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Research Article

Targeted disruption of the mouse testis-enriched gene *Znf230* does not affect spermatogenesis or fertility

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Abstract

The mouse testis-enriched *Znf230* gene, which encodes a type of RING finger protein, is present primarily in the nuclei of spermatogonia, the acrosome and the tail of spermatozoa. To investigate the role of *Znf230* in spermatogenesis, we generated Znf230-deficient mice by disrupting *Znf230* exon-5 and exon-6 using homologous recombination. The homozygous Znf230-knockout (KO) mice did not exhibit *Znf230* mRNA expression and Znf230 protein production. Znf230 KO mice exhibited no obvious impairment in body growth or fertility. Male Znf230 KO mice had integral reproductive systems and mature sperm that were regular in number and shape. The developmental stages of male germ cells of Znf230 KO mice were also normal. We further examined variations in the transcriptomes of testicular tissue between Znf230 KO and wild-type mice through microarray analysis. The results showed that the mRNA level of one unclassified transcript *4921513I08Rik* was increased and that the mRNA levels of three other transcripts, *i.e.*, *4930448A20Rik*, *4931431B13Rik* and potassium channel tetramerisation domain containing 14(*Kctd14*), were reduced more than two-fold in Znf230 KO mice compared with wild-type mice. Using our current examination techniques, these findings suggested that Znf230 deficiency in mice may not affect growth, fertility or spermatogenesis.

Keywords: Znf230, knockout mice, spermatogenesis, Kctd14.

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Introduction

Mammalian spermatozoa development is a complex process that involves the renewal and differentiation of spermatogonia, the meiosis of spermatocytes, and drastic morphological changes accompanying the development from round spermatids to mature spermatozoa (Russell *et al.*, 1990). Many environmental, behavioral and genetic factors affect male fertility. An estimated 50% of human infertility has been attributed to genetic abnormalities (Hwang *et al.*, 2011). Two previous microarray studies (Schultz *et al.*, 2003; Schlecht *et al.*, 2004) showed that haploid germ cells express a large number of germ cell-specific genes (approximately 4% of mammalian genes). Therefore, it is necessary to identify these unique genes and characterize the precisely programmed cell- and stage-specific gene expression that occurs during the regulation of the developmental spermatogenesis process.

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The human gene *ZNF230* (also named *RNF141*), which encodes a type of RING (Really Interesting New Gene) finger protein, was first identified in our laboratory to be restrictively expressed in the testicular tissue of fertile men (Zhang *et al.*, 2001). RING finger proteins, a subfamily of zinc finger proteins (ZFP), often contain the cysteine-rich $CX_2CX_{(9-39)}CX_{(1-3)}HX_{(2-3)}CX_2CX_{(4-48)}CX_2C$ domain and are involved in a variety of biological processes, including transcriptional regulation, signal transduction, cell apoptosis and protein ubiquitination (Borden, 2000; Joazeiro and Weissman, 2000). The mouse homolog of *Znf230* was also identified in our laboratory (Qiu *et al.*, 2003). The expression of the mouse *Znf230* gene is developmentally regulated, and the Znf230 protein functions as an activator module in transcription. Additionally, the mouse Znf230 protein is primarily expressed in the nuclei of spermatogonia but has subsequent expression in the acrosome system and the tails of developing spermatids and spermatozoa (Song *et al.*, 2008). Hence, we wondered whether *Znf230* may play a role in mammalian spermatogenesis.

Animal models have defined key signaling pathways that are involved in reproductive physiology (Li *et al.*, 2001). To date, over 400 genes that are essential for male

fertility have been identified using transgenic, chemically induced, point mutants and KO/knock-in/gene-trap mouse models (Yatsenko *et al.*, 2010; Jamsai and O'Bryan, 2011).

