

Genetics of Male Infertility: Role of Androgen Receptor Mutations and Y-Microdeletions

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Abstract

Introduction: Although infertility affects about 5% of the male population, its cause in most cases is uncertain. Normal spermatogenesis depends on a sequential cascade of genetic events triggered by factors encoded by the sex chromosomes. To determine the contribution of genetic aberrations to male infertility, the X-linked androgen receptor gene and the Y-chromosome were examined for mutations in a large cohort of infertile men. **Methods:** Screening of the androgen receptor (AR) gene for single-stranded conformation polymorphisms, confirmation by DNA sequencing, structure-function studies with androgen-responsive reporter genes and chimeric-protein constructs were performed. Y-chromosome microdeletions screening was done with multiplex polymerase chain reaction (PCR) analyses. **Results:** Genetic screening of over 400 patients and controls showed that defects in the androgen receptor gene lead to the production of dysfunctional receptor protein in 15% of males with abnormally low sperm production. The dozens of mutations and polymorphisms uncovered were associated with reduced intrinsic androgen receptor activity and involve principally two regions of the androgen receptor. Gene-transfer experiments implicated defective intermolecular protein-protein interactions with coactivator molecules as the cause of reduced receptor function. Submicroscopic deletions of the Y-chromosome were also been detected in about 6% of patients with severely reduced spermatogenesis. The deleted segments encoded RNA-binding proteins of unknown function and are not linked to defects in the androgen receptor. **Conclusions:** Mutations and polymorphisms of the AR, and Y-microdeletions cause defective sperm production and male infertility in about 20% of subfertile men. These traits can be transmitted to progeny, and counselling can be offered to affected families. Clarification of the molecular mechanisms of pathogenesis has led to rational hormonal therapy.

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Key words: Androgen receptor, Defective spermatogenesis, Y-chromosome microdeletions

Introduction

Three to four per cent of men have severe defects in sperm production that result in infertility.¹ Most of these men are healthy and the causes of depressed spermatogenesis are rarely identified with certainty. Clear-cut causes of male infertility, including mumps, hypogonadotropic hypogonadism and obstructive or infective conditions of the ejaculatory ducts, account for less than 30% of the cases. In the majority of infertile men, the cause of infertility is tentative and has to be classified as idiopathic. In recent years, the genetic causes of male infertility have come to the forefront.² Our laboratory has been instrumental in proving that polymorphisms and mutations of the androgen receptor (AR) gene, in the long arm of the X-chromosome, cause up to 15% of male infertility.³ In addition, we have also shown that microdeletions in the Y-chromosome

occur in 6% of Singapore patients with male infertility.⁴ Knowledge of the critical role that the AR plays in the pathogenesis of these diverse conditions has led to improvements in diagnostic methods and successful therapeutic strategies.

Androgen Receptor and Male Fertility

The physiological androgens, testosterone and dihydrotestosterone (DHT) mediate differences between man and woman. Androgens sculpture the human embryo *in utero* to cause development of male internal and external genitalia. At puberty, a surge of androgens leads to the initiation of spermatogenesis and growth of accessory sex organs, including the prostate gland. All androgens act through a single intracellular AR, which is encoded by a single-copy gene in the X-chromosome (Fig. 1). Disruption

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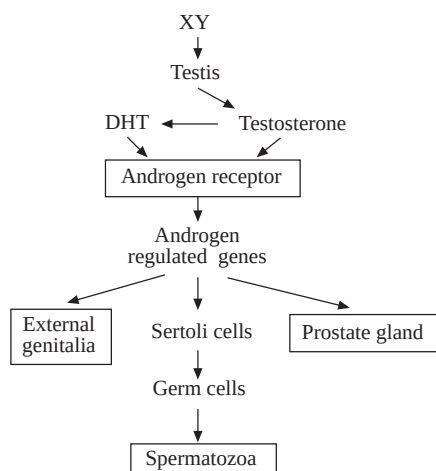
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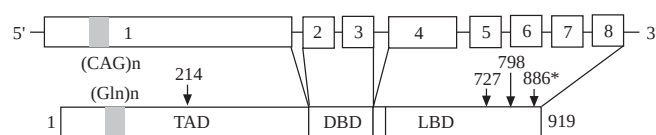
DHT: dihydrotestosterone

Fig. 1. Schematic representation of androgen-regulated events leading to male sexual differentiation, spermatozoa production and growth of the prostate gland. Mutations/polymorphisms of the androgen receptor that render the body insensitive to androgens cause varying degrees of androgen insensitivity leading to defects in male genital development, reduced sperm production and male infertility.

of the AR by genetic mutation results in complete androgen insensitivity syndrome (AIS) and sexual reversal in otherwise healthy 46XY individuals.⁵ Patients with complete AIS have normal male levels of testosterone but are completely female in external appearance. Since the receptor is not essential to life, AR gene mutations are relatively common and over 300 different mutations are presently documented in a mutational database,⁶ making it the most mutated steroid receptor. This review will focus on the role of AR polymorphisms and mutations in minimal androgen insensitivity resulting in defective spermatogenesis and male infertility.

