic DNA Kit (Transgen, Beijing, China). Briefly, the spermatozoa pellet was resuspended in sterile water and mixed with lysis solution containing 20 mg/ml proteinase K and Binding Buffer 3. Lysis was incubated at room temperature for 10 min. Lysates were added to centrifugal column to bind the DNA. Bound DNA was washed and then eluted from the centrifugal column. Quantification of the extracted genomic DNA was conducted by the spectrophotometry analysis. All of the DNA samples were stored at −20 °C until examination.

2.5. Detection of Y microdeletions and a comprehensive gene mutation screening

Multiplex polymerase chain reaction was performed to detect microdeletions in the Azoospermia Factor (AZF) region for patients with idiopathic asthenozoospermia. Using previous studies in the Chinese population and the diagnostic criteria of European Academy of Andrology, we chose 6 sequence-tagged site (STS) markers in the AZF region to detect microdeletions: sY86 and USP9Y in AZFa, sY127 and sY134 in AZFb, and sY254 and sY255 in AZFc (Navarro-Costa et al., 2010; Gong et al., 2015). And the MTHFR, DEFB1/2, HSFY1/2 genes were reported that they were relation with asthenozoospermia. The primers sets as described in Supplementary Table 1 and the products of polymerase chain reaction amplification were detected with agarose gel electrophoresis.