Partial AZFc deletions in infertile men with cryptorchidism

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BACKGROUND: A specific type of partial AZFc deletion, called ‘gr/gr’ deletion, was recently proposed as genetic risk factor for spermatogenic impairment and testis cancer. Since both pathologies can be part of the testicular dysgenesis syndrome (TDS), we aimed to define the role of ‘gr/gr’ deletion in the aethiopathogenesis of another component of the TDS: cryptorchidism. METHODS: A total of 146 cryptorchid and 140 infertile patients without a history of cryptorchidism were screened with a sequence tagged site plus/minus method and further confirmed and characterized by CDY1/DAZ gene dosage and copy analysis. RESULTS: The observed deletion frequency was 4.2% in cryptorchid and 5% in non-cryptorchid patients. Moreover, no differences in the CDY1/DAZ patterns were observed among the two groups. A significant difference in deletion frequency was present only when cryptorchid patients were compared with normospermic controls (P<0.03). CONCLUSIONS: Our data show no relationship between ‘gr/gr’ deletion and cryptorchidism, however, provide further evidence of the deleterious effect of the ‘gr/gr’ deletion on spermatogenesis. The screening for ‘gr/gr’ deletion may therefore be proposed before ICSI to all patients with severe male factor infertility, without the exclusion of those with cryptorchidism, since this genetic risk factor for spermatogenic impairment will be transmitted to the male offspring.

Keywords: cryptorchidism; genetics; ‘gr/gr’ deletions; male infertility; Y chromosome

Introduction

The importance of AZF genes in spermatogenesis is undisputable since a clear cause-effect relationship between microdeletions of the AZF regions of the long arm of the Y chromosome (Yq) and severe spermatogenic disturbances has been ascertained by several reports (for review see Krausz et al., 2004). Recently, a new type of Yq microdeletion—the so called ‘gr/gr’ deletion—which removes approximately half of the gene content of the AZFc region, has been reported by Repping et al. (2003). This specific type of partial AZFc deletion is considered a new genetic risk factor for spermatogenic impairment by a number of research groups (Repping et al., 2003; de Llanos et al., 2005; Ferlin et al., 2005; Giachini et al., 2005; Lynch et al., 2005) but not by others (Machev et al., 2004; Hukkanenbroich et al., 2005; Ravel et al., 2006; de Carvalho et al., 2006a; Lin et al., 2007; Lardone et al., 2007).

Depending on the aetiology, testicular dysfunction may manifest with impaired spermatogenesis alone or in association with other symptoms such as cryptorchidism (failure of the testis to descend into the scrotum), hypospadias and testicular cancer. According to the testicular dysgenesis syndrome (TDS) hypothesis (Skakkebaek et al., 2001), reduced sperm count, cryptorchidism, hypospadias and testicular cancer may share common genetic and environmental factors acting on the Sertoli and Leydig cells. The question of whether Yq deletions are responsible for the aetiopathogenesis of other components of this syndrome has been addressed only by a few studies. According to these studies, classical AZF deletions do not seem to induce cryptorchidism (Kunej et al., 2003) nor testicular cancer (Frydelund-Larsen et al., 2003), whereas the ‘gr/gr’ deletion has been recently reported as a significant genetic risk factor for testicular cancer (Nathanson et al., 2005). The role of ‘gr/gr’ deletions in the aetiopathogenesis of cryptorchidism has not been evaluated so far since all of the earlier studies focused on the comparison of idiopathic infertile men (without abnormal andrological findings) versus fertile or normospermic controls or the general population. Cryptorchidism is the most frequent congenital abnormality of the male reproductive tract, affecting ~2–3% of full-term males. The aetiology of this pathological condition is multifactorial and can be due to endocrine disorders (e.g. hypogonadotrophic hypogonadism, androgen resistance), anatomical abnormalities, or mutations of the INSL3 and LGR8 genes (Foresta and Ferlin, 2004; Bogatcheva and Agoulnik, 2005). Among genetic risk factors, polymorphisms in the ESR1 gene have been proposed, although with opposite effects in Japanese (Yoshida et al., 2005) and Caucasian populations (Galan et al., 2007).

