Complex chromosomal rearrangements in infertile males: complexity of rearrangement affects spermatogenesis

In this report, we describe 10 male cases of complex chromosome rearrangements (CCRs) with fertility problems: seven of them showed impairment of spermatogenesis, oligoasthenoteratozoospermia or azoospermia; in the other three cases, recurrent abortions were observed. The CCRs were characterized by conventional fluorescence in situ hybridization (FISH) and multicolor FISH methods as well as by the routine G-banding technique. CCRs found in three cases with recurrent abortions were double two-way exchanges, which were the simplest forms of CCRs; three oligoasthenoteratozoospermic cases were double two-way exchanges or three-way exchanges. However, the CCRs in four azoospermic cases were much more complicated forms of CCRs. From our results and a review of the literature, we conclude that the complexity of CCRs might affect the severity of spermatogenetic impairment rather than the number of chromosomes involved or the location of breakpoints. (Fertil Steril® 2011;95:349–52. ©2011 by American Society for Reproductive Medicine.)

Key Words: Complex chromosome rearrangement, infertility, male, spermatogenesis, M-FISH
CONVENTIONAL CYTOGENETIC ANALYSES

Conventional cytogenetic studies were performed on lymphocytes from phytohemagglutinin-stimulated peripheral blood cultures. GTG-banded chromosomes were obtained according to a standard protocol, and 20 metaphases were examined and karyotyped using the CytoVision System version 3.6 (Applied Imaging, Thunderland, UK). Chromosomal abnormalities were described according to the International System for Human Cytogenetic Nomenclature 2009.

FISH

Chromosomal abnormalities identified by conventional chromosome analysis were initially reevaluated by conventional FISH using Vysis whole chromosome painting probes (Abbott Molecular Inc. Chicago, IL). Two patients were evaluated by M-FISH and FISH using Vysis locus-specific identifier probes. M-FISH was performed using the 24 XCyte multicolor probe kit (Metasystems, Altussheim, Germany). All FISH procedures were performed.
ANALYSIS OF YQ MICRODELETION

Patients were also evaluated for microdeletions of azoospermic factor (AZF) regions on the long arm of the Y chromosome. Genomic DNAs were extracted from peripheral blood using the QIAamp DNA blood Midi kit (Qiagen, Hilden, Germany). The DAZ gene family (DAZ1, 2, 3, and 4) and sequences-tagged sites in the AZF regions (AZFb, AZFc: sY117, sY127, sY143, sY134, sY138, sY152, sY153, sY147, sY149, sY269, sY157, and sY158) were analyzed by polymerase chain reaction (PCR), performed as described elsewhere (10, 11). The PCR products were electrophoresed on 2% agarose gels and visualized under ultraviolet light.

HISTOLOGICAL EXAMINATION

The testicular biopsies were performed by a urologist, after approval by patients, to search for male gametes. Paraffin-embedded specimens were sectioned and stained with a standard hematoxylin and eosin staining method. The specimens underwent meticulous histological evaluation as well as a thorough examination to collect male gametes of any stage. Photomicrographs were taken using a digital microscope (Coolscope, Nikon, Tokyo, Japan).

Hormone profiles and semen analyses of the seven males with spermatogenetic defects are shown in Supporting Table 1. The level of testosterone was detected to be slightly below the normal range (2.45–18.36 ng/mL) in only one patient. Otherwise, no hormonal abnormalities were uncovered. Three patients (numbers 1, 4, and 9) were diagnosed with oligoasteno-tzoospermia (OAT) and four (numbers 6, 7, 8, and 10) with azoospermia, whereas the other three had recurrent abortions (RA). RA was defined as three or more clinically recognized pregnancy losses before 20 weeks from the last menstrual period. The AZF region deletions were not detected in the seven patients with spermatogenetic defects. For azoospermic males, histological examinations were performed. According to the histopathologic criteria (12), specimens can be classified into five groups: normal, hypospermatogenesis, maturation arrest, Sertoli cells only, and tubular fibrosis. Histopathologic examination of patient 6 showed that he had no germ cells and was defined as having Sertoli cells only (Supporting Fig. 1A), whereas examination of the three other azoospermic males (numbers 7, 8, and 10) showed no evidence of mature sperm, with all germ cells in the seminiferous tubules showing maturation arrest (Supplementary Fig. 1B). Patients 1 and 9 tried to get pregnant with intracytoplasmic sperm injection and preimplantation genetic diagnosis using blastomeres biopsies, but both patients failed to achieve pregnancy.

