FAM9B serves as a novel meiosis-related protein localized in meiotic chromosome cores and is associated with human gametogenesis

Xin-jie Zhuang, Xue Feng, Wen-hao Tang, Jin-liang Zhu, Ming Li, Jun-sheng Li, Xiao-ying Zheng, Rong Li, Ping Liu, Jie Qiao

1 Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Peking University Third Hospital, Haidian District, Beijing, PR China, 2 Key Laboratory of Assisted Reproduction, Peking University, Ministry of Education, Haidian District, Beijing, PR China, 3 Beijing Key Laboratory of Reproductive Endocrinology and Assisted Reproductive Technology, Haidian District, Beijing, PR China, 4 National Clinical Research Center for Obstetrics and Gynecology, Peking University Third Hospital, Haidian District, Beijing, PR China, 5 Department of Urology, The Third Hospital of Peking University, Beijing, China

* Bysylp@sina.com (PL); zhuangxinjie902@163.com (XJZ)

Abstract

Meiosis is a complex process involving the expression and interaction of numerous genes in a series of highly orchestrated molecular events. Fam9b localized in Xp22.3 has been found to be expressed in testes. However, FAM9B expression, localization, and its role in meiosis have not been previously reported. In this study, FAM9B expression was evaluated in the human testes and ovaries by RT-PCR, qPCR, and western blotting. FAM9B was found in the nuclei of primary spermatocytes in testes and specifically localized in the synaptonemal complex (SC) region of spermatocytes. FAM9B was also evident in the follicle cell nuclei and diffusely dispersed in the granular cell cytoplasm. FAM9B had a similar distribution pattern and co-localization as γH2AX, which is a novel biomarker for DNA double-strand breaks during meiosis. All results indicate that FAM9B is a novel meiosis-associated protein that is co-localized with SYCP3 and γH2AX and may play an important role in SC formation and DNA recombination during meiosis. These findings offer a new perspective for understanding the molecular mechanisms involved in meiosis of human gametogenesis.

Introduction

Gametogenesis is a highly complex process in which gametes are produced from germinal stem cells by mitotic and meiotic cell division [1]. During gametogenesis, synaptonemal complex (SC) formation may play a universal role in meiosis. The SC is a complex protein structure with many proteins involved in its formation [2]. During this process, a large number of genes
participate in meiosis. However, human meiosis is still poorly understood on a molecular level.

Many important proteins participate in the assembly of SC, such as SYCP1 [3, 4], SYCE1 [5, 6], SYCE2 [7, 8], TEX12 [9], SYCE3 [10], SYCP2 [11, 12], SYCP3 [13, 14], and SLX2 [15, 16]. SYCP1 is an extended filamentous protein that consists of three domains. It is probably a TF constituent and a major protein component of SC [3]. SYCP1 is associated with infertility in both sexes and leads to synopsis failure in mice [17]. SYCE1 encodes an SC protein that plays an essential role during meiosis. Deleterious SYCE1 mutation is associated with male infertility [6, 18] and autosomal recessive primary ovarian insufficiency [19]. SYCE2 is required for SC assembly [8] and forms a highly stable, constitutive complex within the central element (CE) of the SC [7]. SYCE3 is localized in the CEs and Syce3−/− in mice and is required for male and female fertility [10]. SYCP2 is a component of metazoan SC [20], which is essential for proper chromosome synopsis [11, 21]. SYCP3 is important for SC formation, while SYCP3 mutation plays a role in male fertility [22] and recurrent pregnancy loss [23]. Female germ cell aneuploidy [24] and embryo death have been observed in mice lacking the meiosis-specific protein SYCP3 [25]. SLX2 is also localized in SC and may be involved in SC formation during spermatogenesis [16] and meiotic oocyte maturation [15].

