**Analysis of TATA-box binding protein associated factor 4b gene mutations in a Chinese population with nonobstructive azoospermia**

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## Abstract

Nonobstructive azoospermia (NOA) is a severe form of male infertility. The molecular basis of NOA is still poorly understood. The aim of this study was to explore the associations between single nucleotide polymorphisms (SNPs) of the TATA-box binding protein associated factor 4b (TAF4B) gene and NOA. A total of 100 Han Chinese patients with NOA and 100 healthy men as controls were recruited. Targeted gene capture sequencing was performed. A total of 11 TAF4B SNPs were screened in the NOA and control subjects. Six synonymous and 4 nonsynonymous variants were detected. The c.11G>T (p.G4V) mutation was detected only in NOA patients. Polymorphism Phenotyping v2 and Sorting Intolerant From Tolerant analysis indicated that the p.G4V mutation influenced the protein structure of TAF4B. Haplotype analysis showed that the candidate SNPs did not independently associate with NOA and were found at extremely low frequencies in the subject population. Mutation Taster analysis indicated that the c.11G>T/p.G4V mutation was damaging. WebLogo analysis showed that the residue at amino acid 4 was relatively conserved. The p.G4V substitution may affect the structure of the TAF4B protein. The c.11G>T mutation of the TAF4b gene may be associated with NOA in a Chinese population. Bioinformatics analysis indicated this variation may play an important role in the process of spermatogenesis.

**Abbreviations:** NOA = nonobstructive azoospermia, TAF4B = TATA-box binding protein associated factor 4b.

**Keywords:** azoospermia, infertility, male, polymorphism, single nucleotide

## 1. Introduction

Male infertility is a multifactorial reproductive health problem. Most male infertility is caused by the absence of spermatozoa in the testes (azoospermia) or distinct alterations of sperm quality.[1] Nonobstructive azoospermia (NOA) affects 60% of men with azoospermia.[2] Until now, the etiology of NOA, especially the detailed molecular mechanisms, has remained largely unknown. In the past 10 years, the genetic tests become widespread for the finite etiology. More than 2000 genes are involved in spermatogenesis,[3] and mouse models have identified over 400 genes specifically linked to azoospermia. Although increasing numbers of genes associated with NOA have been reported through case reports and mouse model studies, the molecular basis of NOA is still poorly understood.[4] Only a small number of genes associated with azoospermia proposed by mouse models have been identified in humans, such as testis expressed 11 (TEX11), tudor domain containing 9 (TDRD9), Zinc finger MYND-type containing 15 (ZMYND15), and TATA-box binding protein associated factor 4b (TAF4B).[5]

TAF4B is a cell type-specific TBP-associated factor that may mediate transcription by a subset of activators in B cells. TAF4B is located on human chromosome 18q11.1 and is highly expressed in testis (RPKM 5.0), lymph node (RPKM 3.4), and 24 other tissues.[6] In mice, TAF4B is a gonadal-enriched component of the general transcription factor complex, transcription factors IID (TFIID), which is required for the maintenance of spermatogenesis.[7] TAF4B expression may affect the development of spermatogenic cells spermatogonial stem cells.

In the present study, we performed targeted gene capture sequencing to identify mutations of TAF4B among 100 patients with NOA and 100 controls. Bioinformatic analysis combined with case-control studies was conducted to systematically assess the effects of mutations on TAF4B structure.

## 2. Material and methods

### 2.1. Study population

A total of 100 Han Chinese patients with NOA (age 29.14 ± 4.40 years) and 100 healthy men as a control group (age 25.10 ± 5.68 years) were recruited from the Center for Reproductive Medicine and Center for Prenatal Diagnosis, First Hospital, Jilin University, 71 Xinmin Street, Chaoyang District, Changchun, Jilin Province 130021, China (e-mail: zhanghguo2018@163.com).
Medicine, the First Hospital of Jilin University. Patients with abnormal karyotypes and Y chromosome microdeletions were excluded from the study. Controls were healthy men randomly chosen with a normal sperm count and no known history of infertility. This study was approved by the ethics committee of the First Hospital of Jilin University and all patients gave written informed consent.

