Association of common SNP rs1136410 in PARP1 gene with the susceptibility to male infertility with oligospermia

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Abstract

Purpose This study aims to explore possible associations between polymorphisms of common SNP rs1136410 and rS1805405 in PARP1 gene and male infertility with spermatogenesis impairment.

Methods The polymorphic distributions of SNP rs1136410 and rS1805405 were investigated by polymerase chain reaction and restriction fragment length polymorphism analysis in a Chinese cohort including 371 infertile patients with idiopathic azoospermia or oligospermia and 231 controls.

Results Significant differences in the frequencies of allele and genotype of SNP rs1136410 were observed between patients with oligospermia and controls. The allele C (46.3 % vs. 36.4 %, $P=0.003$) and genotype CC (22.6 % vs. 13.4 %, $P=0.014$) significantly increased, whereas genotype TT (30 % vs. 40.7 %, $P=0.021$) significantly decreased in patients with oligospermia compared with controls at this SNP locus.

Conclusions These results indicated that genotype CC of SNP rs1136410 may increase the risk of oligospermia and genotype TT of rs1136410 may have some protective effect from

oligospermia, suggesting that the polymorphism of SNP rs1136410 in PARP1 gene may modify the susceptibility to male infertility with oligospermia.

Keywords PARP1 · Polymorphism · Spermatogenesis impairment · Male infertility · Oligospermia

Introduction

Infertility is a worldwide reproductive health problem in humans. It has been estimated that about 10–15 % of child-bearing couples suffer from infertility and approximately 50 % of infertile couples are due to male infertility [1]. Spermatogenesis impairment is the most common cause of male infertility, in which many genetic factors have been implicated [2–5]. Spermatogenesis is a unique and complex developmental process regulated by many genes [6] and their mutations or other alterations that may cause spermatogenesis impairment and male infertility [7].

Poly (ADP-ribose) polymerases (PARP) gene family encodes a group of enzymes responsible for catalyzing poly(ADP-ribosyl)ation which plays diverse roles in a series of cellular biological processes, such as DNA damage detection and repair, chromatin modification, cell differentiation, transcription and apoptotic cell death [8–10]. In the last decade, the roles of PARP in spermatogenesis has received much attention. Thus, mounting evidence suggests many roles for PARP family members in male germ cells during spermatogenesis [11, 12].

PARP1, the most abundant member of PARP family, is responsible for the major (85 %–90 %) of PARP activity in cells and highly conserved during evolution [13, 14]. PARP1

Capsule The results of this study suggested that polymorphism of SNP rs1136410 in PARP1 gene may modify the susceptibility to male infertility with oligospermia.

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gene is located at chromosome 1q42.12. It is highly expressed in testis, predominantly in cells of the testicular germ line but not in somatic testicular cells [15, 16]. PARP1 mRNA and protein exhibited a high expression level in primary spermatocytes undergoing meiosis, round spermatids and elongating spermatids both in mice and human, implying that PARP1 gene is essential for regulating meiosis, differentiation and maturation of male germ cell [16, 17]. During spermatogenesis, PARP1 gene plays a key role in repairing DNA damage and maintaining the DNA integrity of germ cell [12, 16, 18], which is vital to normal spermatogenesis and male fertility [11]. In addition, PARP1 is also play an important role in chromatin remodeling required for spermatogenesis [16, 19–21]. These data suggested that PARP1 gene is a crucial gene for spermatogenesis. Therefore, it is reasonable speculated that PARP1 gene may be involved in spermatogenesis and its mutation or polymorphism may modify the susceptibility to spermatogenesis impairment in human.

Although the available data have suggested that PARP1 gene may be implicated in spermatogenesis impairment, there is dearth of the data on the effect of PARP1 gene on human spermatogenesis impairment and male infertility. To redress the deficiencies in this field, this study selected two common single nucleotide polymorphism (SNP) loci (rs1136410 and rS1805405) in PARP1 gene and carried out a case–control study on the association between the polymorphisms of the two SNPs and male infertility with spermatogenesis impairment to explore the possible the role of PARP1 gene in spermatogenesis impairment and male infertility.

Subjects and methods

Subjects

Three hundreds and seventy-one infertile patients were recruited as cases from the Center of Reproductive Medicine, Affiliated Hospital of Dali University, including 141 men with idiopathic azoospermia and 230 with oligospermia (sperm count less than $15 \times 10^6/\text{ml}$). All patients underwent at least two semen analyses according to WHO guidelines [22]. 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 [23]. Two hundreds and thirty-one fertile men with normal semen profile who had at least one offspring conceived without the use of assistant reproduction technique were selected as controls. 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.

