Genetic Variants in Meiotic Program Initiation Pathway Genes Are Associated with Spermatogenic Impairment in a Han Chinese Population

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Abstract

Background: The meiotic program initiation pathway genes (CYP26B1, NANOS1 and STRA8) have been proposed to play key roles in spermatogenesis.

Objective: To elucidate the exact role of the genetic variants of the meiosis initiation genes in spermatogenesis, we genotyped the potential functional genetic variants of CYP26B1, NANOS1 and STRA8 genes, and evaluated their effects on spermatogenesis in our study population.

Design, Setting, and Participants: In this study, all subjects were volunteers from the affiliated hospitals of Nanjing Medical University between March 2004 and July 2009 (NJMU Infertile Study). Total 719 idiopathic infertile cases were recruited and divided into three groups according to WHO semen parameters: 201 azoospermia patients (no sperm in the ejaculate even after centrifugation), 155 oligozoospermia patients (sperm counts <20×10⁶/ml) and 363 infertility/normozoospermia subjects (sperm counts >20×10⁶/ml). The control group consisted of 383 subjects with normal semen parameters, all of which had fathered at least one child without assisted reproductive technologies.

Measurements: Eight single nucleotide polymorphisms (SNPs) in CYP26B1, NANOS1 and STRA8 genes were determined by TaqMan allelic discrimination assay in 719 idiopathic infertile men and 383 healthy controls.

Results and Limitations: The genetic variant rs10269148 of STRA8 gene showed higher risk of spermatogenic impairment in the groups of abnormospermia (including azoospermia subgroup and oligozoospermia subgroup) and azoospermia than the controls with odds ratios and 95% confidence intervals of 2.52 (1.29–4.94) and 2.92 (1.41–6.06), respectively (P = 0.006, 0.002 respective). Notably, larger sample size studies and in vivo or in vitro functional studies are needed to substantiate the biological roles of these variants.

Conclusions: Our results provided epidemiological evidence supporting the involvement of polymorphisms of the meiotic program initiation genes in modifying the risk of azoospermia and oligozoospermia in a Han-Chinese population.

Introduction

Infertility was reported affecting 10–15% of couples, and roughly half of which are due to the man’s problem [1,2]. Spermatogenic impairment is the most common form of male infertility, which is closely related with impaired preimplantation development, low fertilization rate, increased abortion and elevated incidence of disease in the offspring, and as well as child cancer [3]. Spermatogenesis is a highly regulated process which can be subdivided into three main phases: mitotic proliferation, meiosis and sperm morphogenesis, among which, the meiosis is an important event in the process of sexual reproduction of biology.
gametes (including male gametes and female gametes) generation, and its smooth start is key to the final completion of meiosis. But we still lack a detailed understanding of the molecular mechanism for the initiation of germ cell meiosis [4, 5, 6, 7]. Recently, a series of studies have shown that several genes (CYP26B1, NANOS1, STRA8 et al) co-modify the process of meiotic program initiation [5, 6, 8, 9].

Of these genes, the STRA8, stimulated by retinoic acid gene 8, was activated by retinoic acid (RA), which can directly enter the nucleus and bind to the corresponding retinoic acid receptor (RAR) to regulate the expression of specific genes. RA plays an inhibitory role in the development of male germ cells and closely related with somite and germ cell differentiation [5]. Recent studies report that genetic deletion of CYP26B1 leads to increased RA levels and activation of STRA8 in the embryonic testes. As a consequence, male germ cells are prematurely entering into meiosis, arrested at pachytene stage, causing a rapid increase in apoptosis and a lack of spermatogenesis of male mice after birth [15,14,15,16]. If no other STRA8 inhibitory factors exist, the concentration of RA will be increased, and the STRA8 will be re-activated. To ensure the process of meiosis initiation go normally, other inhibitory factors are required.

