Preliminary investigations on androgen receptor gene mutations in infertile men

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A total of 50 men were selected from all patients attending an infertility clinic on the basis of oligozoospermia or azoospermia with concentrations of luteinizing hormone >6 IU/l and testosterone >30 nmol/l. Six of these men responded to written invitation and DNA was extracted from blood leukocytes. Individual exons of the androgen receptor gene were amplified by polymerase chain reaction and screened for the presence of mutations by denaturing gradient gel electrophoresis. The glutamine rich portion of exon 1 was sequenced directly. All of the coding sequence of the gene was examined except the glycine rich portion of exon 1 in all patients. No mutations or deletions were identified. Androgen receptor gene mutations do not appear to be present in infertile men with biochemical disturbances compatible with androgen resistance. It is therefore unlikely that such mutations are a major factor in the pathogenesis of oligozoospermia/azoospermia and infertility.

Key words: androgen/infertility/mutation/oligozoospermia/receptor

Introduction

The syndromes of androgen insensitivity are X-linked genetic disorders characterized by failure of tissue responsiveness to circulating testosterone (Carson-Jurica et al., 1990). Both the complete form of the syndrome (testicular feminization) and the partial form are characterized by a failure of secondary sexual development in the presence of high concentrations of testosterone and normal functioning testicular tissue.

The syndromes are well recognized as having a genetic basis. The androgen receptor (AR) consists of eight exons (Carson-Jurica et al., 1990) in three functional domains (Chang et al., 1988). Point mutations or deletions of the AR gene have been identified in many patients with both complete androgen insensitivity (testicular feminization) and partial androgen insensitivity (Wilson, 1992; McPhaul et al., 1993). These mutations occur throughout the length of the gene, but are concentrated in the hormone binding domain and DNA binding domain of the receptor (McPhaul et al., 1992; Quigley et al., 1995). Some evidence suggests that the nature of a given mutation in a particular site can influence the degree of androgen resistance seen in the patient and hence the final phenotype (Saunders et al., 1992; Kazemi Esfarjani et al., 1993), but it has also been found that the same mutation in different patients can produce different clinical effects, implying that other factors are acting to determine the final functional effect of any mutation (McPhaul et al., 1992).

Atypical cases of androgen resistance indicate that the range of receptor abnormalities extends beyond gross effects such as abolition of ligand binding to include less apparent defects of function. It has been suggested that the spectrum of abnormalities may extend to include some men whose only presenting feature is infertility. It has been suggested (Aiman et al., 1979; Aiman and Griffin, 1982) that a mild form of androgen insensitivity may be responsible for infertility in a subpopulation of infertile men, based upon androgen binding characteristics of genital skin fibroblast (GSF) androgen receptors taken from a group of 18 azoospermic men. The incidence in the infertile population has been estimated to be 40% (Aiman and Griffin, 1982) although other groups have doubted the magnitude of this figure (Eil et al., 1985; Morrow et al., 1987). This theory has apparently been confirmed by the finding of a deletion of exon 4 of the AR gene in one of seven azoospermic men examined (Akin et al., 1991). We wished to examine the incidence of AR mutations in infertile men further and selected a sample of men from those attending a large infertility/andrology clinic based upon their hormonal profiles and semen analysis.

Materials and methods

Patients

Patients were selected from an infertility/andrology clinic in a teaching hospital. The records from each couple have been stored on computer since 1978 and data was available from 1700 couples (Hargreave and Elton, 1986). Follow-up information was available on all couples. We presumed that AR gene mutations would result in a mild degree of abnormality of the testicular/pituitary feedback loop causing mild to moderate elevation of androgen and/or luteinizing hormone (LH) concentrations. We therefore selected the following laboratory parameters for our initial search: testosterone >30 nmol/l (normal range in our laboratory 10-30), LH >6 IU/l (normal range 1.5–9.0) and a sperm density <20×10⁶/ml (normal range >20×10⁶/ml).
**Methods**

The extraction of DNA from peripheral blood leukocytes and polymerase chain reaction (PCR) were performed as previously described (Saunders et al., 1992). Exons of the androgen receptor gene were amplified using the primers described by DeBellis et al. (1992). These primers contain a ‘GC clamp’ to increase the sensitivity of denaturing gradient gel electrophoresis (DGGE) to >95% (Myers et al., 1987; Sheffield et al., 1989). The samples were screened for the presence of mutations using DGGE (Myers et al., 1987) using the gel parameters described (DeBellis et al., 1992). Samples showing different gel mobility relative to control samples of normal sequence were reamplified from the source DNA using the same primer sequences with the GC clamp removed, and sequenced as previously described (Saunders et al., 1992). Samples from the region of exon 1 containing the glutamine repeat region were sequenced to confirm the number of glutamine repeats and then run on DGGE gels alongside samples of normal sequence with the same number of glutamine repeats.