Androgen Receptor Structure-Function Correlates

The AR is a ligand-activated, intracellular transcription factor that belongs to the steroid/nuclear receptor superfamily, which also includes receptors to oestrogen, adrenal hormones, thyroid hormone, retinoid acid and vitamin D.⁷ In common with other steroid receptors, the AR, when activated by androgens, translocates to the nucleus and binds to specific genomic 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 proteins. In conjunction with co-activator proteins like SRC1 or TIF2, the liganded AR form pre-initiation complexes in promoters thereby turning on gene transcription and protein synthesis.⁸ The AR, like other members of the steroid receptor superfamily, has three main functional domains: the transactivation domain (TAD), the DNA binding domain (DBD) and the ligand-binding domain (LBD) (Fig. 2).



*Three unrelated subjects have mutations in this residue.

Fig. 2. Structure of the androgen receptor gene and protein. The gene consists of eight exons which when translated, results in a protein with three main domains: the transactivation domain (TAD), the DNA-binding domain (DBD) and the ligand-binding domain (LBD). The TAD has a polymorphic glutamine (Gln) tract (shaded box) encoded by varying numbers (n) of the trinucleotide repeat, CAG. Arrows indicate residues where amino acid substitutions causing male infertility and depressed spermatogenesis are located.

The CAG Trinucleotide Tract in the Androgen Receptor

The polymorphic trinucleotide repeat segment, (CAG)_n, encoding a polyglutamine tract in the TAD, has been the source of unprecedented interest in recent years because of the discovery that expansion of this tract leads to spinal bulbar muscular atrophy (SBMA), a fatal neuromuscular disease.⁹ Trinucleotide repeat expansions are now implicated in many neuromuscular diseases,¹⁰ including Fragile X syndrome, Huntington's disease, spinocerebellar ataxia type 1, dentatorubral-pallidolucyian atrophy and Machado-Joseph's disease and Friedrich's ataxia. Interestingly, SBMA patients also have evidence of reduced AR function in the form of low virilisation, oligospermia (reduced sperm production) or azoospermia (no sperm production), testicular atrophy and reduced fertility.^{11,12}

Long CAG Trinucleotide Tracts and Male Infertility

Besides the pathological expansions of polyglutamine lengths found in SBMA, short alleles were found to be associated with the androgen-dependent tumour, prostate cancer. Thus, short CAG repeats increase AR androgenicity and resulting in abnormally high stimulation of prostatic tissue with a resultant earlier age of onset, increased tumour grade and increased risk of extra-prostatic extension in prostate cancer.^{13,14} If short CAG lengths can lead to disease in androgen-dependent tissues, we wondered whether at the other end of the spectrum, long CAG repeats might cause reduced AR androgenicity and disease. Such a disease must be one that is extremely sensitive to reduction of AR function. In this light, we decided to investigate spermatogenesis and CAG length. Spermatogenesis, as discussed earlier, is very dependent on high levels of androgens. The lengths of the CAG repeats in 153 patients with defective sperm production were compared to 72 normal controls of proven fertility.¹⁵ The distribution of AR-CAG alleles suggested that patients with reduced sperm counts differed significantly, with respect to the prevalence of CAG repeat length beyond 26, when compared to normal fertile controls. Patients with ≥ 28

CAGs in their AR had more than 4-fold increased risk of reduced spermatogenesis. There was also a trend whereby the more severe the spermatogenic defect, the higher the proportion of patients with the longer polyglutamine alleles. These results have now been re-confirmed in an Australian population, wherein 30 infertile patients with idiopathic azoospermia or oligozoospermia had significantly longer CAG repeat tracts than controls.¹⁶ The odds of having a CAG repeat length of 20 were 6-fold higher for fertile men than for men with a spermatogenic disorder. The data indicate an inverse relationship between the length of the polyglutamine tract in the AR and the risk of reduced spermatogenesis. Interestingly, ARs with CAG lengths of ≤ 22 , the same size that was found to give an excessive risk of prostate cancer,¹³ were associated with a reduced risk of male infertility,¹⁵ suggesting that the greater androgenicity associated with the shorter AR-CAG alleles could boost germ cell replication but at the long-term risk of overstimulating the growth of prostatic tissue (Fig. 3).

