SPANDIDOS PUBLICATIONS

DNA repair gene *XRCC1* and *XPD* polymorphisms and the risk of idiopathic azoospermia in a Chinese population

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Abstract. Genetic polymorphisms in DNA repair genes may influence individual variation in DNA repair capacity and further influence the risk of developing cancer. However, little information is available on these polymorphisms in infertility. To investigate whether polymorphisms in DNA repair genes, *X-ray repair cross-complementing group 1 (XRCC1)* and *xeroderma pigmentosum group D (XPD)*, alone or in combination, are associated with the risk of developing idiopathic azoospermia, the genotype and allele frequencies of three observed polymorphisms (*XRCC1* Arg194Trp and Arg399Gln, and *XPD* Lys751Gln) were examined by polymerase chain reaction-restriction fragment length polymorphism based on a Chinese population consisting of 171 idiopathic azoospermia patients and 247 normal-spermatogenesis fertile controls. Associations between the polymorphisms and the idiopathic azoospermia risk were estimated by logistic regression, and the Statistical analysis system was used to test the gene-gene joint effects. All observed polymorphisms were in agreement with Hardy-Weinberg equilibrium. The *XPD* 751Gln allele seemed to be a risk allele for azoospermia, with a frequency of 11.40% in the cases and 5.67% in the controls (p=0.004). Compared with the Lys/Lys genotype, the *XPD* 751 Lys/Gln+Gln/Gln genotype was associated with a moderately increased risk of azoospermia (OR=2.09), while the risk increased 5.100- or 3.064-fold, respectively, when combined with the *XRCC1* 194 Arg/Arg or 399 Arg/Arg genotype. In conclusion, our study provided the first evidence that the *XPD* and *XRCC1* polymorphisms contributed to the risk of developing idiopathic azoospermia in a selected Chinese population.

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Introduction

Endogenous and exogenous mutagens may cause DNA damage in most cells including somatic and germ cells, and as a result, patients may manifest azoospermia (1). It has been clarified that DNA damage was more frequent in patients with complete spermatogenesis failure as compared to patients with incomplete spermatogenesis failure (2). However, humans have developed a set of complex DNA repair systems to safeguard the integrity of the genome by defending harmful consequences of DNA damage. Among the DNA repair systems, the base excision repair (BER) and nucleotide excision repair (NER) pathways are two crucial mechanisms that correct the localized small lesions and bulky DNA damage, respectively (3).

Up to now, more than 150 human DNA repair genes in several distinct pathways have been identified, and most are known to have genetic variation in humans (4,5). In the present study, we focus on two well-studied DNA repair genes, *X-ray repair cross-complementing group 1* (*XRCC1*) and *xeroderma pigmentosum group D* (*XPD*).

XRCC1 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 (6,7). The *XRCC1* gene expresses conservatively and significantly high in the testis (8,9), especially in pachytene spermatocytes and round spermatids, maintaining the spermatogenesis by repairing some DNA damages during meiogenesis and recombination in germ cells (10). *XPD* encodes a protein which is essential for transcription and the NER pathway (11), and mutations in *XPD* result in a defect in NER (12). The results of the cDNA microarray showed that the expression of the *XPD* gene was significantly down-regulated in azoospermia testes compared with normal testes (13), which indicated that the *XPD* gene was involved in male infertility with idiopathic azoospermia.

Owing to the critical role for maintenance of normal spermatogenesis, mutations or polymorphisms in the *XRCC1* and *XPD* gene might disturb normal spermatogenesis. Several single nucleotide polymorphisms (SNPs) have been described in the XRCC1 and XPD genes, among which, two *XRCC1* polymorphisms (Arg194Trp and Arg399Gln) and one *XPD* polymorphism (Lys751Gln) were shown to alter DNA repair capacity in some phenotypic studies receiving

considerable attention (14,15). However, little information is available on the polymorphisms of *XRCC1* or *XPD* in male infertility so far. In the current study, we compared the genotype and allele frequencies of the *XRCC1* gene polymorphisms, Arg194Trp (rs1799782) and Arg399Gln (rs25487), as well as the *XPD* gene polymorphism, Lys751Gln (rs28365048), between idiopathic azoospermia patients and controls, and we further investigated the associations of these polymorphisms with the risk of developing idiopathic azoospermia.

