Androgen receptor gene and male infertility

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Androgens are critical steroid hormones that determine the expression of the male phenotype. Their actions are mediated by a single androgen receptor (AR) which, upon ligand binding, translocates to the nucleus to regulate the expression of androgen-responsive genes. Mutations that disrupt AR function totally result in the complete feminization of 46 XY individuals and the complete androgen insensitivity syndrome. Studies have revealed that AR mutations that do not lead to complete abrogation of its activity can cause a wide spectrum of milder androgen insensitivity syndromes, from ambiguous genitalia in newborn infants to ‘idiopathic’ male infertility. Recent studies indicate that missense amino-acid substitutions in the ligand-binding domain of the AR result in infertility through a novel mechanism that involves defective protein–protein interactions between receptor domains and coactivator proteins. Independent of missense mutations, studies involving Singaporean, Australian, North American and Japanese subjects indicate that increases in length of a trinucleotide repeat (CAG) tract, encoding a polyglutamine stretch in the transactivation domain of the AR, are associated with increased risk of defective spermatogenesis and undermasculinization. This association was however not observed in European populations, suggesting that the genetic background may play a significant role in the expression of the AR defects.

Key words: androgen receptor/genetic mutations/male infertility/trinucleotide repeat/undermasculinization

Introduction

Defective spermatogenesis is a common observation in subfertile men, but its definitive cause(s) in many men are unclear. However, it is becoming more evident in recent years that a significant proportion of male infertility is associated with a variety of genetic abnormalities. Genetic defects associated with male infertility that have been described include microdeletions of spermatogenic genes on the Y-chromosome (Krausz and McElreavey, 1999), genetic disorders affecting the secretion and action of gonadotrophins leading to androgen deficiency (Bhasin et al., 2000), and mutations in the cystic fibrosis transmembrane conductance regulator gene which is associated with azoospermic men with congenital aplasia of epididymis and vas deferens (Mickle and Cutting, 2000). In addition to these genetic disorders, our laboratory and others have demonstrated that mutations and polymorphisms of the androgen receptor (AR) gene and its expressed protein are significantly associated with depressed spermatogenesis and ‘idiopathic’ male infertility. In this review, we describe the molecular properties of the AR and particularly, studies that support the growing appreciation that pathogenicity of male infertility is transmitted through dysfunctional AR inter-domain and coactivator interactions. We will also discuss the status of CAG trinucleotide repeat lengths in the AR of normal and infertile males and its role in the pathogenesis of male infertility. PubMed was searched for primary papers using [CAG, androgen receptor, infertility] as keywords. Papers published after June 2002 were excluded from the review. Combined data from all published Asian studies are presented to complement existing European analyses.

Molecular biology of the AR

Development of the male phenotype and the initiation of spermatogenesis leading to production of the male gametes are intricately dependent on the cellular events that respond to androgens. The two most important physiological androgens are testosterone and 5α-dihydrotestosterone (DHT). The actions of androgens are mediated by the AR. Despite the existence of two different forms of androgens, only one AR has been identified and cloned (Trapman et al., 1988). Testosterone is crucial for the survival of the Wolffian duct and its subsequent development and differentiation into the epididymis, ductus deferens and seminal vesicles. DHT, a metabolite of testosterone, is involved in the development of the penis and scrotum. At puberty, androgens drive the initiation of spermatogenesis and growth of accessory sex organs, including the prostate. All these androgen-dependent developmental processes culminate in successful spermatogenesis; thus, perturbation to any of these steps can result in spermatogenic failure.
The AR is encoded by a single copy gene in the X-chromosome. The AR gene consists of eight exons, and encodes an intracellular transcription factor that belongs to the steroid/nuclear receptor superfamily; members of which include receptors to estrogen, progesterone, adrenal hormones, thyroid hormones, retinoid acid and vitamin D (Brinkmann and Trapman, 2000). Consistent with other steroid receptors, the AR—when activated by androgens—translocates to the nucleus and binds to specific chromosomal DNA sequences (androgen response elements) in the regulatory regions (promoters/enhancers) of AR-regulated genes. Binding of the androgen–AR complex activates, or represses, the expression of androgen-regulated AR-regulated genes. Binding of the androgen–AR complex facilitates by a ligand-dependent, high-affinity interaction between homodimers, adopting an antiparallel conformation that is drawn in CREB-binding protein (CBP/p300), pCAF and other cofactors to modify chromatin and initiate transcription by RNA polymerase II (Heinlein and Chang, 2002). Of the p160/SRC proteins, TIF2 in particular interacts most strongly with the AR (Ding et al., 1998) by binding to portions of the AR TAD (Irvine et al., 2000) and AR LBD regions (He et al., 1999; Yao et al., 1996; Berrevoets et al., 1998) and, in concert with CBP/p300, brings enhancer and promoter elements together to increase AR transactivation function (Shang et al., 2002). The significance of coactivator-AR function in infertility was demonstrated by AR mutations that lead to defective coactivator binding (see below), and also suggested by studies on genital skin fibroblasts from androgen insensitivity subjects in whom no AR gene mutation was detected (Adachi et al., 2000).

