

—Original—

Infertility Associated with Meiotic Failure in the *tremor* Rat (*tm/tm*) is Caused by the Deletion of *Spermatogenesis Associated 22*

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Abstract: The *tremor* rat is an autosomal recessive mutant exhibiting sterility with gonadal hypoplasia in both sexes. The causative mutation *tremor* (*tm*) is known as a genomic deletion spanning >200 kb in Chr 10q24. *Spermatogenesis associated 22* (*Spata22*) has been shown to be a vertebrate-specific gene essential for the progression of meiosis through prophase I and completion of chromosome synapsis and meiotic recombination using a mouse *repro42* mutant carrying an *N*-ethyl-*N*-nitrosourea (ENU)-induced nonsense mutation in *Spata22*. In this study, we show that *Spata22* was identified as the gene responsible for the failure of gametogenesis to progress beyond meiosis I in *tm* homozygous rats by a transgenic rescue experiment. Meiosis was arrested during prophase I in the mutant testis. Precise mapping of the breakage point revealed that the deleted genomic region spanned approximately 240 kb and comprised at least 13 genes, including *Spata22*. Rat *Spata22* was predominantly expressed in the testis, and its transcription increased with the first wave of spermatogenesis, as seen in the mouse ortholog. These results suggest that *Spata22* may play an important role in meiotic prophase I in rats, as seen in mice, and that the *tm* homozygous rat may be useful for investigating the physiological function of *Spata22*, as an experimental system for clarifying the effect of a null mutation, and may be an animal model for studying the pathogenesis and treatment of infertility caused by impaired meiosis.

Key words: gametogenesis, gonadal development, meiotic failure, spontaneous mutation, sterile rat

Introduction

Human infertility extends to 15% of couples, and 60% of idiopathic infertility cases in males have a recessive autosomal cause [9, 16]. Genetically well-defined animal

models are considered to be useful not only for understanding the pathogenesis of infertility but also for the development of treatments for it. At present, mice are widely used as animal models for human diseases. However, considering the effect of species variation of suit-

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ability for animal experiments and phenotypic differences due to species differences, it is desirable to prepare animal models of various species.

Meiosis is a developmentally programmed complex process that produces haploid germ cells from diploid parental cells in reproducing eukaryotes [20]. *In vivo* experimental systems are indispensable for investigating molecular mechanisms of vertebrate meiosis because of the lack of reproducible *in vitro* experimental systems. Animals exhibiting meiotic defects caused by gene mutations may be important tools not only for investigating molecular mechanisms underlying meiosis but also for studying human infertility.

Spermatogenesis associated 22 (SPATA22), formally known as *NYD-SP20*, was originally cloned from human testes by comprehensive expression analysis [24], and later, novel alternative spliced forms were reported [11]. Recently, *SPATA22* has been shown to be a candidate gene for human oligospermia or aspermia [1] and identified as the causative gene for an *N*-ethyl-*N*-nitrosourea (ENU)-induced autosomal recessive mutation, *repro42*, in mice. This mouse model exhibits arrest of meiosis at prophase I with abnormal chromosome synapsis and double-stranded DNA break (DSB) repair in both sexes [15]. *SPATA22* and the orthologs in mouse and rat encode 363-, 358- and 361-amino acid proteins, respectively. Confirmed or predicted *SPATA22* protein sequences from various vertebrates, including the human, chimpanzee, mouse, rat, dog, cattle, chicken, and zebrafish, display evolutionarily conserved regions in their N- and C-termini [15]. *SPATA22* comprises numerous putative functional sites across the protein sequence, including phosphopeptide motifs interacting with the BRCT domain of breast cancer 1, early onset (BRCA1), Polo-like kinase phosphorylation sites, and phosphoinositide-3-OH kinase-related kinase (PIKK) phosphorylation motifs; however, it has no significant homology with any other previously described proteins or functional domains [4, 15]. Although the essential role of *SPATA22* in the progression of meiosis I has been revealed, its molecular function remains unknown.

The mouse *repro42* mutation is a single nucleotide substitution, resulting in the appearance of a premature stop codon in the C-terminal conserved domain in the deduced amino acid sequence of Spata22 [15]. The deduced protein lacks the C-terminal 275–358-amino acid region. In testes of homozygous mutant mice, the expression level of *Spata22* mRNA is decreased, and the

Spata22 protein cannot be detected [15], suggesting that *repro42* is a loss-of-function mutation and that the resultant phenotype is caused by the low level of expression and/or a structural defect in Spata22.

