A Spontaneous *Smc1b* Mutation Causes Cohesin Protein Dysfunction and Sterility in Mice

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In this paper, we describe a novel spontaneous mutation of the *Smc1b* gene coding a cohesin component, which causes female and male sterility. We have discovered an ICR male mouse with a novel autosomal recessive gene that causes small gonads and sterility in both sexes. Mutant female and male mice homozygous for the novel sterility gene had normal body weights and showed normal mating behavior, but did not produce any offspring. Histological examination showed that Sertoli cells and spermatogonia were present in the testicular seminiferous tubules in 8-week-old mutant male mice, but no spermatids or spermatozoa were observed. Mutant females showed a markedly reduced number of oocytes with age. The novel sterility gene mapped between *D15Mit105* (47.9 cM) and *D15Mit171* (54.5 cM) on chromosome 15. Sequences of three candidate sterility genes, *Dmc1*, *Mei1* and *Smc1b*, which are closely linked to these microsatellite markers, were compared between normal and mutant mice. The *Dmc1* and *Mei1* genes showed the same sequences in both normal and mutant mice, but the *Smc1b* gene had a deletion of 16 nucleotides in exon 5, in the mutant mice. We concluded that this deletion led to a frame-shift, which generated a stop codon at position 761 (amino acid 247) of the *Smc1b* cDNA in mutant mice. Exp Biol Med 234:994–1001, 2009

Key words: *Smc1b* gene; cohesin protein; sterility; apoptosis; mouse

Introduction

It is estimated that about 15% of young couples trying to have children suffer from infertility (1), a situation that is becoming a serious problem in human society. Matzuk and Lamb (2) reported that about 15% of male infertility and 10% of female infertility was caused by genetic defects, such as chromosomal aberrations and mutations of genes involved in reproduction and fertility. Sterility due to chromosomal abnormalities has been reported in humans (3–5) and mice (6–8).

Many recent studies have focused on the proteins involved in cohesion and synapsis, using targeted mutation techniques (knock-in and knock-out) in mice. *Smc1a* (structural maintenance of chromosomes 1A) knock-in mice (9) and *Rad51c* (Rad51 homolog c (*Saccharomyces cerevisiae*)) knock-out mice (10) lacking mitosis-specific cohesin protein components show increased chromosome instability and embryonic lethality. *Rec8* (REC8 homolog (yeast)) knock-out mice lacking one of the meiosis-specific cohesin protein components show female infertility, failure of meiosis, azoospermia, and absence of oocytes and ovarian follicles (11–13). *Smc1b* knock-out mice, lacking another meiosis-specific cohesin protein component, show male and female infertility, abnormal male and female meiosis, and arrest of spermatogenesis (14). Knock-out mice lacking four synaptonemal complex protein components, *Sycp1* (synaptonemal complex protein 1), *Sycp2* (synaptonemal complex protein 2), *Sycp3* (synaptonemal complex protein 3) and *Syce2* (synaptonemal complex central element protein 2), have so far been created. No *Syce1* (synaptonemal complex central element protein 1) knock-out mice have yet been reported. *Sycp1* knock-out mice demonstrate male and female infertility, azoospermia, small ovaries, small testes and seminiferous tubules, absent ovarian follicles, and a failure of synapse formation during

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meiosis (15). In *Sycp2* knock-out mice, males are sterile due to a block in meiosis, whereas females are sub-fertile and produce sharply reduced litter sizes (16). *Sycp3* knock-out males are infertile due to blockage of meiosis at early prophase, with a lack of synaptonemal complex formation, while females are sub-fertile with reduced litter sizes due to the production of achiasmatic oocytes, resulting in aneuploidy and embryonic death (17, 18). *Syce2* knock-out mice are infertile with decreased testis and ovary weights, arrest of spermatogenesis, and abnormal meiosis (19).

Recently, we discovered spontaneous sterile mice with small gonads in both sexes. Mating experiments revealed that the sterility was caused by an autosomal recessive gene that mapped between *D15Mit105* (47.9 cM) and *D15Mit171* (54.5 cM) on chromosome 15. We developed a congenic mouse strain carrying the infertility gene on a C57BL/6JJcl genetic background. We performed phenotypic analyses using B6 congenic mice. Compared to normal mice, mutant mice had normal body weights, life spans and mating behavior in both sexes, but no offspring were produced after mating with normal mice. Sequencing of the *Smc1b* gene in the sterile mice also revealed a deletion of 16 nucleotides in exon 5.

In this study, we performed phenotypic and genetic analyses of a novel sterility mutation, as the first spontaneous *Smc1b* gene mutation.

Materials and Methods

Mice, Environment and Nomenclature. An ICR mouse carrying a novel sterility gene was found in our mutation search, which was conducted to detect spontaneous recessive mutations in a Jcl:ICR mouse colony (CLEA Japan, Tokyo, Japan). Namely, (DBA/2JJcl female × ICR male) F_1 females were crossed to the ICR male parent to obtain mutant mice homozygous for recessive mutation(s). C57BL/6JJcl (hereafter B6) and DBA/2JJcl (hereafter D2) mice were purchased from CLEA Japan.