Spectrum of androgen insensitivity syndromes

Disorder of AR function due to mutations in the AR gene is the cause of various forms of male pseudohermaphroditism known as androgen insensitivity syndromes (AIS). To date, more than 300 mutations have been documented (www.mcgill.ca/androgendb). AR mutations that severely impair the amount, structure or activity in vivo, such as DHT, mibolerone and testosterone, but is not detected in the presence of antagonists that bind to the AR and fail to induce AR transcriptional activation, for example hydroxyflutamide and cyproterone acetate (Kemppainen et al., 1999). The physiological significance of this TAD/LBD interaction was further suggested by findings that several mutations responsible for androgen insensitivity syndrome disrupted this interaction (Langley et al., 1998; Ghadessy et al., 1999; Lim et al., 2000a; Thompson et al., 2001). Upon translocation into the nucleus, the AR also serves as a focus to recruit cofactors which either up-regulate (coactivators) or down-regulate (corepressors) AR activity. The most clearly defined class of coactivators are members of the p160/SRC family. These include steroid receptor coactivator 1 (SRC1), transcriptional intermediary factor 2 (SRC2/TIF2) and SRC3/TRAM1/AIB1/pCIP/ACTR/RAC3 and, which bind hydrophobic grooves in the LBDs of steroid receptors via LXXLL motifs in their nuclear-receptor interacting boxes, draw in CREB-binding protein (CBP/p300), pCAF and other cofactors to modify chromatin and initiate transcription by RNA polymerase II. Studies have shown that the AR activates transcription as homodimers, adopting an antiparallel conformation that is facilitated by a ligand-dependent, high-affinity interaction between the amino TAD and carboxyl LBD domains (Langley et al., 1995, 1998; Doesburg et al., 1997). Significantly, this TAD/LBD interaction is selectively induced by ligands that have AR agonist substitutions, which is a naturally occurring polymorphism.

Male infertility and minimal AIS

Defects in sperm production are found in the male partner in up to 30% of all subfertile couples (World Health Organization, 1991). Data from our laboratory and elsewhere have established that AR missense substitutions contribute about 2% (Wang et al., 1998a,b; Ghadessy et al., 1999; Hiort et al., 2000) and CAG repeat expansion (≥28 repeats) may contribute up to 35% of male infertility cases. The spectrum of male infertility associated with AR mutations is wide and includes disorders of spermatogenesis such as obstructive azoospermia, non-obstructive azoospermia and severe oligospermia. Sperm production is found in the male part of the spectrum, such as DHT, mibolerone and testosterone, but is not detected in the presence of antagonists that bind to the AR and fail to induce AR transcriptional activation, for example hydroxyflutamide and cyproterone acetate (Kemppainen et al., 1999). The physiological significance of this TAD/LBD interaction was further suggested by findings that several mutations responsible for androgen insensitivity syndrome disrupted this interaction (Langley et al., 1998; Ghadessy et al., 1999; Lim et al., 2000a; Thompson et al., 2001). Upon translocation into the nucleus, the AR also serves as a focus to recruit cofactors which either up-regulate (coactivators) or down-regulate (corepressors) AR activity. The most clearly defined class of coactivators are members of the p160/SRC family. These include steroid receptor coactivator 1 (SRC1), transcriptional intermediary factor 2 (SRC2/TIF2) and SRC3/TRAM1/AIB1/pCIP/ACTR/RAC3 and, which bind hydrophobic grooves in the LBDs of steroid receptors via LXXLL motifs in their nuclear-receptor interacting boxes, draw in CREB-binding protein (CBP/p300), pCAF and other cofactors to modify chromatin and initiate transcription by RNA polymerase II (Heinlein and Chang, 2002). Of the p160/SRC proteins, TIF2 in particular interacts most strongly with the AR (Ding et al., 1998) by binding to portions of the AR TAD (Irvine et al., 2000) and AR LBD regions (He et al., 1999; Yao et al., 1996; Berrevoets et al., 1998) and, in concert with CBP/p300, brings enhancer and promoter elements together to increase AR transactivation function (Shang et al., 2002). The significance of coactivator-AR function in infertility was demonstrated by AR mutations that lead to defective coactivator binding (see below), and also suggested by studies on genital skin fibroblasts from androgen insensitivity subjects in whom no AR gene mutation was detected (Adachi et al., 2000).
infertility (Tut et al., 1997; Yoshida et al., 1999; Mifsud et al., 2001). However, it should be appreciated that the genetic background influences the expression of AR dysfunction and that not all patients with minimal AIS are necessarily infertile.