2.2. Sequencing and mutational analysis
Genomic deoxyribonucleic acid was isolated from blood lymphocyte samples. Biotinylated capture probes were designed for TAF4B gene exons and mutation screening of genes was performed by targeted gene capture sequencing using the Illumina HiSeq2000 Next-Generation Sequencing platform (MyGenostics, Beijing, China) according to our previously published paper.[8] The impact of the mutations on TAF4B protein was assessed by Polymorphism Phenotyping v2 (PolyPhen-2) and Sorting Intolerant From Tolerant (SIFT). Detailed mutation information was predicted by MutationTaster (http://www.mutationtaster.org/). Statistical analysis was performed with SPSS Inc., version 19.0 (IBM Corp. Armonk, NY, USA) and P < .05 was considered statistically significant.

2.3. Haplotype and conservation analysis
Haplotype analysis was performed with Haplovew 4.2. Haploview was used to generate linkage disequilibrium blocks to determine which of the 11 SNPs were inherited together as haplotypes. Multiple species amino acid sequence alignment and WebLogo analysis of the TAF4B protein were carried out separately by European Molecular Biology Laboratory-European Bioinformatics Institute (EMBL-EBI) (https://www.ebi.ac.uk/Tools/msa/muscle/) and WebLogo Version 2.8.2.[9]

2.4. Structural analysis
Structural analysis of the TAF4B variant was performed using SWISS-MODEL software (https://www.swissmodel.expasy.org; based on the template of the Transcription initiation factor TFIID subunit 4, 2p6v.pdb) and Protein Fold recognition Server (Phyre2). For protein structure visualization, we used PyMol Version 2.2.0.

3. Results
Targeted gene capture sequencing of TAF4B in 100 NOA patients and 100 controls identified 6 synonymous variants (rs12456749, rs1677016, rs17224558, rs3749691, rs3826624, and rs771186391) and 4 nonsynonymous variants (rs12963653, rs148172329, rs200126045, and rs74947492) (Table 1). SIFT showed that p.G492G, p.N539S, p.Q375H, p.E540A, p.V438L, and p.G4V mutations were tolerable. The p.G4V mutation was only detected in NOA patients and not in the controls. For the sites with significant differences in nonsynonymous mutations,
Figure 2. (A) PolyPhen-2 and SIFT analysis of p.G4V mutation; (B) location of mutations (c.11G>T and c.1619A>C) in the TAF4B gene. SIFT = sorting intolerant from Tolerant, TAF4B = TATA-box binding protein associated factor 4b.

Figure 3. Haplotype analysis of NOA-SNPs on chromosome 18. NOA = nonobstructive azoospermia, SNPs = single nucleotide polymorphisms.
the minimum allele frequency of the mutations is shown in Figure 1. Figure 1 also shows the minimum allele frequency of the novel mutation (c.11G>T/p.G4V) that was detected only in NOA patients. The PolyPhen-2 and SIFT analysis indicated that the p.G4V mutation influenced the protein structure and function (SIFT sensitivity: 0.99, specificity: 0.14; PolyPhen-2 intersection points: 0.00) (Fig. 2A). Bioinformatics analysis indicated that c.11G>T led to an amino acid substitution at the fourth residue (p.G4V) and c.1619A>C led to an amino acid substitution at 540th residue (p.E540A) (Fig. 2B).

The 11 candidate SNPs were distributed on chromosome 18. Two linkage disequilibrium blocks were identified within rs3744961 (Fig. 3, black and green box), and 1 linkage disequilibrium block was identified within rs1677016 (Fig. 3, yellow box) of TAF4B. However, haplotype analysis showed that the candidate SNPs did not independently associate with NOA and created haplotypes that were found at extremely low frequencies in the subject population.