To investigate the role of *Znf230* in mouse spermatogenesis, we used a targeted gene KO strategy to generate Znf230-deficient mice. We had previously constructed a gene-targeting vector based on a modified pPNT vector and generated mutant mice with exon-2 of the *Znf230* gene disrupted (Liu *et al.*, 2013). However, a partial sequence from the pPNT vector acted as an alternative exon-2, thus allowing a new *Znf230* transcript to be produced in the mutant mice and a new protein product, possessing a C-terminal amino acid sequence with a RING finger motif similar to that of the wild-type Znf230 protein, to be generated. Thus, Znf230 function in the mutant mice was not entirely inactivated. In the current study, we changed the targeting strategy such that the region of exon-5 and exon-6, which encodes the essential RING finger domain of the Znf230 protein, was directly disrupted. This strategy successfully generated Znf230-null mice for use in this study.

Materials and Methods

Construction of Znf230 KO targeting vector and generation of Znf230 KO mice

Using a highly efficient recombineering-based method that has been previously described (Liu *et al.*, 2003; Chan *et al.*, 2007), the Znf230 KO targeting vector was constructed from a genomic DNA fragment derived from the C57BL/6J bacterial artificial chromosome clone bMQ-291L21. The targeting construct, which had a 4864 bp left arm containing introns 2 and 3 of the *Znf230* gene and a 2902 bp right arm containing the partial untranslated region of exon-6, was inserted into the ABRLFn-pBR32 vector (Figure 1).

Thirty micrograms of the targeting vector was linearized by *Not* I and transfected into CJ7 (derived from 129SV/J mice) embryonic stem cells (ESCs) by electroporation. Ninety-six ESC clones were selected with 300 μg/mL G418 (Geneticin, Sigma-Aldrich Co., St. Louis, MO, USA). Among these neomycin-resistant cells, 12 ESCs that had undergone homologous recombination were identified by long polymerase chain reaction (L-PCR) analysis with two pairs of primers P1F: 5'-acctctggcctttacaaactcatg-3', P1R: 5'-ggcctacccgcttccattgctc-3' and P2F: 5'-ccgtgccttccttgaccctgg-3', P2R: 5'-caagcagccttattacccagttg-3'.

Two correctly targeted ESC clones were microinjected into C57BL/6J blastocysts to generate chimeras that were then crossed into a C57BL/6J genetic background. The offspring were screened by L-PCR analysis of their genomic DNA using P1F/R and P2F/R primers.

The Znf230 KO mice were generated at the Shanghai Research Center for Model Organisms, Shanghai, China.

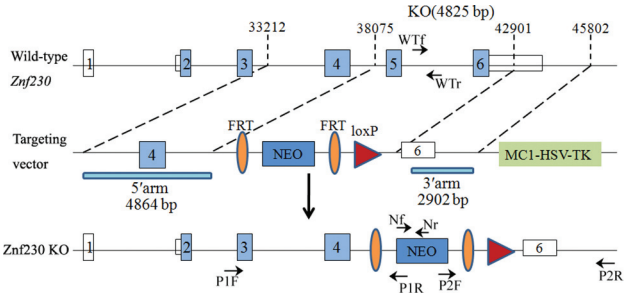

Figure 1 - The targeted knockout strategy for the *Znf230* gene: wild-type allele, targeting construct and targeted allele with the location of primers. Primer pairs P1F/P1R and P2F/P2R, respectively, which flanked the targeted region, were used to select positive ESC clones, and were used to monitor the inheritance of the mutant allele. Primers WTf and WTr were used to amplify the wild-type *Znf230* allele. Primers Nf and Nr were used to amplify the knockout allele.

Znf230 KO mice were crossed into a C57BL/6J background for at least eight generations before use. Germline transmission of the targeted allele was monitored by PCR with primers including Znf230 wild type (WT)-specific primers: WTf: 5'-tgccccttgcccccataat-3', WTr: 5'-gccacccaagaaaaagtcaaaata-3' and Znf230 KO-specific primers: Nf: 5'-ggcgcgagcccctgatgctc-3', Nr: 5'-ttgggtggagaggctattcggctatgac-3', respectively.

The locations of the aforementioned primers are shown in Figure 1.

All animals used in this study were handled in compliance with the National Cancer Center Research Institute's guidelines for the use of animals (USA). All animal experimental protocols were approved by the animal ethics committee of West China Hospital, Sichuan University.

