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·*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

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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 prevalence and phenotype of *AZFc* deletion [11, 16], we investigated the deletion distributions of b2/b4–entire *DAZ* and gr/gr–*DAZ1/DAZ2* in Chinese men with normozoospermia, to evaluate the genetic effect of the deletions on spermatogenesis.

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 $> 20 \times 10^6$/mL with normal motility and morphology, in accordance with the World Health Organization (WHO) criteria. The subjects were divided into three groups: 97 with sperm count $< 50 \times 10^6$/mL; 186 with sperm count 50–100 $\times 10^6$/mL, and 63 with sperm count > 100 and $< 150 \times 10^6$/mL. The study was approved by the Institutional Ethical Review Board of West China Hospital, Sichuan University, and signed informed consent forms were obtained from all participants.

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]. All polymerase chain reaction (PCR) products were separated with 2% agarose gel and visualized by the 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 fragments 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

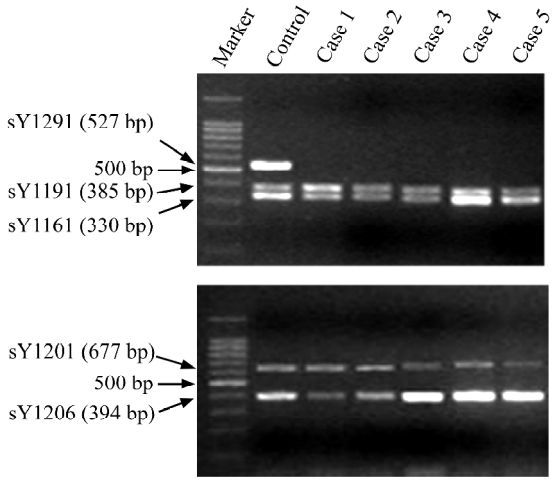

Figure 1. Detection of the gr/gr deletion with specific azoospermia factor c (*AZFc*) sequence-tagged sites (STS). Marker: 100 bp ladder; control: normozoospermic man; Case 1–5: the gr/gr deletion defined by the absence of sY1291 and the presence of other STS.

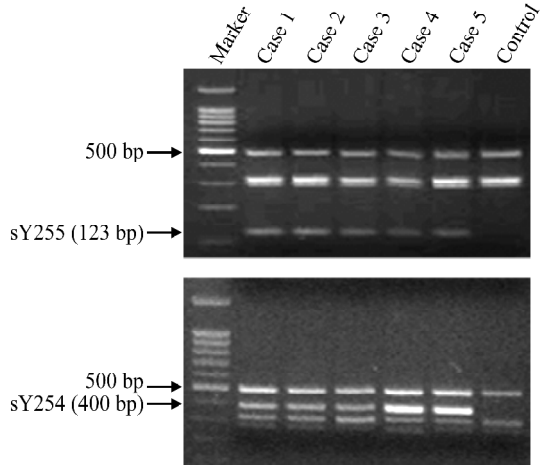

Figure 2. Detection of the b2/b4–entire deleted in azoospermia (*DAZ)* deletion with sY254 and sY255. Marker: 100 bp ladder; control: infertile man with the b2/b4–entire *DAZ* deletion; Case 1–5: the presence of the *DAZ* gene.

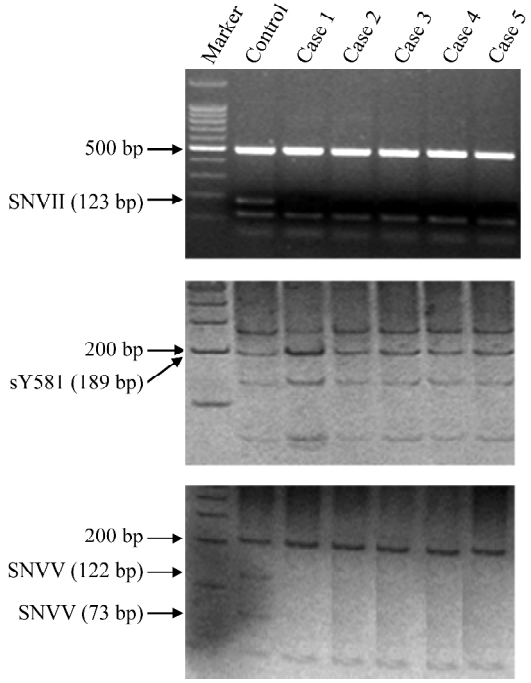

Figure 3. Detection of the deleted in azoospermia 1/deleted in azoospermia 2 (*DAZ1/DAZ2*) deletion with SNVII, SNVV and sY581. Marker: 100 bp ladder; control: normozoospermic man with complete restricted enzyme maps; Case 1–5: the *DAZ1/DAZ2* deletion defined by the restricted fragment absence of 182 bp in SNVII locus and 122 bp/73 bp in SNVV locus.

Table 1. Distribution of gr/gr with deleted in azoospermia (*DAZ*) copies deletion in 346 normozoospermic Chinese men. *n*, number.

Semen sperm density	gr/gr–*DAZ1/DAZ2* *n* (%)	gr/gr–*DAZ3/DAZ4* *n* (%)
⩾ 20 and < 50 × 10^6/mL	3/97 (3.1)	1/97 (1.0)
⩾ 50 and ⩽ 100 × 10^6/mL	2/186 (1.1)	2/186 (1.1)
> 100 and < 150 × 10^6/mL	0/63 (0.0)	0/63 (0.0)
Total	5/346 (1.4)	3/346 (0.9)

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.

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 differencse found in frequencies between two groups of semen sperm counts, (20–50) × 10^6/mL and (50–100) × 10^6/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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