

# Meiotic defects and decreased expression of genes located around the chromosomal breakpoint in the testis of a patient with a novel 46,X,t(Y;1)(p11.3;p31) translocation

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**Abstract.** Balanced translocations are known to be associated with infertility, spontaneous abortions and birth defects in mammals. Spermatocyte spreading and immunostaining were applied to detect meiotic prophase I progression, homologous chromosome pairing, synapsis and recombination in an azoospermic reciprocal translocation 46,X,t(Y;1)(p11.3;p31) carrier. Histological examination of testicular sections revealed a severely reduced number of germ cells with no spermatids or sperm in the carrier. A significant reduction in XY recombination was observed in the patient. The number of MLH1 foci on autosomes that are not involved in the translocation per cell was also significantly decreased in our patient as compared to the controls, which indicates an inter-chromosomal effect (ICE) of the translocation on recombination. An increase in leptotene ( $P<0.001$ ) and zygotene ( $P<0.001$ ) and a

decrease in pachytene spermatocytes ( $P<0.001$ ) were observed in the carrier when compared with the controls, indicating disturbed meiotic progression in the patient. Increased RAD51 foci during pachytene ( $P=0.02$ ) in the spermatocytes of the patient were noted. A decreased expression of the genes (USP1, INSL5, LEPR and MSH4) critical for meiosis/spermatogenesis and located around the breakpoint region of chromosome 1 was observed in the 46,X,t(Y;1) carrier, which may further exacerbate the meiotic failure such as reduced recombination on autosomes and ultimately cause spermatogenesis arrest. In summary, we report a series of events that may have caused infertility in our 46,X,t(Y;1) carrier. To the best of our knowledge, this is the first report shedding light on how, possibly, a reciprocal translocation affects meiosis at the molecular level in azoospermia patients.

## Introduction

The frequency of Y/autosome translocations in the general population is ~1 in 2,000 (1,2). Like any other chromosome, the Y chromosome can be translocated onto an autosome or an X chromosome, either in a balanced or unbalanced way. In the most common form of Y/autosome translocation, the heterochromatic portion of Yq is translocated onto the short arm of an acrocentric chromosome (2). These translocations have been observed in phenotypically normal individuals and have also been reported in multiple families, indicating that fertility is usually not affected (2,3). In contrast, the rare translocation involved in the Y chromosome is that the euchromatic part of this chromosome is translocated onto non-acrocentric autosomal regions, which is frequently associated with azoospermia, a variety of male gamete deformities and idiopathic sterility (4-6). A few cases of reciprocal translocations

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involved in the long arm of the Y chromosome and the short arm of an autosome have been reported (7,8). Almost all of these reported studies focused on the description of translocation breakpoints (6,9), but provide little insight into the underlying relationship between the Y-autosome translocation and meiotic abnormalities (10).

Here, we present the meiotic behavior of spermatocytes in a male 46,X,t(Y;1) carrier with non-obstructive azoospermia. To shed light on possible effects of this reciprocal translocation on human male fertility, we studied synapsis and recombination between homologous chromosomes, meiotic prophase I progression and expression of genes around the breakpoint of chromosome 1 in the testis of our subject by using fluorescence immunocytogenetic approaches.

## Materials and methods

**Patient and karyotyping.** A 29-year-old male was presented to the Center for Reproductive Medicine, Department of Obstetrics and Gynecology, Anhui Provincial Hospital Affiliated to Anhui Medical University, Hefei, China. Semen analysis was conducted according to World Health Organization (WHO Laboratory Manual for the Examination of Human Semen and Semen-Cervical Interaction, 2010) and revealed that the patient was suffering from azoospermia. After obtaining written informed consent from the patient and fertile men, testicular tissues were sampled. Five fertile men, having at least one healthy child, were recruited as normal controls for this study and similar experiments were performed on them as mentioned for the patient. All the procedures of this study were approved by the Institutional Review Board and Ethics Committee of the University of Science and Technology of China.

**Spermatocyte spreading and immunofluorostaining.** Testicular tissues were processed as we described previously (11,12). Rabbit anti-SYCP3 (15093Ab; Abcam, Cambridge, UK), human anti-CREST (HCT-0100; ImmunoVision, Springdale, AR, USA), mouse anti-MLH1 (551092; BD Pharmingen Biosciences, San Diego, CA, USA), mouse anti-Brcal (sc-6954; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), mouse anti- $\gamma$ -H2AX (05-636; Millipore, Billerica, MA, USA) and rabbit anti-Rad51 (sc-8349; Santa Cruz Biotechnology, Inc.) were used as primary antibodies. The primary antibodies were detected using the following secondary antibodies: Alexa 555 donkey anti-rabbit, Alexa 488 goat anti-mouse, Alexa 488 donkey anti-mouse (all from Molecular Probes, Carlsbad, CA, USA) and 1-amino-4-methylcoumarin-3-acetic acid (AMCA) donkey anti-human (Cat. no. 709-155-098; Jackson ImmunoResearch, West Grove, PA, USA).

For the immune-detection of HIT2 and pH3, PFA-fixed testicular sections were incubated overnight at 4°C with primary antibodies: anti-HIT2 (Abcam; ab184838, 1:500) and anti-pH3 (Ser10) (sc-8656-R, 1:500; Santa Cruz Biotechnology, Inc.). After rinsing thoroughly with TBS, the primary antibodies were detected by an Alexa 555 donkey anti-rabbit (1:250; Molecular Probes) secondary antibody for 1 h at 37°C and sections were counterstained using DAPI.

An epifluorescence microscope Olympus BX61 (Olympus Inc., Tokyo, Japan) and Image Pro-Plus version 5.1

software (Media Cybernetics Inc., Bethesda, MD, USA) were used for imaging and cell evaluation.

**Real-time PCR.** A number of genes around the breakpoint on chromosome 1 and Y were selected along with genes on other chromosomes that were not involved in the translocation (as control) to compare their expression in the 46,X,t(Y;1) carrier and control. RNA isolation, RT-PCR, and real-time PCR were performed as previously described (13). All PCR primers used are listed in Table I. For real-time PCR analyses, Ct values of samples were normalized to the corresponding Ct values of glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*). Quantification of the fold-change in gene expression was determined by the comparative Ct method.