Based on the TDS hypothesis and the recently proposed role of ‘gr/gr’ deletions in testis cancer, we now aimed to evaluate
whether cryptorchidism and ‘gr/gr’ deletions have a causal relationship. Considering the intimate intertalk between germ cells, Sertoli cells and Leydig cells, a genetic factor able to influence any of this component may lead to TDS including cryptorchidism. We previously found a significant association between ‘gr/gr’ deletions and reduced sperm count in the Italian population using a combined method based on sequence tagged sites (+/−) deletion screening and further molecular characterization of the deletions (AZFc gene dosage and type of deleted gene copies) (Giachini et al., 2005).

In the present study, we performed ‘gr/gr’ deletion screening in infertile cryptorchid men of Italian origin and data obtained was compared with our own published data (Giachini et al., 2005) and with that published in the literature on idiopathic infertile men without a history of cryptorchidism. Beside the comparison of deletion frequencies, we also aimed to clarify whether specific deletion patterns are observed in this specific group of patients.

Materials and Methods

Subjects

The study population consisted of 146 Italian patients with a history of cryptorchidism. As a control group, we selected 140 men without cryptorchidism from our previously published 150 Italian infertile patients (Giachini et al., 2005). The Y hgr distribution between oligo/azoospermic and normospermic men was similar (M. Mitchell, personal communication) which excludes recruitment bias. Since the same criteria (ethnic and geographic origin) was used for the recruitment of cryptorchid and non-cryptorchid men, significant differences in Y background distribution are not expected between groups. Table 1 reports the clinical characteristics of the study populations, including semen parameters evaluated according to the World Health Organization criteria. All patients underwent a complete andrological investigation for couple infertility at the Andrology Unit of the University Hospital Careggi in Florence (Italy). Cytogenetic analysis and Y chromosome microdeletion screening revealed 46,XY karyotype and the absence of AZF microdeletions in all included patients. Samples were collected using approved protocols and the informed consent of all individual was obtained.

Screening for ‘gr/gr’ deletions

We detected ‘gr/gr’ deletions by a polymerase chain reaction (PCR) amplification of Y chromosome STSs, originally described by Repping et al. (2003): sY1291, sY1191, sY1161, sY1206, sY1201, sY142, sY142 and sY1197 (see GenBank for PCR primers). We identified ‘gr/gr’ deletions by the following STS results: sY1291 negative; sY1191, sY1161, sY1206, sY1201, sY142, sY142 and sY1197 all positive. A positive (normal men) and a negative control (women) were screened with the samples to prevent false results. PCR was carried out in a total volume of 25 ul. The reaction mixture included 100 ng of each DNA sample extracted from peripheral blood cells, 1X PCR buffer (containing 1,5 mM MgCl2), 200 uM of deoxy-nucleotidetriphosphates (dNTPs), 1 uM of each primers and 1 U Taq polymerase (Promega Italia S.r.l.). After an initial denaturation step at 95°C for 5 min, amplification was performed for 35 sequential cycles, each including 1 min denaturation at 95°C, 1 min primer annealing at 55–62°C and 1 min extension at 72°C. The program was followed by a final extension step at 72°C for 7 min. PCR products were then analysed by electrophoresis on 2% agarose gels containing ethidium bromide and visualized under ultraviolet light.

Gene dosage

Quantitative analysis for CDY1 and DAZ, in order to quantify the copy number of these genes, was performed using a PCR-based method, according to Machev et al. (2004): we simultaneously amplified the AZFc locus to be quantified (CDY1 or DAZ) and a homologous locus outside the AZFc interval (CDY2 and DAZL, respectively)—as an internal standard with a known number of copies—using a single primer pair in a PCR reaction with a maximum of 28 cycles (end point into the exponential phase). The primers flank an insertion/deletion difference of 3–5 bp, which allowed the products amplified from the AZFc loci and the control loci to be separated by polyacrylamide gel electrophoresis. One of the primers was labelled at its 5’ end with a fluorochrome (FAM). To overcome the problem of doublet bands—usually referred to as ‘shadow bands’—caused by heterogeneous adenosine addition, PCR for DAZ/DAZL was followed by a treatment with polymerase T4 (1 U for 15 min at 37°C), which removed the extra base added by Taq polymerase at the end of the amplified fragments (Ginot et al., 1996). Then, the reaction was mixed with formamide, denatured at 95°C for 5 min and the different size loci were separated on an automatic sequencer (ABI PRISM 310 Genetic Analyzer PE). Quantification was performed comparing the pick area of the AZFc locus and of its homologous.