Reverse transcription (RT)-PCR analysis

Total RNA was extracted from the testes of at least five 15-week-old mice per genotype using an RNApure kit (Bioteke, Beijing, China). One microgram of total RNA was reverse transcribed using a RevertAid First Strand cDNA Synthesis Kit (Fermentas, Pittsburgh, PA, USA) with oligo(dT) primers. The sequence of interest in the Znf230 gene was amplified using two gene-specific primers: E4f: 5'-cccatcctcggtcacatctt-3', located within the sequence of Exon-4, and E6r: 5'-ccccttctcctctacgacaac-3', the reverse complement primer of a sequence located within Exon-6. A 982 bp fragment corresponding to the mouse *Gapdh* gene was co-amplified as an internal control using the following primers: 5'-tgaaggtcggtgtgaacggatttggc-3' (Forward) and 5'-catgtaggccatgaggtccaccac-3' (Reverse). Three independent RT-PCR analyses were performed to validate the results.

Western blot analysis

The testicular tissues of at least five 15-week-old mice per genotype were removed and homogenized in a

RIPA lysis buffer containing a 1 μL/mL protease inhibitor cocktail (Sigma) to obtain cell lysates. After centrifugation, the supernatants were examined for protein concentration, subjected to 12% SDS-PAGE and transferred to Immobilon-P transfer membranes (Millipore, Bedford, MA, USA). The membranes were incubated with anti-Znf230 primary antibody (Catalog number: ab4542, Abcam, Cambridge, MA, USA) at 4 °C overnight, washed with 1 X PBS containing 0.1% Triton X-100, and incubated with horseradish peroxidase-linked secondary antibodies (1:10,000, Boster, Wuhan, China) at room temperature for 1 h. The respective bands were visualized using an immunostaining kit (Millipore). The anti-Znf230 primary antibody recognizes amino acids 218-230 in the C-terminus of the Znf230 protein. The quality of the antibody was validated by the manufacturer and our previous report (Song *et al.*, 2008). The western blot analysis was repeated again.

Morphological examination

Testes from three mice per post-natal day (4, 8, and 15 weeks of age) were necropsied for histopathological examination. The tissues were routinely processed, embedded in paraffin and stained with hematoxylin and eosin (H&E). Three slides per testis were visualized using a Zeiss Axio Imager Z2 microscope (Carl Zeiss, Jena, Germany).

Analysis of sperm characteristics

Cauda epididymides from five 15-week-old mice per genotype were dissected and immediately mined in 1 X PBS solution (pH 7.4). Sperm were squeezed out with fine forceps and allowed to disperse in PBS at room temperature for 15 min, followed by repeated pipetting. Thereafter, the sperm remaining as a mono-dispersed suspension were counted using a hemocytometer. Sperm counting was performed four times for each sample.

In addition, the sperm were washed three times in 1 X PBS by centrifugation at 500 g for 5 min and then air-dried onto microscope slides. The slides were then stained with H&E and visualized using a Zeiss Axio Imager Z2 microscope.

Serum sex hormone assay

Blood was taken from five 15-week-old male mice per genotype that were generated from different breeding pairs and housed singly in separate cages. The serum testosterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH) levels were measured using an ELISA kit (Uscn Life Science Inc., Wuhan, China), according to the manufacturer's instructions.

Microarray analysis

One hundred micrograms of total RNA from testis tissues from three Znf230 KO and three C57BL/6J wild-type mice was used to generate biotin-labeled cRNA by using a Message Amp Premier RNA Amplification Kit (Ambion, Austin, TX, USA). Following fragmentation, the labeled cRNA of each sample was hybridized to Affymetrix GeneChip® Mouse Genome 430 2.0 Arrays (Santa Clara, CA, USA) and stained according to the manufacturer's instructions.

Processed arrays were scanned using a GeneChip® Scanner 3000 (Affymetrix). The Affymetrix GeneChip® Command Console® Software was used to perform quality assessments and produce data reports. Differences in the expression patterns between groups were analyzed using the R program for significance analysis of microarrays (Irizarry *et al.*, 2003).

Microarray analysis was performed at the CapitalBio Corporation, Beijing, China.

