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·*Original Article*·

Association of XRCC1 gene polymorphisms with idiopathic azoospermia in a Chinese population

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Abstract

Aim: To assess the possible role of genetic polymorphisms in DNA repair gene XRCC1 (X-ray repair cross-complementing group 1) during spermatogenesis by investigating the associations of one promoter polymorphism (T-77C) and two exonic polymorphisms (Arg194Trp and Arg399Gln) in XRCC1 gene with risk of idiopathic azoospermia in a Chinese population. **Methods:** The genotype and allele frequencies of three observed polymorphisms were examined by polymerase chain reaction-restriction fragment length polymorphism based on a Chinese population consisting of 171 idiopathic azoospermia subjects and 247 normal-spermatogenesis controls. **Results:** In our study, all the observed genotype frequencies were in agreement with Hardy-Weinberg equilibrium. The 399A (GA+AA) allele frequency for idiopathic azoospermia subjects and controls was 0.216 and 0.269, respectively. Compared with GG genotype, the AA genotype of *Arg399Gln* showed a significant association with a decreased risk of idiopathic azoospermia (odds ratio = 0.315; 95% confidence interval = 0.12–0.86). However, no significant differences were found between the cases and controls for *T-77C* and *Arg194Trp* polymorphisms. The major haplotypes of XRCC1 gene were TCG, TTG and TCA, whereas no haplotypes appeared to be significantly associated with idiopathic azoospermia based on the cutoff of $P < 0.05$. **Conclusion:** In a selected Chinese population, AA genotype of *Arg399Gln* appears to contribute to a decreased risk of idiopathic azoospermia, while we have not any evidence of involvement of *XRCC1 T-77C* and *Arg194Trp* polymorphisms in idiopathic azoospermia. *(Asian J Androl 2007 Nov; 9: 843–848)*

Keywords: DNA repair; *XRCC1*; polymorphism; male infertility; idiopathic azoospermia

1 Introduction

Endogenous and exogenous mutagens can cause DNA damage in most cells, including somatic cell and germ cells and unrepaired damage can result in apoptosis [1]. Apoptosis and DNA damage can prevent sperm from maturing, and as a result of an imbalance in these pathways, subjects might present with azoospermia [2]. Apoptotic DNA damage is more frequent in subjects with complete spermatogenesis failure as compared to subjects with incomplete spermatogenesis failure [3]. However, humans have developed a set of complex DNA repair systems to safeguard the integrity of genome by defending harmful consequences of DNA damage. Among the DNA repair systems, the base excision repair (BER) pathway is a crucial mechanism that corrects localized DNA damage, such as

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methylated, oxidized or fragmented lesions and non-bulky adducts, that might block DNA replication or cause genetic instability [4].

The gene XRCC1 (X-ray repair cross complementing group 1), which is located on chromosome 19q13.2, encodes a protein involved in DNA BER that is essential in drawing different components of BER to the site of DNA damage and promoting efficiency of the BER pathway [5]. In rodents [6] and primates [7], the expression of XRCC1 gene is significantly higher in testis than other tissues and is involved in male germ cell physiology. Walter *et al.* [8] describe *XRCC1* as being most abundant in pachytene spermatocytes as well as in round spermatids and suggest that it might maintain]spermatogenesis by repairing some DNA damages during meiogenesis and recombination in germ cells.

Owing to its critical role for maintenance of normal spermatogenesis, mutations or polymorphisms in XRCC1 gene might disturb normal spermatogenesis. Several polymorphisms in *XRCC1* have been reported [9], among which three functional polymorphisms *T-77C*, *Arg194Trp* (exon 6, base 26304 C to T) and *Arg399Gln* (exon 10, base 28152 G to A), have been shown to alter DNA repair capacity in some phenotypic studies and have received considerable attention [10, 11]. However, no study has reported the association between any *XRCC1* polymorphisms and male infertility so far. In the current study, we compared the genotype and allele frequencies of three XRCC1 gene polymorphisms, *T-77C* (rs3213245), *Arg194Trp* (rs1799782), *Arg399Gln* (rs25487), between subjects with idiopathic azoospermia and controls, and further investigated the association of *XRCC1* gene polymorphisms with the risk of idiopathic azoospermia.

2 Materials and methods

2.1 Study subjects

In total, 667 infertile men were recruited from the Center of Clinical Reproductive Medicine between April 2004 and July 2006. All of them received physical examinations, semen analyses, serum determination of follicle stimulating hormone, luteinizing hormone and testosterone, karyotyping, and Y-chromosome microdeletions screening, which enabled us to exclude 199 individuals: three obstructive azoospermic cases, 16 with abnormal karyotype (including eight with Klinefelter's syndrome), 16 with Y-chromosome microdeletions, seven with cryptorchidism and 157 secondary sterility cases. The remaining 468 idiopathic infertility subjects were divided into three groups according to semen parameters described in the World Health Organization Laboratory Manual [12]: 176 with non-obstructive azoospermia (no sperm in ejaculation even after centrifugation), 80 with oligozoospermia (sperm count $< 20 \times 10^6$/mL) and 212 with normozoospermia (sperm count $\geq 20 \times 10^6$/mL).