Male and female mice were housed separately in plastic cages lined with sterilized wood shavings in a room at a constant temperature (24 ± 2°C) and with a constant lighting cycle (12 hours light/dark). A solid diet (LabDiet; PMI Nutrition International, IN, USA) and water were provided *ad libitum*.

Our animal facility was maintained under specific pathogen-free (SPF) conditions. In-house monitoring was performed every 3 months using a Monilisa IVA kit (Wakamoto Pharmaceutical Co Ltd, Tokyo, Japan) to detect four major organisms: Sendai virus, mouse hepatitis virus, mycoplasma and Tyzzer's organism. No infection was detected in any animal room in which the mice used in this study were maintained.

Alleles at the sterility gene locus were described as follows: (+) for the wild type allele and (–) for the recessive mutant allele at the sterility gene locus. Normal homozygotes were therefore designated as (+/+), heterozygotes as (+/–), and affected homozygotes for the sterility gene mutation allele as (–/–).

This study was approved by the Hamamatsu University School of Medicine Animal Care and Use Committee.

Gene Mapping. Rough mapping using linkage analyses between the sterility gene and 53 microsatellite DNA markers (Table 1) was performed using 10 mutant F_2 mice (–/–) obtained by crossing (D2 female × ICR male) F_1 females (+/–) and males (+/–). Microsatellite DNA markers were detected using primer sets purchased from Invitrogen Corporation (Carlsbad, CA, USA), with the polymerase chain reaction (PCR) followed by agarose gel electrophoresis, as described elsewhere (20).

Development of a Congenic Strain. By performing backcrosses eight times, we developed a congenic mouse strain carrying the infertility gene on a C57BL/6JJcl genetic background (21).

All mice used in this study were genotyped for the *Smc1b* gene using PCR to discriminate homozygotes (+/+ and –/–) and heterozygotes (+/–). The primer sets (F: forward, R: reverse) detecting exon 5 of the *Smc1b* gene were as follows: F/R 5′-caagatacaactgatgcttttcca-3′/5′-cagttcttttgctgtttgctg-3′. The product sizes were 196 bp for the wild-type allele (+) and 180 bp for the mutant allele (–).

Phenotyping. Phenotyping was performed using normal (+/+ or +/–) and affected (–/–) B6 congenic mice from over 8 backcross generations. Body and organ weights were measured using an electronic balance. We performed mating experiments in order to study the reproductive abilities of mature normal (+/+ and +/–) females (f) and males (m). Testes and ovaries were dissected and fixed in Bouin's solution or 10% neutral buffered formalin for 24 hours. They were embedded in paraffin and sectioned at 5-μm thickness. Histological examination was performed after staining with HE (hematoxylin and eosin). TUNEL (TdT-mediated dUTP-biotin Nick End Labeling)-positive cells showing apoptosis were detected in testes from normal and mutant mice using the ApopTag Plus Peroxidase In situ Apoptosis Detection Kit (CHEMICON International, CA, USA), according to the manufacturer's protocol.

Developmental Markers of Spermatogenesis. Reverse transcription-PCR (RT-PCR) was performed in order to observe the expression of developmental markers of spermatogonia, spermatocytes, spermatocyte-spermatids and spermatids (22). The markers used were *Dazl1* (deleted in azoospermia-like) (23), *Dmc1* (24), *Clgn* (calmegin) (25, 26) and *Gsg2* (germ cell-specific gene 2) (27). The primer sets (F: forward, R: reverse) were as follows (product size in parentheses):

Dazl1: F/R (262 bp), 5′-ttcaggcatatcctccttatc-3′/5′-atgcttcggtccacagacttc-3′

Dmc1: F/R (497 bp), 5′-tgcacgtgcctatactagtg-3′/5′-gcccctaataagcagcaaga-3′

Clgn: F/R (588 bp), 5′-agacaggaagactcacctct-3′/5′-atggaccaaagctctaagcc-3′

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Table 1. Fifty-Three Polymorphic Microsatellite DNA Markers Between the ICR Male Mouse and the DBA/2JJcl Mouse Strain[a]