MutationTaster programs were used to predict the impact of disease-causing variants on protein structure, function, and disease-causing potential of sequence variations. MutationTaster analysis indicated that the c.11G>T/p.G4V mutation was damaging and this mutation is not present in the dbSNP, 1000 Genome, ExAC, and gnomAD databases.

Evolutionary conservation analysis of multiple sequence alignments of TAF4B protein and its homologs showed that the glycine at residue 4 was evolutionarily conserved from the green monkey to human (Fig. 4A), suggesting that Gly4 may play an important role in the function of the TAF4B protein. WebLogo analysis showed that the domain was relatively conserved from the green monkey to humans (Fig. 4B).

Structural analysis of the wild-type and mutant TAF4B proteins (c.11G>T/p.G4V) predicted that the protein folding structure was altered in the mutant protein (Fig. 5). The folder structure of the protein breaks when the amino acid residue 4 changes, which suggests that the p. Gly4Val substitution may affect the structure of the TAF4B protein.

4. Discussion
Approximately 50% of infertility cases are associated with male factors, and azoospermia is prevalent in 5% of infertile men.\textsuperscript{51}
For patients with NOA, the etiology is mostly idiopathic and only a minority of cases carry a defective karyotype or a Y-chromosome microdeletion. The underlying etiology and genetic mechanism of NOA remain largely unclear. Current research has shown that genetic defects are the main causes of abnormal spermatogenesis, especially for NOA.[10–13] Hence, discovering the underlying etiology of NOA is important. A massively parallel sequencing was performed to identify genetic abnormalities in a large cohort including NOA patients and controls.[14] Such studies can help researchers explore the underlying genetic aetiologies of NOA. In this study, we aimed to identify and investigate the genetic mutations of the TAF4B gene in a Chinese population with NOA. A nonsynonymous mutation in exon 1 of the TAF4B gene (c.11G>T/p.G4V) was identified in NOA patients and not detected in 100 healthy men. Our analysis indicates that this mutation may have an irreversible effect on the TAF4B protein. Therefore, this study focused on the application of bioinformatics analysis to explore whether the c.11G>T mutation of TAF4B gene is related to the occurrence of NOA.

The gene encoding TAF4B, also called TAFII105 (RNA polymerase II, TATA box-binding protein-associated factor), has 15 exons and encodes an 862 amino acid protein.[15] The TFIID complex is a core RNA polymerase complex that contains the TATA-binding protein and 14 TBP-associated factors that function in core promoter recognition and activator-dependent RNA polymerase II recruitment.[16] Freeman et al.[17] reported that TAF4B is enriched in mouse gonadal tissues. In addition, TAF4B-null young mice were initially fertile but became infertile after 3 months because of impaired gonocyte proliferation and reduced expression of spermatogonial stem cell markers.[18] Our study evaluated the association between TAF4B variations and NOA in a cohort of Han Chinese patients and controls. We identified 11 known SNPs including 6 synonymous variants and 4 nonsynonymous variants. A novel mutation (c.11G>T/p.G4V) located in exon 1 was detected only in patients with NOA. The mutation was not found in ExAC or 1000G (MutationTaster). ThePolyPhen-2 and SIFT analysis indicated that p.G4V mutations probably altered the protein structure. Evolutionary conservation analysis showed that the residue at amino acid 4 was evolutionarily conserved. TAF4B proteins were modeled by the SWiSS MODel software based on the template of the (2p6v. pdb).[19] We speculate that this mutation may result in altered germ cell function and this should be examined in future studies. Interruption of any of these phases can result in the failure of spermatogenesis and give rise to NOA.[20] Further functional studies are needed to elucidate the effect of these mutations on the changes in the protein structure as well as the biological functions of TAF4B.