The SERMOS (human, NANOS1, mice Nanos2 and Nanos3) is one of the evolutionarily conserved proteins implicated in germ cell development and closely related with somite and germ cell development [17]. It was reported the phenotype of Nanos2 knockout mice’s testis during the embryonic period was similar to that of Cyp26b1 functional defects [18]. It was interesting that Stra8 expression in these two gene knockout mice showed certain period difference. In the testis of Cyp26b1 knockout mice, it begins to express highly in embryonic E13.5, but appears at E14.5 and then reduced at E15.5, prior to mitotic arrest, and then reduced at E15.5 [13]. The degradation effect of CYP26B1 on RA concentration may inactivate STRA8, thus ensuring the normal development of spermatogenesis. Previous study reports that genetic deletion of CYP26B1 leads to increased RA levels and activation of STRA8 in the embryonic testes. As a consequence, male germ cells are prematurely entering into meiosis, arrested at pachytene stage, causing a rapid increase in apoptosis and a lack of spermatogenesis of male mice after birth [15,14,15,16]. If no other STRA8 inhibitory factors exist, the concentration of RA will be increased, and the STRA8 will be re-activated. To ensure the process of meiosis initiation go normally, other inhibitory factors are required.

The study was approved by the Ethics review board of Nanjing medical university. The protocol and consent form were approved by the Institutional Review Board of Nanjing Medical University prior to the study. All participants provided their written informed consent to join in this study. And all the subjects were genetically unrelated ethnic Han-Chinese from East China. Every participant received complete medical history questionnaire, physical examinations and semen analysis. All infertility patients were examined, among which, those with a history of Y chromosome microdeletions, cytogenetic abnormalities, congenital bilateral absence of vas deferens, cryptorchidism and orchitis were excluded [20]. Additionally, subjects having special occupational exposure which may be suspected to affect semen quality were precluded. After completing the questionnaire, each subject donated 5 ml of blood used for genomic DNA extraction.

**Materials and Methods**

**Subject Recruitment and Sample Collection**

The final population consisted of 1102 Han Chinese subjects, composed of 383 fertile controls, 201 azoospermia, 153 oligozoospermia and 363 infertility/normozoospermia. The distributions of
selected characteristics among the case and control subjects were presented in Table 2. All groups had similar patterns of drinking and tea consumption ($p>0.05$). The mean ages were higher in the groups of all infertility, azoospermia and oligozoospermia than the control group (all $p<0.05$). BMI levels in the group of all infertility were significantly higher than those in the control subjects. Smoking prevalence was higher in the azoospermia group than the control group ($p<0.05$).

### Associations between Polymorphisms and Spermatogenic Impairment

The position and minor allele frequency of the 8 potential functional SNPs, were presented in Table 1. All SNPs frequencies were in accordance with HWE, except rs1422627 which had a $p$ value $<0.01$ for deviation from HWE. The associations between meiosis initiation genes SNPs and the risks of male infertility were shown in Table 3. According to the Table 2, 95% CI was adjusted based on the test for deviation from HWE. The associations between the study, we found the rs10269148 significantly increased the risk of idiopathic male infertility with abnormal and/or normal semen parameters.

As to the other SNPs, no significant differences of distribution frequencies were identified among the case and control groups. In our study, due to the low occurrence frequency in Asians, we didn’t analyse homozygous mutant separately.

### Discussion

Although meiosis initiation genes have been recognized as key regulators in sperm function and male fertility [14,18,21], there have been few studies concerning the potential role of genetic variants of meiosis initiation genes in infertility, particularly idiopathic male infertility. STRA8 is required for premeiotic DNA replication, while CYP26B1 is decreased. By preventing STRA8's expression, CYP26B1 and NANOS1 play critical roles in the differentiation of male germ cells [9,13,14,22]. In all, the entry of testicular germ cells into meiotic program may be partly controlled by the stage-specific expression of CYP26B1 and NANOS1 expression in the germ cells [9,13]. To investigate the exact role of meiotic initiation pathway genes, key downstream antagonists (CYP26B1, NAOSI) and effectors (STRA8) of the meiosis-inducing action of RA were analyzed in this study. Through information gained from PubMed and Hapmap searches, eight potential functional polymorphisms in meiosis initiation genes were examined.

