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The polymorphism G4C14-to-A4T14 in p73 gene may affect the susceptibility to male infertility with severe spermatogenesis impairment in Chinese population

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ABSTRACT

Objective: The aim of this study was to explore the association between the polymorphism G4C14-to-A4T14 in the p73 gene and male infertility with severe spermatogenesis impairment in Chinese population.
Study design: Three hundreds and one infertile patients with severe spermatogenesis impairment (including azoospermia and severe oligospermia) and 252 fertile men were recruited in this study. The polymorphism G4C14-to-A4T14 in the p73 gene was genotyped using polymerase chain reaction and restriction fragment length polymorphism assay. The differences in allelic and genotypic frequencies between patients and controls were evaluated by chi-square test.
Results: The frequency of allele AT (28.9% vs. 22.4%, $P = 0.017$, OR = 1.41, 95% CI = 1.07–1.85) in patients with severe spermatogenesis impairment was significantly higher than that in controls, whereas the genotype GC/GC was significantly decreased in patients compared with controls (48.5% vs. 59.1%, $P = 0.048$, OR = 0.65, 95% CI = 0.46–0.91).
Conclusion: The findings of this study suggested that the polymorphism G4C14-to-A4T14 in p73 gene might be associated with severe spermatogenesis impairment and could affect the susceptibility to male infertility with severe spermatogenesis impairment in Chinese population.

Introduction

Male infertility is account for half of human infertility which affects approximately 10–15% childbearing couples [1]. The most causes for male infertility is spermatogenesis impairment in which a myriad of genetic factors involved [2–4]. Spermatogenesis is a complex process highly regulated by numerous of genes and the abnormality of these genes may lead to spermatogenesis impairment and male infertility [5,6]. Thus, it is importance for understanding the genetic etiology of spermatogenesis impartment and male infertility to investigate the genes involved in spermatogenesis.

The p73 gene is located on chromosome 1p36-33 and can be transcribed from two alternative promoters to produce two major isoforms, namely TAp73 with the TA domain and ΔNp73 without the TA domain [7,8]. The p73 protein is a member of p53 family of transcription factors, which has structural and functional homology with p53 [9]. Like p53, the p73 activates the transcription of p53-responsive genes in a p53-like manner and plays roles in cell cycle control, apoptosis, DNA repair and inhibits cell growth [10–12]. Besides its p53-like functions, the p73 also exhibits distinctive functions in neuronal development and differentiation, metabolic control, and spermatogenesis [13]. In recent years, the accumulating data has suggested that TAp73 may play important roles in several critical cellular activities during spermatogenesis. For example, TAp73 (1) is essential for germ cell adhesion and maturation in testis [14]; (2) controls the expression of genes involved in spermatogenesis, and steroidogenesis [15]; (3) regulates the spindle assembly checkpoint to ensure the progression of meiosis and the accuracy of chromosomal segregation [16], and (4) participates in male germ cells apoptosis[17]. In TAp73 knockout mice, the defect of TAp73 will results in severe spermatogenesis impairment and male infertility, which provides evidence that the p73 gene is required for maintaining normal spermatogenesis and male fertility in mice [15]. Therefore, it is

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likely that the variations of the p73 gene may be involved in spermatogenesis impairment and male infertility in human.

Since there is lack of the data about the effect of the p73 gene on human spermatogenesis impairment and male infertility, we carried out a case-controls study on the association between the common functional polymorphism G4C14-to-A4T14 and male infertility with severe spermatogenesis impairment (including azoospermia and svevre oligospermia) in Chinese population to preliminarily explore the possible relationship between the p73 gene and spermatogenesis impairment and male infertility in human.

Materials and methods

Subjects

The patient group consisted of 301 patients with severe spermatogenesis impairment including 194 infertile men azoospermia and 107 with severe oligospermia (sperm count less than 5×10^6) aged from 25 to 40 years. Patients having 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 by chromosome analysis and corresponding molecular analysis respectively [18]. All patients underwent at least two semen analyses according to WHO guidelines [19]. The control group included 252 fertile men with normal semen profiles aged from 26 to 45 years. All participants of the study are of Han nationality that makes up more than 90% of Chinese population and informed approval was obtained from all of them. This study was approved by the Institutional Review Board of Dali University.