One limitation of this study was that the patient group was limited to a Han ethnic population with NOA from Northeast China. Furthermore, the sample size was relatively small in this study. Our bioinformatics results will also need to be confirmed by more cases or animal experiments.

5. Conclusions
The present study showed that the c.11G>T mutation of the TAF4B gene may be associated with NOA in a Chinese population.
population. Bioinformatics analysis indicated this variation might play an important role in the process of spermatogenesis. Further investigations are required to explore the functional and pathological role of the mutated TAF4B in NOA.

Author contributions

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References

[1] Tournaye H, Krausz C, Oates RD. Novel concepts in the aetiology of male reproductive impairment. Lancet Diabetes Endocrinol 2017;5:544–53.
[2] Jarvi K, Lo K, Fischer A, et al. CUA guideline: the workup of azoospermic males. Can Urol Assoc J 2010;4:163–7.
[3] Krausz C, Riera-Escamilla A. Genetics of male infertility. Nat Rev Urol 2018;15:369–84.
[4] Matzuk MM, Lamb DJ. The biology of infertility: research advances and clinical challenges. Nat Med 2008;14:1197–213.
[5] Arafat M, Har-Vardi I, Harlev A, et al. Mutation in TDRD9 causes non-obstructive azoospermia in infertile men. J Med Genet 2017;54:633–9.
[6] Fagerberg L, Hallström BM, Oksvold P, et al. Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics 2014;13:397–406.
[7] Lovasco LA, Gustafson EA, Seymour KA, et al. TAF4b is required for mouse spermatogonial stem cell development. Stem Cells 2015;33:1267–76.
[8] Wang R, Xi Q, Zhang H, et al. Chloride channel accessory 4 (CLCA4) gene polymorphisms and non-obstructive azoospermia: a case-control study. Med Sci Monit 2019;25:2043–8.
[9] Crooks GE, Hon G, Chandonia JM, et al. WebLogo: a sequence logo generator. Genome Res 2004;14:1188–90.
[10] Wosnitzer M, Goldstein M, Hardy MP. Review of azoospermia. Spermatogenesis 2014;4:28218.
[11] Gerlin A, Raucu F, Gatta V, et al. Male infertility: role of genetic background. Reprod Biomed Online 2007;14:734–45.
[12] Mou L, Zhang Q, Gao R, et al. A functional variant in the UBE2B gene promoter is associated with idiopathic azoospermia. Reprod Biol Endocrinol 2015;13:79.
[13] Cirulli ET, Goldstein DB. Uncovering the roles of rare variants in common disease through whole-genome sequencing. Nat Rev Genet 2010;11:415–25.
[14] Li Z, Huang Y, Li H, et al. Excess of rare variants in genes that are key epigenetic regulators of spermatogenesis in the patients with non-obstructive azoospermia. Sci Rep 2015;5:8785.
[15] Ayhan O, Balkan M, Guven A, et al. Truncating mutations in TAF4B and ZMYND15 causing recessive azoospermia. J Med Genet 2014;51:239–44.
[16] Verrijzer CP, Tjian R. TAFs mediate transcriptional activation and promoter selectivity. Trends Biochem Sci 1996;21:338–42.
[17] Freiman RN, Albright SR, Zheng S, et al. Requirement of tissue-selective TBP-associated factor TAFII105 in ovarian development. Science 2001;293:2084–7.
[18] Falender AE, Freiman RN, Geles KG, et al. Maintenance of spermatogenesis requires TAF4b, a gonad-specific subunit of TFIID. Genes Dev 2005;19:794–803.
[19] Wang X, Truckses DM, Takada S, et al. Conserved region I of human coactivator TAF4 binds to a short hydrophobic motif present in transcriptional regulators. Proc Natl Acad Sci USA 2007;104:7839–44.
[20] Zhang Y, Song B, Du WD, et al. Genetic association study of RNF8 and BRDT variants with non-obstructive azoospermia in the Chinese Han population. Syst Biol Reprod Med 2015;61:26–31.