Chromatin reorganization requires formation and repair of DNA double-strand breaks (DSBs). DSB generation rapidly results in the phosphorylation of histone H2A variant H2AX [26]. Because H2AX phosphorylation (γ-H2AX) is abundant, fast, and correlates well with each DSB, it is the most sensitive marker that can be used to examine the DNA damage produced and subsequent repair of the DNA lesion [27]. γ-H2AX appeared to be located in unsynapsed chromosomal segments during male meiotic prophase I. Several waves of H2AX phosphorylation/dephosphorylation coupled to various developmental phases of spermatogonia and spermatocytes, as well as to spermatid differentiation [28]. Previously, we found that SLX2-conserved Cor1 domain was highly homologous with SYCP3. Its co-localization with γH2AX and interaction with TIP60 suggested that SLX2 might be involved in DNA recombination and DSBs during meiosis [2]. Fam9b conserved Cor1 domain is localized in Xp22.3 and expressed exclusively in testes [29]. However, FAM9B expression, localization, and its role in meiosis remain unclear.

The present study demonstrated that FAM9B is abundantly transcribed in human testes and ovaries and is differentially expressed during human gametogenesis. Moreover, FAM9B was found in the nuclei of primary spermatocytes in testes and specifically localized in the synaptonemal complex (SC) region in spermatocytes. FAM9B was also evident in follicle cell nuclei and diffusely dispersed in the granular cell cytoplasm. FAM9B has an expression pattern similar to that of γH2AX and is specifically localized in human SC region as SYCP3 during meiosis. These results strongly indicate that FAM9B plays important roles in the regulation of SC formation during meiosis.

Materials and methods

Sample collection and ethics statement

Twenty testicular biopsy tissue samples were obtained from patients (age: 25–36 years) with obstructive azoospermia, and 50 patients undergoing percutaneous testicular and ovarian biopsy (age: 25–36 years) for IVF treatment agreed to further tests at the Reproductive Medicine Center, Peking University Third Hospital between January 2014 and November 2017. All samples were obtained from donors (age: 25–36 years) who signed the informed consent for...
excision and scientific use of testis tissue voluntarily. The present study was reviewed and approved by the Ethics Commission of the Reproductive Medical Faculty of the Peking University Third Hospital (No. 2013SZ021). All studies and protocols were approved and conducted in accordance with institutional guidelines and the Declaration of Helsinki for research involving human tissue.

**Reagents and antibodies**

All chemicals and electrophoresis and transfer equipment were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and Bio-Rad Laboratories (Carlsbad, CA) unless specified otherwise. Revert Aid First Strand cDNA Synthesis Kit and PCR Cloning Kits were purchased from Clontech (San Jose, CA) and Thermo Fisher Scientific Inc. (San Jose, CA, USA). Anti-SYCP3 antibody and anti-FAM9B antibody were purchased from Abcam (Cambridge, UK), while anti-γ-H2AX antibody was purchased from Upstate Biotechnology (Charlottesville, VA). Primers were synthesized by Invitrogen (Carlsbad, California, USA).

**RNA-seq and real-time PCR validation**

Total RNA from samples was isolated in accordance with the manufacturer’s instructions [30]. For RT-PCR, total RNA was reverse transcription using the Thermo Fisher Scientific Inc. (San Jose, CA, USA) and Superscript III reverse transcriptase as described previously [31]. Three primer pairs were used to detect Fam9b mRNA expression (Table 1). Amplification was carried out, with an annealing temperature of 58.5˚C for Fam9b primers. Reaction was finished at 72˚C for 5 min. The PCR products were analyzed using agarose gel electrophoresis. Relative amounts of cDNA were normalized against GAPDH.

**Protein extraction and western blot analysis**

Western blotting and protein extraction were performed using standard protocols [15]. Human testes and ovarian biopsies were homogenized in RIPA lysis buffer. Protein concentration was determined using the Bradford Reagent (BioRad). Protein lysates were separated by SDS-PAGE. Briefly, 50 μg of total protein were loaded, separated by SDS–PAGE, and immunoblotted with anti-FAM9B antibody. Membranes were blocked in TBST incubated overnight with a 1:200 dilution of anti-FAM9B antibody (Abcam). In addition, membranes were incubated with HRP-conjugated secondary antibody and protein expression levels were measured using Image-Pro plus 5.1.