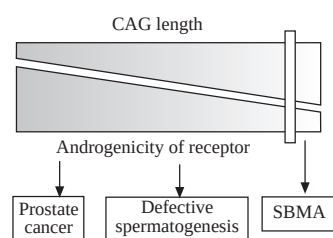


Fig. 3. Diagram showing the inverse relationship between the length of the CAG repeat tract and the function of the AR. Long AR-CAG alleles result in AR with low androgenicity and increase risk of defective spermatogenesis. Expansion of the CAG tract to beyond a threshold (bar) leads to a fatal neuromuscular disorder (SBMA), a condition in which defective spermatogenesis and virilisation are also observed. Conversely, short CAG tracts are associated with increased androgenicity of the receptor and increased risk of prostate cancer, androgenetic alopecia in men and hirsutism in women.

Molecular Basis of Differential AR Activity Associated with CAG Repeats

Short AR-CAG alleles encoding short polyglutamine tracts are associated with high intrinsic AR activity in reporter gene assays.¹⁵ A possible molecular mechanism as to how a change in polyglutamine length can affect the activity of the receptor has been suggested by Hsiao et al.¹⁷ They had identified a novel nuclear G-protein, Ras-related nuclear protein/ARA24 that act as a coactivator with the AR and can bind differentially with different lengths of polyglutamines within AR. AR-CAG/ARA24 interactions become stronger as the number of glutamines decreases, thereby increasing coactivation and AR transactivation capability. Although the likely increase in AR intrinsic activity with each reduction in AR-CAG length is relatively small, these effects are genetically determined and therefore exert its effects over the entire lifetime of the individual.

Small changes can over time have significant cumulative pathological effects. Collectively, the evidence supports the hypothesis that the glutamine repeat has a role in AR function by fine-tuning the balance between excess and deficient receptor function (Fig. 3). The highly polymorphic nature of the glutamine repeat would imply a subtle gradation of AR function among individuals, possibly allowing alleles with evolutionary advantages to be rapidly selected and transmitted to future generations. This function of the AR polyglutamine length could be essential in view of the changing environment and the demands of reproduction versus other androgen-regulated tissues. Besides the polyglutamine tract, some point mutations in the transactivation domain have been related to male infertility.¹⁸

Subtle Mutations in the AR-LBD Cause Male Infertility by a Novel Mechanism

Mutations of the AR-LBD that lead to minimal AIS are the most interesting as there are possibilities for hormonal correction. Systematic screening of AR-LBD by single-stranded conformation polymorphism (SSCP) in 200+ patients with idiopathic male infertility and impaired spermatogenesis³ led to the identification of 5 unrelated subjects with exonic mutations, 3 involving codon 886 (M886V) and 1 each in codon 727 (N727K) and 798 (Fig. 2). The M886V mutation was significantly associated with the severely oligospermic phenotype and was not detected in 400+ control AR alleles.¹⁹ Despite the location of this substitution in the LBD of the AR, the mutant receptor did not have any androgen-binding abnormality. However, the mutant receptor had a consistently reduced capacity to transactivate androgen-inducible reporter genes. Co-expression of AR domain fragments in mammalian and yeast two-hybrid studies suggests that the mutation disrupts protein-protein interactions with the transcriptional intermediary factor, TIF2.¹⁹ This is the first description whereby defective interactions of a steroid receptor with co-regulator proteins cause human disease.

The N727K mutation occurred in a patient who had no evidence of androgen insensitivity, except for reduced testicular volume.²⁰ He had severe oligospermia with sperm counts ranging from 2 to 3 million/mL. In this case 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 million/mL (Fig. 4). His wife conceived during this period and subsequently delivered a healthy infant. Mesterolone was stopped during this time and sperm counts dropped to 5 million/mL. Since the entire spermatogenic process needs about 74 days, the temporal pattern of the sperm densities, rising or falling three months after starting or stopping mesterolone treatment strongly suggests that the androgen analogue was able to

