Investigation of polymorphisms in exon7 of the \textit{NSUN7} gene among Chinese Han men with asthenospermia

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\textbf{ABSTRACT.} Previous studies have shown that genetic polymorphisms in exon7 of the \textit{NSUN7} gene can be used as an infertility marker in Iranian men with asthenospermia. However, there have been no equivalent studies in China. In the present study, we investigated the possible association between the genetic polymorphisms in exon7 of \textit{NSUN7} and asthenospermia in a Chinese Han population. We recruited 240 asthenospermic men as a patient group and 256 normospermic men as a control group, and analyzed the semen parameters on the basis of World Health Organization (WHO) guidelines. The genetic polymorphisms in exon7 of \textit{NSUN7} were detected by DNA sequence analysis. The results were analyzed statistically and a P value < 0.05 was considered significant. There were two genetic polymorphisms, c.906C>T and c.922T>G, in exon7 of \textit{NSUN7}. We found relatively similar genotypes and allele frequencies between the two groups (P = 0.928, P = 0.928, ...
respectively). The combined genotypes of the two polymorphisms did not identify a haplotype associated with asthenospermia ($P = 0.824$, $P = 0.824$, respectively). Our findings revealed that genetic polymorphisms in exon 7 of the $\text{NSUN7}$ gene are not associated with asthenospermia in Chinese Han men.

**Key words:** Genetic polymorphisms; $\text{NSUN7}$ gene; Asthenospermia

**INTRODUCTION**

Parenting is one of the most universally desired goals in adulthood. However, not all people can achieve this desire. Infertility has been recognized as a worldwide health problem. According to incomplete statistics, about 15% of couples suffer from infertility, which can be attributed to male factors in 50% of cases (Ferlin et al., 2006). The main symptoms of male infertility are azoospermia, oligospermia, teratospermia, and asthenospermia. Asthenospermia, also known as poor sperm motility, is one of the important factors for male infertility (Aitken et al., 1982a). Sperm cannot travel through a mucus-filled cervix to reach the site of fertilization if the sperm has low motility (Mortimer et al., 1986; Folgerø et al., 1993).

Early studies mainly focused on the effects of mitochondrial genes on asthenospermia, including point mutations and deletions of mitochondrial DNA, which have been reported in patients with low sperm motility (Kao et al., 1995, 1998; Thangaraj et al., 2003; Selvi et al., 2006; Baklouti-Gargouri et al., 2013). In recent years, some autosomal genes, such as $\text{TEKTIN}$ (Zuccarello et al., 2008a), $\text{DNAI1}$, $\text{DNAH5}$, $\text{DNAH11}$ (Zuccarello et al., 2008b), $\text{AKAP3}$, $\text{AKAP4}$ (Eddy et al., 2003), and $\text{SEPT4}$ (Ihara et al., 2005), have also been confirmed to be correlated with asthenospermia. The $\text{NSUN7}$ gene (NOP1/NOP2/Sun domain family, member 7) encodes an RNA methyltransferase NSUN7, which possess an S-adenosyl methionine binding-domain that may have a role in mitochondrial RNA processing in post-meiotic spermatozoa (Harris et al., 2007). Harris at al. (2007) found that the $\text{NSUN7}$ gene has a role in sperm motility in mice, and revealed a single C to T base substitution at nucleotide 1282 in exon 7 of $\text{NSUN7}$. In humans, the $\text{NSUN7}$ gene (RefSeq NM_024677.4), which encodes putative methyltransferase NSUN7 with 718 amino acids, is located on chromosome 4 and comprises 12 exons and 11 introns (http://www.ncbi.nlm.nih.gov/gene/79730). Exon 7 of $\text{NSUN7}$ is a hot spot and is located in the conserved domains databases (CDD) region (Khosronezhad et al., 2014). A previous study has reported that C26232T (rs2465570) and T26248G (rs2437323) mutations in exon 7 of $\text{NSUN7}$ reduced sperm motility in Iranian men (Khosronezhad et al., 2014). However, there have been no equivalent studies in China. The present study aimed to determine whether the genetic polymorphisms in exon 7 of the $\text{NSUN7}$ gene are associated with asthenospermia in the Han population of China.

**MATERIAL AND METHODS**

**Patients and controls**

We recruited 240 asthenospermic men from the Affiliate Hospital of Sichuan Reproductive Health Research Center between September 2013 and July 2014. As a control group, we included 256 men with normal semen parameters who had at least one child. All the semen
samples were collected and analyzed according to the World Health Organization guidelines (World Health Organization, 1999). The diagnosis of asthenospermia was made on the basis of rapid forward progressive motile sperm (grade A < 25%) and total motile sperm (grades A + B < 50%), with at least one year of infertility. All patients and controls were Han people. The characteristics of the study groups are shown in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Characteristics of groups studied.</th>
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<tr>
<td></td>
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<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Ethnicity</td>
</tr>
<tr>
<td>Motility A (% motile)</td>
</tr>
<tr>
<td>Motility (A+B) (% motile)</td>
</tr>
<tr>
<td>No. of live births</td>
</tr>
</tbody>
</table>

All subjects had the normal karyotype, no azoospermia factor microdeletions, and were without unhealthy lifestyles (smoking, drug use, or chemical exposure); they were asked to complete a questionnaire. The subjects gave their informed consent and the study was approved by the local Research Ethics Committee.