Results of karyotype analyses in all 10 patients are summarized in Supplementary Table 2. Three-way exchanges (group 1) were identified in two patients (numbers 8 and 9); double two-way exchanges or two independent abnormalities (a two-way exchange plus an inversion), which are categorized as group 3, were identified in five (numbers 1–5); and group 2 CCRs were identified in three (numbers 6, 7, and 10). All three patients referred for RA had group 3 CCRs. In contrast, of the seven patients with spermatogenetic defects, two were classified as group 1, three as group 2, and only two as group 3.

The CCRs in the patients 7 and 10, were characterized using M-FISH methods. M-FISH confirmed the routine G-bands and FISH results in patient 7 (Supplementary Fig. 2). In case 10, however, the 16q24-16qter region on der(6) chromosome identified by the Telvysion 16q probe was not fully identified by M-FISH (Supplementary Fig. 3).

We also performed routine checkup of the female partner for infertility, especially when RA was the main outcome, including chromosomal analyses. The karyotypes of all three females with RA were 46,XX, and the hormone profiles and clinical findings of the other seven women showed no abnormalities.

In the present study, all 10 of our patients with CCR showed reproductive impairments, either RA or spermatogenetic failure, with seven (70%) also showing spermatogenetic impairment. In a female carrier of CCR, chromosomal malsegregations during gametogenesis result in abnormal conceptions, with most of these spontaneously aborted. In contrast to female CCR carriers, the male CCR carriers often show impaired spermatogenesis, resulting in oligo- or azoospermia.

The association between CCR and spermatogenesis is not fully understood. In all three of our patients with RA, the CCRs belonged to group 3 CCR, whereas most of the CCRs found in azoo- spermic males were group 1 or 2. These findings indicate that an increase in the number of breakpoints and more complex structural rearrangements have more severe consequences for gametogenesis.

Testicular biopsy results in the four azoospermic males showed maturation arrest in three patients (numbers 7, 8, and 10) and Sertoli cells only in one patient (number 6). The latter patient also showed bilateral varicoceles. Varicoceles, leading to ischemic damage to testicular tissue, have been found to result in impaired sperm quality and reduction in quantity (13). Although a comparably less complex chromosomal rearrangement (involving two chromosomes with four breakpoints), the serious clinical outcome in patient 6 may be due to the combination of chromosomal rearrangement and varicoceles. In the other three azoospermic patients, most spermatogenesis was arrested at the level of spermatocytes, mainly at the late pachytene stage, with only a few cells being early spermatids. There were no mature spermatozoa. The possible mechanisms of spermatogenetic failure in males with CCR have suggested that the number of breakpoints in the autosomes involved in the structural rearrangement is very numerous, increasing the probability of asynapsis as well as of pachytene checkpoint activation. This results in a considerable reduction in the number of postmeiotic cells, including spermatozoa production (14, 15). Moreover, the few spermatocytes that escape pachytene apoptosis are unable to deal with a pentavalent and one univalent in the metaphase I spindle and die before anaphase (6).

Although we did not examine the synaptonemal complexes in any of our azoospermic patients, they may be more complex than those found in individuals with the three-way CCR, or else they failed.