Mutations in the transactivation domain of the AR

Although the almost 400 mutations in the AR are spread along the entire gene, only 19 in the TAD are reported to be associated with some form of androgen insensitivity (Hiort et al., 2000). This is a paradox, as it would be expected that mutations in the TAD, which constitutes more than half of the receptor and harbours the strongest activation function domain, would be a more commonly detected in AIS. One reason for this anomaly is that genetic examination of the TAD is difficult due to its large size and the presence of CG repeat tracts which are resistant to amplification by the polymerase chain reaction. In a large cohort study involving 180 males with variable impairment of spermatogenesis, genetic screening of the AR (Hiort et al., 2000) revealed the presence of a C→T substitution (CCG-TCG) in the TAD of two patients, resulting in substitution of serine for proline at position 390 (Hiort et al., 2000). Both patients had a decreased sperm count and a high percentage of abnormal sperm, but in-vitro functional assessment of this AR mutation showed no gross alterations of transcriptional activity compared with wild-type AR. Despite the limited correlation between in-vitro and in-vivo function, a critical role for Pro390 in maintaining the functional integrity of AR is suggested by the observation that a Pro390Asp AR mutation is associated with complete AIS (Vasiliou et al., 1994). A 20% reduction in AR transactivation capacity was also observed for a glycine-to-arginine substitution at position 214 in the AR TAD region of an infertile male (Wang et al., 1998a). However, this mutation may not be critical for AR function since the same mutation was also observed in a fertile control. It is noteworthy that a conservative nucleotide polymorphism in codon 211 (GAG-GAA) (Vasiliou et al., 1994; Wang et al., 1998a; Hiort et al., 2000), that is related to ethnic origin as it occurs in 10–15% of Caucasian but not Chinese subjects, appeared to be significantly related to male infertility (Mifsud et al., 2001).

Mutations in the LBD

The LBD of the AR is critical for specific binding of androgens and, through interdomain interactions with the TAD, leads to receptor activation through the recruitment of coactivator molecules (McKenna et al., 1999). Most reported mutations of the AR LBD lead to defective androgen binding, loss of receptor function and would be more commonly detected in AIS. Recent studies suggest a new paradigm with respect to AR mutations in the LBD and male infertility: pathogenicity is transmitted through reduced interdomain and coactivator interactions, and androgen analogues that are corrective in vitro may indicate hormonal therapy.

Genetic screening of infertile males has revealed several loci in the AR LBD that are associated with male infertility (Figure 1). An Asp727Lys mutation (N727K) was identified in a man who had no obvious phenotypic abnormality, except for reduced testicular volume and severe oligospermia (Yong et al., 1994). The patient was treated empirically with the androgen analogue mesterolone, and after 6 months of treatment, the sperm counts rose to a normal level of \(28 \times 10^9/\text{ml}\), suggesting that the androgen analogue mesterolone was able to restore normal sperm production. This mutation was particularly interesting because, although located in the LBD, it did not alter any ligand-binding characteristic of the AR. Nonetheless, the mutant AR displayed only half of wild-type transactivation capacity when exposed to physiological or synthetic androgens. Detailed molecular studies using chimeric proteins have established that the Asp727Lys mutation in fact lies outside the ligand-binding pocket of the LBD and disrupted AR function, not through defective ligand binding, but by interfering with external protein–protein interactions with the co-regulatory transcription factors (Lim et al., 2000a). Strikingly, mesterolone, but not the physiological androgen DHT, restored mutant LBD interactions with the TAD and with the coactivator TIF2, when expressed as fusion proteins in the two-hybrid assay.