Chr	cM	Markers	Chr	cM	Markers	Chr	cM	Markers
1	8.0	*D1Mit 58*	6	19.0	*D6Mit207*	14	27.5	*D14Mit37*
	19.5	*D1Mit278*		74.0	*D6Mit15*		40.0	*D14Mit115*
	49.7	*D1Mit46*	7	15.0	*D7Mit155*	15	14.5	*D15Mit7*
	85.0	*D1Mit399*		37.0	*D6Mit250*		25.4	*D15Mit184*
2	9.5	*D2Mit464*		69.0	*D7Mit292*		29.2	*D15Mit63*
	47.5	*D2Mit44*	8	8.0	*D8Mit171*		38.7	*D15Mit235*
	69.0	*D2Mit277*		19.5	*D8Mit125*		47.9	*D15Mit105*
	73.0	*D2Mit304*		59.0	*D8Mit89*		54.5	*D15Mit171*
	91.8	*D2Mit346*	9	18.0	*D9Mit205*		56.6	*D15Mit39*
3	39.7	*D3Mit97*		49.0	*D9Mit197*		60.0	*D15Mit48*
	64.1	*D3Mit110*	10	5.0	*D10Mit246*	16	21.0	*D16Mit154*
4	21.9	*D4Mit111*		21.0	*D10Mit3*		36.0	*D16Mit4*
	31.7	*D4Mit17*	11	55.0	*D11Mit289*	17	51.9	*D17Mit122*
	55.2	*D4Mit278*		63.0	*D11Mit10*	18	6.0	*D18Mit21*
5	10.0	*D5Mit180*	12	13.0	*D12Mit107*		16.0	*D18Mit135*
	41.0	*D5Mit134*		29.0	*D12Mit33*		55.0	*D18Mit128*
	60.0	*D5Mit24*	13	65.0	*D13Mit260*	19	52.0	*D19Mit1*
	81.0	*D5Mit101*	14	12.5	*D14Mit54*			

[a] Map positions (cM) from Mouse Genome Informatics (http://www.informatics.jax.org/javawi2/servlet/WIFetch?page=markerQF).

Gsg2: F/R (440 bp), 5′-gatgggaaacagactaacgg-3′/5′-cagtaaggtgtgctctgttc-3′

Total RNA was extracted from adult testes using RNeasy (Qiagen, MD, USA), according to the manufacturer's instructions. One microgram of total RNA was reverse-transcribed using oligo-dT primer and superscript II RT (Gibco BRL, MD, USA). cDNA was amplified under the following conditions: 28–30 cycles of DNA degeneration for 30 seconds at 94°C, annealing for 30 seconds at 57°C, and extension for 1 minute at 72°C. cDNA obtained from normal and affected testis was adjusted, based on the staining intensity of PCR products from standard glyceraldehyde-3-phosphate dehydrogenase (GADPH) cDNA amplified with a primer set: F/R (259 bp), 5′-cgtggagtctactggtgtgtcttcacc-3′/5′-gatggcatggactgtggtcatgagc-3′.

Sequencing. cDNA coding regions of the *Dmc1*, *Mei1* and *Smc1b* genes from normal and affected mice were sequenced. The entire coding regions of the *Dmc1*, *Mei1* and *Smc1b* genes from normal and mutant mice were amplified using the following primer pairs:

Dmc1: F1/R1 (454 bp), 5′-tttactgccgctctcctttc-3′/5′-tgagctgtcacacagagggta-3′

Dmc1: F2/R2 (637 bp), 5′-actcagctgtctcataccct-3′/5′-aattccccacctactccttg-3′

Mei1: F1/R1 (633 bp), 5′-acacagtgtatgggatcgag-3′/5′-catcagctttggatgcgt-3′

Mei1: F2/R2 (723 bp), 5′-catcccgatggtgatgaggta-3′/5′-aacctgctcatcaaccctggatag-3′

Mei1: F3/R3 (641 bp), 5′-tgaggccgtgatcagtgcaa-3′/5′-tgggtgtggtctgatgaagactc-3′

Mei1: F4/R4 (662 bp), 5′-gagcatgctgtgtgggaagt-3′/5′-gggtgaacagcagaaaccga-3′

Mei1: F5/R5 (729 bp), 5′-gtccatcggacattatctgtggaac-3′/5′-gatgtgagcctgggaaacagac-3′

Mei1: F6/R6 (255 bp), 5′-aaggtgcagctttggttgac-3′/5′-ctgggcctttatgtcctgag-3′

Smc1b: F1/R1 (350 bp), 5′-ctgctgctcgtggagaattt-3′/5′-tccaactgggctacatacaca-3′

Smc1b: F2/R2 (572 bp), 5′-gataaacccgtgagtcgttc-3′/5′-atgtactgaggcctcttctg-3′

Smc1b: F3/R3 (551 bp), 5′-cagcaaacagcaaaagaactg-3′/5′-tttttcagggcctcttcttg-3′

Smc1b: F4/R4 (674 bp), 5′-gcatggattgcttggaagat-3′/5′-tttctcatcccagcacagag-3′

Smc1b: F5/R5 (792 bp), 5′-aggcagtagcacttgatgga-3′/5′-cagccaaaaccttcttgcgt-3′

Smc1b: F6/R6 (634 bp), 5′-tgccacacagagttccaaca-3′/5′-actgttgttcctgcagagcttc-3′

Smc1b: F7/R7 (738 bp), 5′-ggaggtacgatgctttcagtca-3′/5′-ggaatgacactgagcactcctt-3′

Nucleotide sequences of these primers were determined from the Ensembl database (http://www.ensembl.org/Mus_musculus/index.html). Sequencing was performed using the dideoxy chain terminating method with a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, CA, USA), and then applied to an automated DNA sequencer ABI PRISM 3100 (Applied Biosystems, CA, USA).

