

Original Article

DAZ1/DAZ2 cluster deletion mediated by gr/gr recombination per se may not be sufficient for spermatogenesis impairment: a study of Chinese normozoospermic men

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Abstract

Aim: To explore the possible effect of the deleted in azoospermia (DAZ) copy cluster deletion on spermatogenesis in the Chinese population, the deletion of the azoospermia factor c (AZFc) region was analyzed in 346 normozoospermic men.

Methods: Three DAZ single nucleotide variant loci and seven AZFc-specific sequence-tagged sites were examined with polymerase chain reaction (PCR)-restriction fragment length polymorphism and routine PCR. Results: Five (1.4%) of the normozoospermic men were found to have deletion of gr/gr–DAZ1/DAZ2. None of the men were found to have b2/b4–entire DAZ deletion. Conclusion: The presence of gr/gr–DAZ1/DAZ2 deletion in five men with normozoospermia suggests that this deletion per se may not be sufficient for spermatogenic impairment in Chinese men. (Asian J Androl 2006 Mar; 8: 183–187)

Keywords: azoospermia factor c; deleted in azoospermia; gene deletion; normozoospermia

1 Introduction

Impaired spermatogenesis is an essential etiology of male infertility. In the last 20 years, several genetic factors have been correlated to impaired spermatogenesis, in which azoospermia factor (AZF) microdeletion is the second-most frequent cause after Klinefelter syndrome and accounts for approximately 10% of patients with azoospermia or oligozoospermia [1–3].

The AZF locus is mapped to the male-specific region of the Y chromosome and is divided into three discrete regions [1, 4], of which the AZFc region is particularly interesting: approximately 80% of AZF microdeletions occur in this region and most of them result in entire deleted in azoospermia (DAZ) gene deletion [5]. The DAZ gene has four copies, most commonly, and encodes an RNA binding protein exclusively in testicular tissue [6, 7]. Studies have demonstrated that the DAZ gene plays an important role in spermatogenesis [1, 8].

In recent years, studies on the deletion mechanism of the AZFc region and the deletion effect of DAZ gene copies on spermatogenesis have identified that the entire
AZFc deletion, including all DAZ copies, resulting from the recombination event of two massive repeat units b2/b4, is responsible for spermatogenic impairment in some populations [6, 9, 10]. In the past 2 years, at least eight AZFc partial deletion patterns and their clinical significance were reported [11–13]. Of these, only the DAZ1/DAZ2 deletion caused by gr/gr recombination was suggested to be another important etiology for spermatogenic impairment as it was observed exclusively in infertile males with a prevalence of 3% (10/337) in a white study group [14]. However, it is controversial whether DAZ cluster deletion resulting from the recombination of massive repeat units plays any role in spermatogenesis impairment [11, 15], which suggests that such study should be performed in larger and more ethnically diverse populations.

Taking into account the possible different distributions of Y chromosome haplogroups among different ethnic populations, which may result in the various prevalences of Y chromosome haplogroups among different populations [15], which suggests that such study should be performed in larger and more ethnically diverse populations.

2 Materials and methods

2.1 Subjects

Three hundred and forty-six Chinese men aged from 23 years to 34 years were recruited from patients attending the Department of Urology, West China Hospital of Sichuan University (Chengdu, China) from 2003 to 2005. Semen analyses were performed on all subjects three times and their sperm counts were > 20 × 10^6/mL; 186 with sperm count 50–100 × 10^6/mL, and 63 with sperm count > 100 and < 150 × 10^6/mL. All polymerase chain reaction (PCR) conditions were described in Ferlin et al. in 2004 [17]. The GenBank accession numbers are G73166 for SNVII, G63908 for SNVV, and G63906 for sY581. The primer sequences and PCR conditions were as previously described [9, 12].

2.2 Specific sequence-tagged site (STS) analysis of AZFc

Genomic DNA was extracted from peripheral blood lymphocytes using DNA isolation kits (Takara, Ostu, Japan). Seven AZFc-specific STS were used to detect entire and partial AZFc deletions, of which sY1161/sY1191/sY1291 and sY1206/sY1201 were co-amplified separately, and sY254/sY255 was amplified according to the European Academy of Andrology and the European Molecular Genetics Quality Network (EAA/EMQN) best practice guidelines [5]. The partial deletion of DAZ gene copies was detected in SNV sites of the DAZ gene, including SNVII, SNVV, and sY581, by PCR-restriction fragment length polymorphism, in which the MboI digestion fragment of SNVII distinguishes DAZ1 from other DAZ copies, the DraI digestion fragment of SNVV distinguishes DAZ1/DAZ2 from DAZ3/DAZ4, and the Sau3A digestion fragment of sY581 distinguishes DAZ1/DAZ4 from DAZ2/DAZ3. The digestion products of SNVII and SNVV/sY581 were separated with 2% agarose gel and visualized by ethidium bromide staining method. The b2/b4–entire DAZ deletion was identified by the positive result of sY1201 and the negative results of sY254, sY255, sY1191, sY1291 and sY1206 [9]. The gr/gr deletion was identified by the negative result of sY1291 and the positive results of the other STS [12]. The primer sequences and PCR conditions were as previously described [9, 12].