**Statistical analysis.** Statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). A Chi-square test was applied to compare RAD51 foci and meiotic progression between the patient and controls. MLH1 foci were compared between the patient and controls using the Mann-Whitney U test. The Student's t-test was used to compare the expression of studied genes between patient and control.

## Results

Semen analysis revealed that the patient was suffering from azoospermia. Karyotyping on G-banded metaphases from peripheral blood lymphocytes revealed a karyotype of 46,X,t(Y;1)(p11.3;p31) in all the 100 studied cells of the patient (Fig. 1A), which was also confirmed by immunostaining of pachytene spermatocyte spreads (Fig. 1B-D).

**Abnormal homologous chromosome pairing in pachytene spermatocytes of the 46,X,t(Y;1) carrier.** Analysis of immunostained pachytene spermatocyte spreads (n=73) revealed reciprocal translocation on p arms of Y and 1 chromosome. der(Y) was found paired with 1 and X while der(1) was associated with the X chromosome (Fig. 1B-D). It was observed that only 26% of the studied spermatocytes had normal chromosomes pairing while 74% spermatocytes contained either 1 (37%), 2 (19%), 3 (11%), 4 (4%) or 5 (3%) bivalents that showed unpaired regions (Fig. 2A and B).

**Reduced recombination between the homologous region of sex chromosomes in the 46,X,t(Y;1) carrier.** In all the 73 studied spermatocyte spreads from the patient, the association and recombination between the homologous regions of sex chromosomes were determined and compared with the control. X chromosome was found to be associated with translocated part of Y chromosome in 93.2% of the cells (Fig. 3A-D), while in 6.8% of the spermatocytes, they were not found to be associated with each other. In the patient, recombination between the X and translocated part of Y on derived chromosome 1 (represented by MLH1 focus) was observed to be present in the homologous region in 2.7% of the cells (Fig. 3E), which was significantly lower ( $P<0.001$ ) than the recombination in homologous regions on X and Y chromosomes in the control individuals (Table II), indicating that the recombination in the homologous region of XY chromosomes was impaired in the patient.

Table I. List of genes, their location on the chromosomes and the sequence of oligonucleotides used to compare the RT-PCR Ct values between the 46,X,t(Y;1) carrier and control.

| Name    | Gene location      | Direction          | Sequence (5'→3')                                |
|---------|--------------------|--------------------|---|
| USP1    | 1p31.3             | Forward<br>Reverse | GTCCCACCGTCGAGGATTAAC<br>CCAATTAGATGGGCGGGAGC   |
| LEPR    | 1p31               | Forward<br>Reverse | TCCTTCAGTGGGGCTATTGG<br>GGTGGCATGCAAGACAACCTAAA |
| INSL5   | 1p31.3             | Forward<br>Reverse | GCAAGCTGAGACAGGAAACTC<br>GCCATCAGTGCAACACAAAGT  |
| MSH4    | 1p31               | Forward<br>Reverse | GGAGGGGTTCGCTCAGAAAC<br>AGAGACCACCTGGACCGAAG    |
| NFIA    | 1p31.3-p31.2       | Forward<br>Reverse | CGACTTGAAATGTGAACGCA<br>CATCCTGGGTGAGACAGAGC    |
| TEX11   | Xq13.1             | Forward<br>Reverse | ACTTGGCTGTGCAATGTGAC<br>TGCACGACTCAGGAACATGG    |
| CDK2    | 12q13              | Forward<br>Reverse | CTGGACACGCTGCTGGAT<br>GGTTTAAGGTCTCGGTGGAGG     |
| DAZ     | Yq11.223           | Forward<br>Reverse | GTTCTACCTCCGAGGGTTCG<br>TTTGCAGCAGACATGGTGGT    |
| TSPY1   | Yp11.2             | Forward<br>Reverse | TATCGCCGACAGACACCAC<br>AACAACTGGGAGTCCCCTGA     |
| SRY     | Yp11.3             | Forward<br>Reverse | GTGGTCTCGCGATCAGAGG<br>CCTTCCGACGAGGTCGATAC     |
| RPS4Y1  | Yp11.3             | Forward<br>Reverse | TTTGATGTGGTGCATGTGAAGG<br>TGCTGCTACTGCAATTTAGCC |
| SLC25A6 | Xp22.32 and Yp11.3 | Forward<br>Reverse | CATTGCGATCCAACCATCGG<br>GGGCTAGCGCTGAAGTACAA    |

USP1, ubiquitin specific peptidase 1; LEPR, leptin receptor; INSL5, insulin like factor 5; MSH4, MutS homolog 4.