Materials and methods

Study population. In total, 667 infertile men were recruited from the Center of Clinical Reproductive Medicine between April 2004 and July 2006 (16). All of them received physical examination, semen analysis, serum determination of FSH, LH and testosterone, karyotyping, and Y-chromosome microdeletion screening, which enabled us to exclude 199 individuals: 3 obstructive azoospermic cases, 16 with abnormal karyotype (including 8 with Klinefelter's syndrome), 16 with Y-chromosome microdeletions, 7 with cryptorchidism, and 157 secondary sterility cases. The remaining 468 idiopathic infertility patients were divided into three groups according to semen parameters described in the WHO Laboratory Manual: 176 with non-obstructive azoospermia (no sperm in ejaculation even after centrifugation), 80 with oligozoospermia (sperm count <20x10^6/ml) and 212 with normozoospermia (sperm count ≥20x10^6/ml).

The patient group with 176 idiopathic azoospermia subjects between 25 and 38 years old was chosen for this study. The control group included 248 fertile men ranging from 26 to 40 years old who had fathered at least one child without assisted reproductive technologies and who had normal semen with an average sperm density of (53.6±18.7)x10^6/ml.

All participants in this study were of Han nationality which makes up >90% of the Chinese population and all provided informed consent. A short questionnaire was performed, and 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.

Genotype analysis by PCR-RFLP. DNA was extracted from peripheral blood lymphocytes and stored at -20˚C. The Arg194Trp, Arg399Gln and Lys751Gln genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCR products were then digested with the restriction enzymes *Pvu*II, *Nci*I, and *Pst*I (New England BioLabs) respectively and separated on a 3% agarose gel. The primer sequences and the restriction enzymes are shown in Table I.

The wild-type 194Arg allele produced a 485-bp fragment, and the variant 194Trp allele had 396- and 89-bp fragments because it gained a *Pvu*II site. Similarly, the wild-type 399Arg allele generated 2 DNA bands (384 and 133 bp), and the variant 399Gln allele had a single 517-bp fragment. The 751Lys allele produced two fragments of 290 and 146 bp while the 751Gln allele produced three fragments of 227, 146 and 63 bp (Fig. 1).

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.

Statistical analysis. DNA quality or quantity was insufficient for genotyping in 6 subjects (5 cases and 1 control). Thus, the final analysis included 171 cases and 247 controls. We used the Chi-square (χ^2) test to evaluate each allele and genotype frequency of Arg194Trp, Arg399Gln and Lys751Gln polymorphisms among the cases and controls. The associations between the polymorphisms and the risk of idiopathic azoospermia were estimated by odds ratios (ORs) and their 95% confidence intervals (CIs) calculated by unconditional univariate and logistic regression models. A goodness-of-fit Chi-square test was used to study Hardy-Weinberg equilibrium of the observed genotype frequencies. We tested the null hypotheses of multiplicative gene-gene interactions by evaluated departures from multiplicative joint effect models by including main effect variables in the logistic regression model (17). All analyses were conducted using Statistical analysis system (version 9.13, SAS Institute, Cary, NC), and the probability level of <0.05 was used as the criterion of significance.

Results

The genotype and allele frequencies of the Arg194Trp, Arg399Gln, and Lys751Gln polymorphisms among the cases and controls and their associations with the risk of idiopathic

Table I. Primers and restriction enzymes used in this study for genotyping *XRCC1* and *XPD* polymorphisms.

Variant (NCBI SNP Cluster ID)		Primer	Annealing	Restriction enzyme[a]
Arg194Trp (rs1799782)	F	5'-GCCAGGGCCCCTCCTTCAA-3'	57˚C, 35 sec	*Pvu*II, 37˚C, 12 h
	R	5'-TACCCTCAGACCCACGAGT-3'		
Arg399Gln (rs25487)	F	5'-TCCTCCACCTTGTGCTTTCT-3'	61˚C, 35 sec	*Nci*I, 37˚C, 12 h
	R	5'-AGTAGTCTGCTGGCTCTGGG-3'		
Lys751Gln (rs28365048)	F	5'-GCCCGCTCTGGATTATACG-3'	60˚C, 35 sec	*Pst*I, 37˚C, 12 h
	R	5'-CTATCATCTCCTGGCCCCC-3'		

F, forward primer; R, reverse primer. [a]Restriction enzymes for PCR-RFLP analysis.