Statistical Analysis. Statistical analyses were done with Prism software (GraphPad Software, CA, USA). The values were analyzed by one-way analysis of variation (ANOVA) followed by Tukey's post hoc test. P value of < 0.05 was judged as significant.

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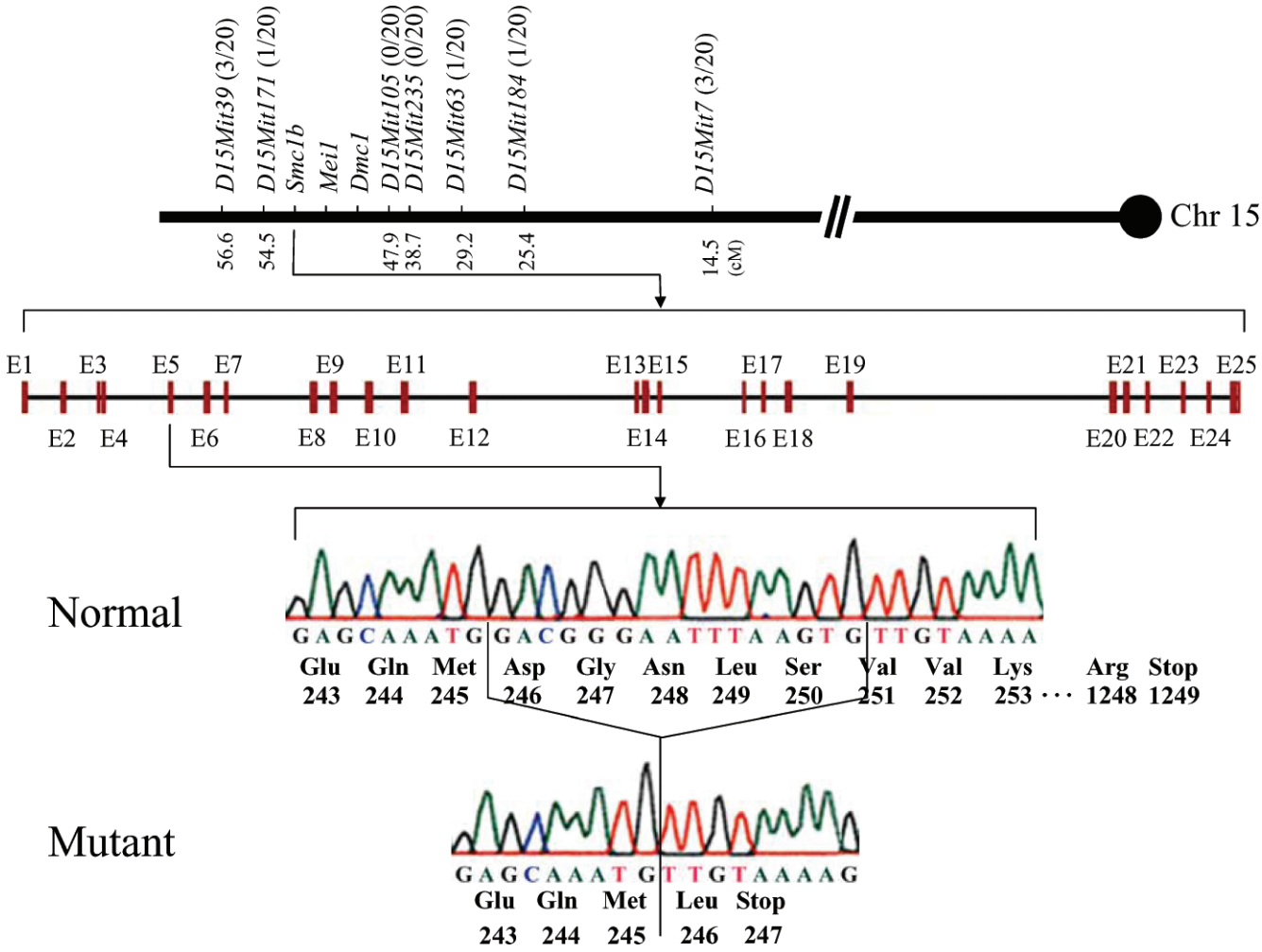

Figure 1. Linkage map of chromosome 15 and comparison of normal and mutant *Smc1b* cDNA sequences. Recombination frequencies are shown in parentheses (No. recombinants/No. chromosomes observed). The *Smc1b* gene spans 67,266 bp (84,895,121–84,962,387 bp) of genomic DNA and is split into 25 exons (E1–E25 in this figure) generating a 4,038-bp transcript encoding a protein of 1,248 amino acids (http://www.ensembl.org/Mus_musculus/ geneview?gene=ENSMUSG00000022432;db=core).