The *tremor (tm)* mutation arose spontaneously in a colony of Kyoto:Wistar rats in 1980 and was initially characterized for its recessive body tremor, hair and whisker abnormalities, and infertility with dysgenesis of gonads in both sexes [29]. A subsequent report indicated that *tm* mutant males and females have recessive and semidominant absence-like seizure and recessive spongiform degeneration in the central nervous system (CNS) [23]. The *tremor* rat had been established as a segregating inbred strain by brother–sister inbreeding of *tm* heterozygous rats. The *tm* mutation has been mapped to rat chromosome 10q24 and has been identified as a deletion spanning a >200-kb genomic region containing *aspartoacylase (Aspa)*, *transient receptor potential vanilloid 1 (Trpv1)*, and several genes encoding olfactory receptors (ORs) [13]. According to the rat genomic sequence (RGSC v3.4), the region deleted in the *tm* locus is considered to comprise *Spata22*. In the present study, we found that gametogenesis failed to progress beyond meiosis I in the *tm* homozygous rat, and that meiosis was arrested during prophase I in the *tm* homozygous male testis. We revealed that the deleted genomic region in the *tm* locus comprised nearly 240 kb and harbored at least 13 genes, including *Spata22*. We identified *Spata22* as the causative gene of infertility and abnormal meiosis in homozygous mutants by a transgenic rescue experiment. This is the first description of meiotic failure caused by a null mutation of *Spata22*, and the first report of infertility associated with meiotic failure caused by a naturally or spontaneously occurring mutation of *Spata22*, including human infertility.

Materials and Methods

Animals

The *tremor* rat TRM/Kyo was supplied by the Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University (Kyoto, Japan), and maintained in the Genome Dynamics Research Center at Hokkaido University. Heterozygous (*tm/+*) females were mated with heterozygous (*tm/+*) males to obtain the affected (*tm/tm*) and control (*+/+*) rats used in this study. All the rats were maintained in a room with controlled temperature and a 12-h light–dark cycle. Food and water were provided *ad*

libitum. All the animal experiments in this study were approved by Institutional Animal Care and Use Committee, Hokkaido University.

Histological analysis

Testes were collected from three each of *tm/tm* and wild-type rats and *tm* homozygous mutants carrying *Spata22* transgenes at 3 months after birth. Ovaries were collected from two each of *tm/tm* and wild-type rats at 7 days after birth. They were fixed using Bouin's fixative solution, embedded in paraffin, cut into 5- μ m-thick sections, and then stained with hematoxylin and eosin (HE).

Chromosome analysis

The preparation of nuclei and chromosomes for light microscopy of spermatogenic cells from two each of *tm/tm* and wild-type rats at 30 days after birth and two *tm* homozygous mutants carrying *Spata22* transgenes at 3 months after birth was conducted using the air-drying method [12], with the omission of colchicine treatment. The preparations were stained with 4% Giemsa in phosphate buffer (pH 6.8).

Physical mapping

We conducted a homology search of the sequence of a breakage point (GenBank entry: AB023434) in the mutant genome using the BLAST program (<http://blast.ncbi.nlm.nih.gov>). PCR amplifications were performed with primer pairs for DNA fragments localized around both ends of the deleted region. Sequences of six primer sets used in sequence-tagged site (STS) analysis and map positions of their target DNA sequences on rat chromosome 10 (RGSC v3.4) are as follows: brk1, CCCATCTCACCATCACCCCAACAGT and ACACCCCACTCTTGGAGCTGTTGAA (60,098,543–60,098,723 bp); brk2, TTGTGCTGGGCTGGGTGC-TATCTCT and TGCCGTGGGTATTAATGAGAAGGA (60,119,939–60,120,055 bp); brk3, GCTCTCACACTA-CAACAAG and GTAGTTGGAAAGGTCTCCC (60,324,857–60,324,992 bp); brk4, TTGTAATAA-CAGGGATTGC and GCCCATTTACAAACCTCAG (60,333,641–60,333,799 bp); brk5, ATGGATA-ACCCAACCTGG and GGTCGTGGTCACCTAACT (60,345,158–60,345,336 bp); and brk6, GTTCCCAC-CATTTGCCTACGAATTT and GCTCCACTAT-GTTCATCGCAGCCT (60,346,178–60,346,348 bp).

