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Case Reports

# Molecular and Cytogenetic Characterization of Two Azoospermic Patients with X-Autosome Translocation

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***Purpose*:** To report two azoospermic patients with reciprocal X–autosome translocations.
***Methods*:** Cytogenetic analysis utilizing GTG-banding and Yq microdeletions shown by polymerase chain reaction (PCR) with 12 sequence-tagged site (STS) markers for Y chromosome microdeletions.
***Results*:** Cytogenetic analysis showed one man with 46,Y,t(X;19)(q22;q13.3) and the other with 46,Y,t(X;8)(p22;q11). Neither had any Yq microdeletions shown. The patient with 46,Y, t(X;8)(p22;q11) showed a slightly lower than normal testosterone level. By NCBI-Blast search, we found four testis-specific genes, t-complex-associated-testis-expressed 1-like (*TCTE1L*), Ferritin, heavy polypeptide-like 17 (*FTHL17*), Testis expressed sequence 13A (*TEX13A*), and Testis expressed sequence 13B (*TEX13B*) located near breakpoints on X chromosome. *FTHL17*, *TEX13A*, and *TEX13B* are spermatogonially-expressed, germ-cell-specific genes.
***Conclusion*:** This is the first clinical report of azoospermia with reciprocal X–autosome translocations on Xp22 and q22. These translocations on Xp22 and q22 may be direct genetic risk factors for azoospermia.

**KEY WORDS:** Azoospermia; infertility; spermatogonia; translocation.

## INTRODUCTION

Reciprocal translocations between a sex chromosome and an autosome have been reported sporadically for the surveys of infertile men (1,2,3,4). In mouse, spermatogenic arrest at the pachytene stage characterizes all such rearrangement (5,6). Madan included, after studying nine male carriers of X-autosome (X;A) translocations, that, regardless of the position of the breakpoint in the X, these men are likely to suffer from a disturbance in spermatogenesis leading to severe subfertility or infertility (7).

In unexplained oligozoospermia and azoospermia males, genetic abnormalities were identified, including chromosomal abnormalities, deletions of the azoospermia factor region (AZF) of the Y chromosome (8,9) and mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene, commonly associated with congenital vas deferens abnormalities (10).

We now report two reciprocal translocation cases with infertility, one a t(X;19) and the other a t(X;8).

## MATERIALS AND METHODS

### Patients

To diagnose the cause of their infertility, the patients underwent routine checkups, including semen, cytogenetic, and Yq microdeletion analyses. Informed consent was obtained from both patients. Patient L was 32 years old and had been married for 2 years;

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Patient C was 37 years old and had been married for 9 years. Semen analysis was performed twice according to the World Health Organization (WHO 1999) guidelines. Serum LH, FSH, PRL, TSH, and T were measured using commercial radioimmunoassay (RIA) kits (DPC, Diagnostic Products Corporation; LA, USA).

### Cytogenetic and Yq Deletion Analysis

Peripheral blood samples were cultured for 72 h in RPMI-1640 medium. Metaphase chromosomes were analyzed by GTG-banding and high resolution staining as previously reported (11). Familial cytogenetic investigations have not yet been possible.

Genomic DNA was extracted according to standard procedure from peripheral blood lymphocytes. The Y-chromosome sex-determining region (SRY) was used as a control. Twelve additional sequence-tagged sites (STS) for Y chromosome AZF regions and other loci were amplified using primers of sY 84, sY 86 (located in AZFa), sY 134, sY 138, MK5 (located in AZFb), sY 152, sY 147, sY 254, sY 255, sPGY1, sY 269, sY 158 (located in AZFc) of the long arm (Yq) of the Y chromosome. Multiplex PCR was carried out using two to five primer sets. PCR was carried out in a total volume of 50$\mu$L containing 100 ng extracted genomic DNA, 200 $\mu$M dNTP, 10 mM Tris-Cl pH 8.3, 1.5 mM $MgCl_2$, and 50 mM KCl. Two units of *Taq* DNA polymerase was added under hot-start conditions, and amplification was carried out for 30 cycles of 94°C for 30s, 58°C for 30s, 72°C for 30s, and a final extension for 7 min at 72°C. The reaction products were analyzed on 2% agarose gel and visualized with ethidium bromide staining.