Most CCRs were identified using routine cytogenetic analyses, and M-FISH was widely used for confirmation of the G-banding results. However, as shown in one of our patients (number 10), subtle rearrangements could not be identified by either of these methods. In this patient, the der(6) chromosome identified by the locus-specific probe could not be fully identified by either G-banding or M-FISH, indicating that, in addition to M-FISH, conventional FISH using locus-specific probes still remains crucial for the detailed identification of cryptic chromosomal rearrangements.
Other than the complexity of rearrangements, gene defects or microdeletions in the long arm of the Y-chromosome, the most common cause of oligo- or azoospermia, may have caused spermatogenetic impairment in our patients. Yq microdeletions were excluded. If the specific breakpoints are associated with spermatogenetic impairment, those regions may contain genes involved in spermatogenesis or chromosomal segregation. We reviewed the chromosomal breakpoints presented in the literature about male cases with a CCR and reproductive failure (Fig. 1) (2, 6, 8, 16–28). Several breakpoints on chromosomes 4q, 5q, 7q, 9p, and 14q have been frequently reported in males with RA, whereas breakpoints on chromosomes 3, 4q, 11q, 12q, and 13q have been reported to be related to spermatogenetic failure. We found, however, that the breakpoints were generally randomly distributed. Although any breakpoints may include genes or gene regions related to spermatogenesis, no particular breakpoint had a decisive effect on spermatogenetic failure.

In conclusion, the complexity of chromosomal rearrangements in patients with CCRs plays a role in male factor infertility and affects the spermatogenetic process rather than the number of chromosomes involved or the location of breakpoints. To corroborate this conclusion, further studies with larger sample sizes and using an advanced technique, such as array-based comparative genomic hybridization, to characterize the breakpoints in detail are required.

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REFERENCES

Histological examination of testicular biopsies from azoospermic males show either Sertoli cells only (A, patient 6) or maturation arrest at the stage of early spermatids (B, patient 10). The other two males with azoospermia (patients 7 and 8) showed the same histological findings as in panel B.

Cytogenetic analyses of patient 7. (A) Partial ideogram from GTG-banding. Derivative chromosomes result from multiple translocations. (B) M-FISH clarification of the derivative chromosomes. (C) Confirmation of the M-FISH results by routine FISH analyses using whole chromosome painting probes. On the basis of these results, the karyotype of this patient was identified as 46,XY,der(1)(9qter→9q22::1p32→1qter),der(4)(1pter→1p32::13q32→13q14::4p14→4pter),der(9)(9pter→9q22::4p14→4pter),der(13)(13pter→13q14::13q32→13qter).

Cytogenetic analyses of patient 10. (A) Partial ideogram from GTG-banding. (B) M-FISH results. (C) 16q24-16qter region on der(6) chromosome identified by the TelVysion 16q probe was not fully identified by GTG-banding and M-FISH. On the basis of these results, the karyotype of this patient was identified as

46,XY,der(3)(3pter→3p23::3q25.3→3p11.1::6q27→6qter),der(6)(6pter→6q27::16q24→16qter),der(12)(12pter→12q24.3::3q25.3→3qter),der(16)(16pter→16q24::3p11.1→3p23::12q24.3→12qter).

## SUPPLEMENTARY TABLE 1

Clinical findings of males with CCRs diagnosed at CHA medical center (2002–2009).