Vivo studies have demonstrated that male mice lacking STRA8 function produce no sperm [8,23] mainly by hampering the homologous-chromosome pairing way [24]. But the exact functional genetic variants of this gene remain unclear. In this study, we found the rs10269148 significantly increased the risk of spermatogenic impairment associated with abnormal semen parameters ($p=0.006$). In the following stratified analysis, compared with homozygous type CC, the rs10269148 C>G increased the risk of azoospermia and displayed 2.92 fold increased risk of oligozoospermia, while the rs707718 AC genotype may be protective factors against the risk of idiopathic male infertility with abnormal and/or normal semen parameters.

### Table 1. The meiotic program initiation pathway genes and polymorphisms evaluated in this study.

| Gene   | SNP ID     | Position | Nucleotide change | Amino acid change | MAF (%) | p value for HWE Test |
|--------|------------|----------|-------------------|-------------------|---------|--------------------|
| Cyp26b1 | rs2241057  | rsSNP    | T>C               | Leu>Ser           | 11.0    | 0.552              |
|        | rs707718   | 3'UTR    | A>C               | –                 | 45.3    | 0.878              |
| Nanos2 | rs1422627  | 3'UTR    | T>C               | –                 | 30.2    | <0.01              |
|        | rs9304651  | 5'near gene | A>G          | –                 | 9.8     | 0.472              |
|        | rs2015728  | 5'near gene | G>T          | –                 | 27.4    | 0.449              |
| Stra8  | rs10269148 | 5'near gene | C>G          | –                 | 3.5     | 0.735              |
|        | rs17168319 | 5'near gene | A>G          | –                 | 23.2    | 0.931              |
|        | rs17168337 | 5'near gene | C>G          | –                 | 31.1    | 0.386              |

Abbreviations: rsSNP, non-synonymous; UTR, untranslated region; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium.

*aMinimum allele frequency in the general Han Chinese population, as reported in dbSNP database.

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Table 2. The distributions of selected variables among cases and control subjects.

| Variables | All infertility (n = 719) | Azoospermia (n = 201) | Oligozoospermia (n = 155) | Infertility/normozoospermia (n = 363) | Infertility/abnormospermia (n = 383) |
|-----------|---------------------------|-----------------------|---------------------------|--------------------------------------|-------------------------------------|
| Age, mean(SD) | 29.79 (6.6) | 29.12 (6.4) | 28.79 (5.8) | 29.22 (6.5) | 29.17 (6.5)* |
| Smoking Ever | 218 (56.92) | 184 (51.69) | 97 (48.26) * | 87 (56.13) | 193 (53.17) |
| Never | 165 (43.08) | 172 (48.31) | 104 (51.74) | 68 (43.87) | 170 (46.83) |
| Drinking Ever | 214 (55.87) | 198 (55.62) | 114 (56.72) | 84 (54.19) | 179 (49.31) |
| Never | 169 (44.13) | 158 (44.38) | 87 (43.28) | 71 (45.81) | 184 (50.69) |
| BMI, mean(SD) | 23.62 (6.4) | 23.21 (3.02) | 23.15 (3.17) | 23.28 (3.17) | 23.21 (3.17) * |

*aIndependent-samples T-test for comparing the mean of the age, BMI and Pack-years of smoking between the cases and controls.

bTwo-sided chi-squared test for either selected variable distributions between cases and controls.

*p, 0.05 for two-sided chi-squared test for either selected characteristics distributions or allele frequencies between control and case group.