**Immunohistochemistry and immunocytochemistry**

Immunohistochemistry and immunocytochemistry were performed via standard manipulations as described previously [32]. The sections were incubated with anti-FAM9B antibody

---

**Table 1. Primer pairs.**

| Name     | Usage   | Sequence (5’-3’)                  |
|----------|---------|-----------------------------------|
| Fam9b-1  | RT-PCR  | Sense: CCGCTGCTGCGAGGTCTGGAGG    |
|          |         | Antisense: GACGAGTGACAGAGGAGCTAGC|
| Fam9b-2  | Q-PCR   | Sense: TACATCACAGACGAGAAAG       |
|          |         | Antisense: TACCTACCTCTCTCTCCTCCAA|
| Fam9b-3  | Q-PCR   | Sense: TAGGAGAGAGGCGTCAGAAAG     |
|          |         | Antisense: AGTTCACTGCTCTTCCATCAAA|

https://doi.org/10.1371/journal.pone.0257248.t001
(diluted 1:200) or pre-immune rabbit serum as a negative control. FITC-conjugated anti-rabbit secondary antibody was used at a dilution of 1:600. The sections were then incubated with DAPI (Sigma-Aldrich) and in PBS instead of primary antibody for negative controls. Sample slides were photographed using a Nikon ECLIPSE 80i microscope with a Nikon DS-Ri1 camera (Nikon Corporation, Japan).

Confocal fluorescence and chromosome spreading

Chromosome spreading of primary human spermatocytes was performed using the drying-down technique [15]. Briefly, human seminiferous tubules were isolated. Subsequently, the seminiferous tubules were torn into small pieces. The spermatocyte cell suspension was mixed with 3.7% PFA solution. The spermatocyte cells were subsequently spread on a clean glass slide. Then, the cell slides were washed, dried, and stained using anti-SYCP3 (1:200, Abcam), γ-H2AX (1:200, Sigma), and/or anti-Fem9b (1:200, Abcam). TRITC- or FITC-conjugated anti-rabbit secondary antibody (Jackson Laboratories, Bar Harbor, ME) was applied at a dilution of 1:600. Protein subcellular localizations were examined using a laser confocal microscope (Zeiss).

Fig 1. Fam9 mapped on human X chromosomes shares homology with SYCP3. (A) Fam9b –conserved Cor1 domain shares homology with SYCP3. (B) Fam9 mapped on Xp22.3 of human X chromosomes.

https://doi.org/10.1371/journal.pone.0257248.g001
Statistical analysis
The results were analyzed using SPSS 17.0 software (Chicago, IL, USA). All experiments were repeated and analyzed at least three times. An independent-sample t-test was performed on the qPCR and immunofluorescence intensity data. P-values <0.05 were considered to be significantly different.

Results
Fam9b is a member of Fam9 family located on chromosome Xp22.3
A FASTA comparison showed that Fam9b DNA sequences demonstrate significant homology with SYCP3, which contains a Cor1 domain (Fig 1A). Fam9b encodes 186AA and also contains a Cor1 domain. FAM9B may have functions that involve spermatogenesis as SYCP3. In addition, Fam9b is located on chromosome Xp22.3 (Fig 1B) and microdeletion/ microduplications at Xp22.3 have been frequently detected with the application of BACs-on-Beads™ and array-comparative genome hybridization technologies in prenatal diagnosis. Xp22.3 deletions

Fig 2. FAM9B expression was determined by qPCR, RT-PCR, and western blotting. (A) qPCR results show that FAM9B mRNAs are highly expressed in testes and ovarian tissue. Lane1. Testis cDNA; Lane 2. Negative control; Lane 3. Ovary cDNA. (B) Western blotting results for FAM9B expression in both testes and ovaries. Lane 4. Testis protein; Lane 5. Negative control; Lane 6. Ovary protein. All experiments were repeated and analyzed at least three times. Actin mRNA and protein were used as internal control. All bar graphs show the mean±s.e.m. *P<0.05, **P<0.001.

https://doi.org/10.1371/journal.pone.0257248.g002
have been associated with clinical features, including mental retardation, short stature, Kallmann syndrome, and infertility.