Results

Inheritance of the Sterility Gene and Chromosomal Mapping. In order to demonstrate inheritance of the sterility gene, we obtained F_2 mice by intercrossing (D2 females × ICR male)F_1. The ratio of normal:mutant male mice was 66:26 (3:1). These results indicated that the novel sterility was caused by a recessive gene.

Rough mapping using linkage analyses based on 53 microsatellite markers selected from all autosomes was performed using 10 mutant F_2 mice. Linkage was observed only between the sterility gene and microsatellite markers on the distal region of chromosome 15 (Fig. 1). No recombination was observed between the sterility gene and three markers, *D15Mit235*, *D15Mit105* and *D15Mit171*. Interestingly, three sterility-associated genes, *Dmc1* (DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast), *Mei1* (meiosis defective 1) and *Smc1b* (structural maintenance of chromosomes 1B), were located between *D15Mit105* and *D15Mit171*.

Phenotypes. The body and gonad weights of mature 8-week-old normal and mutant B6 congenic mice are shown in Table 2. There were no significant differences in average body weight or average ovary weight between normal (+/+ and +/–) and mutant (–/–) mice. However, there was a significant difference in average testis weight between normal and mutant mice (ANOVA, $P < 0.05$). There were no significant differences in average organ weights (kidney, liver, etc.) between normal and mutant mice (data not shown). The life spans of both the normal and mutant mice were > 2 years.

In order to study the reproductive abilities of mature normal (+/+ and +/–) females (f) and males (m), we performed four crosses: +/+ (f) × +/+ (m), +/+ (f) × +/– (m), +/– (f) × +/+ (m), and +/– (f) × +/– (m). The average numbers of pups obtained from these crosses were 6.4 ± 1.5 (nine litters), 6.0 ± 2.4 (six litters), 6.6 ± 2.2 (11 litters) and 6.7 ± 2.0 (11 litters), respectively. There were no significant differences between these values. No pups were obtained

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Table 2. Average Body and Gonad Weights of Normal and Mutant Mice at 8 Weeks Old

	Females			Males		
	+/+ ($n = 6$)*	+/– ($n = 6$)	–/– ($n = 6$)	+/+ ($n = 6$)	+/– ($n = 6$)	–/– ($n = 9$)
Average body weights (g)	20.1 ± 3.5	19.1 ± 2.4	19.5 ± 0.7	22.5 ± 3.1	23.0 ± 3.2	23.7 ± 2.4
Average gonad weights (mg)	2.8 ± 0.6	3.3 ± 0.9	3.2 ± 0.6	140.2 ± 23.7	138.1 ± 14.2	48.0 ± 14.3**

* The number of tested.
** It was significantly (ANOVA, $P < 0.05$) lower than those of normal male mice.

from matings using 12 pairs of normal females (+/+ and +/–) and mutant males (–/–), 12 pairs of mutant females (–/–) and normal males (+/+ and +/–), and six pairs of mutant females (–/–) and mutant males (–/–), even though vaginal plugs were observed.

Histology. Histological examination of testes from normal and mutant B6 congenic mice was performed at 15 and 19 days of age, and at 8 weeks of age. Leydig cells, Sertoli cells, spermatogonia and early spermatocytes were demonstrated by HE staining (Fig. 2) at each stage in both normal and mutant males. Late spermatocytes were observed in 19-day-old normal males, but not in mutant males. Spermatids and spermatozoa were observed in 8-week-old normal males, but not in 8-week-old mutant males. TUNEL-positive cells were arranged concentrically in the lumina of the seminiferous tubules in 19-day-old mutant males. Apoptotic death of seminiferous tubule cells, mainly early spermatocytes, was clearly observed in 8-week-old mutant males.

Histological examination of the ovaries in normal and mutant mice is shown in Figure 3. At 4 weeks old, the ovaries of mutant female mice had developed a large number of growing follicles, Graafian follicles and corpora lutea, as in normal females, but the number of follicles in