Quantitative real time PCR (qRT-PCR)

qRT-PCR analyses were performed with cDNA using a SYBR® Premix Ex Taq™ II kit (Takara, Dalan, China) and 10 mM of the corresponding set of sense and antisense primers of the *Kctd14* gene: 5'-atgggcaccctgatgaagc -3' (Forward) and 5'-gcccagtgcgcaggtagtc -3' (Reverse). The reactions were run on a Bio-Rad MyiQ Cycler (Hercules, CA, USA) using the following parameters: 40 cycles of 95°C for 1 min, 95°C for 10 s and 60°C for 30 s. Three independent assays were performed in duplicate on each sample. The CT values from each run were averaged per sample. The ΔΔCT method was applied for data analysis. To do so, the CT data for the Kctd14 mRNA samples were normalized with that of β-actin (Primers: 5'-aacagtccgcctagaagca-3' (Forward) and 5'-cgttgacatccgtaaagacc-3' (Reverse)). All qRT-PCR data are shown as the mean ± standard deviation (S.D.). The testis sample from Znf230 KO mice was set at 1 as arbitrary unit or 100%.

Statistical analysis

Student's *t*-test was used to compare data between wild-type and mutant mice. A $p < 0.05$ indicated significance. All data analyses were performed using SPSS v17.0.

Results

Generation of Znf230 KO mice

A gene-targeting vector was constructed to delete the genomic segment of Znf230 that includes exon-5 and exon-6, which encode the RING finger motif of Znf230 (Figure 1). The targeting vector was introduced into ESCs. ESCs with successful homologous recombination were confirmed by two L-PCR analyses with primers flanking the targeting region (Figure 2A). The chimeric mice derived from the targeted ESCs transmitted the disrupted *Znf230* allele to their offspring (Figure 2B-D).

RT-PCR analysis was performed to evaluate *Znf230* gene expression, which should be disrupted in Znf230 KO mice. As shown in Figure 2E, the targeting region, *i.e.*,