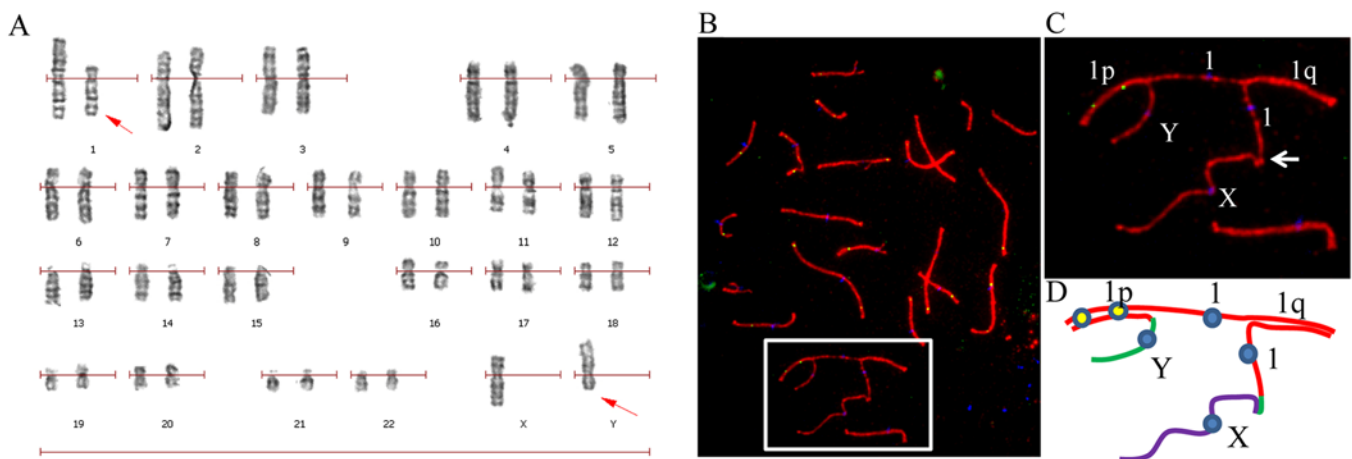


Figure 1. 46,X,t(Y;1) translocation in the patient was identified by karyotyping and confirmed by immunostaining of pachytene spermatocyte spreads. (A) Karyotyping of G-banded peripheral blood lymphocytes identified a 46,X,t(Y;1) karyotype. (B) A representative pachytene spermatocyte spread immunostained for CREST (blue), SYCP3 (red) and MLH1 (green) from the 46,X,t(Y;1) carrier confirms the chromosomal translocation. (C) Enlarged area from B, showing the translocation. (D) A schematic configuration of chromosomes involved in the translocation from the cell shown in B. Chromosomes X, Y and 1 are presented in purple, green and red lines, respectively.

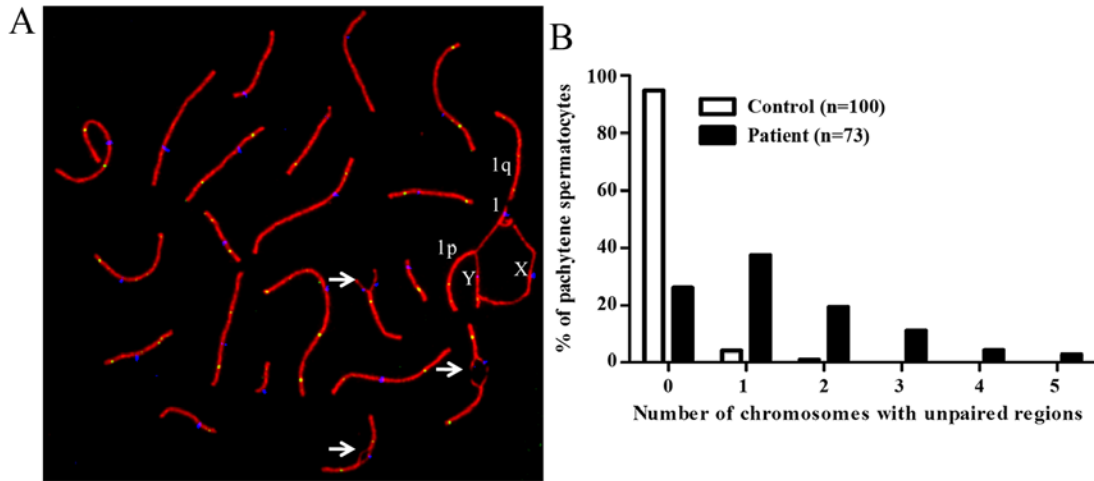


Figure 2. Abnormal pairing between homologous chromosomes in the spermatocytes of the 46,X,t(Y;1) carrier. (A) A representative pachytene spermatocyte spread immunostained for CREST (blue), SYCP3 (red) and MLH1 (green) from the 46,X,t(Y;1) carrier shows abnormal pairing of autosomes. (B) Percentage of spermatocytes with paired and unpaired autosomes in the 46,X,t(Y;1) carrier and control. Arrows indicate the unpaired regions on autosomes. n, the number of cells analyzed.

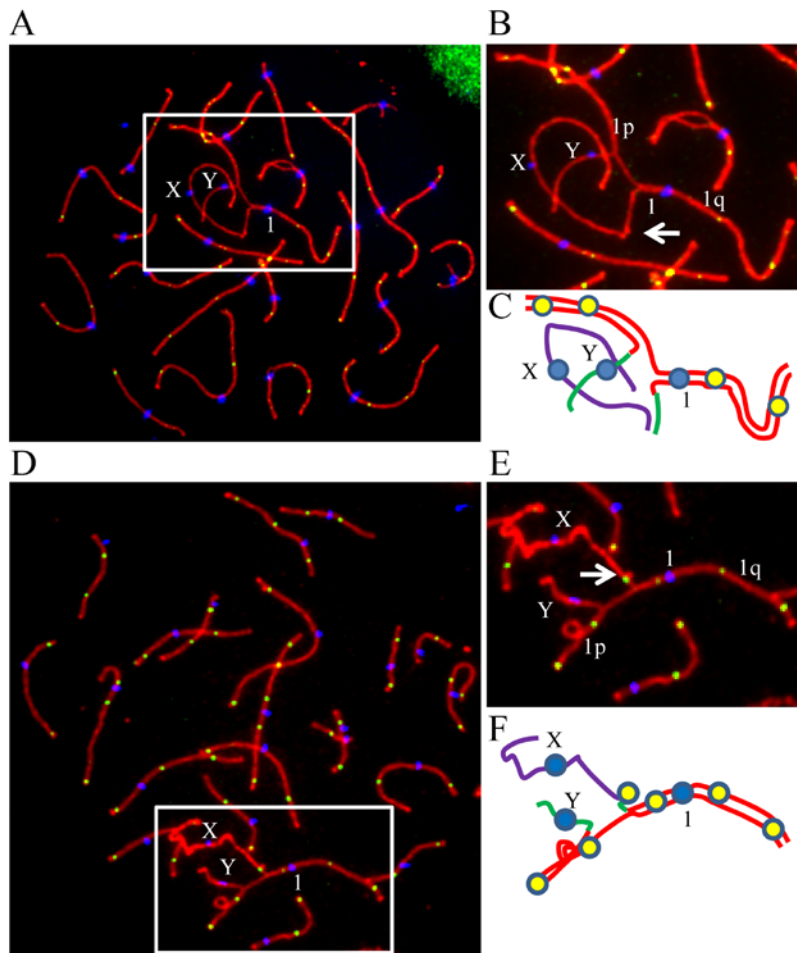


Figure 3. Representative spermatocyte spreads showing pairing and recombination between the homologous regions of sex chromosomes in the 46,X,t(Y;1) carrier. Images of a representative pachytene spermatocyte spread immunostained for CREST (blue), SYCP3 (red) and MLH1 (green). (A and B) Normal pairing but lack of recombination between XY chromosomes. (D and E) Normal pairing and recombination between XY chromosomes. (C and F) Schematic configuration of chromosomes involved in translocation from the cells shown in A and D, respectively. Chromosomes X, Y and 1 are presented in purple, green and red colors, respectively. Arrows indicate the paired regions on X and translocated chromosomes.

