

## The single nucleotide polymorphism of *DMRT1* is associated with oligospermia.

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### Abstract

Male infertility is a common human reproductive defect that can be resulted from spermatogenesis impairment. *DMRT1* encodes a male-specific transcriptional regulator and is important for human spermatogenesis. This study was designed to explore the relationship between the Single Nucleotide Polymorphism (SNP) of *DMRT1* and oligospermia. Two SNPs namely rs3739583 and rs4742608, were screened in 212 infertile men with oligospermia and 217 normal controls by using a PCR-RFLP assay. The genotyping data showed that the frequencies of allele A (64.9% vs. 57.8%,  $p=0.041$ , OR=1.346, 95% CI 1.021~1.773) and genotype AA (39.1% vs. 29.0%,  $p=0.027$ , OR=1.604, 95% CI 1.073~2.398) of SNP rs4742608 were significantly higher in the infertile patients with oligospermia compared with the normal controls. Our data suggest that the SNP rs4742608 in *DMRT1* is associated with oligospermia and may affect the genetic susceptibility to human oligospermia.

**Keywords:** *DMRT1*, Single nucleotide polymorphism (SNP), Male infertility, Spermatogenesis, Oligospermia.

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### Introduction

Male infertility is a common human reproductive defect that accounts for about half of infertile cases [1,2]. A number of genetic factors are involved in male infertility, among which spermatogenesis impairment is one of the most common causes [3-5]. Spermatogenesis is a complex and highly regulated process involving the expression of thousands of genes and the alterations of these genes may cause spermatogenesis impairment [6-14]. Thus, the genes involved in spermatogenesis may be important candidates for spermatogenesis impairment and male infertility. Over the last decades, some genetic abnormalities leading to spermatogenesis impairment, such as chromosomal aberrations, deletions in the AZF regions of Y chromosome and mutations of certain genes, have been identified [15,16]. However, these known genetic abnormalities can only explain 25-30% cases of spermatogenesis impairment [17]. More genes related to spermatogenesis impairment remain to be identified.

Human doublesex and *mab-3* related transcription factor 1 (*DMRT1*) is localized on chromosome 9p24.3 and is homologous to the male sexual regulatory gene *mab-3* from the nematode *Caenorhabditis elegans* and the *Drosophila melanogaster* sex regulatory gene double sex [18]. This gene encodes a male-specific transcriptional regulator containing a conserved zinc finger-like DNA-binding domain (DM domain) and a nuclear localization signal, and plays a key role in sex determination and differentiation. *DMRT1* is mainly expressed in testis including sertoli cells, spermatogonia and spermatocytes, suggesting that *DMRT1* may play an important role in human spermatogenesis [18,19]. In recent year, accumulating evidences demonstrate that human *DMRT1* may be important for gametogenesis and male reproduction [20]. In mice, *DMRT1* is required for the progression of mitosis and meiosis of male germ cells and deletion of *DMRT1* results in spermatogenesis failure and male infertility, indicating that *DMRT1* is critical for spermatogenesis of mice [21-24].

Currently, little is known about whether *DMRT1* is involved in human spermatogenesis impairment. In this study, we selected two common exonic SNPs (rs3739583 and rs4742608) of *DMRT1* from dbSNP and investigated the relationship between these two SNPs and human spermatogenesis in 212 infertile patients with oligospermia and 217 controls in Chinese population.

## Material and Methods

212 infertile patients with idiopathic oligospermia (sperm number less than  $15 \times 10^6/\text{ml}$ ), aged from 24-41 y old, were enrolled in this study. All patients underwent semen analyses at least twice according to the WHO guidelines [25]. Patients with diseases known to affect spermatogenesis, such as orchitis, maldescensus of testis, varicocele and obstruction of vas deferens, were excluded. In addition, patients with chromosomal abnormalities and microdeletions of AZF region on Y chromosome were also excluded after genetic analysis [26]. 217 fertile men aged from 24-48 y old with normal semen profile, were served as normal controls. These men had at least one offspring without technical assistance of reproduction.

This study was approved by the Institutional Review Board of Affiliated Hospital of Dali University and informed approval was obtained from all participants.

## PCR amplification

DNA was extracted from the peripheral blood leucocytes of patients and controls using a TIAN amp Genomic DNA Kit (TIANGEN, Beijing, China). Two pairs of primers were designed to amplify the DNA fragments including the SNPs rs3739583 and rs4742608. Because there was no restriction enzyme site for SNP rs4742608, the forward primer with a mismatched nucleotide was used to generate a restriction site *AccI*. The sequences of primers and the expected size of PCR products are shown in Table 1. PCR was performed in a total volume of 25  $\mu\text{l}$  containing 100 ng of genomic DNA, 200  $\mu\text{mol/L}$  dNTPs, 1.5 mmol/L  $\text{MgCl}_2$ , 10 pmol of each primer, 2.5  $\mu\text{l}$  of  $10 \times$  PCR buffer and 1 U Taq polymerase (Takara, Shiga, Japan). The PCR running condition was: 94°C for 5 min, 94°C for 30 s, 58°C for 30 s, 72°C for 40 s, 35 cycles, and 72°C for 5 min.