A group of 176 idiopathic azoospermia aged between 25 and 38 was chosen for use in the present study. The control group included 248 fertile men with their ages ranging from 26 to 40 years who had fathered at least one child without assisted reproductive technologies and had normal semen with an average sperm density of $(53.6 \pm 18.7) \times 10^6$/mL.

All participants in the present study were of Han nationality, which makes up > 90% of the Chinese population, and had provided informed consent. A short questionnaire was handed out to obtain demographic and medical history information, and the cases and controls were matched by age (± 5 years). The response rate was greater than 90% among the respondents. Each subject donated 5 mL of blood for genomic DNA extraction. The research protocol was approved by the ethics review board of Nanjing Medical University.

2.2 Genotype analysis by polymerase chain reaction (PCR)-restriction fragment length polymorphism

DNA was extracted from peripheral blood lymphocytes. The *XRCC1 T-77C*, *Arg194Trp* and *Arg399Gln* polymorphisms were determined using the PCR-restriction fragment length polymorphism method. The primers used to amplify the target fragments containing three polymorphisms are shown in Table 1. The PCR products were then digested with the restriction enzymes BsrB I, PvuII and NciI (New England BioLabs Inc., Beverly, MA, USA), respectively, and separated on a 3% agarose gel.

The –77T allele produces three fragments of 116, 57 and 46 bp whereas the –77C allele produces two fragments of 173 and 46 bp. The wild-type 194C (194Arg) allele produces a 485 bp fragment, and the variant 194T (194Trp) allele has 396 and 89 bp fragments because it gains a PvuII site. Similarly, the wild-type 399G (399Arg) allele generates two DNA bands (384 and 133 bp), the variant 399A (399Gln) allele has a single 517 bp fragment, and the heterozygote displays all three bands (517, 384 and 133 bp).

Table 1. Primers and restriction enzymes used in the present study for genotyping *XRCC1* polymorphisms. †F, forward primer; R, reverse primer. ‡Restriction enzymes for polymerase chain reaction-restriction fragment length polymorphism analysis. SNC, single nucleotide polymorphisms.

Variant (NCBI SNP Cluster ID)		Primer†	Restriction enzyme‡
T-77C (rs3213245)	F	5'-GGGCTGGAGGAAACGCTC-3'	BsrB I
	R	5'-TGGCCAGAAGGATGAGGTAGAG-3'	
Arg194Trp (rs1799782)	F	5'-GCCAGGGCCCCTCCTTCAA-3'	PvuII
	R	5'-TACCCTCAGACCCACGAGT-3'	
Arg399Gln (rs25487)	F	5'-TCCTCCACCTTGTGCTTTCT-3'	NciI
	R	5'-AGTAGTCTGCTGGCTCTGGG-3'	

The polymorphism analysis was performed by two operators independently in a blind fashion. More than 10% of the samples were randomly selected for confirmation, and the results were 100% concordant.

2.3 Statistical analysis

DNA quality or quantity was insufficient for *XRCC1* genotyping in six subjects (five cases and one control). Therefore, the final analysis included 171 cases and 247 controls. We used the χ^2-test to evaluate each allele and genotype of *XRCC1* polymorphisms between the cases and controls. Unconditional univariate and logistic regression analyses were performed to obtain odds ratios (OR) for the risk of azoospermia and their 95% confidence intervals (CI). A goodness-of-fit χ^2-test was used to determine the Hardy-Weinberg equilibrium of the observed genotype frequencies. 2LD software (http://www.mrc-epid.cam.ac.uk/Personal/jinghua.zhao/software/2ld.zip) was used to calculate the D' value for linkage disequilibrium (LD) among the three *XRCC1* polymorphisms and PHASE software (version 2.0.2; University of Washington, Seattle, WA, USA) was used to reconstruct the haplotypes for each subject on the basis of the known genotypes. The sample power was calculated using the Power Calculator of the UCLA Department of Statistics, based on DSTPLAN 4.2.

3 Results

Genotype and allele frequencies of the *T-77C*, *Arg194Trp* and *Arg399Gln* polymorphisms among the cases and controls and their associations with risk of azoospermia are shown in Table 2. All observed single nucleotide polymorphisms (SNP) were in agreement with the Hardy-Weinberg equilibrium (χ^2 test: $P = 0.995$, 0.655 and 0.606, respectively).

