CAG repeat length in the androgen receptor gene of infertile Japanese males with oligozoospermia

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We analysed the CAG repeat length in exon 1 of the androgen receptor gene in 59 idiopathic Japanese infertile males with oligozoospermia; 36 fertile males were also analysed as controls. The number of CAG repeats in infertile males ranged from 14 to 32 (mean 21.2 ± 4.2), whereas the number of CAG repeats in fertile males ranged from 16 to 31 (mean 21.4 ± 3.5). Among infertile males, six possessed a short form of 14 CAG repeats and three possessed 15 CAG repeats. On the other hand, fertile males did not possess the short form of 14 or 15 CAG repeats. The incidence of infertile males with 14 and 15 CAG repeats was significantly higher (P < 0.05) than that of fertile males. Although the sample size is small, the results suggest that the reduction of CAG repeats in exon 1 of the androgen receptor is closely related to impaired spermatogenesis in infertile Japanese males.

Key words: androgen receptor/CAG repeat/oligozoospermia

Introduction

Mammalian spermatogenesis is absolutely dependent upon androgen produced via the action of luteinizing hormone (LH) on Leydig cells. Androgen acts directly on the Sertoli cells of seminiferous tubules with no direct action on germ cells. Androgen thus promotes the initiation and maintenance of spermatogenesis, although the detailed molecular and cellular mechanisms remain obscure (Vornberger et al., 1994; McLachlan et al., 1996).

As androgen acts on target cells through the androgen receptor (AR), defects of the AR result in complete or partial androgen insensitivity syndrome (AIS) which is observed as a range of phenotypes from complete sex-reversal (female phenotype) to infertility (male phenotype) (McPhaul et al., 1993; Quigley et al., 1995). Since the AR gene was isolated, many AR gene abnormalities have been reported in cases of AIS (McPhaul et al., 1993; Sultan et al., 1993; Quigley et al., 1995; Gottlieb, 1997). The AR gene, one member of the nuclear receptor supergene family, consists of three domains: the N-terminal domain, DNA-binding domain and hormone binding domain. Previously, most abnormalities in the AR gene have been identified with the DNA-binding and hormone-binding domains (McPhaul et al., 1993; Sultan et al., 1993; Quigley et al., 1995; Gottlieb, 1997). On the other hand, only a few abnormalities have been reported in the N-terminal domain which was considered to be related to gene regulation (Gottlieb, 1997). However, since it was observed that the elongation of CAG repeats in exon 1 of the AR gene caused pathogenesis of X-linked spinal and bulbar muscular atrophy (Kennedy’s disease), the N-terminal domain has also been considered to play an important role in AR function (La Spada et al., 1991; Belsham et al., 1992). This finding has led many researchers to focus on the correlation between CAG repeats and various diseases. Since the elongation of CAG repeats lengthens the string of glutamine residues in the AR, it probably affects the structure and function of the AR. On the other hand, males with a shorter CAG repeat length are considered to be at higher risk of prostate cancer because the shorter CAG repeat length is associated with high transcriptional activity of the AR gene (Giovannucci et al., 1997; Stanford et al., 1997). We have also showed that the short CAG repeats of the AR gene suppressed the function of the AR in a case of complete AIS (Komori et al., 1998). In infertile males, the decreased AR binding affinity has been previously reported in some oligozoospermic or azoospermic males (Aiman et al., 1979; Aiman and Griffin, 1981; Akin et al., 1991). Impaired spermatogenesis, such as oligozoospermia and azoospermia, has also been observed in patients with Kennedy’s disease. Puscheck et al. (1994) and Tincello et al. (1997) analysed the mutation of the AR N-terminal domain in infertile males with oligozoospermia and azoospermia, but no mutations in exon 1 were identified. However, they did not report any results related to the CAG repeat length. Therefore, the correlation between CAG repeat length and infertility is still unclear. In this study, we analysed the number of CAG repeats of the AR in Japanese infertile males with oligozoospermia. Our results indicate that a reduction in the CAG repeats is closely related to impaired spermatogenesis in Japanese infertile males.

Materials and methods

Subjects

CAG repeat lengths in exon 1 of the AR were determined for 59 idiopathic Japanese infertile males with oligozoospermia, whose
sperm counts were $<2 \times 10^7$/ml. Semen analyses were performed on three consecutive 4–8 week intervals after at least 2 days of abstinence, and the results were analysed according to World Health Organization guidelines. 36 normal fertile males, who had children, were used as normal controls. The subjects showed normal male phenotype with normal laboratory parameters. Ages ranged from 25 to 55 years. Informed consent was obtained from each subject.

**DNA isolation and polymerase chain reaction of the AR gene**

After the informed consent was obtained, DNA was isolated from peripheral blood samples from fertile and infertile males, and CAG repeats in exon 1 of the AR gene were amplified by the polymerase chain reaction (PCR) (Kasumi et al., 1993). The following primers were used.