RT-PCR

Total RNA was extracted from various organs using TRIzol reagent (Invitrogen, Life Technologies, Carlsbad, CA, USA), and first-strand cDNA was synthesized with oligo-dT primers. The following primer sequences were used for PCR amplification: *Spo11*, CCGAGGCCTC-GTTCTTCGAC and TGCCACCGCAGCCTGGTTC; *Spata22*, TCAACTCGAAGTACAGCAGGCTGTT and GCCACCTTGGCTTCTTTTAGCGTTT; *Msh4*, GCTGT-GTACCATCTGGCTACA and CTCCT-CAGTCTTCTCTGGAAGG; and *Gapdh*, ACCACAGTC-CATGCCATCAC and TCCACCACCCTGTTGCTGTA.

Generation of transgenic rats

Transgenic rats for rescue experiments were generated using the pCAGGS vector (provided by J. Miyazaki). Full-length *Spata22* cDNA was obtained by screening a rat testis cDNA library (Takara Bio, Otsu, Shiga, Japan). Full-length *Spata22* cDNA was inserted into the *EcoRI* site of the vector. The vector plasmid was digested with *SalI* and *HindIII*, electrophoresed, and then purified from agarose gel. Transgenic rats were generated by microinjection of the transgenes into fertilized eggs from Wistar females mated with heterozygous *tm* males. Two lines of transgenic rats (line 2, line 4) were obtained. These transgenic lines were backcrossed to TRM/Kyo. Gross and histological examinations of gonadal development were conducted mainly using line 2. Two each of 7-month-old homozygous mutant males carrying the transgenes (*tm/tm Tg/+*) and 21-day-old homozygous mutant females carrying the transgenes (*tm/tm Tg/+*) were used for gross examinations. Their homozygous (*tm/tm*) and heterozygous (*tm/+*) non-transgenic littermates were used as controls.

Accession number

Sequence data of a full-length rat *Spata22* cDNA has been deposited in DDBJ/EMBL/GenBank databases under accession number AB236891.

Results

Mutant gonads displayed arrest of meiosis I

Testis weights of mutants were markedly reduced; average testis weights (mean \pm SD) were 391 ± 44 mg in three *tm/tm* adult males, $1,228 \pm 254$ mg in three wild type adult males, and $1,395 \pm 198$ mg in three *tm/+* adult males. The average testis weight of the *tm* homozygous

mutants was significantly less than those of the wild types (t -test, $P < 0.001$) and the heterozygotes (t -test, $P < 0.001$). Average testis weights were not significantly different between the wild types and the heterozygotes (t -test, $P > 0.05$). Average ratios (mean \pm SD) of testis weight (mg) to body weight (g) were 1.21 ± 0.15 in the homozygous mutants, 4.23 ± 1.08 in the wild-types, and 3.84 ± 0.39 in the heterozygotes. Pathological examination of the testes from adult mutant males demonstrated the accumulation of spermatocytes and depletion of spermatids and spermatozoa, as shown in Figs. 1A, 1B, 1C, and 1D. The ovaries from juvenile homozygous mutant females (tm/tm) had depleted primordial follicles, with many degenerated oocyte nuclei (Figs. 1E, 1F, 1G, and 1H). Adult mutant females lacked discernible ovarian structures (data not shown). These observations indicated the arrest of meiosis I during gametogenesis in mutant gonads. Subsequent cytogenetic examination of mutant meiosis was carried out using spermatogenic cells.

Mutant male meiosis failed to progress beyond early prophase I

To examine meiotic progression in mutants, we observed spreads of nuclei and chromosomes of spermatocytes. In 30-day-old control testes, zygotene, late zygotene or early pachytene, midpachytene, and metaphase I spermatocyte nuclei were observed (Fig. 2A). In 30-day-old homozygous mutant testes (tm/tm), no normal nuclei that had progressed into midpachytene were observed, whereas nuclei that had nucleus sizes and chromosome morphologies similar to those of control spermatocytes at zygotene and late zygotene or early pachytene stages and many degenerated nuclei were observed (Fig. 2B), indicating that meiosis progressed into the zygotene stage and was arrested at the zygotene or pachytene stage in mutants. This spermatogenesis phenotype, known as the “meiotic arrest” phenotype, is similar to that of the *repro42* homozygous mouse, which carries a nonsense mutation in *Spata22* [15], and has been previously found in many types of sterile mutant mice and rats for genes related to synaptonemal complex formation, meiotic homologous recombination repair, and regulation of DNA damage checkpoint [2, 8, 10, 21].