*Reduced meiotic recombination between autosomes and homologous region of the chromosomes involved in the trans-*

*location in the 46,X,t(Y;1) carrier. In order to determine the effect of chromosomal translocation on recombination during*

Table II. Mean MLH1 foci per cell in the controls and the 46,X,t(Y;1) carrier.

| Donor         | No. of cells analyzed (n) | Mean no. of MLH1 foci on autosomes (except chromosome 1) | SD   | Range of MLH1 foci |
|---------------|---------------------------|--|------|--------------------|
| Control       |                           |  |      |                    |
| C1            | 83                        | 46.4   | 6.8  | 30-59              |
| C2            | 93                        | 43.8   | 4.8  | 32-57              |
| C3            | 100                       | 44.0   | 5.6  | 31-55              |
| C4            | 75                        | 44.2   | 7.9  | 25-63              |
| C5            | 92                        | 44.3   | 4.6  | 33-57              |
| Mean $\pm$ SD |                           | 45.2 $\pm$ 6.3   |      |                    |
| t(Y;1)        | 73                        | 40.2 <sup>a</sup>  | 12.8 | 11-64              |

<sup>a</sup>P<0.01, Mann-Whitney U test. MLH1, MutS homolog 4.

Table III. Comparison of crossovers on the chromosomes involved in the translocation between the controls and the 46,X,t(Y;1) carrier.

| Chromosome arm | No. of MLH1 foci |       |                    |       |
|----------------|------------------|-------|--------------------|-------|
|                | Controls         |       | t(Y;1)             |       |
|                | Mean             | Range | Mean               | Range |
| 1p             | 1.607            | 0-3   | 1.082 <sup>a</sup> | 0-3   |
| 1q             | 1.885            | 0-3   | 1.274 <sup>a</sup> | 0-3   |
| Yp             | 0.849            | 0-1   | 0.027 <sup>a</sup> | 0-1   |
| Yq             | 0                | 0     | 0                  | 0     |

<sup>a</sup>P<0.001, Mann-Whitney U test. MLH1, MutS homolog 4.

meiosis, the MLH1 foci, the meiotic recombination markers, were counted in pachytene spermatocytes of the patient and the results are presented in Tables II and III. A total of 73 spermatocyte spreads were analyzed to calculate the recombination frequencies between autosomes of the 46,X,t(Y;1) translocation carrier. Interestingly, the mean number of MLH1 foci per cell on chromosomes that were not involved in the translocation was also significantly lower (P<0.001) in the patient (40.01) than in the controls (45.22) (Table II), indicating an inter-chromosomal effect (ICE). When the crossover frequencies were compared for homologous region of the chromosomes involved in translocation, a significant decrease in the number of crossovers was observed on p arm of chromosome Y (P<0.001), p arm of chromosome 1 (P<0.001) and on q arm (P<0.001) of chromosome 1 as compared to the controls (Table III).

*Delayed DNA double-strand break repair during recombination in spermatocytes of the 46,X,t(Y;1) carrier.* RAD51 forms cytologically detectable complexes involved in DNA double-strand breaks (DSBs) at sites of ongoing recombination (14,15).

We studied the kinetics of formation and disappearance of RAD51 foci on meiotic chromosomes of the 46,X,t(Y;1) carrier and control (Fig. 4A-D). The following criteria were used to classify the sub-stages of prophase I. During leptotema, the chromatin begins to condense, and proteinaceous axial elements begin to form between sister chromatids. As axial elements of homologous chromosomes align and come into contact during zygonema, a central element and transverse filaments form between homologues, completing the structure called the synaptonemal complex (SC). Homologous autosomes are fully synapsed throughout pachynema, the period during which reciprocal recombination (crossing over) occurs. During diplonema, the central element of the SC disassembles and homologous chromosomes begin to repel one another, but remain held together at chiasmata (crossing-over sites). Prophase concludes with diakinesis, during which further chromatin condensation occurs (16-19).

The mean number of RAD51 foci/cell was significantly higher at middle leptotene (P=0.001) and late leptotene (P=0.01) as well as the pachytene (P=0.02) stage in the 46,X,t(Y;1) carrier (Fig. 4E).

It has been documented that the chromosome or chromosomal regions that have not experienced synapsis undergo inactivation and are decorated by  $\gamma$ -H2AX and BRCA1 signals in spermatocytes (20,21). Upon immunostaining of pachytene spermatocytes, we also observed signals for  $\gamma$ -H2AX (Fig. 5A and C) and BRCA1 (Fig. 5B and D) on unsynapsed regions of autosomes and sex chromosomes of the 46,X,t(Y;1) carrier, indicating silencing of these chromosomal regions.

*Disturbed meiotic prophase progression in spermatocytes of the 46,X,t(Y;1) carrier.* To determine whether t(Y;1) translocation affects the meiotic prophase progression, a total of 369 randomly selected spermatocytes in different sub-stages of meiotic prophase I were studied in the patient and the results were compared with the controls (Fig. 6A-C). An increase in leptotene (P<0.001) and zygotene (P<0.001) while a decrease in the pachytene spermatocytes (P<0.001) were observed in the 46,X,t(Y;1) carrier when compared with the controls (Fig. 6D).