**Fam9b transcripts and protein expression in human testes and fetal ovaries**

Fam9b transcript was measured using real-time quantitative PCR (qPCR) and RT-PCR in the aborted human testes and ovaries (Fig 2A and 2B). Total RNA was extracted from eight human tissue samples and qPCR analysis was performed. The results showed that Fam9b mRNAs are highly expressed in testes and ovarian tissue.

Fam9b protein was detected in human testes and fetal ovaries using western blotting assays. In addition, western blotting also showed that one specific form of protein with a molecular size of ~20 kDa was present in testes and ovaries (Fig 2C).

**FAM9B may play a role in human spermatogenesis and fetal oogenesis**

FAM9B expression analysis was performed on adult testicular sections (Fig 3) using immunostaining. Confocal fluorescence microscopy demonstrated that FAM9B was localized in dense regions that appeared in human germ cell nuclei after immunostaining. Higher expression of FAM9B was found in the spermatocyte nuclei (Fig 3).

FAM9B was detected in human ovaries using immunocytochemical analysis (Fig 4). It was also detected in human fetal ovarian tissue (Fig 4A). FAM9B was distributed in adult ovaries.

---

**Fig 3. Immunostaining results for FAM9B expression in human testicular sections.** (A) FAM9B (green) is clearly present in both testicular cell nucleus and cytoplasm, localized in primary spermatocyte nucleus (red arrow), and evident in sertoli cell cytoplasm. FAM9B (green) is localized in the nucleus of germ cells shown by red arrows. Nuclei are stained with DAPI (blue). Bars = 10 μm.

[https://doi.org/10.1371/journal.pone.0257248.g003](https://doi.org/10.1371/journal.pone.0257248.g003)
and localized in granulosa cells (Fig 4B). FAM9B was evident in follicle cell nuclei and diffusely dispersed in granular cell cytoplasm. Therefore, FAM9B may play a role in oocyte maturation.

**FAM9B is a novel meiosis-associated protein**

FAM9B expression was detected in chromosome spreads of different human spermatocyte cells using immunostaining analysis (Fig 5). FAM9B was localized in the SC region, similar to SYCP3. SYCP3 is a well-known SC marker in the spermatocyte chromosome spreads. Further study investigated whether FAM9B was partly co-localized in SYCP3 and expressed as distinct points along the chromosome axis during meiosis. In leptotene spermatocytes, FAM9B localization matched the SYCP3 staining pattern. In pachytene spermatocytes, SYCP3 staining was restricted to the SC. FAM9B and SYCP3 may participate in the meiosis stage of spermatogenesis and may be involved in SC formation and meiosis in human germ cells. FAM9B may also perform a function similar to SYCP3 in human SC formation, gametogenesis, and fertility.

**FAM9B is partly co-localized with γH2AX**

To confirm that FAM9B was indeed related to meiosis, FAM9B and γH2AX co-localization in the nuclei of primary spermatocytes in meiosis I was evaluated using confocal laser scanning microscope (Fig 6). FAM9B is a nuclear protein that is localized in the spermatocytes in meiosis I and partly co-localized with γH2AX in the spermatocyte nuclei in the first meiotic prophase. It is widely accepted that γH2AX is a novel biomarker that is used to monitor DNA repair in primary spermatocytes during meiosis. The results showed that FAM9B had a
different distribution pattern than $\gamma$H2AX. The level of $\gamma$H2AX was increased in the spermocyte nuclei during meiosis I. In contrast, the protein level of FAM9B decreased gradually. During the first meiotic division, partial co-localization of FAM9B, SYCP3, and $\gamma$H2AX in the nuclei of primary spermatocytes may be associated with DNA recombination in meiosis.