<table>
<thead>
<tr>
<th>Case</th>
<th>Age at diagnosis, y</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>FSH, mIU/mL</th>
<th>LH, mIU/mL</th>
<th>T, ng/mL</th>
<th>Volume, mL</th>
<th>pH</th>
<th>Concentration, x10³/mL</th>
<th>Motility, %</th>
<th>Viability, %</th>
<th>Leukocytes/HPF</th>
<th>Morphology, % with normal form</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>183</td>
<td>83</td>
<td>2.2</td>
<td>8.0</td>
<td>1</td>
<td>9</td>
<td></td>
<td>—</td>
<td>—</td>
<td>16</td>
<td>—</td>
<td>Amorphos head, 40%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>35</td>
<td>155</td>
<td>50</td>
<td>4.3</td>
<td>5.2</td>
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<td>25</td>
<td>8.6</td>
<td>25</td>
<td>8.6</td>
<td>15</td>
<td>—</td>
<td>Amorphous head, most common</td>
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</tr>
<tr>
<td>3</td>
<td>30</td>
<td>165</td>
<td>48</td>
<td>5.9</td>
<td>4.6</td>
<td>2.2</td>
<td>0.1</td>
<td>8.6</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>Amorphous head, most common</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>32</td>
<td>172</td>
<td>68</td>
<td>4.9</td>
<td>5.4</td>
<td>5.23</td>
<td>0.1</td>
<td>8.6</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>Immotile sperm 8/10 µL after centrifuge</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>36</td>
<td>172</td>
<td>68</td>
<td>3.5</td>
<td>2.9</td>
<td>6.36</td>
<td>4.9</td>
<td>8.2</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>Amorphous head, most common</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>172</td>
<td>78</td>
<td>5.4</td>
<td>2.7</td>
<td>5.23</td>
<td>3.0</td>
<td>7.5</td>
<td>—</td>
<td>—</td>
<td>1</td>
<td>—</td>
<td>Amorphous head, most common</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>172</td>
<td>78</td>
<td>6.0</td>
<td>3.0</td>
<td>4.4</td>
<td>2.4</td>
<td>8.1</td>
<td>5</td>
<td>28</td>
<td>5</td>
<td>41</td>
<td>Amorphous, elongated, vacuole head, each 14%</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>34</td>
<td>172</td>
<td>78</td>
<td>3.5</td>
<td>2.9</td>
<td>4.4</td>
<td>2.4</td>
<td>8.1</td>
<td>5</td>
<td>28</td>
<td>5</td>
<td>41</td>
<td>Amorphous, elongated, vacuole head, each 14%</td>
<td></td>
</tr>
</tbody>
</table>

Note: Blank columns represent not analyzed. Normal ranges of hormonal profiles: FSH (1.1–13.5 mIU/mL), LH (0.4–5.7 mIU/mL), T (2.45–18.36 ng/mL).

### Summary of the results of studies of males with CCRs.

<table>
<thead>
<tr>
<th>Case</th>
<th>Karyotype</th>
<th>AZF and DAZ</th>
<th>Testicular biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>46,XY,inv(3)(p21;q11.2),t(6;22)(q22;q13)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P2</td>
<td>46,XY,t(4:5)(q31.3;q32),t(5:11)(q15;p15)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P3</td>
<td>45,XY,t(5:8)(q22;p23),der(13:14)(q10,q10)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>45,XY,t(3:9)(q24;q21.2),der(13:14)(q10;q10)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>46,XY,inv(1)(p13;q21)t(17;22)(p11;p11)</td>
<td>ND</td>
<td>Sertoli cell only</td>
</tr>
<tr>
<td>P6</td>
<td>46,XY,der(3)(14pter::3q10→3qter),der(14) (3pter→3p11.1::14q21→14p11::14q21→14qter)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>46,XY,der(1)(9qter→9q22::1p32→1qter),der(4) (1pter→1p32::13q32→13q14::4p14→4qter), der(9)(9pter→9q22::4p14→4qter),der(13) (13pter→13q14::13q32→13qter)</td>
<td>ND</td>
<td>Maturation arrest</td>
</tr>
<tr>
<td>P8</td>
<td>46,XY,t(2:19:22)(q11.2;p13.2;p11.2)</td>
<td>ND</td>
<td>Maturation arrest</td>
</tr>
<tr>
<td>P9</td>
<td>46,XY,t(2:7:4)(q31;q34;q33)</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>P10</td>
<td>46,XY,der(3)(3pter→3p23::3q25.3→3p11.1::6q27→6qter),der(6) (6pter→6q27::16q24→16qter),der(12) (12pter→12q24.3::3q25.3→3qter),der(16) (16pter→16q24::3p11.1→3p23::12q24.3→12qter)</td>
<td>ND</td>
<td>Maturation arrest</td>
</tr>
</tbody>
</table>

**Note:** ND = no deletion.

*Kim. CCRs in infertile males. Fertil Steril 2011.*