### Gene-Specific PCR Analysis

Primers were picked from 8 gene sequences using Primer 3 program, and their sequences are described in Table I.

PCR was carried out in a total volume of 50 $\mu$L containing 100 ng extracted genomic DNA, and amplification was carried out for 30 cycles of 94°C for 30s, 56°C for 30s, 72°C for 30s, and a final extension for 7 min at 72°C. The reaction products were analyzed on 2% agarose gel and visualized with ethidium bromide staining.

## RESULTS

Cytogenetic analysis showed two azoospermic patients with reciprocal X–autosome translocations. Their karyograms (Fig. 1) show the patient L to be 46,Y,t(X;19)(q22;q13.3) and patient C to be 46,Y,t(X;8)(p22;q11).

**Table I.** The Primer Lists of Genes in p21, p22, and q22 of X Chromosome

| Gene Symbols | | Sequences |
|---|---|---|
| *TCTE1L* | Forward | TTAAGGATTGTTCCACTATGC |
| | Reverse | ATTAACTGCCCTCTGTGC |
| *FTHL17* | Forward | ATCTTGCGCAGGTTGCTCAC |
| | Reverse | AGCCTGCTGGATCTGTACCA |
| *CAGE* | Forward | CCATCTCTTTTGGTGCAGAA |
| | Reverse | GGATAGTGGGAGTATCGGCA |
| *NXF2* | Forward | ATGAGGGATGTCCACAAGGA |
| | Reverse | TTCGTCTTCACTATGCCATT |
| *NGFRAP1* | Forward | ATGGCAAATATTCACCAGGAA |
| | Reverse | GCAGACAATTCCTCAACTGC |
| *TAF2Q* | Forward | TGGAGCAGCCTATGCAGAAT |
| | Reverse | CATATCATCTCCATCTCCAC |
| *TEX13A* | Forward | CTGATGAGGTTCGGAGTATC |
| | Reverse | TCTCAGACATACCCACTAT |
| *TEX13B* | Forward | AGTTGAAAGGCATCTGAGTT |
| | Reverse | CTATTGTAGCAGGCAAATTAG |

Table II shows hormone assay results for each patient. Also per profiles for both patients, patient C had about two-fold increased level of prolactin and slightly lower serum testosterone concentration (2.19 ng/mL) as compared patient L with increased LH level.

Analysis of the AZF a, b, and c regions showed no evidence of deletions on Yq. The 12 STS used are located in the long arm of the Y chromosome, spanning from AZFa to AZFc (intervals 5C–6F). The SRY and the 12 STSs were amplified by Multiplex PCR (Fig. 2). The results show that these two patients have intact Y-chromosomes, so their translocations may be directly related to their azoospermia.

To screen any genomic deletion near breakpoints, we did PCR analysis from genomic DNA of one normal and both patients. We screened 8 genes listed on Table III. We did not find any deletion, which showed that there is at least no entire deletion of gene near breakpoints (Fig. 3).

## DISCUSSION

Cytogenetic anomalies and Yq chromosomal deletions have close associations with male infertility. Karyotypic abnormalities have been reported in up to 13% of men with azoospermia, and Yq chromosomal deletions in up to 20% of men with infertility (7,10). Microdeletions in the Y chromosome thus represent one important cause of nonobstructive infertility in