**Discussion**

Although Fam9b mRNA and protein expression results in human testes have been reported in previous studies [29], some questions remain unanswered, including the location and quantity of FAM9B expression in 8 human tissues. In the present study, FAM9B mRNA and protein levels were detected in the testes and ovaries of fetal tissue using qPCR, RT-PCR, and western blotting assays (Fig 2). Western blotting results revealed that one protein forms in the testes and ovaries, which is recognized by rabbit polyclonal FAM9B antibody. RT-PCR revealed that FAM9B is expressed in the ovarian section follicles. FAM9B plays an important role in male and female meiosis.
SYCP3 is a major component of the chromosome axes. FAM9B shares similarities with Sycep3, suggesting a similar role in meiosis. To further investigate the subcellular localization of FAM9B, chromosome spreading was performed and FAM9B was exclusively located in spermatocyte nuclei using immunohistochemistry and immunocytochemistry. Although FAM9B is generally localized in the nuclei of primary spermatocytes, it is specifically localized during leptotene and diplotene in spermatocyte nuclei. SYCP3 is a well-known SC marker in the spermatocyte chromosome spreads. FAM9B was localized in the SC region, similar to SYCP3. These results showed that FAM9B is a novel meiosis protein that may participate in SC formation similar to SYCP3.

It is widely accepted that γH2AX is phosphorylated at the DSB sites. γH2AX has been detected during multiple developmental steps in adult germ cells. During meiosis, γH2AX participates in recombination and sex chromosome inactivation in the meiotic processes. In addition, γH2AX is an essential signal for the silencing of unsynapsed sex chromosomes during male meiosis. FAM9B is a nuclear protein localized in the spermatocytes in meiosis I and is partly co-localized with γH2AX in the spermatocyte nuclei in the first meiotic prophase. FAM9B acts as a novel meiosis-associated protein and may have a role in regulation of meiotic processes. It is possible that γH2AX may recruit FAM9B in DSBs and homologous recombination during meiosis in human testes. Deletion in FAM9B may be associated with a number of clinical conditions in males, such as infertility.

In conclusion, FAM9B is abundantly expressed and localized in human testes and ovaries, suggesting that FAM9B is a novel meiosis-associated protein that may play an important role in human meiosis.

Fig 6. FAM9B and γH2AX proteins are partly co-localized in spermatocytes. The presence of γH2AX indicates unsynapsed and unrepaired DSB. FAM9B (green) and γH2AX (red) are co-localized in the nuclei of spermatocytes (red arrow). FAM9B is partly co-localized with γH2AX in the spermatocyte nucleus in the first meiotic prophase (white arrow). They have a similar distribution pattern. Scale bar = 10 μm.

https://doi.org/10.1371/journal.pone.0257248.g006
Supporting information

S1 Raw images. (PDF)
S2 Raw images. (PDF)
S3 Raw images. (PDF)
S4 Raw images. (PDF)
S5 Raw images. (PDF)

Author Contributions

Conceptualization: Xin-jie Zhuang, Ping Liu.
Data curation: Jun-sheng Li.
Formal analysis: Xue Feng, Wen-hao Tang, Xiao-ying Zheng.
Funding acquisition: Wen-hao Tang, Rong Li, Jie Qiao.
Methodology: Jin-liang Zhu, Xiao-ying Zheng.
Project administration: Jin-liang Zhu.
Resources: Xue Feng.
Software: Ming Li.
Supervision: Ming Li.
Validation: Xin-jie Zhuang.
Visualization: Jun-sheng Li.
Writing – original draft: Xin-jie Zhuang.
Writing – review & editing: Rong Li, Ping Liu, Jie Qiao.