The *T-77C* genotype frequencies were 80.70% (TT), 18.13% (CT) and 1.17% (CC) in the cases and 79.76% (TT), 19.03% (CT) and 1.21% (CC) in the controls. Similarly, the frequencies of the CC, CT, and TT genotypes of the *Arg194Trp* were 45.03%, 43.27% and 11.70%, respectively, among the test cases and 40.89%, 48.18% and 10.93%, respectively, among the controls. For the *Arg399Gln* polymorphism, the frequencies of the GG, GA and AA genotypes were 59.65%, 37.43% and 2.92%, respectively, among the test cases and 54.66%, 36.84% and 8.50%, respectively, among the controls. However, these differences were not statistically significant using the $P < 0.05$ threshold ($P = 0.972$ for *T-77C*, $P = 0.611$ for *Arg194Trp*, and $P = 0.064$ for *Arg399Gln*).

As shown in Table 2, the AA genotype of *Arg399Gln* showed a significant association with a decreased risk of idiopathic azoospermia compared with GG genotype (OR = 0.315; 95% CI = 0.12–0.86). The allele frequencies of –77C, 194T, and 399A were also showed in Table 2, while no significant differences were found between the cases and controls (χ^2-test: $P = 0.907$ for –77C, $P = 0.666$ for 194T, and $P = 0.1$ for 399A).

The LD analyses suggested that the T-77C locus was in LD with both the *Arg194Trp* locus (D' = 0.933, $P < 0.05$) and the *Arg399Gln* locus (D' = 0.816, $P < 0.05$). The Arg194Trp locus was also in LD with the *Arg399Gln* locus (D' = 0.7186, $P < 0.05$). When we combined the three loci together and performed the haplotype inference using the PHASE 2.0.2 program (University of Washington, Seattle, WA, USA), seven possible haplotypes were derived from the observed genotypes,

Table 2. Genotype and allele frequencies of the *XRCC1* polymorphisms among the cases and controls and the associations with risk of idiopathetic azoospermia. †Two side χ^2-test for three genotype distributions between the cases and controls. ‡Two side χ^2-test for allele frequencies between the cases and controls. § Odds ratios (OR) were obtained from a logistic regression analyses; 95% confidence interval (CI). The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium ($\chi^2 = 0.011$, $P = 0.995$ for *T-77C*, $\chi^2 = 0.848$, $P = 0.655$ for *Arg194Trp*, and $\chi^2 = 1.002$, $P = 0.606$ for *Arg399Gln*).

Genotypes	Cases (n = 171)		Controls (n = 247)		P	OR (95% CI) §
	n	%	n	%		
T-77C						
TT	138	80.70	197	79.76		1.00
CT	31	18.13	47	19.03	0.972†	0.942 (0.57–1.56)
CC	2	1.17	3	1.21		0.952 (0.16–5.77)
CT + CC	33	19.30	50	20.24		0.942 (0.58–1.54)
C allele		10.23		10.73	0.907‡	
C194T (*Arg194Trp*)						
CC	77	45.03	101	40.89		1.00
CT	74	43.27	119	48.18	0.611†	0.816 (0.54–1.24)
TT	20	11.70	27	10.93		0.972 (0.50–1.86)
CT + TT	94	54.97	146	59.11		0.845 (0.57–1.25)
T allele		33.33		35.02	0.666‡	
G399A (*Arg399Gln*)						
GG	102	59.65	135	54.66		1.00
GA	64	37.43	91	36.84	0.064†	0.931 (0.62–1.40)
AA	5	2.92	21	8.50		0.315 (0.12–0.86)
GA + AA	69	40.35	112	45.34		0.815 (0.55–1.21)
A allele		21.64		26.92	0.100‡	

Table 3. Distribution of *XRCC1* haplotypes in the idiopathetic azoospermia cases and controls. OR, odds ratio; CI, confidence interval.

Alleles of *XRCC1* haplotypes			No. of alleles in cases (342 alleles)		No. of alleles in controls (494 alleles)		P	OR (95% CI)
T-77C	C194T	G399A	n	%	n	%		
T	C	G	119	34.85	165	33.39	0.707	1.06 (0.80–1.42)
T	C	A	74	21.60	106	21.42	0.986	1.01 (0.72–1.41)
T	T	G	114	33.30	147	29.87	0.333	1.18 (0.88–1.59)
C	C	G	35	10.19	46	9.27	0.746	1.18 (0.70–1.76)
T	T	A	0	0	23	4.59	–	–
C	C	A	0	0	4	0.90	–	–
C	T	G	0	0	3	0.54	–	–

of which three common haplotypes (TCG, TCA and TTG) represented 89.8% of the chromosomes for the cases and 84.7% for the controls. The distribution was not significantly different between the idiopathetic azoospermia cases and controls (Table 3). We also found that, with the fixed sample of 171 cases and 247 controls and the genotype frequency of 59.11%, the pro-active effect was 57.10%, whereas the risk effect was 1.804, with a significance of 0.05 and power of 80%.