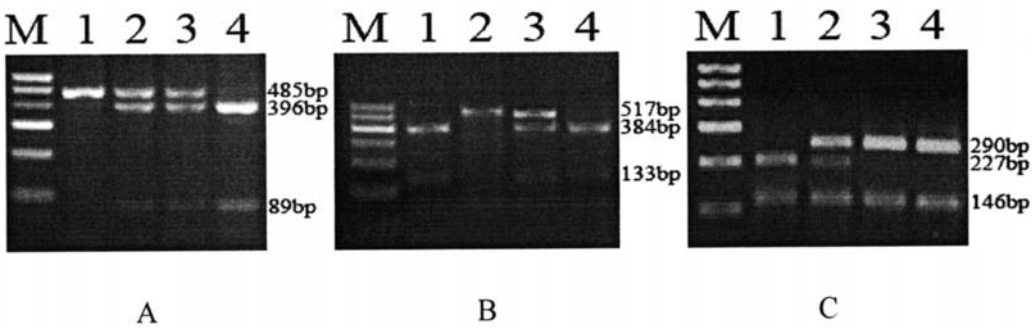

Figure 1. Polymerase chain reaction-restriction fragment length polymorphism assays were performed to genotype the XRCC1 Arg194Trp, Arg399Gln and XPD Lys751Gln. (A) Arg194Trp (3% agarose gel) genotype patterns of Arg/Arg (485 bp) (lane 1), Arg/Trp (485+396+89 bp) (lane 2 and 3), and Trp/Trp (396+89 bp) (lane 4). (B) Arg399Gln genotype patterns of Arg/Arg (384+133 bp) (lane 1 and 4), Gln/Gln (517 bp) (lane 2), and Arg/Gln (517+384+133 bp) (lane 3). (C) Lys751Gln genotype patterns of Gln/Gln (227+146+63 bp) (lane 1), Lys/Gln (290+227+146+63 bp) (lane 2), and Lys/Lys (290+146 bp) (lane 3 and 4); the 63-bp pattern was run into water for the long-time electrophoresis. M, 100-bp DNA marker.

Table II. Genotype and allele frequencies of the Arg194Trp, Arg399Gln, and Lys751Gln polymorphisms among the cases and controls and the associations with the risk of idiopathic azoospermia.

Genotypes	Cases (n=171)		Controls (n=247)		P	OR (95% CI)[c]
	n	%	n	%		
XRCC1 codon 194						
Arg/Arg	77	45.03	101	40.89		1.00
Arg/Trp	74	43.27	119	48.18	0.611[a]	0.816 (0.54-1.24)
Trp/Trp	20	11.70	27	10.93		0.972 (0.50-1.86)
Arg/Trp+Trp/Trp	94	54.97	146	59.11		0.845 (0.57-1.25)
Allele frequency (*Trp*)		33.33		35.02	0.666[b]	
XRCC1 codon 399						
Arg/Arg	102	59.65	135	54.66		1.00
Arg/Gln	64	37.43	91	36.84	0.064[a]	0.931 (0.62-1.40)
Gln/Gln	5	2.92	21	8.50		0.315 (0.12-0.86)
Arg/Gln+Gln/Gln	69	40.35	112	45.34		0.815 (0.55-1.21)
Allele frequency (*Gln*)		21.64		26.92	0.100[b]	
XPD codon 751						
Lys/Lys	135	78.95	219	88.66		1.00
Lys/Gln	33	19.30	28	11.34	0.007[a]	1.91 (1.11-3.31)
Gln/Gln	3	1.75	0	0		---
Lys/Gln+Gln/Gln	36	21.05	28	11.34		2.09 (1.22-3.57)
Allele frequency (*Gln*)		11.40		5.67	0.004[b]	

[a]Two-sided Chi-square test for three genotype distributions between the cases and controls; [b]Two-sided Chi-square test for allele frequencies between the cases and controls. [c]Odds ratios (ORs) were obtained from a logistic regression analyses; 95% CI, 95% confidence interval. The observed genotype frequencies among the control subjects were in agreement with the Hardy-Weinberg equilibrium (χ^2=0.848, P=0.655 for Arg194Trp; χ^2=1.002, P=0.606 for Arg399Gln; and χ^2=0.892, P=0.640 for Lys751Gln).

azoospermia are shown in Table II. All observed SNPs were in agreement with Hardy-Weinberg equilibrium (χ^2 test: P=0.655, 0.606, and 0.640, respectively).