The mutant genome lacked a nearly 240-kb genomic region that comprises at least 13 genes, including Spata22

To identify the gene responsible for the abnormal gametogenesis, we determined the precise location of ends of the deleted region on rat chromosome 10 by searching for the DNA fragment (AB023434) containing the *tm* breakage point using the BLAST program. We verified these results by PCR analysis using STS markers around both ends of the deleted region (Figs. 3A and 3B). The centromere-proximal end of the deleted region was mapped inside the 7th exon of *sedoheptulokinase* (*Shpk*), and the centromere-distal end was located within a LINE1 element downstream of *Olr1472*. Within the determined 240-kb deleted region (60,098,741–60,339,238 bp, RGSC v3.4), entire transcribed regions of 12 genes besides part of the 7th exon of *Shpk* were localized: *transient receptor potential vanilloid 1 and 3* (*Trpv1/3*); *Aspa*; the *Spata22* ortholog; seven OR genes, *Olr1466*, *Olr1467*, *Olr1468*, *Olr1469*, *Olr1470*, *Olr1471*, and *Olr1472*; and one hypothetical gene, *LOC100359760* (Fig. 3A).

High expression of Spata22 that increased with the first meiotic wave

As shown in a previous gene expression analysis [15], RT-PCR analysis revealed that the *Spata22* ortholog was predominantly expressed in the testis tissue (Fig. 3C). The expression levels of mRNA and protein of *Spata22* in mouse testes began to increase with the first wave of spermatogenesis [15]. In laboratory rats, the first meiotic wave begins at 13–14 days after birth [18]. RT-PCR analysis indicated that ortholog expression began to increase between 8 and 14 days after birth, as seen in two other meiosis-related genes, *Spo11* [3, 22] and *Msh4* [14] (Fig. 3D). These results suggest that rat *Spata22* may be involved in the regulation of meiosis I and that the function of this gene may be conserved between the two rodent species: the rat and mouse.

Spata22 overexpression recovered the reproductive phenotype in homozygous mutants

To verify that *Spata22* underlies the mutant reproductive phenotype, we performed transgenic rescue experiments with the minigene (Fig. 4A). All homozygous mutant rats (tm/tm) carrying the transgenes at the fifth generation of backcross (=N5) were rescued from sterility: one male and two females in one of the two trans-