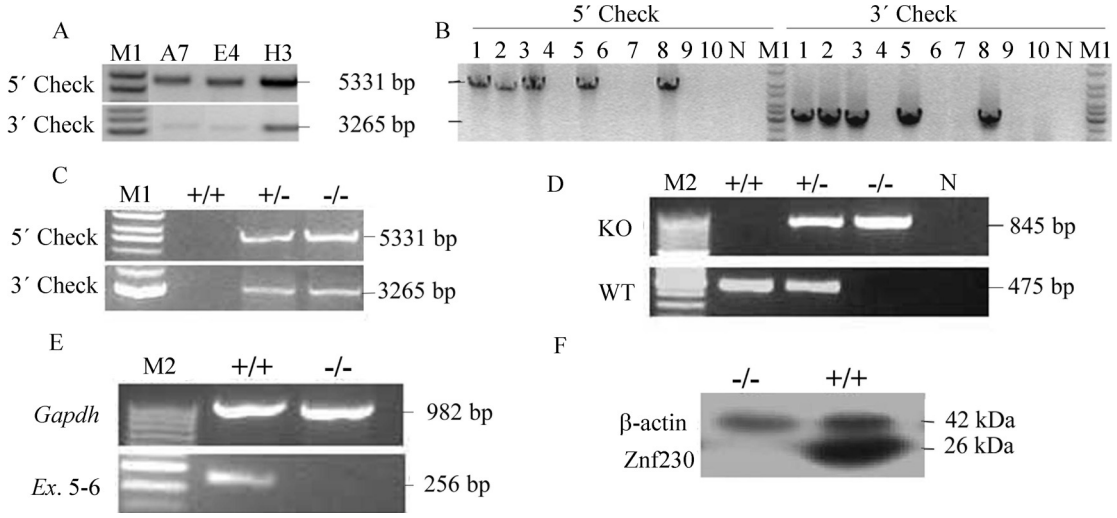

Figure 2 - The generation and identification of Znf230 KO mice. (A) L-PCR analysis using primer pairs P1F/P1R and P2F/P2R to amplify the targeted *Znf230* alleles from genomic DNA extracted from ESCs. ESCs from No. A7, E4 and H3 were the positive clones undergoing targeted homologous recombination. (B) L-PCR analysis of the targeted *Znf230* alleles amplified from genomic DNA derived from the offspring of chimeric mice backcrossed to C57BL/6J. No. 1-10: the offspring members and N: negative control. (C) L-PCR analysis to monitor the inheritance of the targeted *Znf230* allele in the progeny of heterozygous Znf230 KO mice. (D) PCR analysis to monitor the inheritance of the targeted *Znf230* allele in the progeny of heterozygous Znf230 KO mice using primer pairs WTf/WTr and Nf/Nr. (E) RT-PCR analysis of the *Znf230* gene in testes from Znf230 KO and C57BL/6J mice. M1, M2: DNA ladders of 1 kb and 100 bp, respectively. *Gapdh* was used as an internal control. (F) Western blot analysis of the Znf230 protein in the testes from Znf230 KO and C57BL/6J mice. β-actin was used as an internal control.

exon-5 and exon-6, of the *Znf230* gene was not amplified from total RNA of the testes in Znf230 KO mice using the specific primers, in contrast to wild-type mice. Furthermore, western blot analysis with anti-Znf230 antibody showed that the 26-kDa protein representing Znf230 was present in the testes of wild-type mice, as expected, but it was absent in the testes of Znf230 KO mice (Figure 2F). Thus, the *Znf230* gene was indeed disrupted in Znf230 KO mice.

Znf230 KO mice appeared to be normal in growth and fertility

Znf230 KO mice exhibited no obvious impairment in body growth and development because no significant differences were observed in the weights of body or organs, including brain, lung, heart, liver, spleen and kidney, or life spans between Znf230 KO and wild-type mice (Table 1). Because *Znf230* was identified as a testis-enriched gene that likely plays a role in male fertility, we focused our investigation on phenotypes related to male fertility. However, the fertility of Znf230 KO mice appeared to be normal and the offspring of Znf230 KO intercrosses were born at the expected Mendelian ratios. Compared with wild-type mice, Znf230 KO mice displayed no detectable differences in the male reproductive system, including the testis, seminal vesicle, prostate and bladder. There were also no differences in serum testosterone, FSH or LH levels between Znf230 KO and wild-type mice (Table 1, Figure 3A, B). H& E staining demonstrated that the testicular tissue of Znf230 KO mice was intact and that each developmental stage of male germ cells was normal (Figure 3C-H). No significant difference was detected in the shape and number of sperm isolated from the epididymides of Znf230 KO and C57BL/6J wild-type mice (Figure 3I, J and Table 1).

Changes in the mRNA expression profile of Znf230 KO mice

Our previous report proposed that Znf230 was a DNA-binding protein that may function as a transcriptional activator (Qiu *et al.*, 2003). We therefore investigated the differences between the transcriptomes of testes from Znf230 KO and wild-type mice using Affymetrix Mouse Genome 430 2.0 Arrays. The expression levels of over 34,000 genes were assessed, and transcripts with fold changes greater than 2 or less than 0.5 between the two groups were analyzed (Table 2). *Znf230* had the most down-regulated expression level of the analyzed transcripts, thereby confirming the validity of the experimental system. The mRNA level of one unclassified transcript *4921513I08Rik* (GenBank No. AK014883) was detected to be more than two-fold higher, and the mRNA levels of three transcripts including two protein coding genes *Kctd14* (GenBank No. NM_001136235) and *4930448A20Rik* (GenBank No. Ak015411) and a non-coding RNA *4931431B13Rik* (GenBank No. NR_045183) were detected to be more than two-fold lower in Znf230 KO mice compared with C57BL/6J wild-type mice. Because the *Kctd14* gene may encode a functional protein, we performed qRT-PCR analysis to verify the expression changes of *Kctd14* between Znf230 KO and wild-type mice. The results showed that the mRNA level of *Kctd14*

Table 1 - Comparison of phenotypes between Znf230 KO and C57BL/6J mice.