Genetic screening also detected mutations that changed codon 886 in exon 8 from methionine to valine in three unrelated infertile men (Ghadessy et al., 1999). The Met886Val mutation (M886V; Figure 1) was significantly associated with the severely oligospermic phenotype, and was not detected in more than 400 control AR alleles. Like the Asp727Lys above, Met886Val did not have any androgen-binding abnormality but had a consistently reduced capacity to transactivate androgen-inducible reporter genes. Co-expression of AR domain fragments in mammalian and yeast two-hybrid studies suggest that the mutation disrupts interdomain interactions, which are critical for receptor dimerization, and protein–protein interactions with the coactivator, TIF2 (Ghadessy et al., 1999). These data suggest that functional elements centred around residues 727 and 886 have a role, not for ligand binding, but for inter-domain and coactivator interactions culminating in the formation of a normal transcription complex.

Crystallography data of the AR (Matias et al., 2000) and other nuclear receptors indicate that AR residues 727 and 886 are positioned on opposite ends of a TIF2 binding groove, providing a structural explanation as to how these mutations cause defective coactivator binding, minimal androgen insensitivity and impaired spermatogenesis. These two naturally occurring coactivator-defective AR mutants are the first descriptions of human disease caused by defective interactions of a steroid receptor with co-regulator proteins.

Other AR loci associated with male infertility include residues 798 and 712. Studies in two centres have independently identified a Gln798Glu exchange in two patients with severely oligospermia (Q798E; Figure 1) (Wang et al., 1998b) and oligoteratozoospermia with severely deformed spermatozoa (Hiort et al., 2000). Incubation of cells containing the mutated AR showed no receptor transactivation impairment when exposed to DHT but whose activity was reduced in the presence of testosterone. In addition, this defect could not be overcome by increasing testosterone concentrations, suggesting that the Gln798Glu mutation may cause a subtle defect in spermatogenesis due to selective response to different androgens. Pharmacological treatment as a potential therapy for AR LBD dysfunction was also illustrated by the ability to restore mutant AR function in AIS patients carrying a Leu712Phe mutation in the AR LBD (L712F; Figure 1) (Holterhus et al., 2000). Androgen binding studies using in-vitro cultured genital skin fibroblasts from one of the patients indicated that the mutant Leu712Phe AR is deficient at low concentrations of testosterone or DHT, and this was reverted by higher...
testosterone concentrations (>1 nmol/l). When all four affected individuals were administered testosterone therapy, three responded well. These studies suggest that AR LBD mutations resulting in suboptimal AR–ligand interactions may be addressed by judicious selection of androgen analogues. However, not all AR LBD mutations causing reduced sensitivity to androgens result in male infertility. A Gln824Lys mutation in the LBD of AR was reported in three related men who presented with gynaecomastia, although they appeared as otherwise-normal males (Q824K; Figure 1) (Givercman et al., 2000). In-vitro binding studies indicated that the binding of androgen to the receptor was not affected. The transactivation activity of the mutant AR was normal when the synthetic androgen R1881 was used as a ligand, but with DHT (a naturally occurring androgen) its activity was 10–60% compared with that of wild-type AR. Testicular biopsy evaluation revealed that there was ongoing spermatogenesis, despite some reduction in spermatic number. In addition, one of the men possessing the mutation had fathered a daughter, indicating that the Gln824Lys mutation affected somewhat the in-vitro and in-vivo function of the receptor but nevertheless is compatible with fertility. In a recent pedigree study involving 14 affected males from a large Chinese family, an Arg840Cys substitution was identified in exon 7 of the AR (R840C; Figure 1) (Chu et al., 2002). However, some affected males were infertile and showed gynaecomastia and/or hypospadias, but some had fathered children normally. This suggested that androgen action may influence spermatogenesis and genitalia differentiation through different pathways, and that spermatogenesis may be normally accomplished under a wide range of defects in the AR. Intriguingly, hormonal analysis revealed that LH and FSH levels were normal in the patients whose fertility was not affected. Since an increased LH level is a clinical feature of AIS as it reflects impaired androgen action in regulating gonadotrophin secretion, the findings raised the possibility that normal LH and FSH levels may indicate relatively intact testicular function in these AIS patients.