4 Discussion

More than half of male infertility has uncertain causes and a significant proportion of male infertility is accompanied with idiopathic azoospermia, which is generally assumed to be the result of genetic alterations, including chromosomal abnormalities such as Y-chromosome microdeletions and specific gene mutations [13]. Furthermore, genetic polymorphisms might also be factors susceptible to some forms of male infertility [14, 15].

However, up to now, few studies have reported the association of DNA repair gene SNPs with male infertility, although DNA repair system is indispensable in normal spermatogenesis. Indeed, testes produce high levels of reactive oxygen species during the process of spermatogenesis, which induce a variety of DNA lesions [16]. Moreover, the heavy use of agricultural or industrial chemicals and some drugs might also contribute to the DNA damage of spermatogenic cells. Therefore, the reduction of the DNA repair capability might be associated with decreased sperm counts or abnormal sperm [17]. Furthermore, the polymorphism of DNA repair gene BRCA2 was also clarified to be associated with idiopathic azoospermia [18]. As an essential gene in BER pathway, *XRCC1* plays a potential role in single-strand breaks repair in meiotic recombination during spermatogenesis. Qu and Morimoto [9] show that the functional SNP of *XRCC1* gene affects its DNA repair capability and plays an important role in cancer development. However, to the best of our knowledge, no previous studies examine the association between the *XRCC1* polymorphisms and male infertility risk.

Here, we investigated the associations of one promoter polymorphism (*T-77C*) and two well-characterized exonic variants (*Arg194Trp* and *Arg399Gln*) of *XRCC1* gene with risk of idiopathic azoospermia in a Chinese population to detect the possible role of genetic polymorphisms in XRCC1 gene during spermatogenesis. It was found that 399A (GA + AA) allele frequency was 21.6% for idiopathic azoospermia subjects and 26.9% for controls, in agreement with the previously reported value of 0.27 among Asians and 0.34 among Europeans [19], which indicated that the genotype distributions of *Arg399Gln* varied with ethnicity. We also found that the AA genotype of *Arg399Gln* might reduce the risk of developing idiopathic azoospermia. Matsuo *et al.* [20] report a trend that 399 AA genotype might play a protective role for lymphomagenesis. Because *Arg399Gln* is located in the poly (ADP-ribose) polymerase (PARP) binding domain required for efficient SSB repair [5], it is an important polymorphism of XRCC1 gene that might contribute to DNA repair capability. Our results shed some light on the potential protective role of the *XRCC1 Arg399Gln* polymorphism in idiopathic azoospermia, and might provide preliminary information for future studies. However, it is bewildering that a single polymorphic marker with low penetrance provided such an OR value, which might be a result of the relatively small sample size in our study. Additional works with a larger selected population are needed to confirm the effect of *Arg399Gln* in azoospermia.

In the present study, we failed to find any association between *T-77C* and *Arg194Trp* polymorphism and azoospermia risk. Several reports have discussed the relationship between these two polymorphisms and carcinomas, with results being somewhat conflicting. For example, *T-77C* polymorphism is reported to be associated with lung cancer risk in a Chinese population; however, Brem *et al.* [21] found that *T-77C* variant alone showed no association with breast cancer risk in French women. We also combined the three loci to analyze the distribution of *XRCC1* haplotypes and no statistically significant differences were found between the azoospermia cases and controls. Therefore, they can not account for the risk of idiopathic azoospermia. In fact, spermatogenesis is a complex process and a highly coordinated expression of genes is crucial for normal germ cell development. Although our results suggest that the SNP of *XRCC1* do not directly cause idiopathic azoospermia, maybe they affect male infertility by combining some additional polymorphisms in other genes. Further work has been performed to verify this hypothesis (unpublished data).

In conclusion, we have demonstrated that in a selected Chinese population of normal spermatogenesis fertile men and idiopathic azoospermia subjects, the AA genotype of *Arg399Gln* might contribute to a decreased risk of idiopathic azoospermia, whereas *T-77C* and *Arg194Trp* polymorphisms are not significantly associated with idiopathic azoospermia and, therefore, do not appear to be responsible for spermatogenic failure in male infertility. More works with large and different ethnic populations are needed to further validate the contribution of *Arg399Gln* AA genotype to azoospermia and the joint effects of other gene SNPs on idiopathic azoospermia risk.

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