Methods

PCR amplification

DNA was extracted from the peripheral blood leucocytes of patients and controls using a TIANamp Genomic DNA Kit (TIANGEN, Beijing, China). Two primers were designed to amplify the fragments including the SNP rs1136410 and rS1805405. For SNP rs1136410 without restriction enzyme site, a mismatched reverse primer was used to introduce a site of restriction enzyme *Hin*6I. The sequences of primers and the lengths of the PCR products analyzed are shown in Table 1. PCR amplification was carried out in a total volume of 25 μl containing about 100 ng of genomic DNA, 200 $\mu\text{mol/L}$ dNTPs, 10 pmol of each primer, 1.5 mmol/L MgCl_2 and 1 U Taq polymerase and 2.5 μl of $10 \times \text{PCR}$ buffer (Takara, Shiga, Japan). The reaction profile was: predenaturation at 94 °C for 5 min followed by denaturation at 94 °C for 30s, annealing at 55 °C for 30s, extension at 72 °C for 40s for 35 cycles, with a final extra extension at 72 °C for 5 min.

Genotyping

Genotyping for SNP rs1136410 and rS1805405 was carried out using a restriction fragment length polymorphism (RFLP) assay. PCR products were digested overnight with corresponding restriction enzymes (Fermentas, Vilnius, Lithuania) according to the manufacturer protocols and then analyzed by electrophoresis on a 3 % agarose gel. Restriction enzymes and the length of digested fragments are shown in Table 1. The genotypes were further confirmed by DNA sequencing of PCR products of some samples.

Statistical analysis

The allele and genotype frequencies of the SNP rs1136410 and rS1805405 in patients and controls were calculated by counting. The Hardy-Weinberg equilibrium was tested using Hardy-Weinberg equilibrium calculator [24]. The differences in allelic and genotypic frequencies of the two SNPs between patients and controls were evaluated by chi-square test and the level of significance was set at $p < 0.05$.

Results

The polymorphism distributions of SNP rs1136410 and rS1805405 in PARP1 gene were investigated using PCR-RFLP assay in 371 infertile patients with spermatogenesis

Table 1 Primers sequence, products size of PCR, restriction enzyme and length of digested fragments for RFLP analysis

Primer sequence	Annealing temperature	PCR products size	Restriction enzyme	Digested fragment lengths
rs1136410 F:5' –TTCCAGAAAGTCCTTATGAGC- 3' R:5'-TCGATGTCAGCAGGTTGTCAAGCATTG <u>C</u> - 3 ^a	55 °C	220 bp	Hin6I	Allele C: 189 bp+31 bp Allele T : 220 bp
Rs1805405 F: 5'- TTCTAAAGTGTGGGAGGGGC -3' R:5'- CCTTGCTACCAATCCATCCT -3'	55 °C	335 bp	HinfI	Allele C: 296 bp +39 bp Allele A: 335 bp

^a Underlined base indicates a mismatch to create the restriction site

impairment and 231 fertile controls. The distributions of allele and genotype of the two SNPs are listed in Table 2. The distributions of genotypes of the two SNPs were in accordance with the Hardy–Weinberg equilibrium both in patients and controls (data not shown). As shown in Table 2, there were no significant differences in the frequencies of allele and genotype of SNP rs1805405 between patients and controls.

However, the frequency of allele C [43.8 % vs. 36.4 %, $P=0.013$, odds ratio (OR)=1.36, 95 % confidence interval (CI)=1.074–1.732] was significantly higher and the frequency of genotype TT (32.1 % vs. 40.7 %, $P=0.039$, OR=0.69, 95 % CI=0.489–0.968) was significantly lower in total patients than those in controls at SNP rs1136410 locus. After stratifying patients into azoospermia and oligospermia subgroup, the significant differences in allele and genotype distribution of this SNP were only observed between patients with oligospermia and controls. The allele C (46.3 % vs. 36.4 %, $P=0.003$, OR=1.51, 95 % CI=1.159–1.964) and genotype CC (22.6 % vs. 13.4 %, $P=0.014$, OR=1.89, 95 % CI=1.156–3.072) significantly increased, whereas genotype TT (30 % vs. 40.7 %, $P=0.021$, OR=0.625, 95 %

CI=0.425–0.918) significantly decreased in patients with oligospermia compared with controls.

The representative results of genotyping for SNP rs1136410 and rs1805405 in PARP1 gene by electrophoresis were shown in Fig. 1.