The genotype frequencies of the *XPD* codon 751 were 78.95% (Lys/Lys), 19.30% (Lys/Gln) and 1.75% (Gln/Gln) among the cases, while 88.66% (Lys/Lys), 11.34% (Lys/Gln) and 0% (Gln/Gln) among the controls (χ^2=9.85, P<0.01, df=2). As shown in Table II, the *XPD* 751Gln allele was more frequent in the cases than in the controls (11.40 versus 5.67%, P=0.004), which implies that the *XPD* 751Gln allele contributes to the risk of idiopathic azoospermia. As for the *XRCC1* codon 194 and codon 399 genotype frequencies, no significant differences were detected among the cases and controls using the P<0.05 threshold (P=0.611 and 0.064, respectively).

We next examined whether there was a statistical interaction between the *XPD* Lys751Gln and *XRCC1* Arg194Trp polymorphisms (Table III). We found that patients carrying the *XPD* 751 Lys/Gln+Gln/Gln genotype were also more likely to carry the *XRCC1* 194 Gln/Gln genotype than

Table III. Risk of idiopathic azoospermia associated with *XPD* 751 genotypes by *XRCC1* 194 genotypes.

Genotypes		Cases (n=171)		Controls (n=247)		OR (95% CI)
XPD 751	XRCC1 194	No.	(%)	No.	(%)	
Lys/Lys	Arg/Trp+Trp/Trp	77	(45.03)	124	(50.20)	1.000 (reference)
Lys/Lys	Arg/Arg	58	(33.92)	95	(38.46)	0.983 (0.638-1.516)
Lys/Gln+Gln/Gln	Arg/Trp+Trp/Trp	17	(9.94)	22	(8.91)	1.244 (0.622-2.491)
Lys/Gln+Gln/Gln	Arg/Arg	19	(11.11)	6	(2.43)	5.100 (1.951-13.330)

Table IV. Risk of idiopathic azoospermia associated with *XPD* 751 genotypes by *XRCC1* 399 genotypes.

Genotypes		Cases (n=171)		Controls (n=247)		OR (95% CI)
XPD 751	XRCC1 399	No.	(%)	No.	(%)	
Lys/Lys	Arg/Gln+Gln/Gln	56	(32.75)	97	(39.27)	1.000 (reference)
Lys/Lys	Arg/Arg	79	(46.20)	122	(49.39)	1.122 (0.727-1.731)
Lys/Gln+Gln/Gln	Arg/Gln+Gln/Gln	13	(7.60)	15	(6.07)	1.501 (0.666-3.382)
Lys/Gln+Gln/Gln	Arg/Arg	23	(13.45)	13	(5.26)	3.064 (1.440-6.523)

the controls (11.11% versus 2.43%; P<0.001). The presence of the *XPD* 751 Lys/Gln+Gln/Gln genotype was associated with an increased risk of idiopathic azoospermia (OR=1.244, 95% CI=0.622-2.491), while the presence of both the *XPD* 751 Lys/Gln+Gln/Gln and *XRCC1* 194 Gln/Gln genotypes was associated with an even higher elevated risk for azoospermia (OR=5.100, 95% CI=1.951-13.330), which indicated a super-multiplicative interaction (17) between the *XPD* 751 Lys/Gln+Gln/Gln and *XRCC1* 194 Gln/Gln genotype in the risk of developing idiopathic azoospermia.

The combined effects of the polymorphisms in *XRCC1* codon 399 and XPD codon 751 on idiopathic azoospermia risk were also explored (Table IV). Similarly, the patients carrying the *XPD* 751 Lys/Gln+Gln/Gln genotype were also more likely to carry the *XRCC1* 399 Arg/Arg genotype than the controls (13.45 versus 5.26%, P<0.001). The presence of one risk genotype (*XPD* 751 Lys/Gln+Gln/Gln or *XRCC1* 399 Arg/Arg) was associated with a moderate increase in the risk of developing idiopathic azoospermia (OR=1.501, 95% CI=0.666-3.382 or OR=1.122, 95% CI=0.727-1.731, respectively). However, the OR increased to 3.064 (95% CI, 1.440-6.523) among subjects carrying both risk genotypes (P=0.001, test for homogeneity). These results clearly indicate a more than multiplicative joint effect between the *XPD* 751 Lys/Gln+Gln/Gln and *XRCC1* 399 Arg/Arg genotype in the risk of developing idiopathic azoospermia according to the statistical model.