## CAG repeat polymorphisms and male infertility

Glutamine-rich regions of proteins occur widely in both prokaryotes and eukaryotes. They are frequently found as polyglutamine tracts, encoded by CAG repeats, often of considerable length. Despite this wide distribution, the functions of CAG repeats are often unclear. However, expansion of CAG repeats in genes has been implicated in the pathogenesis in a number of progressive neurodegenerative diseases including Huntington’s disease, spinocerebellar ataxia and dentatorubral-pallidoluysian atrophy (Lieberman and Fischbeck, 2000). More importantly, expansion of the CAG tract in the AR leads to spinal bulbar muscular atrophy (SBMA), an adult-onset neuromuscular disease that also presents with endocrinological abnormalities including low virilization, oligospermia or azoospermia and testicular atrophy (La Spada et al., 1991). The CAG tract in exon 1 of the AR is expanded in all SBMA patients studied (range 40–60 repeats) (Fischbeck, 1997), and endocrinological studies have confirmed a significant correlation between CAG repeat length and age of onset of gynaecomastia (Dejager et al., 2002). Of the 22 patients studied, 19 showed clinical signs of partial AIS, including elevated testosterone levels with elevated LH levels in 15 cases. Furthermore, a consistent positive correlation was found

### Table I. Published data on androgen receptor (AR) polymorphic trinucleotide (CAG) repeat numbers in infertile men and normal controls.

<table>
<thead>
<tr>
<th>Study population</th>
<th>Infertile/ fertile controls</th>
<th>Infertile category</th>
<th>Infertile (CAG) repeat mean(range)</th>
<th>Control (CAG) repeat mean(range)</th>
<th>Significant difference? (P-value)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Singapore Chinese</td>
<td>153/72</td>
<td>Varying degrees of impaired spermatogenesis (varied)</td>
<td>ND</td>
<td>ND</td>
<td>4-fold increased risk of depressed spermatogenesis if &gt;28 CAGs</td>
<td>Tut et al. (1997)</td>
</tr>
<tr>
<td>Japanese</td>
<td>41/48</td>
<td>Azoospermic</td>
<td>26.5(20–34)</td>
<td>23.9(17–30)</td>
<td>S (0.0013)</td>
<td>Yoshida et al. (1999)</td>
</tr>
<tr>
<td>Australian</td>
<td>35/32</td>
<td>Varied</td>
<td>23.2(15–34)</td>
<td>20.5(17–25)</td>
<td>S (0.0001)</td>
<td>Dowsing et al. (1999)</td>
</tr>
<tr>
<td>UK</td>
<td>78/850</td>
<td>Moderate/severe undermasculinization</td>
<td>25(23–26)</td>
<td>23(22–26)</td>
<td>S (0.002)</td>
<td>Lim et al. (2000b)</td>
</tr>
<tr>
<td>American</td>
<td>69/45</td>
<td>Severe/extreme severe oligospermic</td>
<td>23.5(18–39)</td>
<td>22(12–30)</td>
<td>S (0.03)</td>
<td>Patrizio et al. (2001)</td>
</tr>
<tr>
<td>American</td>
<td>95/55</td>
<td>Oligo/azoospermic</td>
<td>21.95(14–31)</td>
<td>20.72(8–27)</td>
<td>S (0.034)</td>
<td>Mifsud et al. (2001)</td>
</tr>
<tr>
<td>Singapore Chinese</td>
<td>33/87</td>
<td>Oligo/azoospermic</td>
<td>23.82(18–33)</td>
<td>22.38(11–29)</td>
<td>S (0.043)</td>
<td>Mifsud et al. (2001)</td>
</tr>
<tr>
<td>French</td>
<td>37/50</td>
<td>Oligo/azoospermic</td>
<td>23.91(13–28)</td>
<td>22.17(17–27)</td>
<td>S (0.008)</td>
<td>Wallerand et al. (2001)</td>
</tr>
<tr>
<td>Swedish</td>
<td>33/294</td>
<td>Varied</td>
<td>21.9(16–27)</td>
<td>23.2(8–30)</td>
<td>NS</td>
<td>Givercman et al. (1998)</td>
</tr>
<tr>
<td>German</td>
<td>180/53</td>
<td>Varied</td>
<td>23(13–30)</td>
<td>24(17–39)</td>
<td>NS</td>
<td>Hiort et al. (1999)</td>
</tr>
<tr>
<td>German</td>
<td>119/22</td>
<td>Varied</td>
<td>22(16–34)</td>
<td>20(15–26)</td>
<td>NS</td>
<td>Dadde et al. (2000)</td>
</tr>
<tr>
<td>German</td>
<td>30/62</td>
<td>Azoospermic</td>
<td>20.7(17–27)</td>
<td>20.6(12–25)</td>
<td>NS</td>
<td>von Eckardstein et al. (2001)</td>
</tr>
<tr>
<td>Japanese</td>
<td>30/51</td>
<td>Azoospermic</td>
<td>23.4(19–30)</td>
<td>23.7(17–28)</td>
<td>NS</td>
<td>Sasagawa et al. (2001)</td>
</tr>
<tr>
<td>Dutch</td>
<td>75/70</td>
<td>Varied</td>
<td>22.2 ± 3.1*</td>
<td>21.7 ± 3.4*</td>
<td>NS</td>
<td>Van Golde et al. (2002)</td>
</tr>
</tbody>
</table>