		Znf230 KO	C57BL/6J	P[a]
Total body weight (g)[b]		30.18 ± 1.45	30.31 ± 2.07	NS
Organ weight (mg) [b,c]	Brain	434.55 ± 30.71	405.38 ± 44.78	NS
	Heart	151.8 ± 16.08	139.54 ± 8.48	NS
	Lung	171.21 ± 12.93	166.14 ± 11.21	NS
	Liver	1382.53 ± 82.92	1386.72 ± 192.7	NS
	Kidney	212.32 ± 25.95	204.1 ± 21.18	NS
	Spleen	60.37 ± 7.17	63.64 ± 8.29	NS
	Seminal vesicle	268.2 ± 14.91	236.1 ± 40.69	NS
	Prostate and Bladder	127 ± 25.22	127.5 ± 22.3	NS
	Testis	108.5 ± 15.46	109.6 ± 23.67	NS
Total no. of sperm ($x10^6$)[b]		24.4 ± 3.6	23.7 ± 2.8	NS
Serum sex hormone (ng/mL)[b]	Testosterone	1.290.25	1.2 ± 0.34	NS
	Luteinizing hormone (LH)	8.02 ± 1.37	8.21 ± 1.72	NS
	Follicle-stimulating hormone (FSH)	10.2 ± 3.05	9.56 ± 2.45	NS
Litter size [d]		7.5 ± 1.4	8.1 ± 1.2	NS
Life Span (days) [e]		713.2 ± 147.6	722.5 ± 168.5	NS

[a]Statistical analysis was carried out by Student's *t* test, NS: Not significant.
[b]Fifteen-week-old mice were examine, n = 5 per group, Values are means ± S.D.
[c]Wet weights of paired organs were averaged for each mouse, and the single value was used to calculate mean ± S.D among same genotype.
[d]Data are mean values derived from six breeding pairs for each genotype.
[e]Data are mean values of 10 mice (5 male plus 5 female) per group.

was reduced by more than 10-fold in Znf230 KO mice compared with wild-type mice (Figure 4).

Discussion

The present work was undertaken in an effort to define the physiological role of the testis-enriched gene *Znf230* in mammalian spermatogenesis. We generated a null mutation in the *Znf230* gene by homologous recombination in mouse ESCs, which were used to produce homozygous Znf230 KO mice. Mice that were homozygous for the mutation lacked the intact mRNA and protein in germ cells, but did not exhibit any detectable abnormality in body growth or spermatogenesis. The absence of abnormality in Znf230-null testes was unexpected because of the testis-enriched expression pattern of *Znf230*. However, several explanations may account for the lack of a clear phenotype in Znf230 KO mice. First, the function of Znf230 may be dispensable for male fertility. As an example, H1t is an H1 histone variant that is unique to late spermatocytes and round spermatids, but H1t-null mice have no discernible phenotype (Fantz *et al.*, 2001). Another example is SPAG5, which is an Odf1-interacting protein that is specifically expressed during meiosis. The disruption of SPAG5 does not affect spermatogenesis or fertility (Xue *et al.*, 2002). Other examples include *testicular orphan nuclear receptor 2* gene (Shyr *et al.*, 2002), *testicular haploid expressed* gene (Mannan *et al.*, 2003), *transition protein 2*, *proacrosin* and *histone H1.1* genes (Nayernia *et al.*, 2003), the tumor suppressor *LRP1b* gene (Marschang *et al.*, 2004), the *UBC4*-testis gene (Bedard *et al.*, 2005) and the testis-enriched

Table 2 - Genes differentially expressed in the testes of Znf230 KO mice

Gene ID	Gene symbol	Gene name	Fold change[a]	ProbeSet ID[b]
70875	4921513108Rik	RIKEN cDNA 492151308 gene	2.9705	1432299_at
67150	Znf230	ring finger protein 141	0.445/0.1841/0.158	1449086_at/ 1433655_at/ 1449087_at
233529	Kctd14	potassium channel tetramerisation domain containing 14	0.4276/0.4054	1426632_at/1426633_at
70971	4931431B13Rik	RIKEN cDNA 4931431B13 gene	0.4054	1430416_at
73993	4930448A20Rik	RIKEN cDNA 4930448A20 gene	0.2394	1454205_at

[a]The ratio of signal values between Znf230 KO and Wild-type mice detected by every probe. Statistical p-values < 0.001.
[b]All probes hit one known transcript.