CDY1 versus CDY2 (primers: oMY953a/o1023)

There are two identical copies of each CDY1 and CDY2, which share 98% nucleotide identity. We amplified CDY1 and CDY2 across 3 bp difference in the coding region, to give fragments of 134 bp for CDY1 and 137 bp for CDY2.

DAZ versus DAZL (primers: o1130/0131)

We co-amplified a fragment of intron 10 from DAZ (214 bp) and DAZL (217 bp). This intron is present in one copy per DAZ gene (in a normal 46,XY men there are four copies of DAZ and two copies of DAZL). Some samples present a 40 bp insertion polymorphism in the DAZL intron 10, resulting in an extra band at 260 bp, which could be in heterozygosity or, more rarely, in homozygosity.

Table 1: Seminal patterns of the two study populations

<table>
<thead>
<tr>
<th>Cryptorchid group (n = 146)</th>
<th>Azoospermia</th>
<th>&lt;5 × 10⁶ spermatozoa/ml</th>
<th>≥5 × 10⁶ spermatozoa/ml</th>
<th>Asteno and/or teratozoospermia</th>
<th>Normozoospermia</th>
<th>Not available</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unilateral (n = 77)</td>
<td>2</td>
<td>47</td>
<td>10</td>
<td>1</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td>Bilateral (n = 66)</td>
<td>17</td>
<td>41</td>
<td>4</td>
<td>3</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Total number</td>
<td>19</td>
<td>88</td>
<td>14</td>
<td>4</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Infertile group (n = 140)</td>
<td>33</td>
<td>72</td>
<td>27</td>
<td>8</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Partial AZFc deletions and cryptorchidism
**Gene copy type**

Qualitative analysis for CDY and DAZ, in order to determine which copies of these genes have been removed by the ‘gr/gr’ deletion, was performed according to Machev et al. (2004). For DAZ, we chose the sequence family variant (SFV) at STS sY587 in intron 10, which discriminates DAZ1/2 from DAZ 3/4. For CDY1, we used a C/A SFV situated 7750 bp 5’ of the CDY1 translation start codon (CDY7750), which distinguishes CDY1a from CDY1b. SFVs were scored by PCR followed by enzyme digestion (5 U for at least 4 h): dAZ, sY587, DraI (DAZ1/2 cut); CDY1-7750, PsvII (CDY1b cut).

Digestion products were then analysed by electrophoresis at 100 V under ultraviolet light. Primers pair: sY587, o912/o913; CDY1-7750, o1025/o1026 (Machev et al., 2004).

**Statistical analysis**

Statistical analysis was performed using the standard package SPSS for Windows (version 12.0.1; SPSS, Chicago, IL, USA). We tested the significance of the observed difference in the incidence of ‘gr/gr’ deletion between our two study groups using Fisher’s exact test. Our null hypothesis was that incidence is the same in cryptorchid and infertile men without cryptorchidism.

All variables were checked for normal distribution by Kolmogorov–Smirnov one-sample test. For comparisons of means between groups of different genotypes, Student’s t-test for independent samples, when normal distribution was observed, was applied. Sperm concentration is expressed as mean ± standard deviation. Logarithmic transformation of data was performed in order to normalize the distribution when the presence of log normal distribution was observed, was applied. The arithmetic transformation of data was performed in order to normalize the distribution when the presence of log normal distribution was observed. The number of gene copies remained by the ‘gr’ deletion events. The number of false deletions and deletion/duplication events was the same in all subjects (one CDY1 copy and two DAZ copies), indicating the absence of false deletions and deletion/duplication events.

**Results**

**Frequency and types of partial AZFc deletions in patients with and without cryptorchidism**

Using the STS +/− analysis it is possible to distinguish between different types of partial AZFc deletions. We observed only the ‘gr/gr’ deletion (absence of the STS sY1291) in the cryptorchid group, whereas in the group of 140 infertile men we also found one subject with ‘b2/b3’ deletion (absence of the STS sY1191). The third possible deletion, called ‘b1/b3’, was absent in both study populations.

The frequency of ‘gr/gr’ deletions observed in the cryptorchid group was 4.2% (6/143), which is not significantly different from that observed in the re-analysed 140 infertile men without cryptorchidism (7/140; 5%).