Genotyping

Genomic DNA was extracted from peripheral blood using an Easy Pure Blood Genomic Kit (TransGen, Beijing, China) and stored at -20°C until required for the assay.

The polymerase chain reaction (PCR) primers, 5’AGGTGACAAGGTAATGGGGAAA G-3’ (forward) and 5’GCAACAAGGACAGCTGATAAGG-3’ (reverse), were designed using primer premier 5.0, and synthesized at Sangon Biotech (Shanghai, China). PCR products were processed to a total volume of 25 μL, containing 100 ng genomic DNA, 2.5 μL Easy-Tag Buffer, 0.2 mM dNTPs, 2 U Taq DNA polymerase (TransGen, Beijing), and 0.3 mM of each primer. The amplification conditions were: denaturing at 95°C for 5 min followed by 35 amplification cycles of 30 s denaturation at 95°C, 30 s annealing at 59°C, 30 s extension at 72°C, and a final extension at 72°C for 5 min. The PCR products were sequenced using an ABI 3730xl DNA Analyzer, and sequence analyses were performed using Chromas Pro 2.33 and DNAMAN 8.0.

Statistical analysis

The genotypes and allelic frequencies between patients and controls were assessed using the chi-squared (χ²) test. The odds ratios (ORs) and 95% confidence intervals (95%CIs) were used to estimate the differences in the genotypes and allelic frequencies using a logistic regression model. The statistical analyses described above were performed using the SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). In addition, Hardy-Weinberg equilibrium was determined using the online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php) (Li et al., 2009). The association between the combined genotypes of exon7 of NSUN7 polymorphisms and the risk of asthenospermia were also assessed by the study of haplotypes using Phase 2.1.

All P values were two-tailed and P values < 0.05 were considered to be statistically significant.
RESULTS

In our study, we found that two genetic variations in exon7 of the NSUN7 gene, c.906 C>T (rs2465570) and c.922T>G (rs2437323), were present in both the patient and control groups. The results of direct sequencing of exon7 from samples are shown in Figure 1. The results of statistical analysis of the genetic variants in exon7 of the putative NSUN7 gene are summarized in Table 2. Haplotype analysis of the two polymorphisms is shown in Table 3.

Figure 1. Direct sequencing of exon7 of the NSUN7 gene. (a) CC (c.906C>T) and TT (c.922T>G); (b) TT (c.906C>T) and GG (c.922T>G); (c) CT (c.906C>T) and TG (c.922T>G).
Polymorphisms in the NSUN7 gene and asthenospermia

Table 2. Genotype and allele frequencies in exon 7 of the Nsun7 polymorphisms among patients and controls.

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Subjects</th>
<th>N</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>P value</th>
<th>OR (95%CI)</th>
<th>HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.906 C&gt;T</td>
<td>Patients</td>
<td>240</td>
<td>CC (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>TT (%)</td>
<td></td>
<td></td>
<td>234 (48.7)</td>
<td>246 (51.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CT (%)</td>
<td></td>
<td></td>
<td>266 (51.9)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Controls</td>
<td>256</td>
<td>66 (25.0)</td>
<td>114 (47.5)</td>
<td>134 (52.3)</td>
<td>928</td>
<td>1.029 (0.723-1.463)</td>
</tr>
<tr>
<td>c.922 T&gt;G</td>
<td>Patients</td>
<td>240</td>
<td>TT (%)</td>
<td>G (%)</td>
<td>T (%)</td>
<td>234 (48.7)</td>
<td>246 (51.3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>GG (%)</td>
<td></td>
<td></td>
<td>266 (51.9)</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>TG (%)</td>
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<td>928</td>
<td>1.029 (0.723-1.463)</td>
</tr>
</tbody>
</table>

OR = odds ratio; CI = confidence interval; HWE = Hardy-Weinberg equilibrium.

For the c.906C>T polymorphism, the frequencies of CC, TT, and CT genotypes were 25, 27.5, and 47.5%, respectively, in the patient group, and 21.9, 25.8, and 52.3%, respectively, in the control group. For the c.922T>G polymorphism, the frequencies of TT, GG, and TG genotypes were 25, 27.5, and 47.5%, respectively, in the patient group, and 21.9, 25.8, and 52.3%, respectively, in the control group.

According to the Hardy-Weinberg principle, the c.906C>T and c.922T>G polymorphisms were in equilibrium in the patient group (P = 0.444, P = 0.438, respectively) and the control group (P = 0.444, P = 0.438, respectively).