Snps Are Associated with Spermatogenic Impairment

Considering the function of STRA8, the gene might be moderate and other macromolecules might participate in the initiation of meiosis. The precise mechanism of the variants of STRA8 in male infertility needs further investigation. In conclusion, the results of our study demonstrated for the first time that some representative genetic variants in the meiosis initiation genes might regulate the risk of male infertility. Although the statistical power of our study was limited by the small sample size in the subgroups, our findings might be helpful to understand the mechanism of male infertility. Larger sample size studies and in vivo or in vitro functional studies will be needed to substantiate the biological roles of these variants.
### Table 3. Associations of selected meiotic program initiation pathway gene polymorphisms and the risk of idiopathic male infertility.

| Genotype | Control | Infertility<sup>b</sup>/ abnormospermia | Azoospermia<sup>c</sup> | Oligozoospermia<sup>d</sup> | Infertility<sup>e</sup>/ normozoospermia | All infertility |
|----------|---------|---------------------------------|-----------------|-----------------|-----------------|----------------|
|          | N       | N | OR<sup>g</sup> (95% CI) | p<sup>9</sup> | N       | N | OR<sup>g</sup> (95% CI) | p<sup>9</sup> | N       | N | OR<sup>g</sup> (95% CI) | p<sup>9</sup> | N       | N | OR<sup>g</sup> (95% CI) | p<sup>9</sup> |
| CYP26B1  |         |   |                     |       |         |   |                     |       |         |   |                     |       |         |   |                     |       |
| rs2241057 |          | TT | 318 | 308 | 1.00 | 0.401 | 0.40 | 169 | 1.00 | 0.949 | 0.129 | 321 | 1.00 | 0.099 | 0.116 |
|          |          | CT | 63  | 46  | 0.75(0.50–1.14) | 0.096 | 0.56 | 0.53(0.29–0.96) | 0.051 | 0.40 | 0.61(0.40–0.94) | 0.035 | 0.68(0.48–0.97) | 0.043 |
|          |          | CT/CC | 65  | 48  | 0.76(0.51–1.14) | 0.188 | 0.096 | 0.50(0.30–0.98) | 0.051 | 0.62 | 0.61(0.41–0.94) | 0.035 | 0.68(0.48–0.97) | 0.043 |
| rs707718  |          | AA | 97  | 111 | 1.00 | 0.206 | 0.20 | 55  | 1.00 | 0.740 | 0.746 | 99  | 1.00 | 0.039 | 0.035 |
|          |          | AC | 190 | 161 | 0.74(0.52–1.04) | 0.188 | 0.96 | 0.61(0.40–0.95) | 0.051 | 0.62 | 0.61(0.41–0.94) | 0.035 | 0.68(0.48–0.97) | 0.043 |
|          |          | AC/CC | 286 | 245 | 0.75(0.54–1.03) | 0.077 | 0.92 | 0.62(0.36–1.36) | 0.051 | 0.62 | 0.61(0.41–0.94) | 0.035 | 0.68(0.48–0.97) | 0.043 |
| NANOS1   |          | AA | 330 | 297 | 1.00 | 0.579 | 0.58 | 164 | 1.00 | 0.347 | 0.746 | 306 | 1.00 | 0.740 | 0.600 |
|          |          | AG | 50  | 56  | 1.24(0.82–1.88) | 0.188 | 1.38 | 0.86(2.21) | 0.188 | 1.08 | 1.08(0.62–1.83) | 0.188 | 1.22(0.81–1.84) | 0.188 |
|          |          | AG/GG | 53  | 49  | 1.24(0.83–1.85) | 0.300 | 1.38 | 0.87(2.19) | 0.188 | 1.07 | 1.07(0.62–1.83) | 0.188 | 1.22(0.81–1.84) | 0.188 |
| rs2015728 |          | GG | 207 | 191 | 1.00 | 0.282 | 0.28 | 112 | 1.00 | 0.530 | 0.215 | 206 | 1.00 | 0.117 | 0.127 |
|          |          | GT | 153 | 133 | 0.94(0.69–1.28) | 0.88 | 0.61(1.16) | 0.53 | 73  | 0.91(0.64–1.29) | 0.177 | 177 | 0.91(0.64–1.29) | 0.338 |
|          |          | GT/TT | 176 | 165 | 1.02(0.76–1.36) | 0.914 | 0.93 | 0.66(1.31) | 0.699 | 76  | 0.90(0.65–1.25) | 0.546 | 509 | 0.81(0.61–1.08) | 0.171 |
| STRA8    |          | AA | 370 | 332 | 1.00 | 0.006 | 0.006 | 184 | 1.00 | 0.002 | 148 | 1.00 | 0.124 | 344 | 1.00 |
|          |          | CG | 13  | 29  | 2.52(1.29–4.94) | 0.94 | 2.92(1.41–6.06) | 0.002 | 10  | 1.89(0.81–4.44) | 0.124 | 23  | 1.86(0.93–3.74) | 0.52 |
|          |          | CG/GG | 13  | 29  | 2.52(1.29–4.94) | 0.006 | 19  | 2.92(1.41–6.06) | 0.002 | 10  | 1.89(0.81–4.44) | 0.124 | 23  | 1.86(0.93–3.74) | 0.52 |
| rs1716831 |          | AA | 217 | 188 | 1.00 | 0.500 | 0.50 | 104 | 1.00 | 0.440 | 0.84 | 1.00 | 0.847 | 210 | 1.00 |
|          |          | AG | 143 | 148 | 1.19(0.88–1.62) | 1.28 | 0.90(1.83) | 0.62 | 62  | 1.13(0.76–1.68) | 1.27 | 0.92(0.68–1.25) | 2.75 |
|          |          | AG/GG | 166 | 168 | 1.17(0.87–1.56) | 0.293 | 0.97 | 0.89(1.76) | 0.527 | 71  | 1.10(0.76–1.61) | 0.602 | 153 | 0.95(0.71–1.28) | 0.742 |
| rs1716837 |          | CC | 160 | 165 | 1.00 | 0.440 | 0.44 | 91  | 1.00 | 0.620 | 0.74 | 1.00 | 0.247 | 164 | 1.00 |
|          |          | CG | 181 | 153 | 0.82(0.60–1.11) | 0.92 | 0.91(0.63–1.31) | 0.61 | 61  | 0.72(0.48–1.08) | 1.61 | 0.86(0.63–1.17) | 3.14 |
|          |          | CG/GG | 223 | 191 | 0.83(0.62–1.11) | 0.211 | 1.10 | 0.89(0.63–1.26) | 0.417 | 81  | 0.78(0.53–1.14) | 0.206 | 199 | 0.86(0.65–1.16) | 0.349 |

