

## COMMENT

# X-Linked Sex-Determining Region Y Box 3 (SOX3) Gene Mutations Are Uncommon in Men with Idiopathic Oligoazoospermic Infertility

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The X-linked sex-determining region Y box 3 (*SOX3*) gene is expressed in the developing gonads and brain. *Sox3*-null mice developed according to genetic sex, but the hemizygous null males were hypogonadal, with extensive Sertoli cell vacuolization, loss of germ cells, and reduced sperm count. We hypothesized that *SOX3* mutations might occur in a subset of infertility patients. Genomic DNA samples from 56 infertile men with idiopathic oligo-azoospermia were screened for *SOX3* mutations. Three nucleotide substitutions (609 T→C,

732 A→C, and 978 G→A) were identified, none of which altered the amino acid sequence, suggesting that they are polymorphic variants. The 609 T→C substitution was in the HMG box, and the two other substitutions were identified within the polyalanine repeat regions. Three patients had 609 T→C, 2 patients had 609 T→C and 732 A→C, and one had 978 G→A. These data indicate that mutations in the *SOX3* gene are not a common cause of male infertility. (*J Clin Endocrinol Metab* 89: 4146–4148, 2004)

MALE AND FEMALE factors contribute about equally to infertility, which affects about 10–15% of couples (1). Although many causes (varicocele, cryptorchidism, genital infection, and antisperm antibodies) of male infertility have been identified, about 50% of patients are still assigned a diagnosis of idiopathic infertility. Microdeletions of regions of the Y chromosome required for effective spermatogenesis have been found in approximately 15% of these cases (2). Although mutations in a considerable number of genes can cause pathology in the male reproductive system (3), few have been identified as common causes of male infertility.

The sex-determining region Y box 3 (*SOX3*) has been hypothesized to play a role in the sex-determining process (4, 5). It is a member of the high mobility group (HMG) family of transcription factors and is located on the X chromosome (6). *SOX3* was cloned based on its HMG box homology to sex-determining region Y (*Sry*) (7), the male-determining gene. In a recent study, *SOX3*-null mice developed according to genetic sex, but the hemizygous males had small testes and a reduced number of sperm (8). Testicular histology revealed extensive Sertoli cell vacuolization, loss of germ cells, and disruption of the seminiferous tubules. Small testes were also observed in a human 46,XY male with complete loss of *SOX3*

(6, 9). These findings suggest that *SOX3* plays an important role in testis development and possibly sperm maturation. We hypothesized that *SOX3* mutations might cause oligoazoospermic male infertility and analyzed the *SOX3* gene in 56 male patients with idiopathic oligoazoospermic infertility.

### Subjects and Methods

#### Subjects and DNA isolation

Fifty-six unrelated infertile French men with oligo- or azoospermia were included in this study. Oligoazoospermia (sperm count of  $<20 \times 10^6$  sperm/ml or undetectable) was confirmed by at least two independent semen analyses performed according to the World Health Organization 1999 guidelines (10). Subjects were subjected to detailed clinical investigations, including physical examination, cytogenetic analysis, and endocrinological studies. Subjects were not included if they had a plausible cause of infertility (greater than grade 2 varicocele, cryptorchidism, karyotypic abnormality, Y microdeletion, or obstructive infertility). The majority of patients investigated ( $n = 45$ ) had primary infertility (without any previous pregnancy); 11 patients had secondary infertility (defined by having at least one child, but now unable to conceive after trying for 1 yr or more).

Genomic DNA was extracted from patients' blood leukocytes for DNA sequence analysis. The study design and procedures were approved by the institutional review board of Northwestern University, and informed consent was obtained from each subject.

#### Sequence analysis

A total of 1575 bp of the single exon *SOX3* gene, comprising 113 bp of the 5'-flanking sequence, 121 bp of the 3'-untranslated sequence, and the entire coding sequence, was determined by direct sequencing of five

Abbreviations: HMG, High mobility group; *SOX3*, X-linked sex-determining region Y box 3; *Sry*, sex-determining region Y.

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partially overlapping amplicons obtained by PCR with genomic DNA. The primer sequences and lengths of amplified fragments are listed in Table 1.

Thermal cycling was performed with an initial predenaturation at 94 C for 4 min, followed by 32 cycles of denaturation at 94 C for 1 min, primer annealing at 56 C for 1 min, and primer extension at 72 C for 1 min. The final extension was carried out at 72 C for 15 min. Buffer conditions have been described previously (11). Amplicons were purified on a 1.5% agarose gel using a QIAquick gel extraction kit (Qiagen, Valencia, CA), then subsequently sequenced in forward and reverse directions using dRhodamine Terminator Cycle Ready Reaction kits (Applied Biosystems, Foster City, CA) and automated sequencers ABI PRISM 3100 genetic analyzer (Applied Biosystems). The sequences obtained were compared with the reference sequence (GenBank accession no. NM\_005634). The A of the ATG translation initiation codon was designated +1 (12).

## Results

Sequencing of the single exon, 113 bp of the 5'-flanking sequence and 121 bp of the 3'-untranslated sequence of *SOX3* did not reveal any mutations in the 56 patients studied. A nucleotide substitution was detected at three sites in six patients, none of which altered the predicted amino acid sequence: 609 T→C (five patients, 8.9%), 732 A→C (two patients, 3.6%), and 978 G→A (one patient, 1.8%). The first variant (609 T→C), located in the HMG box, did not change the tyrosine residue. The two other variants (732 A→C, 978 G→A), located in two different polyalanine tracts, did not change the alanine residue (Fig. 1). The first two variants have been described previously (13). The third variant, 978 G→A, has not been previously described and was found in only one patient. We cannot exclude the possibility that these silent mutations might alter DNA methylation, RNA stability, or other features that regulate *SOX3* expression. How-

ever, there was no correlation between these nucleotide variants and the clinical manifestations (Table 2).