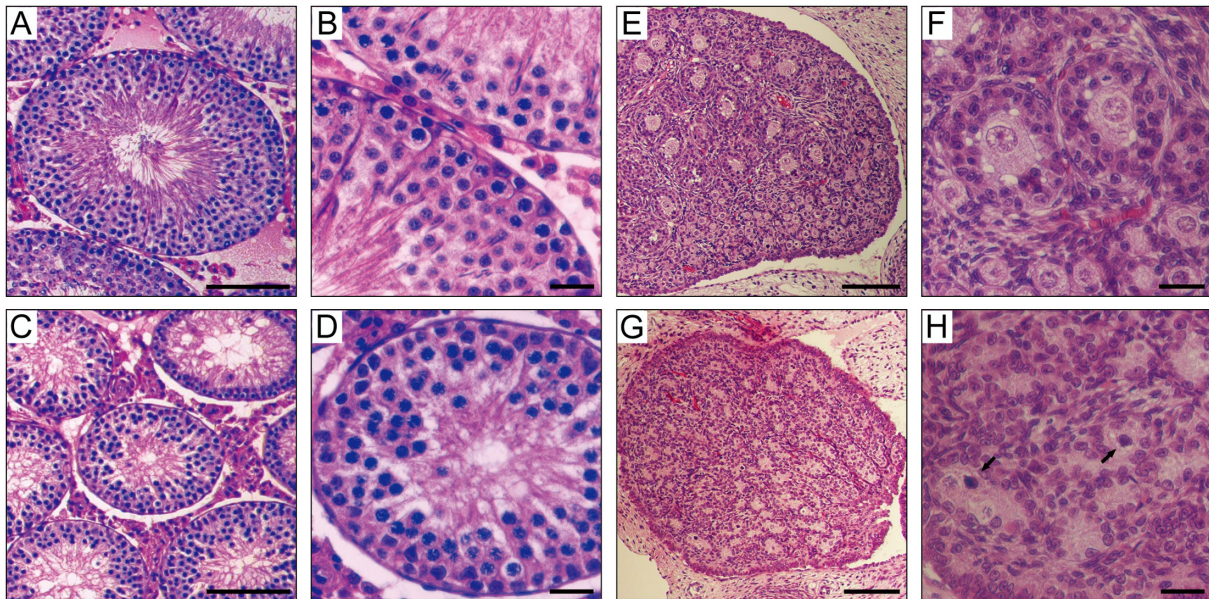


Fig. 1. Abnormal gametogenesis in mutant rats. Upper panels, wild-type. Lower panels, homozygous mutant. Hematoxylin and eosin (HE)-stained sections of seminiferous tubules at 3 months after birth (A–D) and ovaries at 7 days after birth (E–H). Accumulation of spermatocytes and depletion of spermatids and spermatozoa were observed in adult homozygous mutants (C and D). Oocyte was depleted in juvenile homozygous mutants (G and H). Arrows indicate degenerated nuclei. Scale bar: 100 μm in A, C, E, and G and 25 μm in B, D, F, and H.

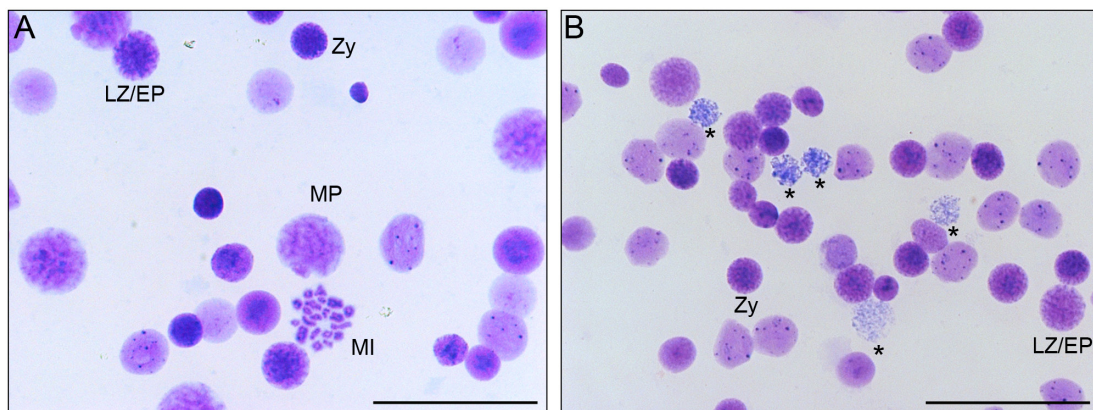


Fig. 2. Failure of meiotic progression during prophase I in the mutant testis. A: Giemsa-stained preparation of testicular cell nuclei or chromosomes from 30-day-old wild-type rats indicating the presence of spermatocytes at zygotene (Zy), late zygotene or early pachytene (LZ/EP), midpachytene (MP), and metaphase I (MI) stages. B: Giemsa-stained preparation of testicular cell nuclei or chromosomes from 30-day-old homozygous mutant rats showing the presence of spermatocyte nuclei similar to control spermatocyte nuclei at zygotene (Zy) and late zygotene or early pachytene (LZ/EP) stages. Many degenerated nuclei were observed (asterisks), whereas no normal midpachytene nuclei were present. Scale bar: 50 μm .

genic lines (line 2) and one male in the other transgenic line (line 4), although they were not rescued from the anomalies in hair, whiskers (Figs. 4B and 4C), and the CNS or from body tremor (data not shown). *Spata22* overexpression induced no apparent changes in developmental, morphological, and behavioral phenotypes in

homozygous mutants, except for those in the reproductive phenotype. Testes and ovaries of the transgenic rats showed normal development upon gross examination (Figs. 4B and 4C). Histological analysis consistently showed that spermatogenesis proceeded normally in the rescued transgenic rats (Fig. 4D). Cytogenetic examina-