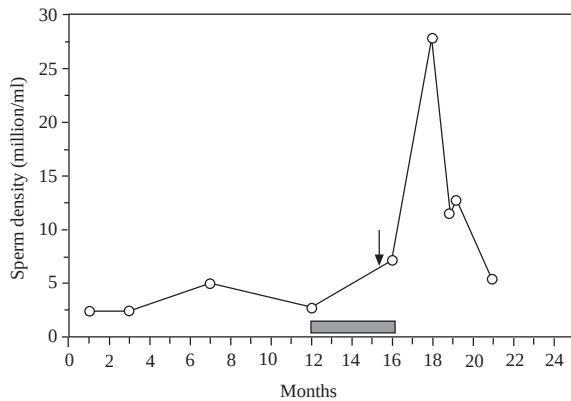


Fig. 4. Treatment of androgen receptor mutations associated with depressed spermatogenesis.²⁰ A patient with severely impaired sperm production, associated with an amino acid substitution (N727K) in the AR, was treated with the androgen analogue mesterolone, for a period of 4 months as indicated by the shaded bar. His sperm counts rose to normal levels and his wife conceived spontaneously (arrow) at the end of treatment period.

restore normal sperm production. This case suggests that hormonal treatment is possible for male infertility associated with mild androgen insensitivity.

Y-chromosome Microdeletions and Male Infertility

Since the first cytogenetic report of Y-deleted segments in 1976,²¹ mapping studies on the Y-chromosome have been greatly facilitated by the use of polymerase-chain-reaction (PCR) techniques to amplify sequence-tagged-sites (STS) spanning the deleted regions.²² Using these STS, the presence of submicroscopic deletions in the Y-chromosome has been detected in a number of azoospermic and severely oligospermic men and it was postulated that azoospermic factors (AZF) are present in three non-overlapping deleted subregions, AZFa, AZFb and AZFc, located in the proximal, central and distal segments of Yq11 respectively.²³ Candidates for azoospermic factors belong to two gene families that encode testis-specific, RNA-binding proteins: the RNA-binding-motif (RBM) gene family from the AZFb, and the deleted-in-azoospermia (DAZ) from the AZFc subregions. Microdeletions in the AZFb and AZFc subregions result in azoospermia or severe oligospermia, but the reported proportions of infertile men with deletions in one or the other subregion have varied widely, ranging from 3% to 30%.⁴ Initial studies have concentrated on azoospermia or severely oligospermic men, but one large study of 400 infertile men and fertile controls has suggested that Y-deletions can be detected in men with mild sperm abnormalities and even in fertile controls.²⁴ Although some of these differences were imputed to ethnic factors, patient selection criteria or the use of different STS probes; the presence of deletions in fertile men throws doubt on the cause and effect relationship between Y-microdeletions and male infertility.

To clarify some of these ambiguities, we screened 303

Singapore men for Y-microdeletions using STS probes that span the entire AZFb and AZFc regions, including probes specific for the coding regions of RBM1 and DAZ.⁴ Of the 202 patients with male infertility and 101 fertile controls examined, 6.8% (3/44) of azoospermic and 3.5% (3/86) of severely oligospermic patients were found to have Y-microdeletions. The prevalence of Y deletions was at the lower end of the 3% to 37% range in previous studies. Microdeletions were about twice as frequent in our azoospermic patients compared to the severely oligospermics. Remarkably, this 2:1 ratio had been consistent in 9 of 10 studies which showed that the combined prevalence of Y-microdeletions in azoospermic subjects was 11%, compared to 5% in severely oligospermic subjects.⁴ Overall, combined data totalling over 1600 subjects worldwide indicate that Y-microdeletions were most common in azoospermic males, and were not found in less severely affected patients and in normal fertile males; strongly indicating that the deleted segments encode factors essential for spermatogenesis.

Clinical Implications

Our findings that up to 20% of infertile males have AR mutations or Y-microdeletions have important implications for the management of male infertility. Definite diagnoses and prognoses can now be provided for patients that were previously miss-classified as idiopathic. A good case can be made for genetic screening in all men with severely affected sperm production, since the genetic defect can be precisely defined in a significant number of cases. Genetic counselling and risk assessment for transmission to progeny can be provided, providing relief from the debilitating and frustrating psychological state of "not knowing why", and allowing the patient to decide on treatment options with full knowledge of its likely consequences. Discovery of the molecular mechanisms whereby AR mutations cause disease has led to effective treatment as discussed above. Rational therapy for AR mutations, whereby specific ligands are designed to fit different mutant receptors, is currently being explored and should be a viable treatment option in the near future.

The Future

Although AR polymorphisms/mutations and Y-chromosomal deletions contribute to about 20% of male infertility cases, the cause of low sperm counts in a large proportion of subjects is still unknown. There is every likelihood that new genetic mutations relating to male infertility will be uncovered, as mice in diverse gene-knockout experiments exhibit the infertile male phenotype only.²⁵ Another concern is that people with very low sperm counts due to genetic mutations can have offspring with the advent of *in vitro* fertilisation and sperm-injection

techniques using immature germ cells. Genetic mutations associated with male infertility will be transmitted with largely unknown long-term consequences.

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