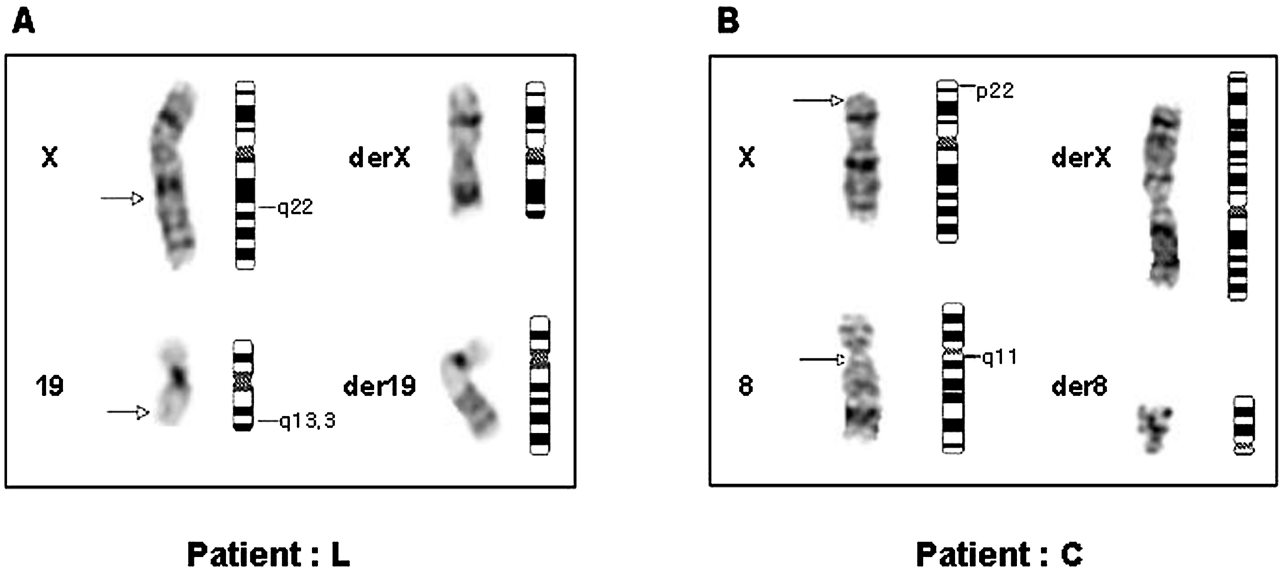


**Fig. 1.** Partial karyotypes of translocations. An ideogram at high resolution is at the right of each chromosome pair. (A) Patient L, 46,Y,t(X;19)(q22;q13.3); (B) Patient C, 46,Y,t(X;8)(p22;q11).

azoospermic and severely oligozoospermic men. The average frequency of Yq microdeletions is estimated to be 10% among such men in our hospital (unpublished observation).

Quack *et al.* (6) reported two infertile men with reciprocal X–autosome translocations, t(X;12) and t(X;2). Each had inherited the translocation from his mother. In general, human reciprocal X–autosome translocations with a breakpoint in the short arm of the X chromosome or without involving the critical region q13 → q26 in the long arm are not associated with female infertility, although some oocytes losses may be inferred from comparisons with the mouse (7).

In our study, 12 STS on Yq along with SRY were analyzed from two azoospermic patients with X–autosome translocations. The patients showed no evidence of microdeletions on Yq, so the translocation may be a direct genetic cause of their infertility. Unfortunately, familial cytogenetic investigations have not yet been possible.

Basrur *et al.* (17) showed that testicular activity and semen characteristics of bulls carrying an X-autosome translocation could have contributed to the sperm head malformation and oligozoospermia in X-autosome translocation (Xq-AT) carrier bulls which have the Pseudo-autosomal region (PAR) at the distal end of Xq. PAR is at the distal ends of Xp on

**Table II.** X-Autosome Translocations Associated with Male Infertility

| | | | | | Hormone levels | | | |
|---|---|---|---|---|---|---|---|---|
| Reference | Rearrangement | Seminal analysis | Meiotic investigation | Microdeletion | FSH | LH | Prolactin | Testosterone |
| (12) | $Xp^-$ , $14q^+$ | Azoospermia | Atrophic testes | Not done | | | | |
| (13) | t(X;5)(q28;q29) | Azoospermia | Testis biopsy refused | Not done | | | | |
| (14) | t(X;22)(q25;qter) | Azoospermia | Not done | Not done | | | | |
| (15) | t(X;15)(p11;p1) | Severe oligo-zoospermia | Not done | Not done | | | | |
| (15) | t(X;22)(q26;q11) | Azoospermia | Not done | Not done | | | | |
| (16) | t(X;21)(q23;q11) | Azoospermia | Pachytene arrest | Not done | | | | |
| (6) | t(X;12)(p22;q15) | Azoospermia | Some cells reaching MI | Not done | | | | |
| (6) | t(X;2)(q27;p1229) | Azoospermia | Some MIs and rare MIIs | Not done | 3.5 | | | |
| *Patient C* | *t(X;8)(p22;q11)* | *Azoospermia* | *Not done* | *No deletion* | 3.3 | < 1.0 | 33.3 | 2.19 |
| *Patient L* | *t(X;19)(q22;q13.3)* | *Azoospermia* | *Not done* | *No deletion* | 8.4 | 58 | 12.7 | 3.70 |