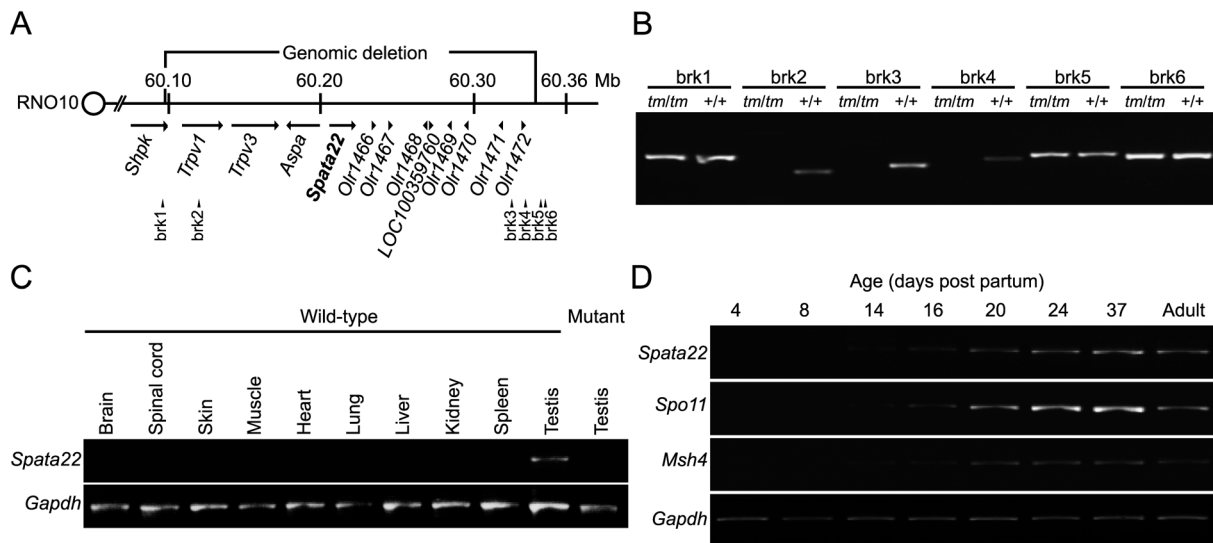


Fig. 3. Positions of ends of the genomic deletion in the *tm* locus and expression analysis of *Spata22*. A: Schematic diagram of the *tm* critical region of rat chromosome 10 (RNO10) indicating positions of the genomic deletion, *Spata22*, and other genes within the deleted region. The breakage sites are mapped to *sedoheptulokinase* (*Shpk*) and an L1 retrotransposon element in the large cluster of olfactory receptor genes, respectively. The distance between the breakage sites is approximately 240 kb. Arrows above gene names represent approximate gene size and indicate the direction of transcription. Positions of STS markers (brk1–brk6) used in PCR analysis are shown below the chromosome. Map positions in Mb are shown above the chromosome. B: Comparison of PCR amplification of sequence-tagged site (STS) markers between genomic DNA of control and homozygous mutant rats demonstrating loss of PCR amplification of STS makers (brk2–brk4) within the predicted deleted region in mutants. C: mRNA expression of *Spata22* and *Gapdh* in various organs of adult controls and testes of adult homozygous mutants showing enhanced expression of *Spata22* in control testes. Weak expression was detected in other organs when the number of PCR cycles was increased. D: mRNA expression of *Spata22*, *Spo11*, *Msh4*, and *Gapdh* in testes of wild-type rats between the ages of 4 and 37 days and in testes of adult wild-type rats. *Spata22* expression begins to increase between the ages of 8 days and 14 days, as shown for *Spo11* and *Msh4*.

tion of meiotic progression also confirmed that meiosis was recovered in homozygous mutant males carrying the transgenes (Fig. 4E). These results indicate that *Spata22* is the causative gene for infertility and abnormal gametogenesis in the homozygous mutant rat.

Discussion

In the present study, we have shown that infertility with abnormal gonadal development was associated with the arrest of meiosis I in the *tm* homozygous rat. Meiotic cell division was blocked during the zygotene or pachytene stage of prophase I in the *tm* homozygous male rat, similar to the findings in mutant mice and rats for genes related to chromosome synapsis, meiotic recombination, and cell cycle control [2, 8, 10, 21] as well as the *repro42* homozygous mouse, which carries a nonsense mutation in *Spata22* [15]. Analysis of progression of meiosis in the mutant ovary will be the subject of future study. We revealed that an approximately 240-bp

genomic region comprising at least 13 genes, including *Spata22*, which is known to be a candidate gene for human aspermia or oligospermia and an essential gene for the progression of meiosis I, was lost in the *tm* genome. Using a transgenic complementation test, we showed that *Spata22* is responsible for infertility and abnormal gametogenesis.