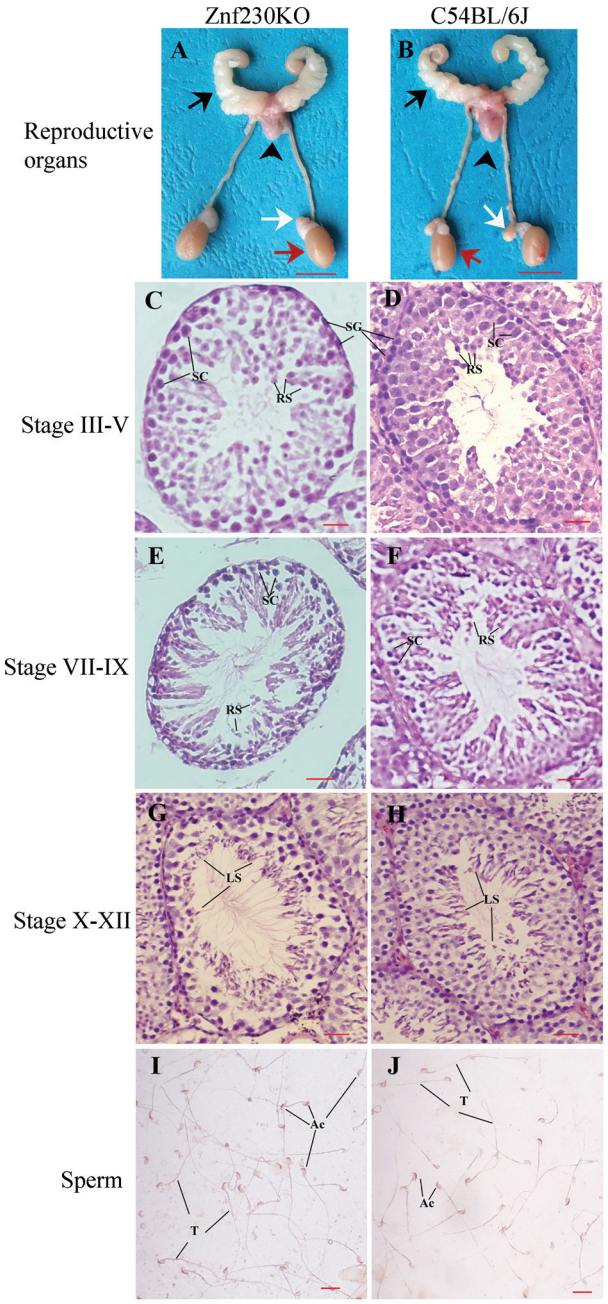

Figure 3 - Characteristics comparison of reproductive organs and sperm shape between Znf230 KO and C57BL/6J mice. Morphology of reproductive organs from Znf230 KO (A) and C57BL/6J (B) mice. The seminal vesicles (black arrows), bladder (black arrowheads), epididymis (white arrows) and testes (red arrows) were highlighted. Scale bar = 1 cm. Histological analysis of H&E stained testes from Znf230 KO (C,E,G) and C57BL/6J (D,F,H) mice. SG: Spermatogonia, SC: Spermatocyte, RS: Round spermatids, and LS: elongated spermatids. Scale bar = 100 μm. Characteristics of H&E stained sperm from Znf230 KO (I) and C57BL/6J (J) mice.. Ac: acrosome, T: sperm tail. Scale bar = 50 μm.