*Note*. Hormone levels of normal males: FSH; 1.1∼13.5 mIU/mL, LH; increased in case of Klinefelter syndrome, prolactin; 1.75∼16.5 ng/mL, testosterone; 2.36∼9.96 ng/mL.

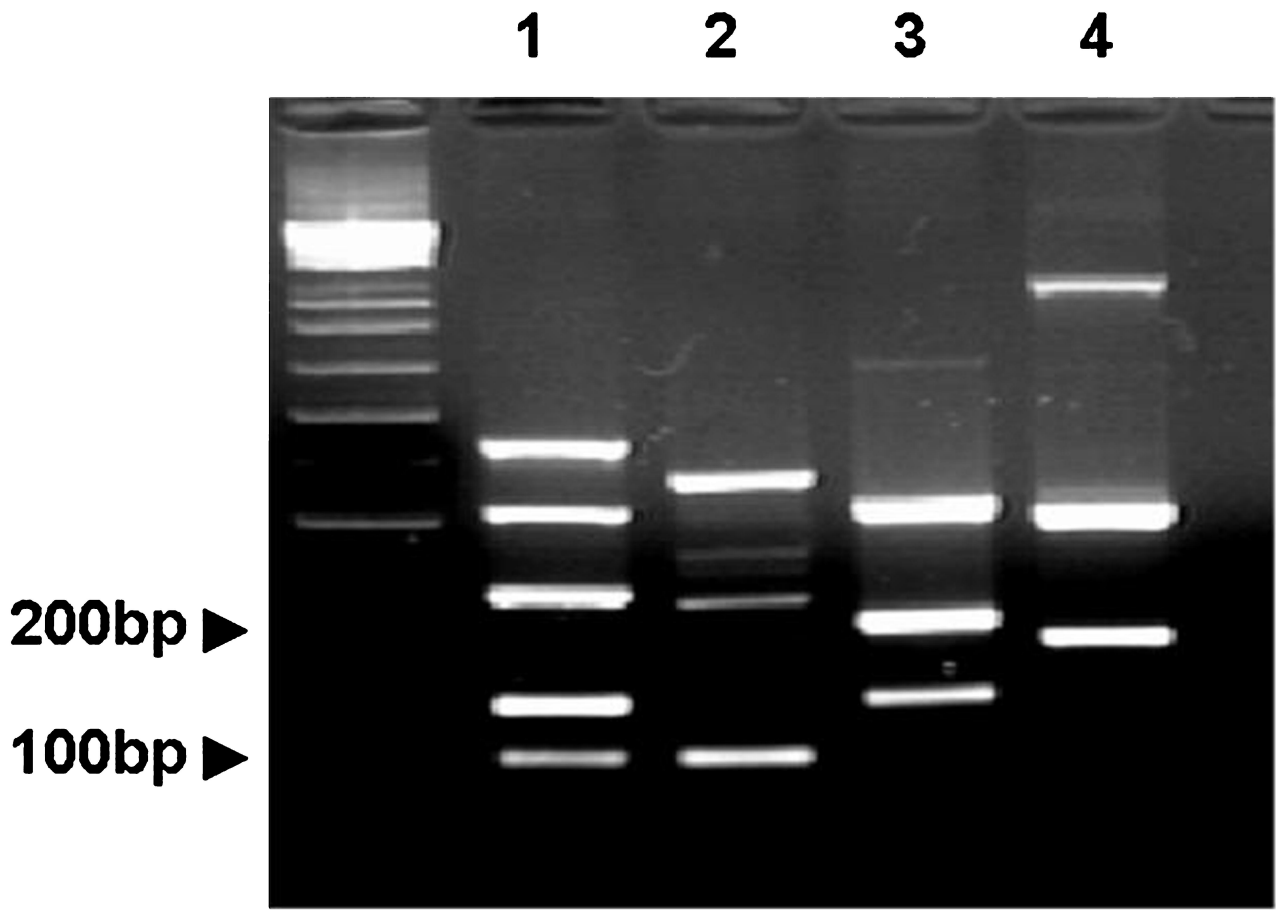


**Fig. 2.** STS multiplex PCR of patient L. Lane 1; SPGY1 (460bp), sY 86 (318bp), sY 158 (215bp), sY 152 (125bp), sY 147 (100bp), lane 2; sY 254 (401bp), SRY (203bp), sY 269 (94bp), lane 3; sY 84 (326bp), MK5 (182bp), sY 255 (123bp), lane 4: sY 134 (301bp), sY 138 (164bp). The result of patient C was same (data not shown).

human, and so the reciprocal translocation between Xp22 and autosome of Patient C may be the other factor for azoospermia through the complex meiotic pairing.

Wang *et al.* (18) isolated 25 novel germ-cell-specific genes from mouse spermatogonia, which showed 3 are Y-linked and 10 are X-linked. Their 10 X-linked orthologs are also located in human X chromosome,

**Table III.** The Gene Lists in p21, p22, and q22 of X Chromosome by NCBI-Blast Search

| Gene Symbol | Description | Position |
|---|---|---|
| *TCTE1L* | t-complex-associated-testis-expressed 1-like | Xp21 |
| *FTHL17* | Ferritin, heavy polypeptide-like 17 | Xp21 |
| *CAGE* | Cancer-associated gene | Xp22.13 |
| *NXF2* | Nuclear RNA export factor 2 | Xq22 |
| *NGFRAP1* | Nerve growth factor receptor (TNFRSF16) associated protein 1 | Xq22.1 |
| *TAF2Q* | TBP-associated factor II Q | Xq22.1 |
| *TEX13A* | Testis expressed sequence 13A | Xq22.2 |
| *TEX13B* | Testis expressed sequence 13B | Xq22.3 |