**Table 1.** Primers, size of PCR product, restriction enzymes and size of alleles for RFLP analysis.

Primer sequence*	Size	Enzyme	Sizes of alleles
rs3739583	325 bp	<i>TaqI</i>	Allele T: 202 bp+123 bp Allele A: 325 bp
F: 5'-TTCATCCCTCGCAGCAGTCT-3' R: 5'-AGGCGTAGCCGTGGTTCCT-3'			
rs4742608	260 bp	<i>AccI</i>	Allele A: 233 bp+27 bp Allele G: 260 bp
F: 5'-GAAACTAGTCTAAAAAATTCATTGGTCT-3' R: 5'-CTAAAACCACTGGTGGATGA-3'			

\*Base in bold indicates a mismatch to create the restriction site.

## Genotyping

A Restriction Fragment Length Polymorphism (RFLP) assay was performed to genotype SNP rs3739583 and rs4742608. PCR products were digested overnight with corresponding restriction enzymes (Fermentas, Vilnius, Lithuania) according to the manufacturer's protocols and then electrophoresed on a 3% agarose gel. Restriction enzymes and the size of digested fragments of alleles were shown in Table 1. The genotypes were further confirmed by direct sequencing of the PCR products.

## Statistical analysis

The allele and genotype frequencies of rs3739583 and rs4742608 in patients and controls were manually quantified. The Hardy-Weinberg equilibrium was tested by using Hardy-Weinberg equilibrium calculator [27]. The differences in allelic and genotypic frequencies of the two SNPs between patients and controls were assessed by chi-square test. Odds Ratios (ORs), 95% Confidence Intervals (CIs) and specificity were

calculated by using unconditional logistic regression analyses. The difference between two groups was considered to be statistically significant if the p value was smaller than 0.05. All statistical analyses were performed by using IBM SPSS 19.

## Results

The polymorphic distributions of SNPs rs3739583 and rs4742608 in *DMRT1* were analyzed for 212 infertile patients with oligospermia and 217 fertile controls by using a PCR-RFLP assay. The frequency distributions of the allele and genotype of the two SNPs were shown in Table 2. The genotypic distributions of the two SNPs were consistent with the Hardy-Weinberg equilibrium in both patients and normal controls (data not shown). As shown in Table 2, there was no significant difference in frequencies of allele and genotype of SNP rs3739583 between patients and controls.

The significant difference in the polymorphic distribution of rs4742608 was detected between the patients with oligospermia and controls. The frequencies of allele A (64.9%

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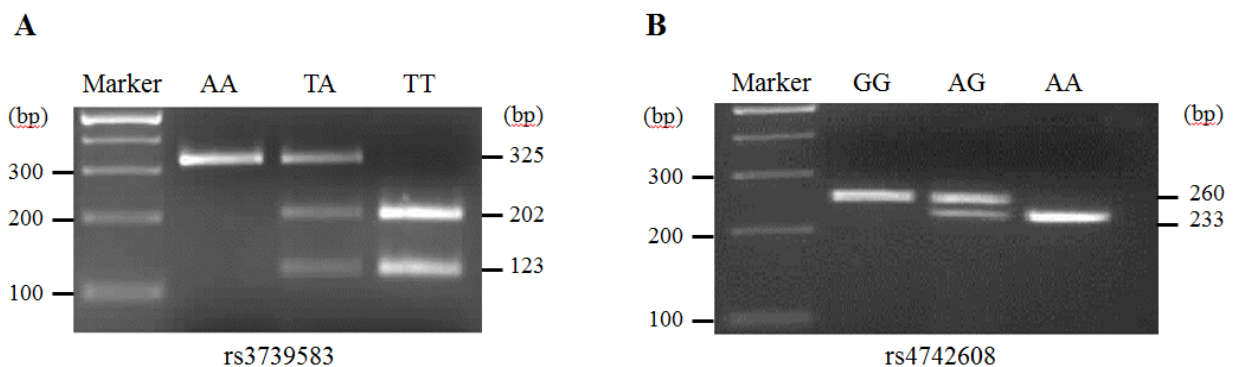
vs. 57.8%, p=0.044, OR=1.346, 95% CI 1.021~1.773) and genotype AA (39.1% vs. 29.0%, p=0.027, OR=1.604, 95% CI 1.073~2.398) at rs4742608 locus were significantly higher in the patients with oligospermia than those in the controls.

Representative genotyping images for the SNPs rs3739583 and rs4742608 in *DMRT1* were shown in Figure 1. The genotypes of AA, TA, TT and GG, AG, AA were displayed for rs3739583 and rs4742608, respectively.

