ORIGINAL ARTICLE: GENETICS

Genetic variants in Ser-Arg protein–coding genes are associated with the risk of nonobstructive azoospermia in Chinese men

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Objective: To evaluate the association between genetic variants in Ser-Arg (SR) protein–coding genes and the susceptibility of nonobstructive azoospermia (NOA) in Chinese men.
Design: Case-control study.
Setting: State Key Laboratory of Reproductive Medicine in Nanjing Medical University conducted the genotyping and examined the expression levels of genes.
Patient(s): The study included 962 NOA patients and 1,931 control subjects.
Intervention(s): None.
Main Outcome Measure(s): Genotyping of 16 single-nucleotide polymorphisms (SNPs) of eight "canonic" SR protein–coding genes were performed with the use of the Illumina Infinium Beadchip platform. Odds ratios were calculated by logistic regression analysis in the additive model. Expression levels were measured by quantitative reverse-transcription polymerase chain reaction.
Result(s): Rs17431717 near *SFRS9* and rs12046213 near *SFRS4* were significantly associated with a decreased risk of NOA, whereas rs10849753 near *SFRS9* and rs6103330 in *SFRS6* were associated with an increased risk of NOA. Of the two SNPs in *SFRS9*, only rs17431717 remained significant after conditioning on another. Combined analysis of three promising SNPs (rs17431717, rs12046213, and rs6103330) showed that compared with individuals with "0–2" risk alleles, those carrying "3," "4," and "≥5" risk alleles had 1.22-, 1.38-, and 1.90-fold increased risk of NOA, respectively.
Conclusion(s): Polymorphisms in SR protein–coding genes may contribute to the risk of NOA in Chinese men. The findings of this study can help us to further understand the etiology of spermatogenic impairment, and they provide more evidence for the role of splicing activity in human spermatogenesis. (Fertil Steril® 2014;■:■–■. ©2014 by American Society for Reproductive Medicine.)
Key Words: NOA, susceptibility, alternative splicing, SR protein family

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Infertility, a growing severe health problem, is affecting ~15% of the couples in the world (1). Studies have shown that one-half of all fertility problems are due to male reproductive defect, including azoospermia (2, 3). Azoospermia is known to be the major cause of male infertility in humans and can be categorized into two major categories: obstructive and nonobstructive. Nonobstructive azoospermia (NOA) is characterized by

no or little sperm in semen as a result of congenital dysfunction in spermatogenesis (4, 5), and it occurs in >1% of men. Many studies have explored the underlying mechanism of NOA in the past few years. As reported, well known genetic causalities of NOA involve chromosomal alterations, Y chromosome microdeletions, and autosomal chromosome mutations (6–10). However, other genetic factors contributing to the predisposition of NOA remain to be clarified.

Alternative splicing is a crucial mechanism for gene regulation, and predicted outcomes of alternative splicing include extensive proteome diversity, introduction of premature termination codons, which causes mRNA down-regulation by nonsense-mediated decay (NMD); and variability in mRNA untranslated regions (11–13). Recent estimates indicate that the expression of nearly 95% of human multiexon genes involves alternative splicing (14–16). The numbers of alternative splicing increase from invertebrates to vertebrates, suggesting that the generation of new alternative exons could be a driving force in evolution (17, 18). Therefore, it is not surprising that alternative splicing links to multiple human biologic processes and diseases, such as heart and brain development, spermatogenesis, cancers, human immunodeficiency virus, and so on (19–25). Furthermore, several studies have reported that splicing mutations of certain genes are associated with male infertility. For example, analysis of sperm RNA revealed that novel splicing mutations in kelch-like 10 (*KLHL10*) were associated with reduced sperm count in infertile men (26), and splicing mutations in the gene encoding zona pellucida–binding protein 1 (*ZPBP1*) were predicted to cause splicing defects in the *ZPBP1* mRNA and subsequently lead to abnormal sperm head morphology in infertile men (27).

