Integrative Molecular Medicine

Case Report ISSN: 2056-6360

SOX30 might not be associated with Sertoli cell-only syndrome in azoospermic Japanese men

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Abstract

Purpose: Members of the Sry-related HIGH Motility Group (HMG box (SOX) gene family encode transcription factors that are highly conserved and are critical for a range of developmental processes. In mice, loss of function of the *Sox30* gene results in male infertility with failure of spermatogenesis. Here, we investigated the relevance of this gene to human male infertility manifested as Sertoli cell-only syndrome (SCOS) with azoospermia.

Methods: A total of 138 Japanese men with SCOS were included, along with 95 fertile Japanese men as healthy controls. All patients underwent testicular microdissection and sperm extraction; however, no spermatozoa were found. Mutation analysis of the *SOX30* coding region was performed.

Results: Six single nucleotide polymorphisms (SNPs) were identified in the coding region in the patient group. However, the frequency and distribution of SNPs in this allele were not significantly different between patient and control groups.

Conclusions: This study suggests a lack of association of *SOX30* with azoospermia in infertile Japanese men with SCOS.

Introduction

Infertility affects about 15% of couples wishing to have children, and approximately half of these cases are associated with male factors. Genetic causes of azoospermia include chromosomal abnormalities, Y chromosome microdeletions, and specific mutations/deletions of several genes [1-3]. Approximately 10% of all cases of male infertility (<1% of all men) exhibit nonobstructive azoospermia (NOA) [4,5]. NOA is a histopathological diagnosis based on testicular biopsy findings including hypospermatogenesis, maturation arrest, or Sertoli cell-only syndrome (SCOS). Germ cells are completely absent in men with SCOS, which therefore represents the most severe type of NOA. Although the pathogenesis of SCOS remains unknown, it is postulated to occur before or during the premeiotic proliferation phase of spermatogonia [6-8].

Members of the *Sry*-related HIGH Motility Group [HMG) box gene (*SOX*) family encode transcription factors that are highly conserved and critical for a range of developmental processes, including sex determination, neuronal development, and regulation of stem cell pluripotency [9]. Within the *SOX* gene family, *SOX30* is the sole representative of group II and is a relatively divergent member, with an HMG domain only 46% identical to the SOX–HMG box consensus sequence [10,11]. It has been reported that *Sox30*-null male mice are heathy but sterile, whereas female mice with this deletion are fertile. In the absence of *Sox30*, meiosis initiates normally in both sexes; however, germ cell development arrests during the postmeiotic round spermatid period in male mice, leading to azoospermia. Therefore, a *SOX30* mutation might underlie some instances of unexplained NOA in humans [12].

Here, we describe an analysis of *SOX30* single nucleotide polymorphisms (SNPs) in 138 infertile Japanese men showing SCOS.

Materials and methods

This study was approved by the Ethics Committee of Asahikawa Medical University, Japan. Written informed consent was obtained from each study participant. Japanese men with azoospermia secondary to SCOS without any chromosomal abnormalities were selected. Those with defective spermatogenesis caused by infections, seminal tract obstruction, pituitary gland dysfunction, or other causes of testicular disorder were excluded from the study. A total of 138 men with SCOS were included, together with 95 fertile Japanese men who had fathered children as healthy controls. All SCOS patients underwent testicular microdissection and sperm extraction to confirm the absence of spermatozoa. A final diagnosis of SCOS was decided by two pathologists. All fathers of the patients were obviously fertile, and none of their brothers suffered from azoospermia. Direct sequencing of the *SOX30* coding region was performed on fragments amplified by PCR using peripheral leukocyte DNA and gene-specific

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Key words: *azoospermia, mutation, Sertoli cell-only syndrome, single nucleotide polymorphism, SOX30*

Received: April 04, 2020; **Accepted:** April 23, 2020; **Published:** April 28, 2020

Table 1. Genotype and allele frequencies for six coding SNPs in the human SOX30 gene identified in 138 azoospermic Japanese patients with SCOS and 95 normal controls

SNP	Alteration	Genotype frequency	Allele frequency
	Nucleotide Amino acid	Genotype/Total no. of samples (%)	Minor allele/Total no. of chromosomes (%)
(G) SCOS Control	p-value	(A) SCOS Control	p-value
SNP1	c105G>A	(GA) 2/138 (1.4%) 0/95 (0.00%) 0.515	(A) 2/276 (0.72%) 0/190 (0.00%)0.516
SNP2	c359C>T	NS (CT) 2/138 (1.4%) 0/95 (0.00%)0.515	(T) 2/276 (0.72%) 0/190(0.00%)0.516
SNP3	c526G>A	NS (GA) 1/138 (0.70%) 0/95 (0.00%) 1.00	(A) 1/276(0.36%) 0/190 (0.00%) 1.00
SNP4	c1284C>A	NS (CA) 15/138 (10.9%)10//95 (10.5%) (AA) 2/138 (1.4%) 1/95 (1.05 %) 1.00	(A) 19/276 (5.80%) 12/190 (6.32%)0.852
SNP5	c1457G>C	NS (GC) 1/138 (0.70%) 1/95 (1.05%) 1.00	(C) 1/276 (0.36%) 1/190 (0.53%) 1.00
SNP6	c1811C>T	(CT) 29/138 (21.0%) 18/95 (19.0%) 0.742	(T) 29/276 (10.5%)18/190 (9.47%)0.757