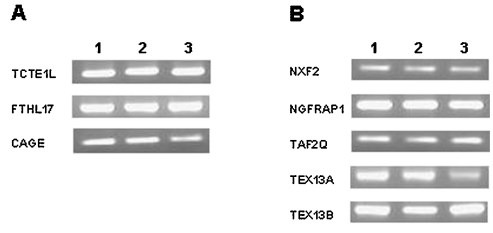


**Fig. 3.** PCR analysis of genomic DNA from azoospermic patients and normal control. Lane 1; patient C, 46,Y,t(X;8)(p22;q11), lane 2; patient L, 46,Y,t(X;19)(q22;q13.3), lane 3; control male. (A) Genes located on Xp21 and p22; (B) Genes on Xq22.

which suggested X-linked genes are most strongly implicated in male germ-cell development (18). We surveyed the genes located at the breakpoint of X chromosome (Table III) by NCBI-Blast search. We found total 8 genes, three (*TCTE1L*, *FTHL17*, *CAGE*) on Xp21–22 and five (*NXF2*, *NGFRAP1*, *TAF2Q*, *TEX13A*, *TEX13B*) on Xq22. Four genes, *TCTE1L* (t-complex-associated-testis-expressed 1-like), *FTHL17* (Ferritin, heavy polypeptide-like 17), *TEX13A* (Testis expressed sequence 13A), and *TEX13B* (Testis expressed sequence 13B) are testis-specific, where *TCTE1L* and *FTHL17* genes are located on Xp21, and *TEX13A* and *13B* genes on Xq22. Specially, *FTHL17*, *TEX13A*, and *TEX 13B* genes are spermatogonially expressed, germ-cell-specific genes in mouse (18). The alteration of these human spermatogonially specific genes may cause human germ-cell development because these orthologs are present in human. In this study, we did not show the direct evidence of biological implication of these X-autosome translocations. Further studies with knock-out of these four genes may be interesting for animal model of azoospermia.

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