References

1. Vertika S, Singh KK, Rajender S. Mitochondria, spermatogenesis, and male infertility—An update. Mitochondrion. 2020; 54:26–40. Epub 2020/06/14. https://doi.org/10.1016/j.mito.2020.06.003 PMID: 32534048.
2. Shi YQ, Zhuang XJ, Xu B, Hua J, Liao SY, Shi Q, et al. SYCP3-like X-linked 2 is expressed in meiotic germ cells and interacts with synaptonemal complex central element protein 2 and histone acetyltransferase TIP60. Gene. 2013; 527(1):352–9. Epub 2013/07/03. https://doi.org/10.1016/j.gene.2013.06.033 PMID: 23810942.
3. Liu JG, Yuan L, Brundell E, Bjorkroth B, Daneholt B, Hoog C. Localization of the N-terminus of SCP1 to the central element of the synaptonemal complex and evidence for direct interactions between the N-termini of SCP1 molecules organized head-to-head. Exp Cell Res. 1996; 226(1):11–9. Epub 1996/07/10. https://doi.org/10.1006/excr.1996.0197 PMID: 8660934.
4. Ollinger R, Alsheimer M, Benavente R. Mammalian protein SCP1 forms synaptonemal complex-like structures in the absence of meiotic chromosomes. Mol Biol Cell. 2005; 16(1):212–7. Epub 2004/10/22. https://doi.org/10.1091/mbc.e04-09-0771 PMID: 15496453.
5. Pashaei M, Rahimi Bidgoli MM, Zare-Abbodliah D, Najmabadi H, Haji-Seyed-Javadi R, Fatehi F, et al. The second mutation of SYCE1 gene associated with autosomal recessive nonobstructive azoospermia. J Assist Reprod Genet. 2020; 37(2):451–8. Epub 2020/01/10. https://doi.org/10.1007/s10815-019-01660-1 PMID: 31916078.

6. Hernandez-Lopez D, Geisinger A, Trovero MF, Santinaque FF, Brauer M, Folle GA, et al. Familial primary ovarian insufficiency associated with an SYCE1 point mutation: defective meiosis elucidated in humanized mice. Mol Hum Reprod. 2020; 26(7):485–97. Epub 2020/05/14. PMID: 32402064.

7. Davies OR, Maman JD, Pellegrini L. Structural analysis of the human SYCE2-TEX12 complex provides molecular insights into synaptonemal complex assembly. Open Biol. 2012; 2(7):120099. Epub 2012/08/08. https://doi.org/10.1098/rsob.120099 PMID: 22870393.

8. Bolcun-Filas E, Costa Y, Speed R, Taggart M, Benavente R, De Rooij DG, et al. SYCE2 is required for synaptonemal complex assembly, double strand break repair, and homologous recombination. J Cell Biol. 2007; 176(6):741–7. Epub 2007/03/07. https://doi.org/10.1083/jcb.200610027 PMID: 17339376.

9. Boroujeni PB, Sabbaghian M, Totonchi M, Sodeifi N, Sarkardeh H, Samadian A, et al. Expression analysis of genes encoding TEX11, TEX12, TEX14 and TEX15 in testis tissues of men with non-obstructive azoospermia. J Biomed. 2018; 22(3):185–92. Epub 2018/06/23. PMID: 29932616.

10. Dunne OM, Davies OR. A molecular model for self-assembly of the synaptonemal complex protein SYCE3. J Biol Chem. 2019; 294(23):9260–75. Epub 2019/04/27. https://doi.org/10.1074/jbc.RA119.008404 PMID: 31023827.

