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Analysis of *CDK2* mutations in chinese men with non-obstructive azoospermia who underwent testis biopsy

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KEY MESSAGE

The genotype and allele frequencies of four known single nucleotide polymorphisms in the *CDK2* gene, identified in Chinese men with non-obstructive azoospermia (NOA) who underwent testis biopsy, were similar in patients and controls. Mutations in the coding sequence of the *CDK2* gene may not, therefore, be responsible for idiopathic NOA.

A B S T R A C T

To examine whether mutations of the *CDK2* gene exist in Chinese men with non-obstructive azoospermia (NOA) with different histopathology, we recruited 175 Chinese men with idiopathic NOA who underwent testis biopsy, including hypospermatogenesis, germ cell maturation arrest and Sertoli cell only syndrome. Genomic DNA was extracted from peripheral blood samples. Subsequently, the seven exons of the *CDK2* gene were amplified using polymerase chain reaction with specific primers, respectively. The polymerase chain reaction products were sequenced on an automated sequencer. We identified four known single nucleotide polymorphisms: c.324G>A in exon 1; c.363T>C in exon 2; c.*570G>A; and c.*1160G>C in the 3' UTR of the *CDK2* gene. Comparison of the genotype and allele frequencies showed no significant differences between NOA cases and controls for the four single nucleotide polymorphisms. Furthermore, no significant differences were found between each pathological group and control group, respectively. The results indicate that mutations in the coding sequence of the *CDK2* gene may not be responsible for idiopathic NOA in Chinese men. Future studies in large cohorts of different ethnic populations are warranted to establish whether associations exist between the *CDK2* gene and NOA.

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Introduction

According to the World Health Organization, around one in 10 couples of reproductive age is unable to conceive (Ammar-Khodja et al., 2014). Among these infertile couples, about one-third could be attributed to male factors and an additional one-third to both male and female problems (Bhasin et al., 1994). Several causes of male infertility have previously been proposed, including endocrine disorders, environmental factors, spermatic duct obstruction, anti-sperm antibodies, cryptorchidism, testicular trauma, chromosomal abnormalities and hormone factors (Bonarriba et al., 2013).

Azoospermia, a lack of spermatozoa in the seminal fluid, is a type of male infertility. Incidence of azoospermia is 10–20% (Lee et al., 2011). Types of azoospermia include obstructive azoospermia and non-obstructive azoospermia (NOA) (Xu et al., 2017; Yafi and Zini, 2013). According to testicular biopsy, azoospermia can be categorized into several histopathologies. Hypospermatogenesis is a mild form of azoospermia, with a variable degree of germ-cell loss and spermatozoa in the seminiferous tubule. Germ-cell maturation arrest is a form of infertility, with a cessation at the stage of germ-cell formation. The most severe form of azoospermia is the Sertoli cell only syndrome, which is defined as the complete absence of germ cells in the seminiferous tubule (Alhalabi et al., 2013; Ammar-Khodja et al., 2014; Yatsenko et al., 2015).

Although idiopathic azoospermia remains unexplained (Aboutaleb et al., 2014; Giannouli et al., 2004), nearly 50% of idiopathic infertility cases are thought to have a genetic basis (Bozhedomov, 2016; O'Flynn et al., 2010). Numerous mouse models have linked hundreds of genes with azoospermia, but only a few studies have identified gene mutations in humans with idiopathic azoospermia, such as *TEX11*, *SYCP3*, *PRM1*, *PRM2, and NR5A1* (Abbas et al., 2014; Imken et al., 2009; Miyamoto et al., 2003; Niederberger, 2016; Ropke et al., 2013; Yatsenko et al., 2015; Zheng et al., 2012). Other genes that might be involved in idiopathic azoospermia remain to be determined. Furthermore, it has been reported that ethnic diversity exists in related genes that lead to spermatogenic failure, such as *ESR1*, *ESR2*, *eNOS* and *DAZL* (Ge et al., 2014; Krausz et al., 2015). For instance, a missense variant (rs121918346) in the *DAZL* gene was found to be significantly associated with spermatogenic failure in Chinese men, but not in other populations (Becherini et al., 2004; Chen et al., 2016).