The SR protein family has many important roles in splicing mRNA precursors. These proteins contain either one or two RNA recognition motifs (RRMs) at the N-terminus and an arginine/serine–rich (RS) domain at the C-terminus. The RRMs bind to RNA sequence to determine splicing specificity and commit pre-mRNA to the splicing pathway, and the RS domains promote specific protein-protein interactions in early steps of spliceosome assembly (28, 29). So far, nine proteins (SRp20, SC35, ASF/SF2, SRp40, SRp55, SRp75, 9G8, SRp30c, and SRp38) with structural similarity have been identified as "canonic" SR proteins, all of which function as pivotal regulators of constitutive and alternative splicing process (30).

The human testis displays the highest number of skipped exons and more frequently diverged alternative splicing (31, 32). In addition, the differentiation of germ cells is characterized by a powerful wave of transcriptional events, which is also accompanied by high levels of splicing activity (33). According to the expressed sequence tags profile in the public database Unigene (www.ncbi.nlm.nih.gov/UniGene), all of the nine SR protein–coding genes are expressed in human testis tissues with transcripts ranging from 13 to 546 per million (Supplemental Fig. 1, available online at www.fertstert.org). Additionally, several reports have indicated that some SR proteins, such as SRp38 and 9G8, play an essential role in spermatogenesis (34, 35). Therefore, it is plausible that changes of SR proteins may affect the genesis of mature sperm and link to the development of NOA. However, to date, there is no report about the relationship between genetic variations in SR protein–coding genes and NOA susceptibility.

In the present study, we hypothesized that potentially functional single-nucleotide polymorphisms (SNPs) of "canonic" SR protein–coding genes might modify NOA risk in the Chinese population. To test the hypothesis, we performed a case-control study in Han Chinese men by genotyping 16 SNPs of eight "canonic" SR protein–coding genes in 962 NOA cases and 1,931 male control subjects with healthy birth history.

MATERIALS AND METHODS

Ethics Approval

This study was approved by the Institutional Review Board of Nanjing Medical University. The design and performance of the study involving human subjects were clearly described in a research protocol. All participants were voluntary and completed the informed consent in writing before taking part in this research.

Study Populations

The case-control analysis included 962 NOA cases and 1,931 male control subjects. The cases were recruited from the Center of Reproductive Medicine from April 2005 to January 2012. All infertile subjects were genetically unrelated Han Chinese men recruited from Jiangsu Province, China, and determined to have idiopathic NOA on the basis of comprehensive andrologic testing, including examination of medical history, physical examination, semen analysis, scrotal ultrasound, hormone analysis, karyotyping, and Y chromosome microdeletion screening. Those with a history of cryptorchidism, vascular trauma, orchitis, obstruction of the vas deferens, vasectomy, abnormalities in chromosome number, or microdeletions of the azoospermia factor region on the Y chromosome were excluded from the study. Semen analysis for sperm concentration, motility, and morphology was performed following World Health Organization criteria (1999). Subjects with NOA were defined as those without detectable sperm in the ejaculate after evaluation of the centrifuged pellet. To ensure the reliability of the diagnosis, each individual was examined twice, and the absence of spermatozoa from both samples was considered to be azoospermia. NOA-free male control subjects were also Han Chinese men, who were randomly selected from more than 30,000 participants in a community-based screening program for noninfectious diseases in Jiangsu Province, China. Each of the male control subjects had fathered at least one healthy child without assisted reproductive technologies and were frequency matched to the case subjects on the basis of age and area of residence. At recruitment, informed consent was obtained from each subject. For each participant, 5 mL whole blood was obtained to extract genomic DNA for further genotyping analysis.

SNP Selection and Genotyping

According to a review by Chen and Manley (30), nine proteins (9G8, ASF/SF2, SC35, SRp20, SRp30c, SRp40, SRp55, SRp75,