11. Kolas NK, Yuan L, Hoog C, Heng HH, Marcon E, Moens PB. Male mouse meiotic chromosome cores deficient in structural proteins SYCP3 and SYCP2 align by homology but fail to synapse and have possible impaired specificity of chromatid loop attachment. Cytogenet Genome Res. 2004; 105(2–4):182–8. Epub 2004/07/09. https://doi.org/10.1159/000078188 PMID: 15237206.

12. Schilt SLP, Menon S, Friedrich C, Kammin T, Wilch E, Hanscom C, et al. SYCP2 Translocation-Mediated Dysregulation and Frameshift Variants Cause Human Male Infertility. Am J Hum Genet. 2020; 106(6):3054–65. Epub 2020/04/01. https://doi.org/10.1016/j.ajhg.2019.11.013 PMID: 31860047.

13. Escobar ML, Echeverria OM, Valenzuela YM, Ortiz R, Torres-Ramírez N, Vazquez-Nin GH. Histochemical Study of the Emergence of Apoptosis and Altered SYCP3 Protein Distribution During the First Spermatogenic Wave in Wistar Rats. Anat Rec (Hoboken). 2019; 302(11):2082–92. Epub 2019/06/07. https://doi.org/10.1002/ar.24187 PMID: 31168949.

14. Roos A, von Kaisenberg CS, Eggermann T, Schwantz G, Loffler C, Weise A, et al. Analysis of SYCP3 encoding synaptonemal complex protein 3 in human aneuploidies. Arch Gynecol Obstet. 2013; 288(5):1153–8. Epub 2013/05/17. https://doi.org/10.1007/s00404-013-2861-5 PMID: 23677416.

15. Zhuang XJ, Shi YQ, Xu B, Chen L, Tang WH, Huang J, et al. SLX2 interacting with BLOS2 is differentially expressed during mouse oocyte meiotic maturation. Cell Cycle. 2014; 13(14):2231–7. Epub 2014/05/30. https://doi.org/10.4161/cc.29265 PMID: 24870619.

16. Zhuang XJ, Tang WH, Feng X, Liu CY, Zhu JL, Yan J, et al. Trim27 interacts with Slx2, is associated with meiotic processes during spermatogenesis. Cell Cycle. 2015; 14(19):2576–84. Epub 2016/10/18. https://doi.org/10.1080/15384101.2016.1174796 PMID: 27612028.

17. Bolcun-Filas E, Hall E, Speed R, Taggart M, Grey C, De Rooij DG, et al. Mutation of the mouse Syce1 gene disrupts synapsis and suggests a link between synaptonemal complex structural components and DNA repair. PLoS Genet. 2009; 5(2):e1000393. Epub 2009/02/28. https://doi.org/10.1371/journal.pgen.1000393 PMID: 19247432.

18. Maor-Sagie E, Cinnamon Y, Yaacov B, Shafi A, Goldsmith H, Zevirt S, et al. Deleterious mutation in SYCE1 is associated with non-obstructive azoospermia. J Assist Reprod Genet. 2015; 32(6):887–91. Epub 2015/04/23. https://doi.org/10.1007/s10815-015-0445-y PMID: 25899990.

19. de Vries L, Behar DM, Smirn-Yosef P, Lagovsky I, Tzur S, Basel-Vanagaite L. Exome sequencing reveals SYCE1 mutation associated with autosomal recessive primary ovarian insufficiency. J Clin Endocrinol Metab. 2014; 99(10):E2129–32. Epub 2014/07/26. PMID: 25062452.

20. Feng J, Fu S, Cao X, Wu H, Lu J, Zeng M, et al. Synaptonemal complex protein 2 (SYCP2) mediates the association of the centromere with the synaptonemal complex. Protein Cell. 2017; 8(7):538–43. Epub 2017/02/06. https://doi.org/10.1007/s13238-016-0354-6 PMID: 28150150.

21. Winkel K, Alzheimers M, Ollinger R, Benavente R. Protein SYCP2 provides a link between transverse filaments and lateral elements of mammalian synaptonemal complexes. Chromosoma. 2009; 118(2):259–67. Epub 2008/11/27. https://doi.org/10.1002/0001-6194-09 PMID: 19034475.