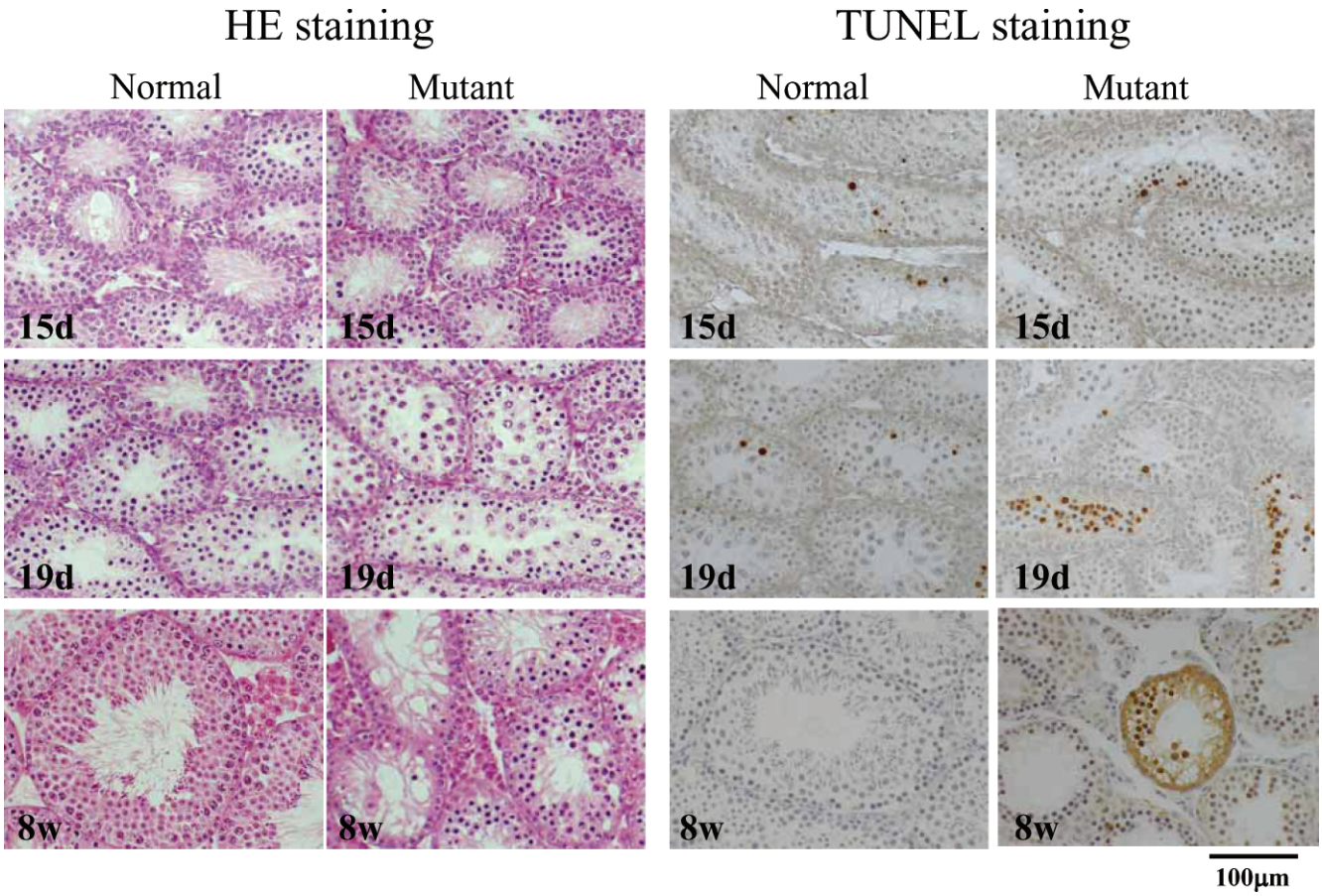

Figure 2. Histology of testes in normal and mutant mice. Hematoxylin-eosin staining (HE) and TdT-mediated dUTP-biotin nick end labeling (TUNEL) of testes sections obtained from normal and mutant mice at 15 and 19 days after birth (15 d and 19 d, respectively) and 8 weeks of age (8 w). Normal testes have normal seminiferous tubules. Mutant seminiferous tubules show meitic defects and no elongating spermatids. TUNEL staining of apoptotic cells in sections of 19 d and 8 w mutant mice.