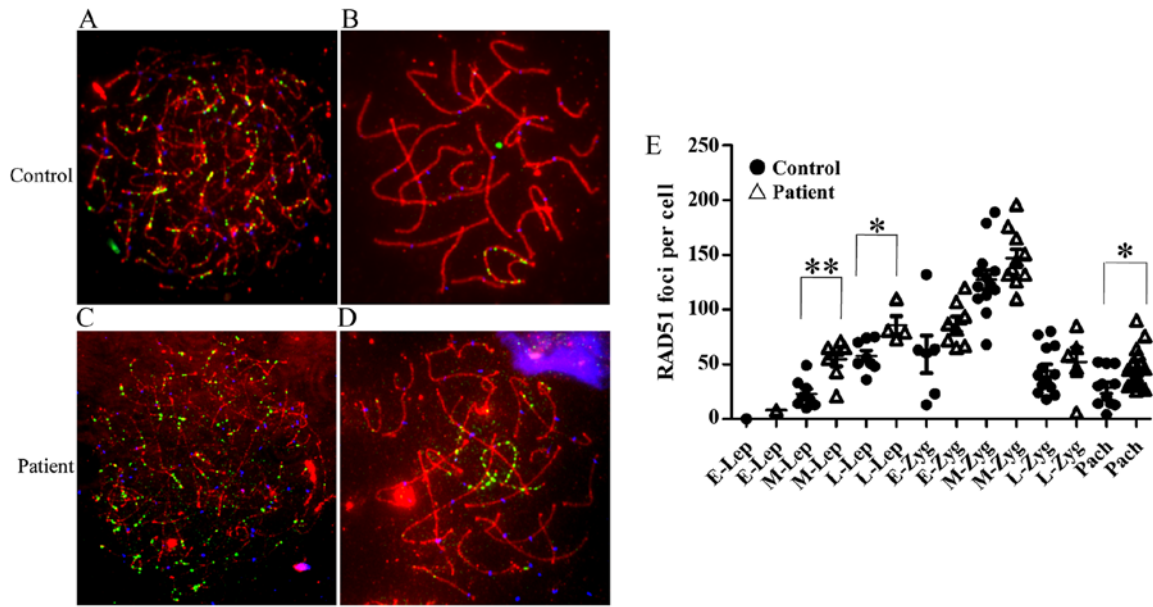


Figure 4. Representative pachytene spermatocyte spreads of the 46,X,t(Y;1) carrier and control showing RAD51 foci on meiotic chromosomes. Representative spermatocyte spreads of the 46,X,t(Y;1) carrier and control immunostained for SYCP3 (red) and RAD51 (green) during (A and C) zygotene and (B and D) pachytene stages in prophase I. (E) RAD51 foci per cell were quantified during various stages in prophase I. Each ball or triangle stands for a cell. E, M and L represents early, mid and late stage for leptotene (Lep), zygotene (Zyg) and pachytene (Pach), respectively. \*P<0.05 and \*\*P<0.01, Chi-square test.

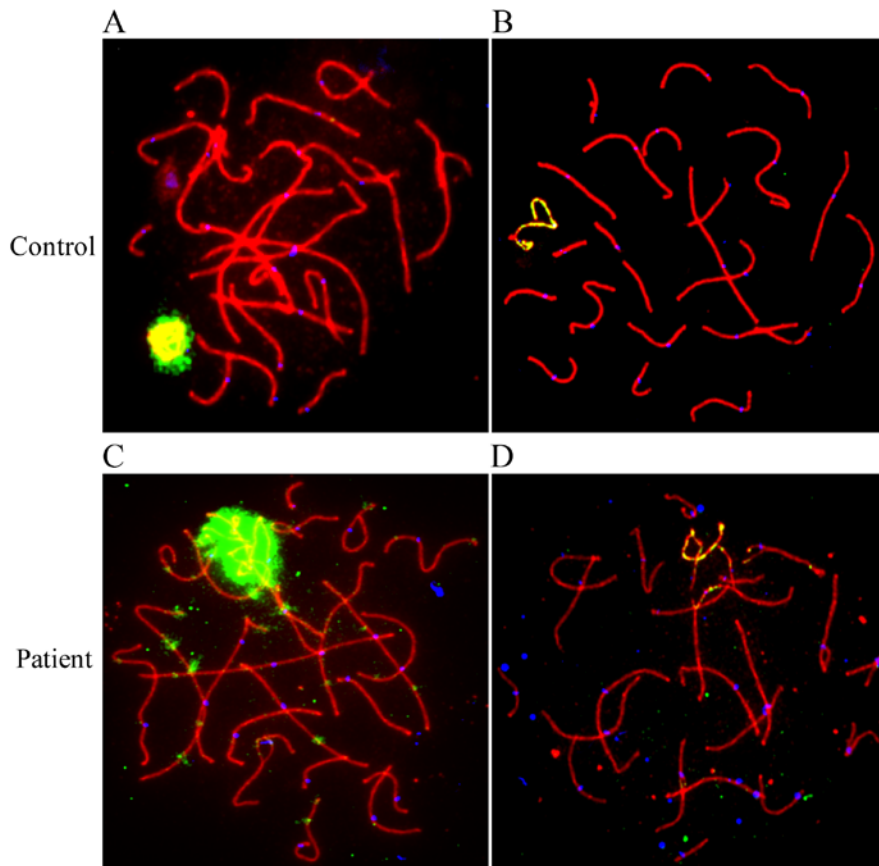


Figure 5. Unsynapsed regions of meiotic chromosomes were stained positive for  $\gamma$ -H2AX and BRCA1 in pachytene spermatocytes of the 46,X,t(Y;1) carrier and controls. Representative pachytene spermatocyte spreads immunostained for BRCA1 (green),  $\gamma$ -H2AX (green), CREST (blue) and SYCP3 (red) showing  $\gamma$ -H2AX (A and C) and BRCA1 signals (B and D) on ununsynapsed regions and axes of autosomes and sex chromosomes in the control and the 46,X,t(Y;1) carrier, respectively.