*Values are mean ± SD. Abbreviations: oligo = oligospermic; ND = not determined; S = significant; NS = not significant. # This study suggested that short AR CAG repeats was associated with impaired spermatogenesis.
between the degree of endocrine dysfunction (as defined by LH×testosterone) and the number of CAG repeats wherein the longer the CAG, the more pronounced the androgen insensitivity. The CAG repeat length associated with SBMA patients is in contrast with the reported AR CAG repeat distribution for normal fertile populations. Depending on ethnic origin, the observed CAG repeat length for fertile populations can vary from 8 to 30 among American whites, from 8 to 39 among Europeans (Rajpert-De Meyts et al., 2002), and from 11 to 31 among Asians (Table I; Figure 2). Indeed, in-vitro studies have demonstrated an inverse relationship between CAG repeat length and AR function: progressive expansion of the CAG repeat in AR caused a linear decrease of transactivation function (Chamberlain et al., 1994). In addition, short alleles were found to be associated with the androgen-dependent tumour, prostate cancer. Short CAG repeats increase AR androgenicity and result in abnormally high stimulation of prostatic tissue, leading to an increased risk (Hsing et al., 2000), earlier age of onset, increased tumour grade and extra-prostatic extension in prostate cancer (Giovannucci et al., 1997; Stanford et al., 1997).