**Results**

Using the selection criteria detailed above a group of 50 men was selected (2.9% of the clinic population), 12 of whom wished no further follow-up and 32 of whom failed to respond to three standard letters inviting them to attend. A final group of six men (12% of the identified cohort) was successfully recruited and blood samples were taken from them. Relevant details of the patients involved in the study are shown in Table I.

All exons of the gene from 2 to 8 were amplified in all patients and found to be normal. Exon 1 proved difficult to amplify in many of the patients, especially across the glutamine and glycine repeat regions. The glycine repeat region of exon 1 did not amplify from the DNA of any patient, and the glutamine repeat region failed to amplify in patient 2. Of the exons of the gene examined by DGGE no samples with altered mobility were detected. The glutamine repeat region of exon 1 (A1, bases 250–312) showed variation in size between patients of 20–27 residues, all of which were within the normal range for this region.

**Discussion**

This study was undertaken to examine the hypothesis that infertile men with elevated testosterone and LH concentrations may possess mutations of the AR gene. It has been suggested that mutations of the AR gene may be a cause of infertility in men with unexplained infertility and elevated serum hormone parameters suggestive of mild androgen resistance (Aiman et al., 1979; Aiman and Griffin, 1982). We selected a cohort of men on the basis of elevated hormone parameters similar to those described by Aiman and Griffin (1982) in order to further investigate the hypothesis that mild AR abnormalities may be implicated in subfertility. No mutations were identified in six men studied. We were unable to examine the glycine rich region of exon 1, which is technically difficult to sequence. The function of this region is poorly understood, and we acknowledge that mutations may reside in this region.

We realise that this study is hampered by small numbers, but the patients studied were selected on biochemical grounds from a large database of patients. The criteria of elevated testosterone and LH with low or absent sperm density are comparable with the biochemical findings of patients with recognized androgen insensitivity associated with impaired receptor function. The LH–testosterone product (LH–T) can be used as an index of androgen resistance (Aiman et al., 1987) and in all of our patients LH–T was elevated well above the normal range (15–270). Using these criteria we selected 3% of 1700 men who were theoretically most at risk of an abnormality of AR function on the basis of their endocrine profile. We feel that the absence of detectable mutations in these men indicates that AR mutations are unlikely to be a common cause of idiopathic infertility, but realize that the sample size should be increased substantially to be confident of this conclusion. However, it is still possible that AR mutations may occur which cause subfertility in the absence of detectable biochemical disturbances. The next step would be to screen the AR gene from a large cohort of azoospermic and oligozoospermic men with normal biochemical parameters using the same methods as we have used and to combine this with in-vitro assays of receptor function.

On the basis of this small study we conclude that AR gene mutations are not likely to be a significant factor in the aetiology of male factor infertility in men with endocrine markers of androgen resistance. The findings in this study need to be verified by a larger study of azoospermic and oligozoospermic men with and without endocrine markers of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Testosterone (nmol/l)</th>
<th>LH (IU/l)</th>
<th>LH–T (× 10⁶/ml)</th>
<th>Spermatozoa</th>
<th>Comment</th>
</tr>
</thead>
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</tr>
<tr>
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<td>400.14</td>
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</tr>
<tr>
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<tr>
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<tr>
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<td>6.90</td>
<td>298.08</td>
<td>0.00</td>
<td>Azoospermic brother</td>
</tr>
</tbody>
</table>

LH = luteinizing hormone; LH–T = LH testosterone product.

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**Table I.** Hormonal profile of infertile patients

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D.G. Tincello, P.T.K. Saunders and T.B. Hargreave
Androgen receptor mutations in infertility

hypothalamic–pituitary disturbance. Furthermore, expression of the AR gene is controlled by an upstream promoter region which has recently been characterized (Faber et al., 1993). It will be of value to examine this region in infertile men with endocrine markers of androgen resistance to determine whether defects in the expression of a normal gene may play a role in the aetiology of unexplained infertility.

References


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