**Table 2.** The allele and genotype frequency of the SNPs in patients and controls.

SNP	Genotype/Allele	Controls (n=217)	Patients (n=212) total		P value (OR, CI% 95) <sup>a</sup>
			Azoospermia	Oligozoospermia	
rs3739583	TT	0.273 (59)	0.264 (56)		0.943 (0.961, 0.627~1.474)
	TA	0.483 (105)	0.533 (113)		0.357 (0.833~1.779, 1.218)
	AA	0.244 (53)	0.203 (43)		0.361 (0.787, 0.499~1.242)
	T	0.514 (223)	0.531 (225)		0.671 (0.935, 0.715~1.222)
	A	0.486 (211)	0.469 (199)		
rs4742608	AA	0.290 (63)	0.391 (84)		0.027 (1.604, 1.073~2.398)
	AG	0.576 (125)	0.505 (107)		0.166 (0.75, 0.513~1.100)
	GG	0.134 (29)	0.104 (21)		0.334 (0.713, 0.393~1.294)
	A	0.578 (251)	0.649 (275)		0.041 (1.340, 1.021~1.773)
	G	0.422 (183)	0.351 (149)		

Values are shown as frequency (No. of individuals). <sup>a</sup>Controls versus patients.



**Figure 1.** Genotyping was performed for the SNPs rs3739583 and rs4742608 by a PCR-RFLP assay. (A) Genotyping for the SNP rs3739583. Three different genotypes, AA, TA and TT, were displayed. The expected sizes were as follows: AA, 325 bp; TT, 202 bp and 123 bp; (B) Genotyping for the SNP rs4742608. Three different genotypes, GG, AG and AA, were displayed. The expected sizes were as follows: GG, 260 bp, AA 233 bp and 27 bp (The products of 27 bp in genotype AA and AG of rs4742608 were not shown in the figure). M: DNA Marker.

**Discussion**

The genetic causes of spermatogenesis impairment are very complex and the majority of genes involved in spermatogenesis impairment remains unknown. By using the gene knockout technique, hundreds of mouse models with spermatogenesis impairment have been established and a number of candidate genes for human spermatogenesis impairment were identified [28,29]. Given the genetic background difference between mouse and human, whether these candidate genes also regulate spermatogenesis in human needs to be further investigated. The polymorphisms or variations in candidate genes are potential risk factors for

human spermatogenesis impairment. Thus, the polymorphic analysis in candidate genes represents an interesting area of research to investigate the relationship between the candidate gene and spermatogenesis impairment in human [17,30].

*DMRT1* is an important candidate gene for spermatogenesis impairment and its role in spermatogenesis impairment in mice has been proven [24]. In recent years, the role of this gene in human spermatogenesis impairment has caught more attention. A recent study showed that the coding sequence deletion of *DMRT1* was detected in some men with azoospermia, suggesting that the genetic abnormality of *DMRT1* is associated with azoospermia and affects spermatogenesis in

human [31]. Thus, it was speculated that the polymorphism of *DMRT1* might be also involved in human oligospermia. To test this hypothesis, we investigated the polymorphic distributions of the SNPs rs3739583 and rs4742608 in *DMRT1* in patients with oligospermia and normal controls in this study. As the result shown, the polymorphic distribution of SNP rs3739583 had no significant difference between patients with oligospermia and normal controls, suggesting that the polymorphism of SNP rs3739583 is not associated with oligospermia and this SNP may not affect the susceptibility to oligospermian. However, the allelic frequency and genotype of rs4742608 had significant difference between the patients with oligospermian and the controls. The frequencies of allele A and genotype AA were significantly higher in patients than those in controls, indicating that the polymorphism of SNP rs4742608 is associated with oligospermia, and genotype AA may increase the risk of oligospermia (OR=1.6). These results suggest that SNP rs4742608 may affect the susceptibility to oligospermia. The SNP rs4742608 is located within the 3'-untranslated region (3' UTR) of *DMRT1*. Thus, it is possible that this SNP affects *DMRT1* protein translation and then causes abnormal spermatogenesis. Of course, whether this SNP does really affect the translation of *DMRT1* needs to be further investigated. It cannot be excluded that the SNP rs4742608 is only a genetic marker of oligospermia, which is in linkage disequilibrium with other locus that plays a role in oligospermia.

## Conclusion

In this study, we first report the relationship between the polymorphism of *DMRT1* and human oligospermia. Our results indicated that polymorphism of SNP rs4742608 in *DMRT1* is associated with oligospermia and may affect the susceptibility to oligospermia in Chinese population, suggesting that *DMRT1* may be involved in human spermatogenesis impairment. However, our findings need to be validated in further studies with larger size of sample and other ethnic populations, since the sample size in this study is limited and also restricted to Chinese population.

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## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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