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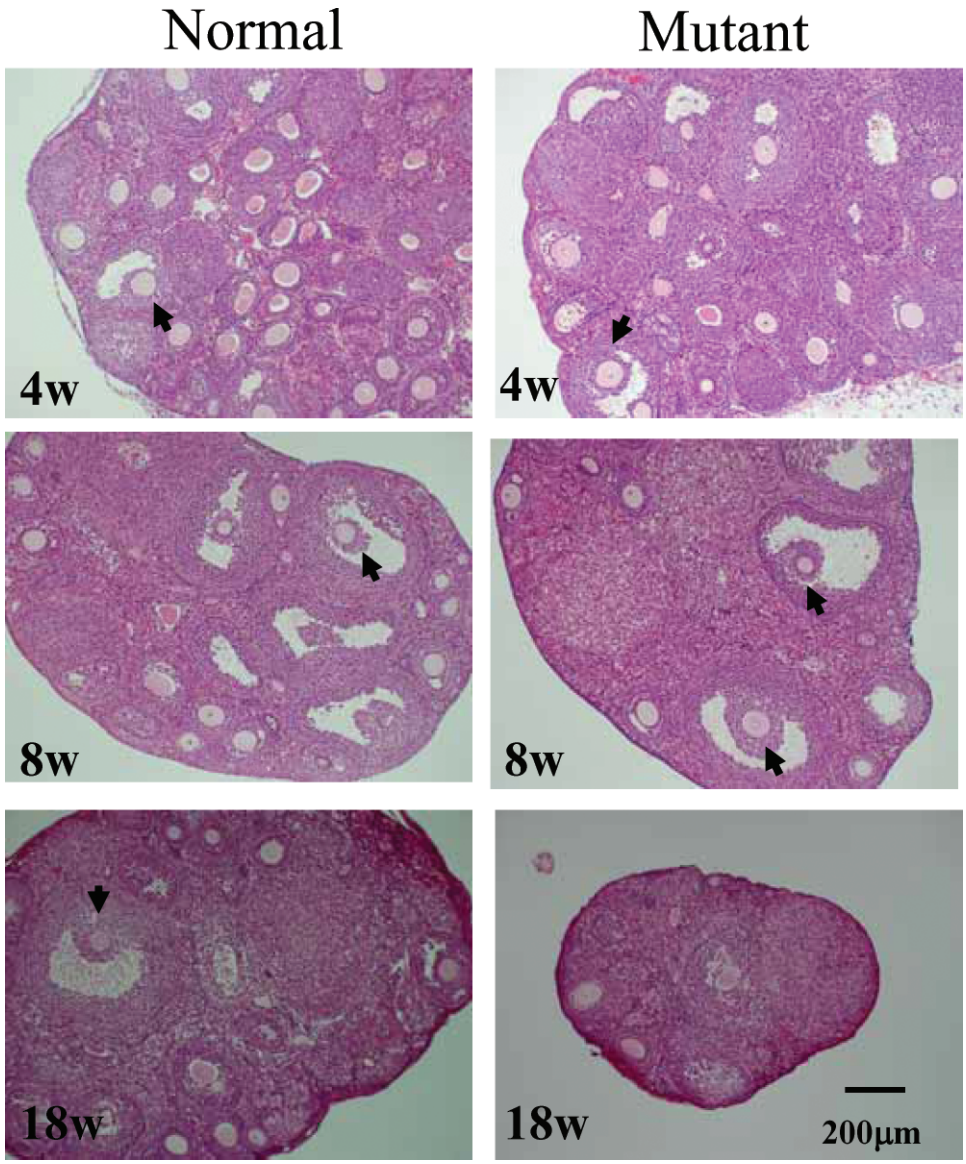

Figure 3. Histology of ovaries in normal and mutant mice. Hematoxylin-eosin staining of ovary sections obtained from normal and mutant mice at 4, 8 and 18 weeks of age (4 w, 8 w and 18 w, respectively). Black arrow indicates representative mature Graafian follicle. Normal ovaries contain many follicles, but the number of follicles in the mutant ovaries gradually decreases with increasing age.

mutant mice appeared to decrease from 4 to 8 weeks of age.

Expression of Developmental Markers in Spermatogenesis. We attempted to demonstrate the stage of developmental arrest of spermatogenic cells in mutant B6 congenic mice using semi-quantitative RT-PCR analyses to detect transcripts of genes expressed during spermatogenesis (22). It is known that *Dazl1* is expressed in spermatogonia (23), *Dmc1* during leptotene to mid-pachytene of spermatocytes (24), *Clgn* during early-pachytene of spermatocytes to spermatids (25, 26), and *Gsg2* (synonym to Haspin) during late-pachytene of spermatocytes to spermatids (27). Transcripts of the *Dazl1* and *Dmc1* genes were found in testes of both normal and mutant mice, as shown in Figure 4. However, expression of the *Clgn* gene was apparently reduced, and that of the *Gsg2* was absent, in mutant mice. These results strongly suggest that spermatogenesis in spermatocytes was mutant at the pachytene stage, but not during zygotene, in mutant mice.

Sequencing. Coding regions of the three candidate genes, *Dmc1*, *Mei1* and *Smc1b*, from normal and mutant B6 congenic mice were sequenced. No mutations were observed in *Dmc1* and *Mei1* genes from mutant B6 congenic mice (data not shown). However, a 16-bp deletion, from 756–771 bp, was observed in the *Smc1b* gene from mutant B6 congenic mice, as shown in Figure 1. This deletion was not observed in normal B6 congenic mice (+/+), C57BL/6JJcl or DBA/2JJcl mice. This deletion mutation was predicted to cause a frame-shift, generating a stop codon (TAA) at codon 247 of the mutated gene. This deletion mutation at the *Smc1b* gene locus was named

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	Expression of marker genes							
	Dazl1		*Dmc1*		*Clgn*		*Gsg2*	
	N	M	N	M	N	M	N	M
Spermatogonia	+	+	−	−	−	−	−	−
Spermatocytes								
Leptotene	−	−	+	+	−	−	−	−
Zygoten	−	−	+	+	−	−		
Pachytene (Early)	−	−	±	±	+	+	−	−
Pachytene (Mid)	−	−	−	−	+	±	−	−
Pachytene (Late)	−	−	−	−	+	−	+	−
Spermatids	−	−	−	−	+	−	+	−