Discussion

This molecular epidemiologic study examined whether genetic polymorphisms in *XRCC1* and *XPD*, alone or in combination, were associated with the risk of developing idiopathic azoospermia. On the basis of analyzing 171 idiopathic azoospermia patients and 247 frequency-matched controls in a Chinese population, we found that the *XPD* 751 *Gln/Gln* genotype was rarely observed in the controls, which was in agreement with previous reports that the *Gln/Gln* genotype was uncommon in China (18), South Korea (19), and Japan (20). However, the *Gln* allele was common in Europe and North America; approximately 50% carried the heterozygous *Lys/Gln* genotype and 10-15% had the homozygous *Gln/Gln* genotype (21). All of these studies indicate that the genotype distribution of *XPD* 751 varies with ethnicity.

The *XPD* 751Gln allele seemed to be the risk allele for developing azoospermia, which was more frequent in the cases than the controls. Compared with the Lys/Lys genotype, the *XPD* 751 Lys/Gln+Gln/Gln geno-type showed a significant association with an increased risk of idiopathic azoospermia (OR=2.09; 95% CI=1.22-3.57). It was biologically plausible to assume that the *XPD* polymorphism at codon 751 might have functional significance. This polymorphism occurred at a conserved evolutionarily site and resulted in amino acid substitution (Lys→Gln), a change from a basic to a polar amino acid, which might alter the function of the *XPD* protein (22). Our results also confirmed previous findings demonstrating that the *XPD* 751Gln allele was associated with a higher DNA adduct level or lower DNA repair efficiency (22,23), while contrasting results also existed (15,24). Clearly, it is difficult to detect subtle differences in DNA repair capacity due to a single polymorphism of a single gene in a very complex pathway. The inconsistency in the effect of the *XPD* polymorphism at codon 751 in these studies may be also ascribed to the exposure and interaction with other genes participating in DNA damage recognition, repair and cell cycle regulation (11).

The combined effects of polymorphisms in DNA repair genes *XRCC1* and *XPD* on idiopathic azoospermia risk

ier explored. The results suggested that individuals h the *XRCC1* 194 Arg/Arg and *XPD* 751 Lys/Gln+Gln/Gln genotypes seemed to synergistically have an increased risk of idiopathic azoospermia (OR=5.100), compared with those having either of them. Similarly, we also found a greater than multiplicative interaction between *XPD* codon 751 and *XRCC1* codon 399 polymorphisms; when both risk genotypes were present, the risk of developing idiopathic azoospermia increased 3.064-fold.

Whereas mechanistically genetic interactions were thought to be more likely between genes involved in the same biological pathways, it was not unprecedented to find an increased joint effect between genes acting in different pathways. In the case of *XRCC1* and *XPD*, because DNA damage caused by a mixture of environmental exposures might require either the BER or NER pathway, a reduction in the efficiencies of only one of these pathways might not increase the disease risk to the same extent as when both pathways are compromised. Furthermore, our findings are not unprecedented because at least 15 previous studies reported the *XRCC1-XPD* interaction in relation to the risk of lung cancer, prostate cancer, breast cancer, colorectal adenoma, and bladder cancer. However, up to now, no previous study has examined either of these SNPs in relation to the risk of idiopathic azoospermia.

During the process of spermatogenesis, the testis produces high levels of reactive oxygen species which induce a variety of DNA lesions (25). Moreover, the heavy use of agricultural or industrial chemicals and some drugs may also contribute to the DNA damage of spermatogenic cells (26,27). Therefore, DNA repair systems (especially BER and NER pathways) are indispensable in normal spermatogenesis, and the reduction of DNA repair capability might lead to decreased sperm counts or abnormal sperm. Our results shed some light on the potential effects of the *XRCC1* and *XPD* polymorphisms on the risk of idiopathic azoospermia, and to our knowledge, this is the first report of DNA repair gene polymorphisms in relation to the risk of idiopathic azoospermia in a case-control study in the Chinese population. Additional studies with a larger selected population are needed to further explore the biological mechanism of these functional SNPs in azoospermia.

In summary, our data provide the first evidence that DNA repair gene *XPD* and *XRCC1* polymorphisms contribute to the risk of developing idiopathic azoospermia in a selected Chinese population. Further studies with a larger selected population are required to validate our findings.

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