## Discussion

Despite advances in assisted reproductive technologies, infertility is a major health problem. Approximately 15% of couples are unable to conceive within 1 yr of unprotected intercourse. A plausible etiology is found in about 50% of cases. Few genetic causes of infertility have been identified in humans (14); nevertheless, genetic etiologies are thought to underlie many cases of idiopathic infertility. As described by Wang and co-workers (15) in a systematic search for genes expressed in mouse spermatogonia, but not in somatic tissue, the X chromosome has a high concentration of spermatogenesis genes.

*SOX3*, located on the X chromosome, is expressed in the urogenital ridge (7), from which the gonads develop, and it continues to be expressed in the adult gonads of both sexes (8, 16). *SOX3* is present in gonads lacking germ cells (7), consistent with expression in somatic cells. However, in chickens, *SOX3* is detected in the primordial germ cells of both sexes (17), and expression in germ cells cannot be excluded. In the adult, *SOX3* is still expressed in the testis.

*SOX3*-null mice developed according to genetic sex. However, female *SOX3*-null mice displayed high rates of follicular atresia and severely reduced fertility. Male *SOX3*-null mice have Sertoli cell dysfunction with extensive vacuolization, loss of germ cells, and reduced sperm count.

The pattern of *SOX3* expression and its location on the X chromosome make it a plausible gene candidate for various pathologies, such as sex reversal (13), primary ovarian failure (18), or Rett syndrome (19). However, no *SOX3* mutations were found in these disorders.

In humans, a large deletion (including *SOX3*) of the X chromosome has been described in men with varying degrees of mental retardation (9, 20, 21). In at least two cases, small testes were associated with these deletions (9, 20). In a different model of gonadal dysgenesis, deletion of *Ahch* (*Dax1*) caused a severe testicular phenotype in mice (22) that was only subsequently confirmed in humans (23).

The current study indicates that *SOX3* mutations are not a common cause (<2%) of idiopathic male infertility. It is possible, however, that *SOX3* mutations might be associated with specific testis pathologies or with mental retardation. Thus, *SOX3* should still be considered in future studies of male infertility until other more commonly affected genes are identified.

**TABLE 1.** Primer pairs and corresponding PCR product size

Pair no.	Name	Primer sequence (5'–3')	Size (bp)
1	F1	TGCGAACCTGTCAATCAC	440
	R1	CGCACTACTCTTGCCTGC	
2	F2	CTTCTGGAGACTGAACTC	420
	R2	GAGCAGCGTCTTGGTCTT	
3	F3	GACTTCGCGCCGTGCACA	450
	R3	GCCGCGGCTGCTGTGGCT	
4	F4	GCGCTCAGAGCTACATGA	270
	R4	GGTACATGCTGATCATGT	
5	F5	GAAGTCTGAGCCCAGCTC	340
	R5	CATCGGTACAAGGCAACA	

**TABLE 2.** Clinical data for patients with nucleotide variants

Patient	Variant	Diagnosis	Sperm conc. (million/ml)	Testis volume (ml)		FSH (mIU)
				Left	Right	
Z	609 T→C	Idiopathic	3.4	20	20	6.9
BE	609 T→C	Idiopathic	1.5	13	12	3.6
BK	609 T→C	Idiopathic	0.9	14	16	12.6
AU	609 T→C	Idiopathic	6.5	9	11	12.3
BC	732 A→C	Idiopathic	0.1	12	14	8.4
	609 T→C					
AS	978 G→A	Idiopathic	5.1	8	6	4.3

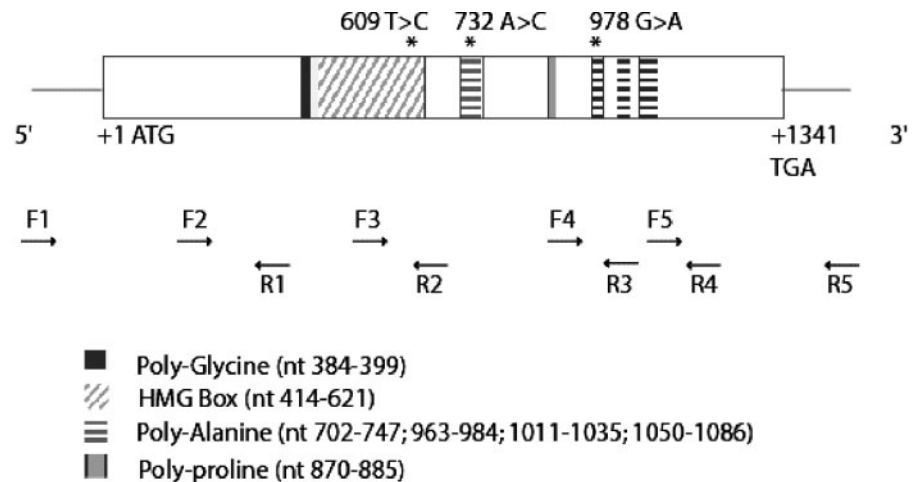


FIG. 1. Position on the *SOX3* gene of the primer pairs and the nucleotide (nt) variants identified.

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