Discussion

As an important nuclear enzyme encoded by PARP1 gene, PARP1 has important functions in the maintenance of genomic integrity, the regulation of chromatin structure and transcription, the establishment of DNA methylation patterns as well as cell death pathways, which contributes to many physiological and pathological outcomes [25, 26]. In recent years, the association of PARP1 gene with human diseases has been investigated extensively. Epidemiological studies have indicated that the variations or polymorphism of PARP1 gene can affect the risk of some human disease, such as cancers, tourette syndrome, Alzheimer's disease, asthma and allergic rhinitis [27–31]. However, the effect of PARP1 gene on human

Table 2 The allele and genotype frequencies of the two SNPs studied in infertile patients and controls

SNP	Genotype/Allele	Controls (n=231)	Patients			P value ^a		
			Total (n=371)	Azoospermia (n=141)	Oligozoospermia (n=230)	[1]	[2]	[3]
rs1136410	TT	0.407 (94)	0.321 (119)	0.355 (50)	0.300 (69)	0.039	0.371	0.021
	TC	0.459 (106)	0.482 (179)	0.495 (70)	0.474 (109)	0.631	0.550	0.818
	CC	0.134 (31)	0.197 (73)	0.150 (21)	0.226 (52)	0.062	0.808	0.014
	T	0.636 (294)	0.562 (417)	0.603 (170)	0.537 (247)			
	C	0.364 (168)	0.438 (325)	0.297 (112)	0.463 (213)	0.013	0.355	0.003
rs1805405	CC	0.398 (92)	0.426 (158)	0.404 (57)	0.439 (101)	0.560	0.996	0.427
	CA	0.437 (101)	0.412 (153)	0.397 (56)	0.422 (97)	0.607	0.515	0.809
	AA	0.165 (38)	0.162 (60)	0.199 (28)	0.139 (32)	1.000	0.487	0.529
	C	0.617 (285)	0.632 (469)	0.603 (170)	0.650 (299)			
	A	0.383 (177)	0.368 (273)	0.397 (112)	0.350 (161)	0.639	0.761	0.330

Values are frequency (no. of individuals). The number of alleles is based on the genotype

The bold entries emphasize that P value <0.05

^a Controls versus total infertile patients [1], azoospermia [2] and oligozoospermia [3]

Fig. 1 The genotyping results of SNP rs1136410 and rs1805405 by electrophoresis (31 bp band for genotype CC and TC of rs1136410 and 39 bp band for genotype CC and AC of rs1805405 not shown in figure). M: DNA size marker

spermatogenesis impairment and male infertility remains unknown. In view of the key roles of PARP1 in meiosis, differentiation, maintaining the DNA integrity and chromatin remodeling of male germ cell [11, 12, 16], it is likely that the variations of PARP1 gene play a role in the spermatogenesis impairment.

In this study, the possible associations between SNP rs1136410 and rs1805405 in PARP1 gene and male infertility with spermatogenesis impairment were investigated in Chinese infertile patients with azoospermia or oligospermia and fertile controls to test whether the polymorphism of PARP1 gene is also involved in human spermatogenesis impairment. As a result, there were no significant differences in frequencies of allele and genotype of SNP rs1805405 between patients with spermatogenesis impairment and controls, which indicated that this SNP in PARP1 gene is not associated with spermatogenesis impairment.

However, the significant differences in frequencies of allele and genotype between patients with oligospermia and controls were detected at SNP rs1136410 locus. The frequencies of allele C and genotype CC were significantly higher in patients with oligospermia than those in controls, suggesting that allele C and genotype CC were associated with oligospermia and genotype CC may be a risk factor of oligospermia ($OR=1.89$). Meanwhile, the genotype TT of SNP rs1136410 significantly decreased in patients with oligospermia compared with controls, which indicated that there is a negative association between the genotype TT and oligospermia, and that genotype TT may have some protection effect from oligospermia ($OR=0.625$). These results suggested that the polymorphism of SNP rs1136410 in PARP1 gene may modify the susceptibility to male infertility with oligospermia.

Although this study revealed that the polymorphism of SNP rs1136410 in PARP1 gene may affect the susceptibility to male infertility with oligospermia, the underlying reasons for this finding are not clear yet. SNP rs1136410 is a T to C change at the codon 726 of PARP1 gene, which will lead to the substitution of alanine for valine in the catalytic domain of PARP1. It has been reported that this substitution of amino acid residue will affect the activity of PARP1. For example, PARP1-Ala762 (allele C) can reduce the activity of PARP1 compared with PARP1-Val762 (allele T) and individuals with genotype CC (Ala/Ala) displayed significantly lower activity of PARP1 than individuals with genotype TT (Val/Val) [32, 33]. Thus, the altered activity of PARP1 caused by SNP rs1136410 may affect the roles of PARP1 during spermatogenesis, and then result in the alteration in spermatogenesis, which may be one of the possible explanations for that this SNP can modify the susceptibility to male infertility with oligospermia in this study.

In conclusion, this study is the first, according to our knowledge, to investigate the relationship between the polymorphism of PARP1 gene and male infertility with spermatogenesis impairment in human. The results of this study revealed that the polymorphism of SNP rs1136410 in PARP1 gene is associated with male infertility with oligospermia and may modify the susceptibility to oligospermia in Chinese population, which suggested that PARP1 gene may be implicated in pathology of male infertility with oligospermia. Considering that the sample size of this study is limited and is restricted to Chinese population, the findings of this study is needed to validate in further studies with larger samples and other ethnic population.

Conflict of interest The authors declare that they have no conflict of interest.

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