A previous study on the *repro42* mutant mouse has shown that testicular weights of mutant males are markedly reduced; the average testis weight of *repro42* homozygous adults is approximately 20% of that of wild type littermates [15]. Histological analysis of adult *repro42* mutant testes showed complete lack of spermatids and spermatozoa despite presence of spermatogonia and spermatocytes [15]. Histological analysis of 10 dpp *repro42* mutant ovaries revealed an almost complete depletion of oocytes and presence of degenerating or degenerated oocyte-containing follicles [15]. These findings are quite similar to our present findings concerning the gonadal phenotype of *tm* mutant rats. In most patients with

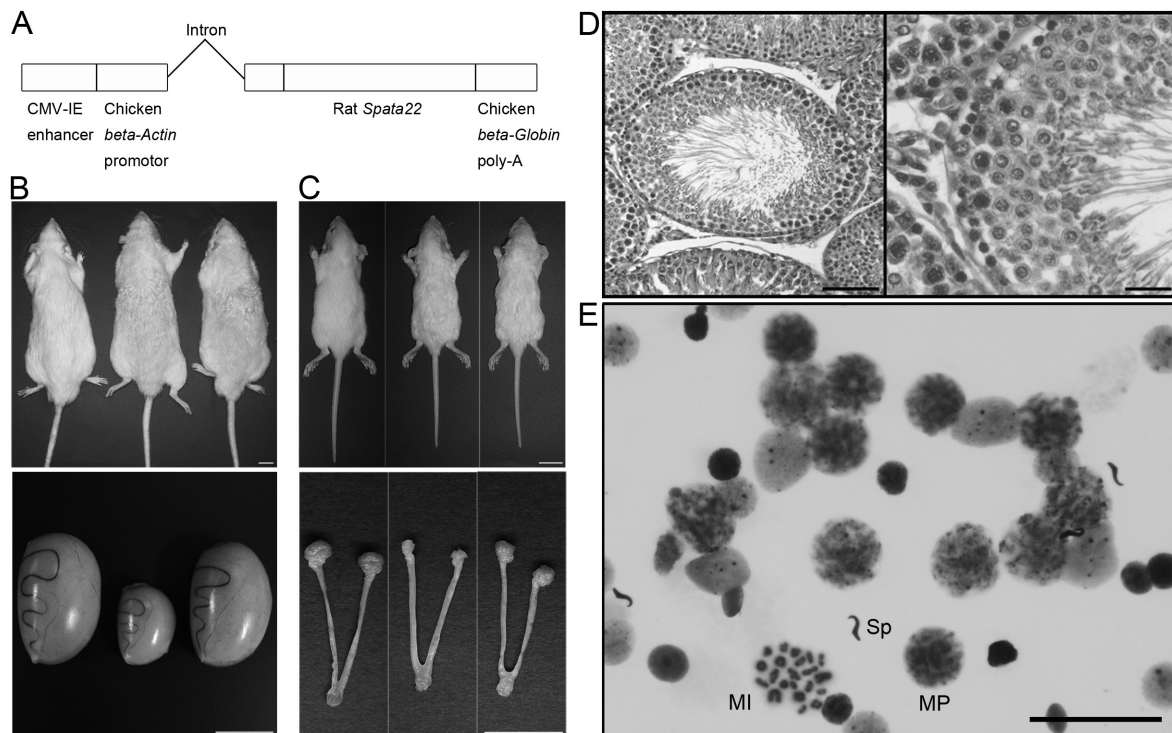


Fig. 4. Recovery of spermatogenesis by transgenic rescue. A: Transgenic vector constructed by insertion of full-length rat *Spata22* cDNA into pCAGGS. B and C: Gross examination of 7-month-old testis (B) and 21-day-old ovary (C) in mutants carrying the transgenes. Upper panels: dorsal views of animals. Lower panels: gross morphologies of gonads, oviducts, and uteri. In each panel, the genotypes of the animals and the organs are as follows: *tm/+* (left), *tm/tm* (middle), and *tm/tm Tg/+* (right). The homozygous mutant testis was small, and the homozygous mutant ovary could not be found despite the presence of oviducts and uteri. The testis and the ovary of the *tm/tm Tg/+* rats appeared to develop normally, whereas the wavy coat phenotype of the *tm/tm Tg/+* rats was similar to that of the *tm/tm* littermates in both sexes. Scale bar: 2 cm in upper panels, 1 cm in lower panels. D: Hematoxylin and eosin (HE)-stained section of testes from adult homozygous mutants that carry the transgenes, showing normal morphology of the seminiferous epithelium. Scale bar: 100 μm and 25 μm for the low- and high-magnification images, respectively. E: Giemsa-stained preparation of testicular cells, cell nuclei, or chromosomes from adult homozygous mutants carrying the transgenes, showing normal progression of meiosis. Spermatocyte nuclei at midpachytene and metaphase I stages and a spermatozoon head are indicated by MP, MI, and Sp, respectively. Scale bar: 50 μm .