*Disturbed spermatogenesis in testicular sections of the 46,X,t(Y;1) carrier.* Histological examination of the

hematoxylin and eosin (H&E)-stained testicular sections revealed normal spermatogenesis in the testicular sections of a



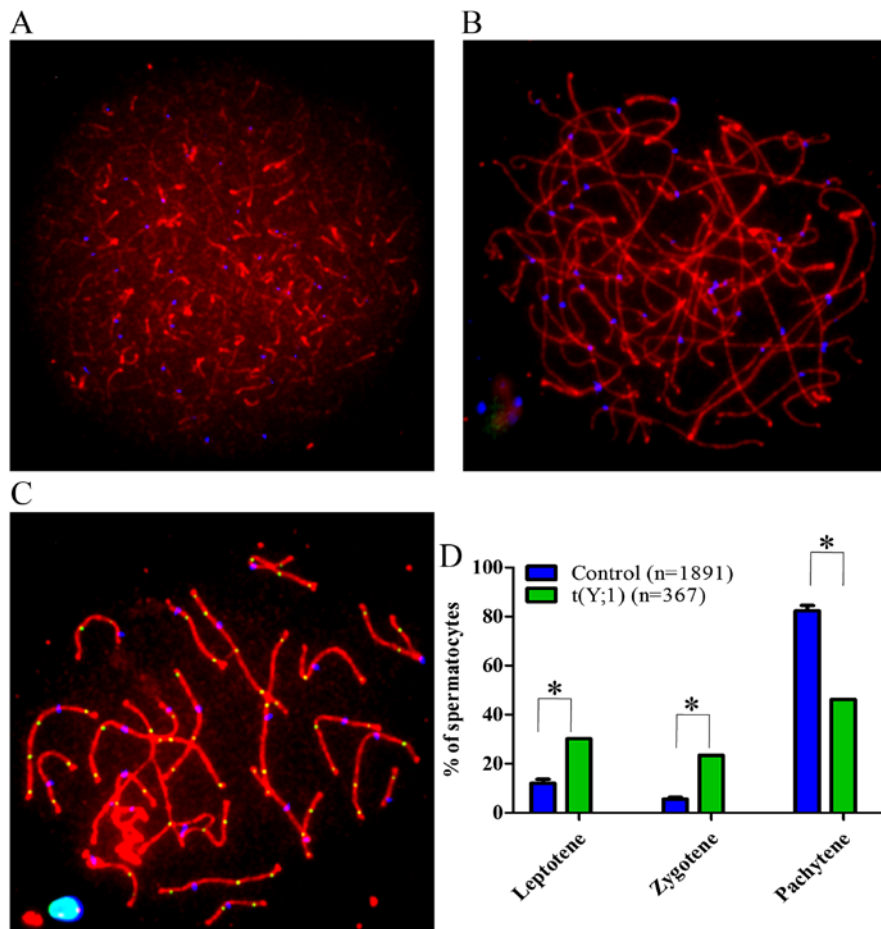


Figure 6. Meiotic prophase progression was disturbed in the 46,X,t(Y;1) carrier. Representative spermatocyte spreads immunostained for SYCP3 (red), MLH1 (green) and CREST (blue) show (A) leptotene (B) zygotene and (C) pachytene in the 46,X,t(Y;1) carrier. (D) Comparison of the cell count at various sub-stages of prophase I between the 46,X,t(Y;1) carrier and controls. n, the number of cells analyzed; \*P<0.001, Chi-square test.

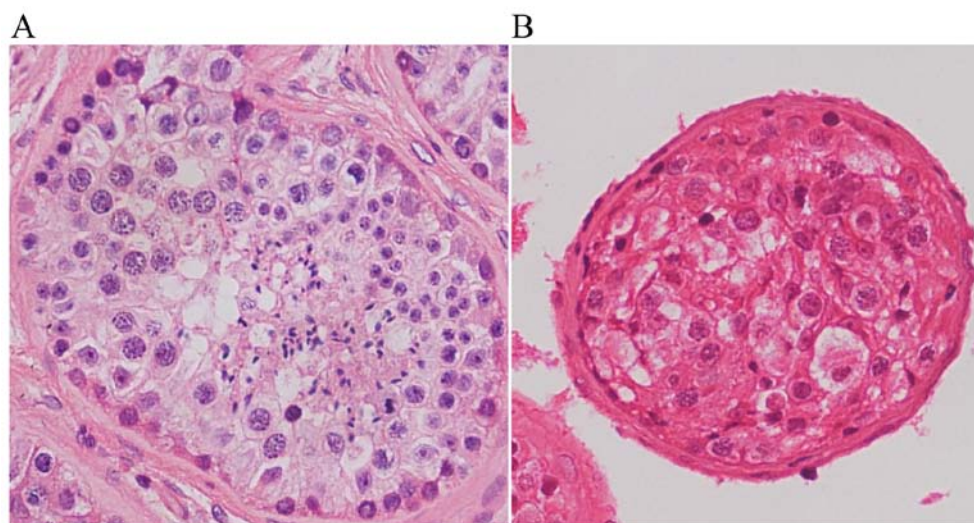


Figure 7. Abnormal testicular histology and spermatogenesis in the 46,X,t(Y;1) carrier. Testicular sections stained with hematoxylin and eosin (H&E) show (A) normal histology and spermatogenesis in control and (B) severely reduced germ cells in testis of the 46,X,t(Y;1) carrier.

control man (Fig. 7A), while reduced germ cells with no spermatids or sperm were observed in the patient (Fig. 7B). These observations were consistent with the findings in testicular sections immunostained for pH3 (phosphorylated histone H3),

a specific marker of chromosomes in cells at diplotene and/or metaphase stage, and HIT2, a marker for spermatids (Fig. 8). Numerous pH3- and HIT2-positive cells were observed in the control testis (Fig. 8A and C), however, no such cells were