SNP: Single nucleotide polymorphism; SCOS: Sertoli cell-only syndrome; NS: Nonsynonymous substitution

forward (Fw) and reverse (Rv) primers as follows: SOX30-cds1-Fw (5′-AGGGTTCTGGGTAGCAAAGG-3′) and SOX30-cds1-Rv (5′-GTTTAGACTTGGGAACTGCTGAC-3′); SOX30-cds2-Fw (5′-ATAGACGGAGGTGCACTTTACC-3′) and SOX30-cds2-Rv (5′-CTCATGCCCAGTGTCATTTCA-3′); SOX30-cds3-Fw (5′-CCGGAGCTGGAAAAGATACTTTAG-3′) and SOX30-cds3-Rv (5′-ACCAAGTGTTTTGATCTCTACTGAA-3′); SOX30-cds4-Fw (5′-GTGATTGATGAAGGCCTCTGCA-3′) and SOX30-cds4-Rv (5′-TTGTCACAGGTAAAAACTCATTAGC-3′); SOX30-cds5-Fw (5′-ACTTTCCAAACCCCAAAGAGG-3′) and SOX30-cds5-Rv (5′-TCACGACTGAAAACTTTCAACAA-3′).

Fisher's exact test was used to evaluate the statistical significance of differences in the distribution of *SOX30* variants among patients and controls. The potential pathogenicity of *H3T* mutations was predicted by *in silico* analysis using the software package MutationTaster (http://www.mutationtaster.org/).

Results

The *SOX30* coding and flanking intronic regions were amplified and subjected to direct sequencing in all 138 patients with SCOS. Six variants were detected in this patient group (Table 1): SNP1, c105G>A, Chr5:157651974, rs767435597; SNP2, c359C>T, Chr5:157651720, rs763607355; SNP3, c526G>A, Chr5:157651553, rs549735716; SNP4, c1284C>A, Chr5:157646739, rs12188040; SNP5, c1457G>C, Chr5:157638652, rs778628194; and SNP6, c1811C>T, Chr5:157638298, rs35793864. All six SNPs have been reported previously. Only the SNP4 change was predicted to be "disease causing" in an *in silico* analysis using MutationTaster; the other SNPs were predicted to be polymorphisms. However, we found no information about their frequencies in the Japanese male population. Allele and genotype distributions of the identified six SNPs in the patients and controls are listed in Table 1. A statistically significant association with SCOS could not be found in the identified SNPs.

Discussion

We found no critical mutations or differences in genotype and allele frequencies between patient and control groups. The lack of a critical mutation in *SOX30* suggests that the phenotype in *Sox30* null mice might not apply to humans. However, the sample size was not sufficient for a comprehensive mutational analysis because the occurrence and/or frequency of mutations in *SOX30* is not known for the general Japanese population; therefore, these results should be considered preliminary. Additionally, this is the first report on the possible effect of *SOX30* variants on human male infertility. The small sample size might be one of the reasons we did not observe any statistically significant differences between the two groups. Furthermore, we only analyzed Japanese men, so other ethnic groups should be analyzed to confirm our findings.

While many genes and processes involved in spermatogenesis are conserved between mice and humans [7], functional mouse orthologs are not always representative of their corresponding human genes. *Sox30* null male mice are sterile with complete arrest of spermiogenesis, but we were only able to analyze patients with azoospermia and SCOS here because we lacked DNA samples from patients with azoospermia caused by complete arrest of spermiogenesis. Therefore, we only studied patients with azoospermia secondary to SCOS. Future studies should analyze patients with azoospermia caused by defective or arrested spermiogenesis, and include more Japanese patients and men from other ethnic groups.

In conclusion, the genotypes and allele frequencies of these six SNPs in *SOX30* were not shown to be connected with azoospermia associated with SCOS in this Japanese population. The lack of significant mutations in *SOX30* in these patients indicates that mutations in this gene are not likely to be responsible for this form of male infertility. However, it must be noted that this is a preliminary report because the sample size was small and we could not evaluate the regulation of this gene or the level of SOX30 protein in testicular tissues.

Acknowledgments

This work was supported by Grants-in-Aid for Scientific Research (Nos 19K09770 and 19K18550) from the Japan Society for the Promotion of Science, Japan. We thank James Cummins, PhD, and Sarah Williams, PhD, from EDANZ Group (www.edanzediting.com/ac) for editing drafts of this manuscript.

Disclosures

All authors declare that they have no conflict of interest. The study was approved by the Institutional Review Board of Asahikawa Medical University. Written informed consent was obtained from all participants. All procedures followed were in accordance with the Helsinki Declaration of 1964 and its later amendments.

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