22. de la Fuente R, Parra MT, Viera A, Calvente A, Gomez R, Suja JA, et al. Meiotic pairing and segregation of achiasmate sex chromosomes in eutherian mammals: the role of SYCP3 protein. PLoS Genet. 2007; 3(11):e198. Epub 2007/11/07. https://doi.org/10.1371/journal.pgen.0030198 PMID: 17983272.
23. Bolor H, Mori T, Nishiyama S, Ito Y, Hosoba E, Inagaki H, et al. Mutations of the SYCP3 gene in women with recurrent pregnancy loss. Am J Hum Genet. 2009; 84(1):14–20. Epub 2008/12/27. https://doi.org/10.1016/j.ajhg.2008.12.002 PMID: 19110213.

24. Mizutani E, Suzumori N, Ozaki Y, Oseto K, Yamada-Namikawa C, Nakanishi M, et al. SYCP3 mutation may not be associated with recurrent miscarriage caused by aneuploidy. Hum Reprod. 2011; 26(5):1259–66. Epub 2011/03/02. PMID: 21357605.

25. Stouffs K, Lissens W, Tournaye H, Van Steirteghem A, Liebaers I. SYCP3 mutations are uncommon in patients with azoospermia. Fertil Steril. 2005; 84(4):1019–20. Epub 2005/10/11. https://doi.org/10.1016/j.fertnstert.2005.04.033 PMID: 16213863.

26. Blanco-Rodríguez J. gammaH2AX marks the main events of the spermatogenic process. Microsc Res Tech. 2009; 72(11):823–32. Epub 2009/05/01. https://doi.org/10.1038/sj.mr.6704370 PMID: 19499919.

27. Blanco-Rodríguez J. Programmed phosphorylation of histone H2AX precedes a phase of DNA double-strand break-independent synapsis in mouse meiosis. Reproduction. 2012; 144(6):699–712. Epub 2012/10/05. https://doi.org/10.1530/REP-12-0326 PMID: 23035256.

28. Jiang HW, Gao Q, Zheng W, Yin S, Wang L, Zhong LW, et al. MOF influences meiotic expansion of H2AX phosphorylation and spermatogenesis in mice. PloS Genetics. 2018; 14(5). ARTN e1007300 https://doi.org/10.1371/journal.pgen.1007300 PMID: 29795555.

29. Martínez-Garay I, Jablonka S, Sutajova M, Steuernagel P, Gal A, Kutsche K. A new gene family (FAM9) of low-copy repeats in Xp22.3 expressed exclusively in testis: implications for recombinations in this region. Genomics. 2002; 80(3):259–67. Epub 2002/09/06. https://doi.org/10.1006/geno.2002.6834 PMID: 12213195.

30. Zhuang XJ, Hou XJ, Liao SY, Wang XX, Cooke HJ, Zhang M, et al. SLXL1, a novel acrosomal protein, interacts with DKKL1 and is involved in fertilization in mice. PLoS One, 2011; 6(6):e20866. Epub 2011/06/24. https://doi.org/10.1371/journal.pone.0020866 PMID: 21698294.

31. Zhuang XJ, Tang WH, Liu CY, Zhu JL, Feng X, Yan J, et al. Identification and Characterization of Xlr5c as a Novel Nuclear Localization Protein in Mouse Germ Cells. PLoS One. 2015; 10(6):e0130087. Epub 2015/06/16. https://doi.org/10.1371/journal.pone.0130087 PMID: 26075718.

32. Zhuang XJ, Huang J, Li M, Wang YP, Qiu X, Zhu WW, et al. Role of tripartite motif protein 27 as a gametogenesis-related protein in human germ cells. Int J Clin Exp Pathol. 2017; 10(9):9427–35. Epub 2017/09/01. PMID: 31966815.