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ARTICLE

Polymorphism in *DNMT1* may modify the susceptibility to oligospermia

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Abstract As an important methyltransferase, *DNMT1* plays a key role in DNA methylation that is essential for normal spermatogenesis, which suggests that it may be involved in male infertility with spermatogenesis impairment. To explore the relationship between *DNMT1* and spermatogenesis impairment, polymorphic distributions of single-nucleotide polymorphisms (SNP) rs16999593, rs2228612 and rs2228611 in *DNMT1* were investigated in 342 infertile patients with idiopathic azoospermia or oligospermia and 232 fertile controls in a Chinese population. As a result, no significant differences in allele and genotype frequencies of the three SNP between total patients and controls were observed. However, after stratifying the patients, significant differences in allele and genotype frequencies were detected between oligospermia subgroup and control group. The frequencies of rs16999593 allele A (83.6% versus 77.6%, $P = 0.033$) and genotype AA (69.2% versus 59.0%, $P = 0.037$) and SNP rs2228611 genotype AA (18.4% versus 9.9%, $P = 0.016$) in patients with oligospermia were significantly higher than those in control group. These findings suggest that the polymorphism in *DNMT1* might be associated with oligospermia and could modify the susceptibility of oligospermia.

KEYWORDS: DNMT1, male infertility, oligospermia, polymorphism, SNP, spermatogenesis impairment

Introduction

Infertility is a common and complex reproductive health problem that affects approximately 10–15% of couples seeking to conceive, and in half of these couples the cause is male factor infertility (De Kretser and Baker, 1999). Spermatogenesis impairment is the most common disorder leading to male infertility and can be caused by genetic

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deficiency and environmental factors (Massart et al., 2012; Skakkebaek et al., 1994; Toshimori et al., 2004). Genetic deficiency was considered as one of the major aetiologies for spermatogenic impairment (Ferlin et al., 2006, 2007).

Spermatogenesis is a complex process, including proliferation and differentiation of the spermatogonia, meiosis and differentiation from round spermatids to spermatozoa, in which highly regulated expression of numerous genes are involved. DNA methylation can repress the expression of genes, which plays an important role in gene regulation during spermatogenesis (Dada et al., 2012; Hisano et al., 2003; Li, 2002). DNA methylation of certain genes is essential for normal spermatogenesis (Marchal et al., 2004; Rajender et al., 2011). It has been demonstrated that abnormal DNA methylation might lead to spermatogenesis impairment (Hartmann et al., 2006; Takashima et al., 2009). For instance, hypomethylation of DNA could disrupt the differentiation of germ cells into spermatocyte and lead to sperm count decrease (Kobayashi et al., 2007; Raman and Narayan, 1995). In addition, DNA methylation is also important for germ-cell-specific organization of chromatin required for meiosis and spermatogenesis (Oakes et al., 2007a,b). Therefore, it is speculated that the genes playing roles in DNA methylation may be implicated in spermatogenesis impairment and male infertility.

DNA methylation is a biochemical process where methyl groups are added from S-adenosyl-methionine to the 5′-position of cytosine residues of CpG dinucleotides, which is catalysed by DNA methyltransferases (DNMT). Three main DNA methyltransferases, including DNMT1, DNMT3A and DNMT3B, are responsible for DNA methylation and play crucial roles in establishing specific DNA methylation during gametogenesis (Biermann and Steger, 2007; Rodriguez-Osorio et al., 2010). DNMT1 methylates hemimethylated DNA and maintains already-established methylation patterns (Bestor and Ingram, 1983; Gruenbaum et al., 1982), whereas DNMT3A and DNMT3B act to establish de-novo methylation patterns (Lei et al., 1996; Okano et al., 1999). *DNMT1* is located at 19p13.2. It is expressed in all stages of human spermatogenesis including proliferation, meiosis, spermatids differentiation and in ejaculated spermatozoa (Marques et al., 2011), which indicates the importance of DNMT1 for spermatogenesis. Decreased expression of *DNMT1* in mouse germline stem cells could result in apoptosis and spermatogenic defects (Takashima et al., 2009). In humans, abnormal *DNMT1* expression was also observed in testis of patients with impaired spermatogenesis (Omisanjo et al., 2007). These data suggested that *DNMT1* is crucial for spermatogenesis and may play a role in spermatogenesis impairment and male infertility.

Since there is dearth of data about the effect of *DNMT1* on male infertility with spermatogenesis impairment, the current study selected three exonic single-nucleotide polymorphisms (SNP; rs16999593, rs2228612 and rs2228611) in *DNMT1* according to the SNP database (dbSNP) at NCBI and carried out a case–control study on the association between the polymorphisms of these SNP and male infertility with spermatogenesis impairment in a Chinese cohort, including 342 patients with idiopathic azoospermia or oligospermia and 232 controls, to explore the possible role of *DNMT1* in spermatogenesis impairment and male infertility.

Materials and methods

Subjects

The patient group included 342 patients with idiopathic azoospermia or oligospermia (sperm count $<15 \times 10^6$/ml) aged 25–42 years. Of these patients, 141 were azoospermic and 201 were oligospermic. For patients with oligospermia, the ejaculate volume, sperm concentration, sperm motility and sperm morphology were 1.75 ± 0.64 ml, 9.38 ± 4.89 million/ml, 65.15 ± 18.9% and 45.46 ± 15.3%, respectively. Patients with diseases known to affect spermatogenesis, including 42 with orchitis, eight with maldescensus of testis, 58 with varicocele and 61 with obstruction of vas deferens, were excluded. In addition, 121 patients with chromosomal abnormalities and microdeletions of the AZF region on the Y-chromosome were also excluded by chromosome analysis and corresponding molecular analysis respectively (Simoni et al., 2004). All patients underwent at least two semen analyses according to WHO guidelines (World Health Organization, 2010).

The control group consisted of 232 fertile men aged 26–45 years who had at least one offspring conceived without the use of assisted reproduction techniques. All participants of the study were of Han nationality, which makes up >90% of the Chinese population and informed approval was obtained from all of them. This study was approved by the Institutional Review Board of Dali University (IRB approval reference no. 2012–05–1, approved 5 October 2012).

Choice of SNP

According to the dbSNP and the genomic sequence of *DNMT1*, this study selected exonic SNP, including missense SNP and synonymous SNP with high frequency in the Asian population and near to the splicing site of heterogeneous nuclear RNA (hnRNA). As a result, three SNP (rs16999593, rs2228612 and rs2228611) were chosen.

PCR amplification

DNA was extracted from the peripheral blood leukocytes of patients and controls using a TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). Three pairs of primers were designed and synthesized to amplify DNA fragments containing the three SNP. For SNP rs2228612 and rs2228611 without restriction enzyme sites, mismatched primers were used to introduce restriction enzyme sites. The sequences of primers and the lengths of the PCR products are shown in Table 1. PCR amplification was carried out in a total volume of 25 μl containing about 100 ng genomic DNA, 200 μmol/l dNTP, 10 pmol each primer, 1.5 mmol/l $MgCl_2$, 1 U Taq polymerase and 2.5 μl of 10× PCR buffer (Takara, Shiga, Japan). The reaction cycle was denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 30 s, annealing at 54–58°C for 30 s (detailed in Table 1) and extension at 72°C for 40 s, with a final extra extension at 72°C for 5 min.