Although a number of papers have been published on ‘gr/gr’ deletions in infertile and control men, only a minority of them confirmed the deletion with gene dosage analysis and ruled out deletion/duplication events. The ‘gr/gr’ deletion frequency in each study is reported in Table 2. All three studies were performed in Caucasian populations and the cumulative deletion frequency is 4.96%, which is not different from the deletion frequency of our cryptorchid (4.2%) and infertile (5%) groups.

**Molecular analysis and clinical findings in cryptorchid men with ‘gr/gr’ deletions**

**Definition of ‘gr/gr’ deletion subtypes**

The second step of molecular characterization is of particular importance since the currently used STS+/− PCR method is not able to exclude deletion/duplication events and other rearrangements in the AZFc region.

In order to confirm and to characterize ‘gr/gr’ deletions, we defined the gene dosage of CDY1 and DAZ and the type of missing DAZ (DAZ1/DAZ2/DAZ3/DAZ4) and CDY1 (CDY1a/CDY1b) copies. The number of gene copies remaining after the ‘gr’ deletion events was the same in all subjects (one CDY1 copy and two DAZ copies), indicating the absence of false deletions and deletion/duplication events.

Based on the type of deleted DAZ and CDY1 copies, we found different subtypes of deletion patterns (Fig. 1) in the two study groups. Accordingly, ‘gr/gr’ deletion may be divided into at least four subtypes. The most frequent deletion subtypes (defined on the basis of the missing copies) both in cryptorchid and infertile groups was CDY1a+DAZ1/2 followed by CDY1a+DAZ3/4. The deletion subtype CDY1b+DAZ1/2 was present only in one cryptorchid patient, and CDY1b+DAZ3/4 was present only in one infertile man.

The frequency of ‘gr/gr’ deletions with missing CDY1a in the cryptorchid group was 83%, which is similar to that observed in the infertile men (86%). The loss of DAZ1/2 is more frequent, although not significantly different, in cryptorchid men compared with infertile men (67 and 43% respectively).

**Genotype-phenotype association**

The mean values of sperm concentration were similar in the infertile patients with and without cryptorchidism (4.0 ± 10.3 × 10^6 spermatozoa/ml and 4.3 ± 8.6 × 10^6 spermatozoa/ml, respectively). Similarly, the comparison between ‘gr’ deleted men with cryptorchidism (2.1 ± 3.3 × 10^6 spermatozoa/ml) and without cryptorchidism (2.5 ± 3.5 × 10^6 spermatozoa/ml) shows no differences. The clinical description of men with ‘gr/gr’ deletion is given in Table 3 along with the indication of the CDY1/DAZ gene patterns. The sperm concentration in the cryptorchid group ranges between azoospermia and 8 × 10^6 spermatozoa/ml, whereas in the non-cryptorchid group it was between 0.01 and 10 × 10^6 spermatozoa/ml. The ‘gr’ deletion was observed both in men with unilateral and bilateral cryptorchidism.

In order to define if the presence of ‘gr/gr’ deletion in cryptorchid men has an additional negative effect on sperm concentration, we compared the mean values of sperm concentration

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**Table 2: Frequency of ‘gr/gr’ deletion in infertile men of Caucasian origin, reported in studies in which gene dosage analysis was also performed**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Total analysed, n</th>
<th>‘gr/gr’ deleted, n</th>
<th>‘gr/gr’ deletion, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Repping et al. (2003)</td>
<td>246</td>
<td>9</td>
<td>3.7</td>
</tr>
<tr>
<td>Machev et al. (2004)</td>
<td>300</td>
<td>18</td>
<td>6</td>
</tr>
<tr>
<td>Giachini et al. (2005)</td>
<td>140</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>686</td>
<td>34</td>
<td>4.96</td>
</tr>
</tbody>
</table>

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between ‘gr/gr’ deleted and not deleted cryptorchid patients. Although the mean value was lower in cryptorchid men with ‘gr/gr’ deletion \(2.1 \pm 3.3 \times 10^6\) spermatozoa/ml compared with those without \(4.1 \pm 10.5 \times 10^6\) spermatozoa/ml, the difference was not statistically significant. In our previous publication, we observed ‘gr/gr’ deletion with missing CDY1a copy only in the infertile group and not in normospermic control men. This observation leads us to propose a possible pathogenetic effect of these deletion subtypes on spermatogenesis. However, since all our cryptorchid patients with ‘gr/gr’ deletions were oligospermic and we found only one cryptorchid patient with CDY1b deletion, the comparison of the mean values of sperm concentration in the ‘gr/gr’ deleted cryptorchid men with loss of CDY1a versus CDY1b has no clinical relevance.