The allele frequencies of c.906C>T among patients and controls were 48.7 and 48.1%, respectively, for the C allele, and 51.3 and 51.9%, respectively, for the T allele. The allele frequencies of c.922T>G among patients and controls were 48.7 and 48.1%, respectively, for the C allele, and 51.3 and 51.9%, respectively, for the T allele. The statistical analysis revealed no association between c.906C>T and c.922T>G polymorphisms (P = 0.928, P = 0.928, respectively) between the studied groups.

Haplotype analysis of c.906C>T and c.922T>G polymorphisms showed that there were only two haplotypes (C-T and T-G) in our study, without the C-G and T-T haplotypes, and none of the haplotypes was associated with asthenospermia (P = 0.824, P = 0.824, respectively).

DISCUSSION

Sperm motility is an attribute of semen quality, and is very important for fertilization (Aitken et al., 1982b; Bedford, 1983). Sperm dysfunction has been identified as one of the major factors in male infertility (Hull et al., 1985). Wilson et al. (2005) mutagenized the mouse genome with N-ethyl-N-nitrosourea and found that males homozygous for the Ste5Jcs1 mutation were prone to low sperm motility. Subsequently, Harris et al. (2007) mapped and cloned the Ste5Jcs1 mutation and identified a candidate gene for Ste5Jcs1 called Nsun7, which has a role in sperm motility. The NSUN7 gene encodes an RNA methyltransferase with a Sun do-
main that is homologous to tRNA and rRNA cytosine methyltransferases. Microarray analysis of the mouse testicular transcriptome indicated that Nsun7 RNA is abundant in spermatocytes and haploid spermatids (Shima et al., 2004; Harris et al., 2007). The exon 7 of NSUN7 is a hot spot and is located in the CDD region (Khosronezhad et al., 2014). Harris et al. (2007) first found a mutation in the exon 7 of Nsun7 in mice. In humans, Khosronezhad et al. (2014) found two mutations (C26232T and T26248G) in exon7, which can cause deficits in sperm motility in Iranian men with asthenospermia.

We investigated the genetic polymorphisms in exon7 of the NSUN7 gene in the present study and believe we are the first to report c.906C>T and c.922T>G polymorphisms in Chinese Han people. The polymorphisms we found are the same as in the previous study by Khosronezhad et al. (2014) (c.906C>T = C26232T, c.922T>G = T26248G). The c.906C>T polymorphism is a silent mutation that converts codon GGC to GGT, both being glycine codons. The c.922T>G polymorphism is a missense mutation that leads to the replacement of serine arising from the TCC codon with alanine from codon GCC.

We evaluated the association of the c.906C>T and c.922T>G polymorphisms and their possible association with the risk of asthenospermia. We found relatively similar genotypes and allele frequencies of c.906C>T and c.922T>G polymorphisms among patients and controls. Haplotype analysis also failed to identify any association with asthenospermia. It is possible that mutations in other genes affect sperm motility in Chinese men. However, Khosronezhad et al. (2014) confirmed that in Iran the two polymorphisms in exon7 of NSUN7 existed only in asthenospermic men but not in the control group.

Prior to this study, only one study has reported the polymorphisms of the NSUN7 gene in humans. Owing to the limited number of relevant studies, the inconsistency in results between our study and the previous study may be attributed to the differences in the studied populations, the sample sizes, and environmental factors. Khosronezhad et al. (2014) studied 100 patients with asthenospermia, but only 30 controls, and the study focused on an Iranian population. In the present study, we included 240 asthenospermic men and 256 controls from a Chinese Han population. Therefore, in the future it would be useful to study a greater variety of ethnicities from different geographical populations comprising large numbers of infertile men with asthenospermia.

Moreover, in the present study we only investigated one exon of the NSUN7 gene; possibly polymorphisms or mutations in other exons of NSUN7 are associated with asthenospermia, so it would be useful to analyze the genetic variants in the whole sequence of the NSUN7 gene in a future study.

In addition, Chi and Delgado-Olguín (2013) studied Nsun7 expression during early embryogenesis in mice in detail and found that Nsun7 was broadly expressed in embryonic and extra embryonic tissues. It is possible that these polymorphisms are related to other diseases. In the future, we will also study the NSUN7 gene and its genetic polymorphisms in relation to other diseases.

Interestingly, although none of the haplotypes was associated with asthenospermia, we found that in two haplotypes (C-T and T-G) the two sites of each haplotype were highly linked, and the two haplotypes had similar frequencies in patients and controls.

In summary, the results of our study showed that c.906C>T and c.922T>G polymorphisms of exon7 of the NSUN7 gene are not significantly related to asthenospermia in the Chinese Han population. Further studies with larger groups of subjects of different ethnicities
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are required to clarify the association between these polymorphisms and asthenospermia. We plan to investigate all polymorphisms of NSUN7 with regard to their associations with asthenospermia and other diseases, and we will continue to research other factors for asthenospermia in Chinese men.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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