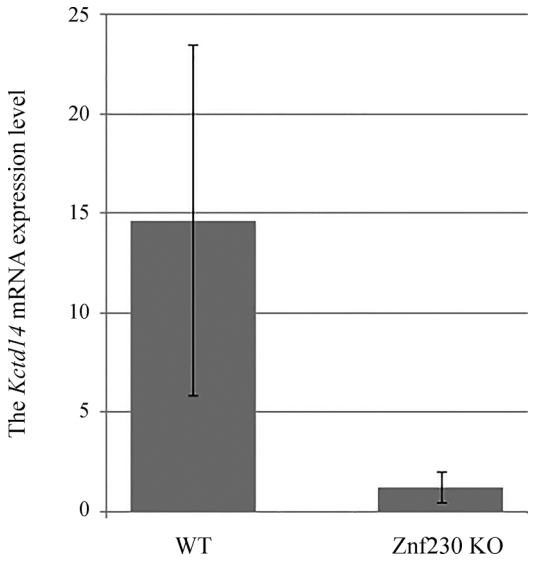

Figure 4 - Comparative qRT-PCR analysis of mRNA levels of the *Kctd14* gene between Znf230 KO and C57BL/6J (WT) mice. Bars represent the means ± S.D., Statistical p-values < 0.001.

histone demethylase *KDM4D* gene (Iwamori *et al.*, 2011). One explanation for such phenomena is that an unidentified protein may compensate for the loss of function of these genes. It is reasonable that other RING finger proteins may compensate for the loss of *Znf230* function because RING finger proteins belong to one of the largest zinc finger protein families. Second, the defects in Znf230 KO mice may be too small to be detected using the techniques employed here. A detailed ultrastructural examination of testicular tissues and spermatozoa may be needed to confirm eventual minor changes in the mutant mice. Third, because spermatogenesis is a very complex process that involves many genes, the inactivation of one gene may not be sufficient to produce a detectable phenotype. For example, *Tyro 3*, *Axl*, and *Mer* encode three structurally related receptors that possess tyrosine kinase activity. Mice that lack any single receptor or any combination of two receptors are viable and fertile, but males that lack all three receptors produce no mature sperm (Lu *et al.*, 1999). Therefore, it will be of interest to explore the interaction between Znf230 and its related proteins by ablating their network and dissecting the resulting phenotypes to help clarify the biological role of the *Znf230* gene in male fertility.

Indeed, we found that the mRNA levels of four transcripts were changed more than two-fold in Znf230 KO mice compared with C57BL/6J wild-type mice. Three of the four transcripts were unclassified. However, *Kctd14* encodes a putative member of the KCTD protein family that contains the bric-a-brac/tramtrak/broad (BTB) complex domain, which resembles the tetramerization domain of voltage-gated potassium channels. The KCTD protein family, which comprises 22 members, has been implicated in many important biological processes (Schwenk *et al.*, 2010; Seddik *et al.*, 2012; Cao-Ehlker *et al.*, 2013; Skoblov *et al.*, 2013). However, because no report has yet described the biological function of the Kctd14 protein, Znf230 KO mice may provide a clue for investigating the pathways in which these unclassified transcripts are involved.

Conversely, the Znf230 protein may act as a transcriptional factor, and the disruption of the Znf230 protein may cause the related transcriptional complex to be destroyed, this directly affecting the expression of the four transcripts. It is interesting to note that *Znf230* and the four transcripts are all located on mouse chromosome 7 (Chr7). As shown in Figure S1, the transcripts of *4921513I08Rik* and *Kctd14* are located upstream of *Znf230*, while the other two transcripts are located downstream of *Znf230*. It is thus possible that *cis*-acting elements may have been destroyed during the targeted disruption of the *Znf230* gene. In addition, changes in the chromatin structure in the KO region of Chr7 may be the cause for the changes in the expression levels of the four transcripts.

In conclusion, we generated Znf230-deficient mice that exhibited normal body growth and fertility based on our current examination techniques. Using microarray analysis to compare the transcriptomes of testicular tissue from Znf230 KO and wild-type mice, we observed changes in the expression levels of four transcripts in Znf230 KO mice. In the future, the Znf230 KO mouse model may be used to uncover the biological roles and explore the interaction between Znf230 and its related transcripts.

Acknowledgments

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Supplementary Material

The following online material is available for this article:

Figure S1- The locations of the transcripts of *Kctd14*, *Znf230*, *4921513I08Rik*, *4930448A20Rik* and *4931431B13Rik*.

This material is available as part of the online article from http://www.scielo.br/gmb.

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