**Discussion**

The human Y chromosome has a unique structure, rich in very long near identical direct and indirect repeats which makes the chromosome prone to rearrangements (Skaletsky et al., 2003). The AZFc region, which is made up almost entirely of amplicons, is the most frequently deleted Yq region in infertile men (Kuroda-Kawaguchi et al., 2001). Thanks to the presence of amplicons with the same orientation, deletion break points may occur at different sites leading to complete (‘b2/b4’) or a number of possible partial AZFc deletions. The most frequent partial AZFc deletions are the ‘gr/gr’ and the ‘b2/b3’, both removing approximately half of the AZFc region gene content. Despite a similar loss of gene number, ‘b2/b3’ deletions are not associated with spermatogenic impairment, whereas ‘gr/gr’ deletions have been reported as a risk factor for male infertility by the majority of the authors (Repping et al., 2003; de Llanos et al., 2005; Ferlin et al., 2005; Giachini et al., 2005; Lynch et al., 2005). However, the pathogenic role of this genetic anomaly in male infertility is still under debate and unfortunately, it is impossible to perform a meta analysis of the published data due to methodological differences and different inclusion criteria applied for the selection of patients and controls (for review see Krausz and degl’Innocenti, 2006).

**Figure 1:** Schematic representation of the deletion patterns found in the cryptorchid and infertile subjects [from the paper by Giachini et al. (2005) with the exclusion of one monolateral cryptorchid man] with ‘gr/gr’ deletion, based on the type of CDY1 and DAZ copies deleted and on the analysis based on STS +/− described by Repping et al. (2003) Open and filled boxes indicate the absence or presence of a given marker or gene, respectively.
The appropriate selection of control group in association studies is of fundamental importance and should be represented by a group of subjects free of the disease under study. Therefore, in order to understand whether ‘gr’ deletions are able to influence spermatogenesis, the controls should be normospermic subjects. In the literature, only a few studies used exclusively normospermic controls (Repping et al., 2003; Ferlin et al., 2005; Giachini et al., 2005; Hukkanenbroch et al., 2005) and among them only two (Repping et al., 2003; Giachini et al., 2005) confirmed the deletions by dosage analysis. Recent East Asian studies, based on an STS þ /− method, show a relatively high frequency of ‘gr’ deletion in these populations (de Carvalho et al., 2006b; Fernando et al., 2006) and a lack of association with infertility. On the contrary, a study from Yen’s group reports that partial duplication and not partial deletion of the AZFc region is a risk factor for impaired spermatogenesis in Han Chinese (Lin et al., 2007). It is likely that ethnic background plays a determinant role in the phenotypic expression of this genetic risk factor. Therefore, the exclusion of recruitment bias (i.e. exclusion of significant differences in Y hgr distribution between controls and cases) is of fundamental importance especially in ethnically mixed populations.

In our previous study on the Italian population (with homogeneous inclusion criteria, normospermic controls, exclusion of recruitment bias, i.e. similar Y background in cases and controls), we observed that men with congenital ‘gr’ deletions have a 10-fold increased risk of being oligospermic compared with men without such a deletion (Giachini et al., 2005). A similar conclusion was made also by another Italian study in which the selection criteria of the control group was based on normozoospermia (Ferlin et al., 2005). With the aid of additional molecular characterization (gene dosage and type of deleted DAZ and CDY1 copies), we also showed that ‘gr’ deletion is not a uniform entity, and it can be divided into four major subtypes. We suggested that subtype 1, which is characterized by the loss of DAZ1/DAZ2 and CDY1a copies, may represent a more severe variant, since this subtype is absent in normospermic controls with ‘gr’ deletions. The recently reported association between testis cancer and ‘gr’ deletion (Nathanson et al., 2005) opened the question about the role of this polymorphism in other TDS-related pathologies such as cryptorchidism.