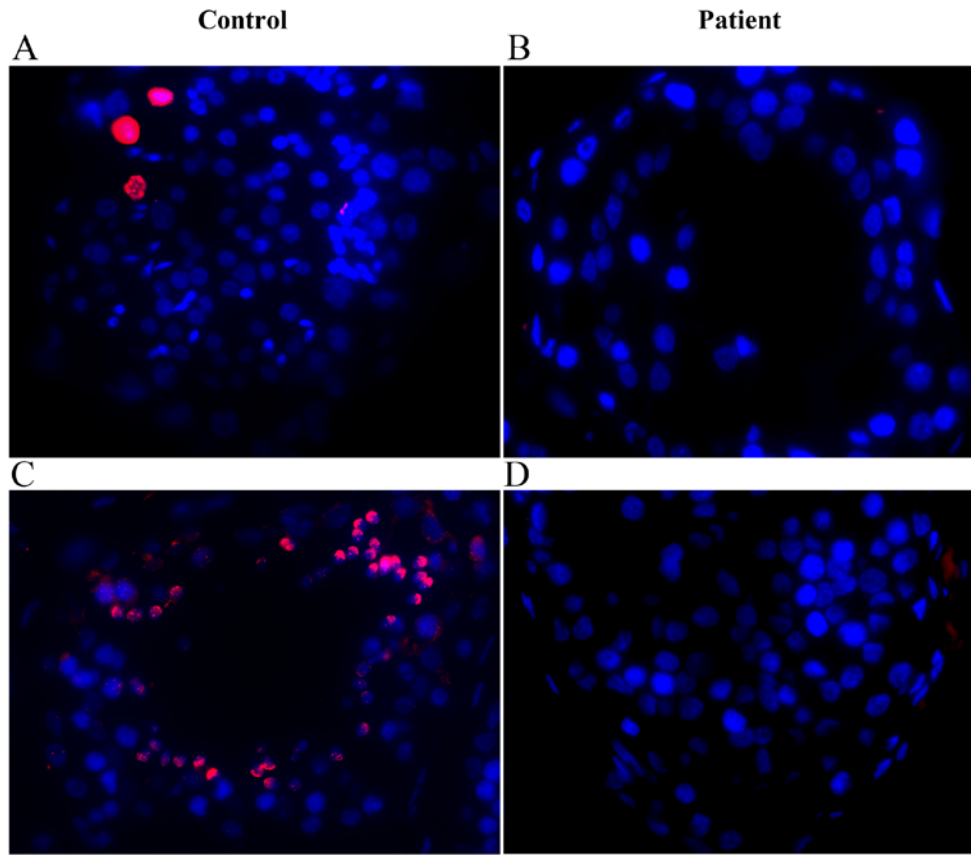


Figure 8. Spermatogenesis failed to progress beyond prophase in the 46,X,t(Y;1) carrier. Representative spermatocyte spreads immunostained for p33 (red), HIT2 (red) and DAPI (blue) show presence of metaphase cells [p33-positive, (A)] and spermatids [HIT2-positive (C)] in the control, but not in the 46,X,t(Y;1) carrier (B and D), respectively.

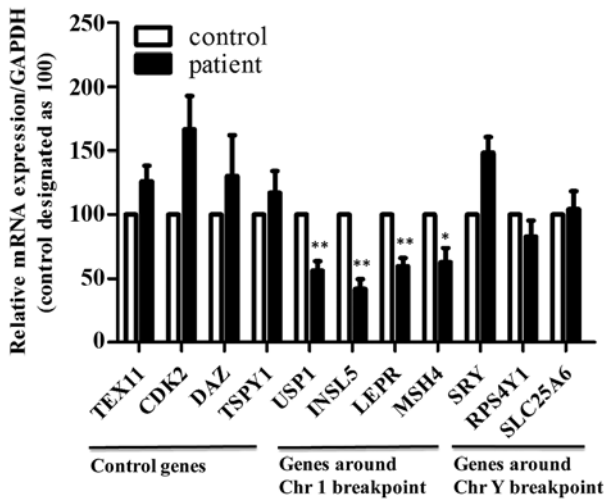


Figure 9. Comparison of relative mRNA expression of various genes located around the breakpoint on translocated (Y and 1) and untranslocated chromosomes between the control and the 46,X,t(Y;1) carrier. Relative mRNA levels were compared for selected genes around the breakpoint on chromosome Yp11.3 and 1p31 along with some genes on chromosomes that were not involved in translocation between the control and the 46,X,t(Y;1) carrier by real-time PCR. GAPDH was used as internal control. Data are presented as mean ± SEM. \*P<0.05 and \*\*P<0.01, Student's t-test.

observed in the translocation carrier's testis (Fig. 8B and D), indicating spermatogenesis did not progress beyond the pachytene stage in the patient.

*Downregulation of genes present around the breakpoint on chromosome 1 in the 46,X,t(Y;1) carrier.* CT for various studied genes critical for meiosis/spermatogenesis and located around the breakpoint of chromosome 1 and Y, and on the chromosomes that are not involved in the translocation were compared between the patient and control, to determine the effect of chromosomal translocation on gene expression in the testes, if any. Our results revealed decreased expression of ubiquitin specific peptidase 1 (USP1), insulin like factor 5 (INSL5), leptin receptor (LEPR) and MutS homolog 4 (MSH4) present around the breakpoint of chromosome 1, and no change for those on Y chromosome and other chromosomes in the testis of the 46,X,t(Y;1) carrier as compared to the control (Fig. 9). These results indicate that downregulation of these genes may have affected the spermatogenesis in this patient.

**Discussion**

Balanced reciprocal translocations are the structural abnormalities involving mostly two autosomes while a few involve a gonosome (X or Y chromosome) and an autosome (22). These rearrangements are usually associated with infertility and/or a higher risk of chromosomal imbalances among offspring (23). The relationship between Y-autosome translocation and male sterility has been reported but the mechanism of the sterilizing effect remains elusive (1,10,23). This is the first report of immunofluorescence meiotic analysis on a reciprocal translocation t(Y;1)(p11.3;p31) carrier with an azoospermic phenotype.



Homologous chromosome pairing, synapsis and recombination are critical to meiosis, and failure in either of these processes has been associated with meiotic arrest and infertility (24). Meiotic studies on infertile male carriers of reciprocal translocations have shown that the regions surrounding the breakpoints often fail to completely pair and synapse (24,25). Furthermore, these asynapsed regions have been found to be associated with the sex chromosomes (11,26). In the present study, we also observed abnormal chromosomal pairing in most of the studied spermatocytes in our patient (Fig. 2B). As expected, the recombination between homologous regions of the chromosomes involved in the translocation was significantly decreased in our patient (Fig. 3 and Table III). Interestingly, the mean number of MLH1 foci per cell on chromosomes that were not involved in the translocation were also significantly lower ( $P < 0.001$ ) in the patient (40.01) than in the controls (45.22) (Table II), highlighting an ICE.