Canavan disease, point mutations in *ASPA* are causative [5]. Deletion mutations comprising *ASPA* and adjacent genes, such as the *tm* mutation, have been reported in a few cases [5, 27, 30], and gonadal phenotypes of Canavan patients carrying such mutations have not been reported. The similarity in gonadal phenotype between *repro42* mutant mice and *tm* mutant rats suggests that genomic deletion involving lack of *SPATA22* in humans may induce gametogenic failure as seen in these mutants.

Concerning the 12 genes other than *Spata22* in the deleted region, targeted disruption of *Trpv1*, *Trpv3*, and *Aspa* has no apparent effect on the reproductive system in mice [6, 19, 26], while *Trpv3*- and *Aspa*-deficient mice exhibit an abnormal coat and skin [7] and spongiform degeneration in the CNS [26], respectively. Whether the

loss of *Shpk* [28], seven OR genes [25], and a function-unknown gene, *LOC100359760*, affects the reproductive system has not been known. Although the functions of the seven OR genes are unknown, olfactory receptors are known to be responsible for the recognition and G protein-mediated transduction of odorant signals in cells, such as olfactory receptor neurons and sperms [25]. Therefore, it is not likely that loss of the seven OR genes affects spermatogenesis seriously. In this study, the transgenic rescue experiment showed that deficiencies in *Trpv1*, *Trpv3*, and *Aspa*, partial deletion of *Shpk*, loss of the other eight genes, and genomic deletion at the *tm* locus *per se* had no apparent effect on meiosis. These findings, taken together with a finding that the meiotic phenotype in the *tm* homozygous rat is similar to a mei-

otic phenotype caused by only a single nonsense mutation induced in *Spata22* in the *repro42* homozygous mouse [15], suggest that the deleted genes, except for *Spata22*, may have no effect or minor effects on the meiotic arrest phenotype caused by the loss of *Spata22*.

As shown in a previous study on mouse *Spata22* [15], the rat *Spata22* ortholog was highly expressed in the testis, and its expression level began to be enhanced in conjunction with the initiation of meiosis. Together with the meiotic phenotype in the mutant rat, these results suggested that, like mouse *Spata22*, rat *Spata22* has an important role in the regulation of meiotic prophase I [15]. Despite its importance in the progression of meiosis through prophase I, the molecular function of *Spata22* has been poorly understood. The *Spata22* allele of the *tm* genome is a null mutation resulting from complete removal of the gene from the genome. Therefore, the *tremor* rat (*tm/tm*) could contribute to further functional analysis of *Spata22*, as a null mutant, in conjunction with the transgenic rescued mutant.

This is the first report of a deletion mutation of *Spata22*, which leads to infertility and abnormal gametogenesis. However, in some patients with Canavan disease, which is caused by *ASPA* deficiency, large genomic deletions involving loss of several genes, including *ASPA*, were found at Chr 17p13 [5, 27, 30]. In two types of such deletions, approximately 190 kb and 439 kb in size, *SPATA22* is located in the deleted regions and seems likely to be removed from the genome with *ASPA*, although the gonadal phenotypes in the patients were not reported. The sequences around ends of the deleted region in the *tm* genome have no significant homology with each other, suggesting that the genomic loss may be caused by a nonhomologous recombination event, such as a 190-kb-sized deletion in human Chr 17p13 [27]. The genomic region around the *SPATA22* locus comprises no known chromosomal fragile site [17]. Although it is unclear whether these large genomic deletions found at nearly the same map position in Canavan patients and the *tremor* rat are caused by a common mechanism, similar deletions could also occur in other mammalian species as well as humans and rats. Therefore, the *tremor* rat could be a useful animal model for studying diseases resulting from such deletions around the *Spata22* locus.

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