In order to define the role of ‘gr’ deletion in the aetio-pathogenesis of cryptorchidism, we compared the deletion frequency in patients with and without undescended testis. The ‘gr’ deletion frequency in 146 cryptorchid men (4.2%) compared with that of our control population based on 140 infertile men without cryptorchidism (5%) showed no significant differences. Moreover, we found a similar distribution of deletion patterns, defined on the basis of CDY1 and DAZ gene copy losses. The recently reported association between testis cancer and ‘gr’ deletion (Nathanson et al., 2005) opened the question about the role of this polymorphism in other TDS-related pathologies such as cryptorchidism.

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<table>
<thead>
<tr>
<th>Code</th>
<th>Phenotype</th>
<th>Deleted gene copies</th>
<th>DAZ</th>
<th>CDY1</th>
<th>Number (sperm/ml × 10⁶)</th>
<th>Progressive Motility (%)</th>
<th>Morphology (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘gr’/‘gr’ deleted cryptorchid men</td>
<td>CRA46</td>
<td>Crypt. monolat.</td>
<td>1/2</td>
<td>a</td>
<td>0.01</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CRA258</td>
<td>Crypt. bilat.</td>
<td>1/2</td>
<td>a</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CRA568</td>
<td>Crypt. bilat.</td>
<td>1/2</td>
<td>b</td>
<td>1.40</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>CRMMP193</td>
<td>Crypt. monolat.</td>
<td>1/2</td>
<td>a</td>
<td>8.00</td>
<td>23</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>CRMMP263</td>
<td>Crypt. bilat.</td>
<td>3/4</td>
<td>a</td>
<td>1.00</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>CH10</td>
<td>Crypt. monolat.</td>
<td>3/4</td>
<td>a</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*excluded one subject with monolateral cryptorchidism.*
frequency was found only when the comparison is made between normospermic and cryptorchid, but not between infertile and cryptorchid men, 'gr/gr' deletion is more likely to affect sperm parameters than testicular descent. Although the majority of cryptorchid men (especially in case of bilateral undescended testes) will develop severe impairment of spermatogenesis in adulthood, seminal parameters in ex-cryptorchid men may range from azoospermia to normozoospermia. Since cryptorchidism is a multifactorial disease, the reason for such a heterogeneous phenotype can be related to specific aetiological factors which underlie cryptorchidism. Although on the basis of our results, we cannot consider 'gr/gr' deletion as a common aetiological risk factor for both spermatogenic failure and cryptorchidism, its presence in the cryptorchid group was associated with a risk factor for both spermatogenic failure and cryptorchidism, cannot consider 'gr/gr' deletion as a common aetiological risk factor for both spermatogenic failure and cryptorchidism. Although on the basis of our results, we cannot consider 'gr/gr' deletion as a common aetiological risk factor for both spermatogenic failure and cryptorchidism, its presence in the cryptorchid group was associated with a lower sperm concentration (2.1 ± 3.3 × 10^6 spermatozoa/ml versus 4.1 ± 10.5 × 10^6 spermatozoa/ml). This observation, needs to be confirmed, but may suggest an additional modulating effect of 'gr/gr' deletion on the final testicular phenotype of cryptorchid patients.

In conclusion, in analogy to classical AZF deletions (Kunej et al., 2003), 'gr/gr' deletion is not associated with cryptorchidism but it is present at a similar frequency in infertile patients with and without history of maldescended tests. The comparison with normospermic men has further confirmed the deleterious effect of this type of AZFc deletion on spermatogenesis. Consequently, the screening for 'gr/gr' deletions can be extended to all severely oligospermic patients attending to assisted reproductive techniques, without the exclusion of those with cryptorchidism, since this test is able to provide the identification of a transmissible genetic risk factor for reduced sperm count.

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References
Lin YW, Chia-Ling Hsu L, Kuo PL, Huang WJ, Chiang HS, Yeh SD, Hsu TY, Yu YH, Hsiao KN, Cantor RM et al. Partial duplication at AZFc on the Y chromosome is a risk factor for impaired spermatogenesis in Han Chinese in Taiwan. Hum Mutat 2007 (Epub ahead of print).
Skakkebaek NE, Raipert-De Meyrs E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. Hum Reprod 2001;16:972–978.

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