The *CDK2* gene, which belongs to the Cyclin-dependent kinases (CDKs), has been reported to be critical for the mammalian cell cycle both at the G1 to S phase transition and throughout the S phase of gamatogenesis (Ashley et al., 2001; Baudat et al., 2000; Malumbres, 2005). Interestingly, *Cdk2*$^{-/-}$ mice did not have severe consequences for embryonic development, but both males and females were infertile (Lopes et al., 2013; Satyanarayana et al., 2008). *Cdk2*$^{-/-}$ male mice showed a reduced testis volume, and an apparent blocking of meiosis took place in prophase I that led to spermatocyte apoptosis and, consequently, the total absence of mature spermatids (Berthet et al., 2003; Sierant et al., 2015). In the present study, we investigated whether perturbations of the *CDK2* gene were present in Chinese idiopathic NOA patients with different histopathology.

Materials and methods

Participants

This study enrolled adult male patients with newly diagnosed NOA who had visited the Center for Reproductive Medicine, Shandong University, from January 2014 to December 2015. All patients were selected on the basis of an andrological examination that included medical history, physical examination, semen analysis, hormone analysis, scrotal ultrasound, karyotype testing, and Y chromosome microdeletion screening. Participants whose infertility was related to known causes or who had any relevant history that could account for their infertility (childhood disease, varicocele, cryptorchidism, environmental exposure, radiation exposure, prescribed drug use, chromosomal abnormalities, obstructive azoospermia, hypogonadotropic hypogonadism, recurrent infections, testis trauma, iatrogenic infertility, karyotype anomalies, or Y-chromosome microdeletions) were excluded in the study. On the basis of World Health Organization recommendations and standards (Shu et al., 2013), testicular biopsies were conducted in patients without available sperm after two or more inspections of semen. The participants comprised 175 Chinese men with NOA. Mean age was 28 ± 4.2 years. All samples were handled in accordance with the National Regulation of Clinical Sampling in China. Informed consent was obtained from all participants. The control group comprised 46 men who are from normal Han Chinese population in Beijing. The genotype and allele frequencies of the control group were obtained from Ensemble database (Http://asia.ensembl.org/index.html). The study was approved by the Institutional Review Board of Reproductive Medicine of Shandong University on 11 October 2014 (reference number 42).

Polymerase-chain-reaction and sequencing analysis

Genomic DNA of 175 patients was obtained from peripheral blood. The seven exons coding for *CDK2* were amplified using polymerase-chain-reaction (PCR) with seven pairs of primers (**Table 1**). Detailed information of PCR conditions is available upon request. The PCR products were first analysed by agarose gel electrophoresis and then sequenced on an automated sequencer (PRISM 310; Applied Biosystems).

Table 1 – The *CDK2* gene-specific primer sequences.

Primer identifier	primer sequences(5'-3')	Product size (bp)
Exon1-F	ACCAATAGAAAGGCCTGGGG	416
Exon1-R	GGAGTCCCGGGTACAGAGG	
Exon2,3-F	TCTTCTCACTTTCCTAGGGG	491
Exon2,3-R	CCCATGATGAGAGGAGCTGA	
Exon4-F	GCAAACCCAGTCTGC	271
Exon4-R	CTCTTGGGAAGCTCAGAGAAA	
Exon5-F	TACCCTATAAACCACCCCGC	205
Exon5-R	GTTCTTGGATGTGGGGAGGA	
Exon6-F	GTCAAGGTGGGTCTTGGTAT	267
Exon6-R	GAAAACAGGTGCCCACTCTC	
Exon7-1-F	CTGCTGCCCATTTAGTCCAC	633
Exon7-1-R	ACTCCTCCCAGTGGTTTTGT	
Exon7-2-F	GGGGCTAAGTTGGTGCTTTT	793
Exon7-2-R	TCTGTCTCCCCACCATTCTTTCA	

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