PCR amplification

Only the polymorphism G4C14-to-A4T14 in the p73 gene was investigated in this study. DNA was extracted from the peripheral blood leucocytes of patients and controls using a TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). Primers 5′-CAGGAGGACAGAGCACGAGTT-3′ and 5′-TGATGAGGGTGGCTAAGGCTA-3′ were used to amplify a 433 bp fragments including the polymorphism G4C14-to-A4T14 in the p73 gene. PCR amplification was carried out in a total volume of 25 μL containing about 100 ng of genomic DNA, 200 μmol/L dNTPs, 10 pmol of each primer, 1.5 mmol/L $MgCl_2$ and 1U Taq polymrease and 2.5 μL of 10 × PCR buffer (Takara, Shiga, Japan). The reaction profile was: predenaturation at 94 °C for 5 min followed by denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 40 s for 35 cycles, with a final extra extension at 72 °C for 5 min.

Genotyping

A restriction fragment length polymorphism (RFLP) assay was used to genotype the polymorphism G4C14-to-A4T14 in the p73. PCR products were digested overnight with restriction enzyme *StyI* (Fermentas, Vilnius, Lithuania) according to the manufacturer protocols and then analyzed by electrophoresis on a 3% agarose gel. The wild type homozygote (genotype GC/GC), heterozygote (genotype GC/AT) and mutant homozygote (genotype AT/AT) showed one band (433 bp), three bands (433,364 and 69 bp) and two bands (364 and 69 bp), respectively, because allele AT produced a cut site for *StyI*. The genotypes were further confirmed by DNA sequencing of PCR products of some samples.

Statistical analysis

The allele and genotype frequencies of the polymorphism G4C14-to-A4T14 in patients and controls were calculated by counting. The Hardy–Weinberg equilibrium was tested using Hardy–Weinberg equilibrium calculator [20]. The differences in allelic and genotypic frequencies of the polymorphism G4C14-to-A4T14 between patients and controls were evaluated by chi-square test and the level of significance was set at $P < 0.05$.

Results

The polymorphic distribution of the polymorphism G4C14-to-A4T14 in the p73 gene was investigated using PCR-RFLP assay in 301 infertile patients with severe spermatogenesis impairment and 252 fertile controls. The frequency distributions of allele and genotype of the polymorphism G4C14-to-A4T14 are summarized in Table 1. The distributions of genotypes of the polymorphism G4C14-to-A4T14 were in accordance with the Hardy–Weinberg equilibrium (data not shown) in both patients and controls (data not shown). As from Table 1, the significant differences in allele AT and genotype GC/GC were observed between patients and controls. The frequency of allele AT (28.9% vs. 22.4%, $P = 0.017$, OR = 1.41, 95% CI = 1.07–1.85) in patients was significantly higher than that in controls, whereas the genotype GC/GC was significantly decreased in patients compared with controls (48.5% vs. 59.1%, $P = 0.048$, OR = 0.65, 95% CI = 0.46–0.91).

The representative results of genotyping for the polymorphism G4C14-to-A4T14 in the p73 gene by electrophoresis and DNA sequencing of genotypes are shown in Figs. 1 and 2, respectively.

Discussion

As an important transcription factor encoded by the p73 gene, the p73 plays key roles in many biology activities, such as tumorigenesis, neuronal development and differentiation, metabolic control, and male and female reproduction [13,15,21,22], suggesting that the variations or polymorphisms of the p73 gene may be associated with different diseases. In recent years, the effects of the common functional polymorphism G4C14-to-A4T14 in the p73 gene on cancers were extensively investigated. Some Epidemiologic studies have indicated that the polymorphism G4C14-to-A4T14 in the p73 gene may affect the risks of different types of cancer [23–26]. However, whether the polymorphism G4C14-to-A4T14 in the p73 gene is associated with spermatogenesis impairment and male infertility is unknown. Given the critical roles of p73 in maintaining normal spermatogenesis and male fertility [15], it is reasonable speculated that the polymorphism G4C14-to-A4T14 in the p73 gene may also affect the susceptibility to spermatogenesis impairment and male infertility.

Table 1
Allele and genotype frequencies of the polymorphism G4C14-to-A4T14 in patients with severe spermatogenesis and controls.

	Controls ($n = 252$)	Patients ($n = 301$)	OR, 95% CI, P value[a]
Genotypes			
GC/GC	0.591(149)	0.485(146)	0.65, 0.46–0.91, 0.048[b]
GC/AT	0.369(93)	0.452(136)	NS
AT/AT	0.040(10)	0.063 (19)	NS
Alleles			
GC	0.776(391)	0.711(428)	
AT	0.224(113)	0.289(174)	1.41, 1.07–1.85, 0.017

Values are frequency (no. of individuals). The number of alleles is based on the genotype. NS = not statistically significant.
[a] Controls versus patients.
[b] Corrected P value.