SNPs, single-nucleotide polymorphisms; OR, odds ratios; CI, confidence interval.

<sup>a</sup>Subjects consisted of proven fertility men with semen volume ≥2 ml, sperm counts ≥20 x 10⁶/ml and sperm motility ≥50% motile sperm.

<sup>b</sup>Subjects consisted of idiopathic infertile men with sperm counts < 20 x 10⁶/ml.

<sup>c</sup>Subjects consisted of idiopathic infertile men with sperm counts ≤20 x 10⁶/ml and sperm motility <50% motile sperm.

<sup>d</sup>Subjects consisted of idiopathic infertile men with sperm counts from 0.1 to 20 x 10⁶/ml.

<sup>e</sup>Subjects consisted of idiopathic infertile men with sperm counts ≥20 x 10⁶/ml.

<sup>f</sup>Subjects consisted of idiopathic infertile men with sperm counts < 20 x 10⁶/ml.

<sup>g</sup>Two-sided χ² test for genotype distributions between cases and controls.

<sup>h</sup>Odds were obtained from multivariate logistic regression analysis.

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Author Contributions

Study supervision: XW. Conceived and designed the experiments: XW. Performed the experiments: CL, MX. Analyzed the data: CL, YQ. Contributed reagents/materials/analysis tools: GD, WW, XH, CJ, YY, AG. Wrote the paper: CL, YW.