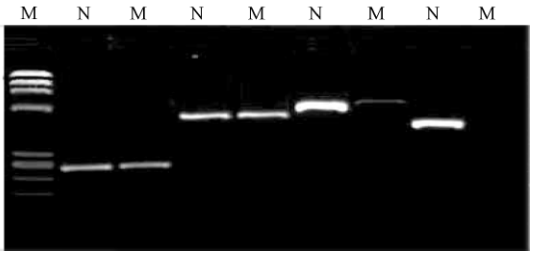

Figure 4. Expression of four developmental markers at three different stages in normal (N) and mutant (M) mice. *Dazl1* is expressed in spermatogonia, *Dmc1* during leptotene to early-pachytene of spermatocytes, *Clgn* during early-pachytene of spermatocytes to spermatids, and *Gsg2* (synonym to Haspin) during late-pachytene of spermatocytes to spermatids (22). Dotted lines show expression of each developmental marker at three cell stages. M is a DNA size marker, φX174 digested with *Hae*III.

Smc1b$^{m1\text{-}Ham}$, according to the nomenclature rule (http://www.informatics.jax.org/mgihome/nomen/gene.shtml).

Discussion

It is known that cohesin and condensin proteins play important roles in the maintenance of sister chromatid cohesion during both mitosis and meiosis. Synaptonemal complex proteins also play important roles in pairing, synapsis and recombination (crossing-over) between homologous chromosomes during meiosis (28). Abnormal meiotic chromosome dynamics due to a lack of these protein components is thought to lead to azoospermia in males and the absence of oocytes and ovarian follicles in females. Recently, mutated alleles of *SYCP3* (29) and *MEI1* (30) have been reported in relation to infertility in humans. These recent studies on cohesion and synapsis suggest that currently unexplained causes of infertility will be accounted for at the molecular level in the future.

We performed phenotypic analyses in order to reveal the cause of sterility in both sexes of the novel mutant mice. Histological studies with HE staining revealed that early spermatogenesis (spermatogonia and spermatocytes) in mutant male mice was normal at 15 days of age, but late spermatogenesis (late spermatocytes and spermatids) was apparently abnormal at 19 days and 8 weeks after birth. No spermatids were observed in the testes of 8-week-old mutant male mice, suggesting that arrest of spermatogenesis occurred at the pachytene stage in spermatocytes. In order to confirm this hypothesis, expression of the *Dazl1*, *Dmc1*, *Clgn* and *Gsg2* genes, which are expressed at different cell cycle stages of spermatogenesis (22), was studied. Reduced *Clgn* gene expression and the lack of *Gsg2* gene expression in mutant male mice confirmed that spermatogenesis was completely arrested at the pachytene stage of meiosis prophase I. Thus, data on the expression of developmental genes in spermatogenesis supported the histological data. We also observed a marked, age-dependent reduction in the number of follicles in mutant female mice.

To identify the gene causing the novel sterility, we performed chromosomal mapping and found that the sterility gene was associated with microsatellite markers on chromosome 15. Sequencing of the candidate genes, *Dmc1*, *Mei1* and *Smc1b*, revealed that the *Smc1b* gene had a deletion of 16 nucleotides in exon 5 in mutant mice, which generated a frame-shift and a premature stop codon at amino acid position 247. We therefore concluded that *Smc1b* gene function was disrupted in mutant mice.

Over 200 mouse models of defects in reproduction and fertility are believed to have been developed for studies of gametogenesis and reproduction in humans (2). We retrieved the MGI phenotype database to search for mutations showing the same or similar phenotypes as those of our sterile mice. As a result, we found a *Smc1b* gene knock-out mouse (14). We compared our mutant mice and the knock-out mice, but we could not observe any phenotypic differences between them, even though our *Smc1b* mutant gene had a C57BL/6JJcl background, and the targeted *Smc1b* mutation had a mixed background of 129S6/SvEvTac and C57BL/6 (14). Although genetic background has been shown to influence the phenotype of a gene (31), it may have almost no effect on the phenotype of the *Smc1b* gene.

There is considerable evidence showing that phenotypes of mutations depend on mutated exons. Homologous recombination for gene targeting was designed in the tenth exon in *Smc1b* knock-out (*Smc1b*^{tm1Jess}) mice (14) and a deletion mutation naturally occurred in the fifth exon in our mutant mice. Nonetheless, they did not show any phenotypic differences. These findings suggest that the phenotypes seen in both mutations were caused by deletion of the tenth exon to 25th exon, but not by deletion of the fifth to ninth exon. We expect that gene targeting reveals which exon(s) among the 16 exons from the tenth exon to the 25th exon contributes critically to the phenotypes of both infertile mice and causes milder or severer infertility phenotypes by disruption downstream from the tenth exon.

In conclusion, we reported the first spontaneous infertility mutation which was caused by the *Smc1b* mutation. Our genetic studies demonstrated that a deletion of 16 nucleotides generated a stop codon at position 761 of *Smc1b* cDNA in mutant mice. Our mutant mice and *Smc1b* knock-out mice (14) will provide both useful animal models for the study of human infertility and gene therapy models using transgenic technology.

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