RAD51 forms cytologically detectable complexes involved in DSBs at sites of ongoing recombination (14,15). In our translocation carrier, we observed increased RAD51 foci during the pachytene as compared to controls (Fig. 4), which may indicate an inefficient DSB repair mechanism in the patient (Fig. 5).

During male meiosis, the X and Y chromosomes are transcriptionally silenced, forming a condensed sex body. Meiotic sex chromosome inactivation (MSCI) is characterized by the localization of phosphorylated histone H2AX on the sex chromosomes, which is thought to be critical for chromatin condensation and transcriptional inactivation (27). BRCA1 is also critical for MSCI, recruiting ataxia telangiectasia (ATR and RAD3 related) to phosphorylate H2AX (28). Recent studies, however, have suggested that the phenomenon of meiotic inactivation is not limited to the sex chromosomes; unsynapsed autosomal chromosomes have been shown to undergo a similar transcriptional silencing in germ cells of mice (21,29). Our results complement the above studies as upon immunostaining of pachytene spermatocytes, we also observed signals for  $\gamma$ -H2AX (Fig. 5C) and BRCA1 (Fig. 5D) on unsynapsed regions of autosomes and sex chromosomes in spermatocytes of the 46,X,t(Y;1) carrier.

The local consequences of asynapsis may include persistence of DNA DSBs (30), silencing of genes in the unsynapsed chromosomal segment (21) and intrusion of the rearranged autosomes in the sex body (31). The ultimate physiological consequence of asynapsis in the male is subfertility or sterility due to partial or complete meiotic arrest at the first meiotic prophase (32), even though in many instances, as in multiple heterozygotes for Robertsonian translocation, pachytene block can be leaky or missing and spermatogenesis may fail at metaphase I of meiosis (33-35). Here, we studied spermatocytes in different sub-stages of meiotic prophase I from our patient and observed an increased proportion of leptotene ( $P < 0.001$ ) and zygotene ( $P < 0.001$ ) and decreased proportion of pachytene spermatocytes ( $P < 0.001$ ) as compared to the controls, indicating that the meiotic progression in our translocation carrier was disturbed (Fig. 6). After immunostaining of the spermatocytes with anti-pH3, a specific marker for chromatin of diplotene and metaphase cells (36,37), we observed the absence of pH3-positive cells in the testis of the 46,X,t(Y;1) carrier, which indicate that meiosis failed to progress beyond the pachytene stage in our patient (Fig. 8A and B). These find-

ings are also consistent with the studies that translocation can activate the pachytene checkpoint that is responsible for the delay/arrest of pachytene progression when either the process required for crossover is defective or when there are defects in the structure of the meiotic chromosome axis (38). Obviously, the chromosomal rearrangements in our patient may have also activated the pachytene checkpoint in spermatocytes as the meiosis did not progress beyond the pachytene stage (Figs. 7 and 8).

It has been reported that balanced chromosomal rearrangements are associated with aberrant gene expression, which have many underlying causes, e.g. direct disruption of genes or their regulatory elements by chromosomal rearrangement at breakpoints, or silencing of genes due to failure in synapsis between homologous chromosomes around breakpoint (39,40). To test these, we detected the expression of a series of genes that are required for spermatogenesis and located around the breakpoints. USP1 is located at 1p31.3 and responsible for deubiquitination of FANCD2, an important member of the Fanconi anemia (FA) pathway that regulates the repair of DNA crosslinks (41). Targeted deletion of mouse *Usp1* results in male infertility by interfering with homologous recombination repair (42). INSL5 is a member of the insulin superfamily and is located at 1p31.3. It has been reported that *Ins15<sup>-/-</sup>* male mice display impaired fertility due to marked reduction in sperm motility (43). LEPR belongs to the cytokine receptor class I superfamily located at 1p31 (44). De Luca *et al* reported that homozygous LEPRB mutant mice are sterile (45). MSH4, another important gene present in the breakpoint region on chromosome 1 (1p31), is a member of the mammalian mismatch repair gene family and is responsible for post replicative DNA mismatch repair as well as the control of meiotic recombination (46,47). Disruption of the MSH4 gene in mice results in male and female sterility due to meiotic synapsis and recombination failure (48). Significant decreases in the expression of all these genes were observed in the testis of our 46,X,t(Y;1) patient as compared to the control (Fig. 9), which indicates that failure in synapsis of the homologous region around the breakpoint of chromosome 1 is responsible for the decreased expression of these genes. These findings are complementary to our results as abnormal pairing, synapsis, recombination and delayed DSB repair were observed, and confirmed by the presence of  $\gamma$ -H2AX (Fig. 5C) and BRCA1 (Fig. 5D) signals on unsynapsed regions of chromosome 1 and sex chromosomes in spermatocytes of the 46,X,t(Y;1) carrier.

We thus propose that in our patient, the translocation results in the failure in pairing and synapsis of homologous regions around breakpoints and consequently cause inefficient DSB repair, and silencing of the genes in this region. Some of the silenced genes, such as MSH4, are required for meiotic synapsis, recombination and spermatogenesis; their decreased expression will further exacerbate meiotic and spermatogenic aberration in translocation carriers, which is also consistent with our finding that our patient showed ICE in recombination as MSH4 was downregulated.

In conclusion, we report a potential mechanism that caused azoospermia in our 46,X,t(Y;1) carrier. We observed that the studied chromosomal translocation disturbed the process of meiotic pairing, synapsis and recombination, which resulted in the silencing of genes located in the unsynapsed chromosomal

regions. As some of the silenced genes are required for meiosis and spermatogenesis, their reduced expression could further exacerbate meiotic and spermatogenesis failure, and ultimately cause meiotic arrest and azoospermia in translocation carriers.

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