Forward primer: AGAGCGTGCGGAAGCGATCAGAACCCG.

Reverse primer: AACGTGGA TGGGGCAGCTGAGTCA T.

The amplified DNAs were sequenced by an autossequencer (ABI 373A DNA sequencer; Applied Biosystems Japan, Tokyo, Japan) and conventional gel electrophoresis with dideoxynucleotide chain termination methods (Sanger et al., 1977). Statistical analysis was performed with Fisher’s Exact Test.

**Results**

Sequence analysis indicated that the CAG repeat length in infertile males ranged from 14 to 32 (mean $21.2 \pm 4.2$), whereas the number in fertile males ranged from 16 to 31 (mean $21.4 \pm 3.5$) (Figure 1). Among infertile males, six males possessed 14 CAG repeats and three males possessed 15 CAG repeats. On the other hand, no fertile males possessed fewer than 16 CAG repeats (Table I). The incidence of 14 and 15 CAG repeats in infertile males was significantly higher ($P < 0.05$) than those in fertile males. This result suggests that the reduction in the CAG repeats is closely related to impaired spermatogenesis.

**Table I. Correlation between the number of CAG repeats and infertile and fertile males**

<table>
<thead>
<tr>
<th>No. of CAG repeats</th>
<th>Idiopathic infertile males with oligozoospermia</th>
<th>Fertile males with normozoospermia</th>
</tr>
</thead>
<tbody>
<tr>
<td>$&lt;16$</td>
<td>9$^a$</td>
<td>0$^a$</td>
</tr>
<tr>
<td>$\geq16$</td>
<td>50</td>
<td>36</td>
</tr>
</tbody>
</table>

*Incidence of infertile males and fertile males compared using Fisher’s exact test ($P < 0.05$).

**Discussion**

During the process of spermatogenesis, androgen plays an important role in round spermatid development, attachment to Sertoli cells and elongation of spermatids (van Roijen et al., 1995; McLachlan et al., 1996). As androgen acts on target cells through the AR, impaired function of the AR induces abnormalities in androgen action resulting in abnormal conditions such as AIS and infertility. Many abnormalities of the AR gene have been identified in AIS (McPhaul et al., 1993; Sultan et al., 1993; Quigley et al., 1995; Gottlieb, 1997) and decreased AR binding activity has also been reported in infertile males (Aiman et al., 1979; Aiman and Griffin, 1981; Akin et al., 1991). In this study, we have shown that the incidence of infertile males with short CAG repeats is significantly higher than that of fertile males.

There have been many reports on the correlation between CAG repeat length and diseases such as Kennedy’s disease (La Spada et al., 1991, 1992; Biancalana et al., 1992; Igarashi et al., 1992; Doyu et al., 1993). These results have shown that in Kennedy’s disease the elongation of CAG repeats is closely related to pathogenesis. Men who possess exceptionally long CAG repeat lengths also experience androgen insensitivity.
presumably related to reduced transcription of the AR (La Spada et al., 1991). Recently, Tut et al. (1997) demonstrated that long CAG repeat length was related to impaired sperm production. In AIS, Komori et al. (1998) have shown that short CAG repeats affected AR function in in-vitro transfection experiments. McPhaul et al. (1991) also demonstrated that the short CAG repeats might be related to the pathogenesis of AIS. In prostate cancer, the variability in the CAG repeat length, probably through mediation of transcriptional activation of the AR, is associated with a risk of developing prostate cancer (Giovannucci et al., 1997; Stanford et al., 1997). In-vitro experiments have shown that the length of the polyglutamine chain, which is encoded by CAG repeats, is inversely correlated with the transcriptional activity of AR (Mhatre et al., 1993; Chamberlain et al., 1994; Kazemi-Esfarjani et al., 1995). Taken together, these results indicate that a suitable CAG repeat length is required for the maintenance of proper AR function. Therefore, it seems highly probable that short CAG repeats also affect sperm concentration in spermato genesis.

The CAG repeat length is highly polymorphic between races with the average CAG repeat length, ranging from 17 to 26 (Edwards et al., 1992). As African Americans generally have shorter CAG repeats (La Spada et al., 1991; Edwards et al., 1992; Giovannucci et al., 1997), they are therefore considered to be a high risk group for prostate cancer. In Japanese, the CAG repeat length ranges from 17 to 29 (Yamamoto et al., 1992; Doyu et al., 1993). In this study, nine infertile males with 14 or 15 CAG repeats were identified, whereas no fertile males were identified. Therefore, the high incidence of Japanese infertile males with the short CAG repeats seems to be significant, although the sample number examined is small. The short CAG repeat length most likely affects AR function and, as a result, causes the problems with spermatogenesis observed in Japanese infertile males.

References

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