Consistent with the inverse relationship between CAG repeat length and AR function, our laboratory and several other groups have shown that longer CAG repeats are found in the AR of patients with defective spermatogenesis. In a Singaporean population of mixed Asian origin, patients with a CAG tract of more than 28 repeats in their AR had a more than 4-fold increased risk of reduced spermatogenesis (Tut et al., 1997). This trend was also observed in Australian (Dowsing et al., 1999), Japanese (Yoshida et al., 1999), American (Mifsud et al., 2001; Patrizio et al., 2001) and French (Wallerand et al., 2001) populations wherein subfertile men with idiopathic azoospermia or oligo-azoospermia had significantly longer CAG repeat tracts than controls (Table I). Strikingly, longer AR CAG repeats are also associated with moderate to severe undermasculinization in male infants (undermasculinized: n=78, median 25, range 23–26; control: n=850, median 23, range 22–26, P=0.002) (Lim et al., 2000b). Taken together, the data support the hypothesis that longer CAG tracts have reduced intrinsic AR activity, leading to depressed spermatogenesis and male infertility. However, these findings could not be corroborated in studies involving either Swedish (Giwercman et al., 1998), German (Dadze et al., 2000; von Eckardstein et al., 2001), Dutch (Van Golde et al., 2002) or Danish (Rajpert-De Meyts et al., 2002) population studies wherein no statistically significant relationship was found between the size of CAG repeats and idiopathic defective spermatogenesis. Combined data from previously published European studies also did not find any difference in the CAG repeat length distribution between fertile and infertile males (Rajpert-De Meyts et al., 2002). However, although a German study did not observe correlation with infertility, it documented an inverse correlation between sperm counts and CAG length in those whose sperm counts are within normal limits (von Eckardstein et al., 2001). The relationship between CAG repeats and defective spermatogenesis is strongest in those with azoospermia, and least in those who are mildly affected. Similarly, in a large study involving 223 infertile and 181 normal Belgian men in whom there was no correlation with infertility, it was also observed that more males with spermatogenesis problems had longer CAG repeats than expected for the control males (Legius et al., 1999). The cause of the disparities from different studies is unclear at present, but may be related to the influence of ethnicity differences. To shed some light as to whether ethnic differences could be a contributing factor, we have analysed combined CAG repeat length data from all published Asian studies, including two reports that did not show any significant differences between control and patient groups. Figure 2 shows that the distribution of CAG repeat length between fertile controls and infertile men was significantly different (Mann–Whitney U-test, P<0.005). The median, mean and range values for normal fertile controls were 23, 23.05 ± 3.11 and 11–31; and corresponding values for infertile patients were 24, 24.39 ± 3.69 and 14–34. The mean difference in CAG length between fertile and subfertile groups was 1.34 [95% CI 0.80 to 1.88], t-test, P<0.005). In comparison to the European studies (Rajpert-De Meyts et al., 2002), although normal fertile Asians have a higher mean CAG repeat length (mean 23.05 versus 22.4), Europeans have a bigger range in their AR CAG repeat lengths (range 8–39 versus 11–31). As a result, it is conceivable that variations in CAG repeat lengths may be better tolerated in European populations where the CAG repeat range is wider as compared to Asian populations where variations within a narrower CAG repeat range may be more significant in terms of functional disruption of the AR. Alternatively, because impaired spermatogenesis has a multifactorial aetiology, a long CAG repeat length may be a significant factor only when other factors that negatively influence spermatogenesis are also present.
Recent studies have provided more insights into the molecular mechanism of how the CAG tract affects the functional competence of AR. The transactivation domain of AR is important for normal AR function, providing the essential AF-1 function and coactivator interactions. It was shown (Irvine et al., 2000) that with increasing CAG repeats length, p160-mediated coactivation (such as AIB1, SRC-1a, GRIP1) of AR is repressed. It was further demonstrated that the partial loss of AR function (with 65 CAG repeats) is due to decreased mutant AR protein that has been preferentially targeted for degradation via the ubiquitin-proteasome pathway (Lieberman et al., 2002). The mutant receptor also undergoes altered post-translational modifications such as hyperacetylation and phosphorylation, thereby targeting AR for ubiquitilation and degradation (Lin et al., 2002).

The action of the AR depends also on the level of bioactive androgen available for binding to the receptor. To understand the factors in the androgen economy which regulate levels of bioactive androgen, the levels of testosterone (T), sex-hormone binding globulin (SHBG) and the AR CAG repeat length were measured and compared to total and free prostate-specific antigen (PSA) in 91 subjects with proven fertility, and 112 subfertile men with defective spermatogenesis (Mifsud et al., 2001). PSA is an important tumour marker, the expression of which is thought to be androgen-dependent. Total T, T adjusted for SHBG, and free T were 17–20% lower in subfertile men compared to their fertile counterparts. This subtle, but highly significant, difference in T between fertile and subfertile men was accentuated by the positive correlation between T and AR CAG length in fertile, but not subfertile subjects. In subfertile subjects, testosterone strongly correlated with PSA levels, and independent of T, total PSA negatively correlated with AR CAG length. Overall, these data suggest that, first, PSA correlates with T only in an environment of relatively low androgenicity, such as in subfertile men. Second, in such a low-androgenic environment, short CAG tracts (associated with high AR activity) correlate positively with PSA levels, thereby suggesting that interpretation of PSA is best made in conjunction with T levels and AR CAG length.

Conclusion

The genetic screening of patients has uncovered mutations and polymorphisms of the AR that lead to abnormal AR function resulting in ambiguous genitalia, undermasculinization and male infertility. The picture that emerges is that of a complex interplay between the genome and proteome. Nevertheless, detailed characterization of the molecular mechanisms of AR dysfunction in AIS, together with a thorough phenotype profiling, may lead to effective therapy and usefull genetic counselling for affected individuals and families. In view of the success of testicular sperm aspiration and the prospects of successful conception following ICSI in azoospermic men (Cram et al., 2000), screening for AR mutations and appropriate pre-conception counselling should be offered to all subfertile men.

References


Hiort, O., Holterhus, P.M., Horter, T., Schulze, W., Kremke, B., Bals-Patrach, E.L.Yong, C.J.Loy and K.S.Sim
Androgen receptor mutations and infertility