2.3 Single nucleotide variant (SNV) analysis of the DAZ gene

The partial deletion of DAZ gene copies was detected in SNV sites of the DAZ gene, including SNVII, SNVV and sY581, by PCR-restriction fragment length polymorphism, in which the MboI digestion fragment of SNVII distinguishes DAZ1 from other DAZ copies, the DraI digestion fragment of SNVV distinguishes DAZ1/DAZ2 from DAZ3/DAZ4, and the Sau3A digestion fragment of sY581 distinguishes DAZ1/DAZ4 from DAZ2/DAZ3. The digestion products of SNVII and SNVV/sY581 were separated with 2% agarose gel and 8% polyacrylamide gel and visualized by ethidium bromide and silver staining methods. The DAZ1/DAZ2 deletion was identified by the presence of pair-fragments of 195 bp/49 bp and 122 bp/60 bp with the SNVV-DraI and SNVII-MboI tests, respectively, together with integrated restriction fragmentation of sY581-Sau3A. The primer sequences and PCR conditions were described in Ferlin et al. in 2004 [17]. The GenBank accession numbers are G73166 for SNVII, G63908 for SNVV, and G63906 for sY581.

3 Results

With specific STS analysis of AZFc, eight of 346 men showed partial AZFc deletions (2.3%), and all of them were the gr/gr deletion (Figure 1). The b2/b4–entire DAZ deletion was not found in any subject according to the positive results of sY254 and sY255 (Figure 2).

In the SNV analysis of the DAZ gene, 21 men were found to have lost sequence family variants in the SNV loci, 12 of which were identified as presenting two fragments of 195 bp and 49 bp with SNVV-DraI, and three
fragments of 489 bp, 122 bp and 60 bp with SNVII-MboI, together with integrated restriction fragments of sY581-Sau3A, indicative of the deletion of DAZ1/DAZ2 (Figure 3) and the prevalence was 3.5% (12/346). Nine out of 21 males were identified by the three fragments of 122 bp, 73 bp and 49 bp with SNVV-DraI, and four fragments of 489 bp, 182 bp, 122 bp and 60 bp with SNVII-MboI, in addition to the integrated restriction fragments of sY581-Sau3A, suggesting that the deletion frequency of DAZ3/DAZ4 was 2.6% (9/346).

Taking specific AZFc STS and SNV analysis together, we found that, of eight men with gr/gr recombination, five showed the doublet deletion of DAZ1/DAZ2 and three showed the DAZ3/DAZ4 deletion. These deletion patterns in the three groups of subjects categorized by their sperm counts are shown in Table 1.

<table>
<thead>
<tr>
<th>Semen sperm density</th>
<th>gr/gr–DAZ1/DAZ2 n (%)</th>
<th>gr/gr–DAZ3/DAZ4 n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥20 and &lt;50×10⁶/mL</td>
<td>3/97 (3.1)</td>
<td>1/97 (1.0)</td>
</tr>
<tr>
<td>≥50 and ≤100×10⁶/mL</td>
<td>2/186 (1.1)</td>
<td>2/186 (1.1)</td>
</tr>
<tr>
<td>&gt;100 and &lt;150×10⁶/mL</td>
<td>0/63 (0.0)</td>
<td>0/63 (0.0)</td>
</tr>
<tr>
<td>Total</td>
<td>5/346 (1.4)</td>
<td>3/346 (0.9)</td>
</tr>
</tbody>
</table>
4 Discussion

In the present study, b2/b4–entire DAZ deletion was not detected in any of the 346 subjects. This result, in addition to the approximately 10% of entire DAZ deletion frequency reported in a previous study in Chinese infertile men with azoospermia or oligozoospermia [18], suggests that the b2/b4–entire DAZ deletion may be a major AZFc abnormality responsible for impaired spermatogenesis in Chinese men, which is in agreement with reports in other populations [9].

One study has suggested that the gr/gr–DAZ1/DAZ2 deletion represents an important genetic defect leading to impaired spermatogenesis in white men [14]. However, as shown in Table 1, the gr/gr–DAZ1/DAZ2 deletion was observed in 1.4% (5/346) of normozoospermic Chinese men with no significant difference found in frequencies between two groups of semen sperm counts, (20–50) × 10⁹/mL and (50–100) × 10⁹/mL, suggesting that the gr/gr–DAZ1/DAZ2 deletion on its own may be insufficient to result in spermatogenic impairment in Chinese men. This is in contrast to previous studies in which gr/gr–DAZ1/DAZ2 deletion was only found in patients with impaired spermatogenesis, but not in the 263 normal men examined [14]. The reason for the different results in different studies could be manifold, such as environmental factors, genetic modification, as well as Y chromosome haplogroups in different ethnic populations [19]. As for gr/gr–DAZ3/DAZ4 deletion, it occurs with a frequency of 0.9% (3/346), which is similar to that reported in white men [14].

In summary, we confirmed that the b2/b4–entire DAZ deletion might be the essential genetic abnormality for impaired spermatogenesis in Chinese men. However, the gr/gr–DAZ1/DAZ2 deletion was identified in Chinese normozoospermic men, which suggests that the gr/gr–DAZ1/DAZ2 deletion per se is not an essential cause of spermatogenic failure in the Chinese population. Further study on partial AZFc deletion should be performed in infertile Chinese men to compare the gr/gr–DAZ1/DAZ2 deletion frequency, so as to reveal the genetic effect of the cluster deletion and to explore novel deletion patterns contributing to spermatogenic impairment.

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