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An Increased CAG Repeat Length in the Androgen Receptor Gene in Azoospermic ICSI Candidates

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ABSTRACT: The androgen receptor gene has a polymorphic trinucleotide repeat that encodes a polyglutamine tract in its N-terminal transactivation domain. We started this study in order to find out whether a correlation existed between the length of this polymorphic tract and the presence of azoospermia in candidates for intracytoplasmic sperm injection (ICSI). The CAG repeat length in exon 1 of the androgen receptor (AR) gene was directly sequenced in 102 patients with azoospermia and in 96 fertile controls. Hormone levels were also measured in patients with azoospermia. The mean AR gene CAG repeat length was significantly larger in azoospermic subjects than it was in control fertile men (23.25 ± 2.7 versus 22.42 ± 2.8; $P = .033$). A receiver operating characteristic analysis evidenced a cutoff point at 22/23 CAG repeats at which the probability of being azoospermic increased 2.2 times. Subsequent logistic regression analysis of the data showed that the odds for azoospermia increased with the number of CAG repeats. Men with more than 26 CAG repeats have a 4.09 greater risk of being azoospermic. Therefore, in our candidates for ICSI, a direct correlation exists between the CAG repeat length in the exon 1 of the AR gene and the risk of being azoospermic.

Key words: Assisted reproduction, male infertility, spermatogenesis.
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Androgens are essential for normal sperm production and it has been demonstrated that decreasing levels of intratesticular androgens results in defective spermatogenesis (Zirkin et al, 1989). However, most patients with idiopathic azoospermia exhibit normal serum androgen levels, suggesting that defects in the androgen response pathway may be involved in the etiology of this condition (Yoshida et al, 1999). All androgens act through a single intracellular androgen receptor (AR), which is encoded by a single-copy gene in the X-chromosome (Chang et al, 1988). The AR protein, when activated by androgen binding, translocates into the nucleus and binds to androgen response elements (AREs) in the promoter regions of androgen responsive genes, causing specific gene transcription (Quigley et al, 1995). The AR has three main functional domains: the transactivation domain (TAD), the DNA binding domain (DBD), and the ligand-binding domain (LBD) (Yong et al, 2000). Most abnormalities in the AR gene have been previously identified in the DBD and LBD (McPhaul et al, 1993; Quigley et al, 1995; Gottlieb et al, 1997). However, the polymorphic trinucleotide repeat segment (CAG)n, in exon 1 of the AR gene, which encodes a polyglutamine tract in the TAD of the AR protein, 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) (La Spada et al, 1991), a fatal neuromuscular disease associated with low virilization, oligozoospermia or azoospermia, testicular atrophy, and reduced fertility (Arbizu et al, 1983; Nagashima et al, 1988). On the other hand, short AR CAG alleles are associated with prostate cancer and androgen-dependent tumors (Irvine et al, 1995; Hardy et al, 1996). Thus, it is possible that spermatogenesis, a very androgen-dependent process, could be affected by changes in the lengths of this polymorphic tract. However, this correlation between CAG repeat length and infertility is still unclear.

Some authors have reported no association between the length of the CAG repeat and impairment of sperm production (Giwercman et al, 1998; Dadze et al, 2000; Sasagawa et al, 2000, 2001; von Eckardstein et al, 2001; Yu and Handelsman, 2001). On the other hand, findings by other authors suggest that long androgen receptor gene CAG alleles are associated with male infertility and defective spermatogenesis (Tut et al, 1997; Dowsing et al, 1999; Yoshida et al, 1999; Mifsud et al, 2001; Patrizio et al, 2001; Wallerand et al, 2001). However, consensus on the number of CAG repeats that increases the risk of the defective spermatogenesis is still lacking. Moreover, a

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high incidence of the short form of 14 or 15 CAG repeats has been found in a population of infertile Japanese men with oligozoospermia (Komori et al, 1999). This variability of results in independent studies may be attributed to 1) the different ethnic origins, and hence different genetic modifiers of the populations studied, 2) the possibility that these infertile men may represent a heterogeneous group with respect to the causes of defective spermatogenesis (Dadze et al, 2000), and 3) the differences in the number of subjects included in each study.

Longer trinucleotide repeats are unstable and might either expand or contract between generations. If they expand, conception through the use of intracytoplasmic sperm injection (ICSI) could result in a son of an ICSI daughter being affected not only by infertility but they may also exhibit Kennedy disease (Patrizio et al, 2001).

We started this work in order to clarify the extent to which the variation in the CAG repeat length of the AR gene is related to azoospermia. Specifically, we investigated the relationship between variations in the length of CAG repeats of the AR gene and the impairment of spermatogenesis in a group of Spanish men with azoospermia who were candidates for ICSI, a population for which these type of data were so far lacking.

Materials and Methods

Patients

Our pool of candidates consisted of 102 patients with azoospermia who participated in an ICSI protocol and 96 control men who had fathered at least 1 child by natural conception. Sperm parameters for infertile men were evaluated according to recommendations published by the World Health Organization (1999) and were the mean of at least 2 analyses performed 3 months apart. The participants were white and from Spain. The project was approved by the ethics committee of the hospital and each participant gave informed consent. All samples were anonymous throughout the procedure.

Serum concentrations of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured with an immunoenzymatic assay (Immuno 1; Technicon, Bayer, Tarrytown, NY). A karyotype analysis, a screening for microdeletions in the long arm of the Y chromosome, and the detection of mutations in the CFTR gene were performed for all initial participants. All patients with a clear explanation for their azoospermia (such as Y chromosome microdeletions, CFTR mutations, or karyotype abnormalities) were excluded from the study. In addition, the diagnosis of nonobstructive azoospermia was based not only on the lack of CFTR mutations, but also on the histology available for the majority of samples, the clinical history, the clinical examination, and FSH and inhibin B levels.

DNA Isolation, Amplification, and Sequencing of CAG Repeats

DNA was isolated from peripheral blood samples from fertile and infertile men according to standard protocols. The CAG repeats in exon 1 of the AR gene were amplified in 2 subsequent polymerase chain reactions (PCRs). The components for each PCR were 1× buffer, 200 μM dNTP, 1.5 mM $MgCl_2$, and 0.5 U of Expand High Fidelity *Taq* polymerase (Roche Diagnostics, Madrid, Spain) in a total reaction volume of 20 μL. The first PCR was performed with 50–100 ng of genomic DNA as a template and 2 μM of each outside primers (Irvine et al, 1995). Thirty-five cycles of amplification were performed (denaturation at 94°C for 1 minute, annealing at 55°C for 1 minute, and extension at 68°C for 1.3 minutes). Subsequently, 2.5 μL of a 1:500 dilution of the first PCR was then subjected to a nested PCR. The nested PCR was performed in a total volume of 20 μL with 2 μM of each inside primer (Irvine et al, 1995). Twenty cycles of amplification were performed (denaturation at 94°C for 1 minute, annealing at 71°C for 1 minute, and extension at 68°C for 1.3 minutes). The nested PCR products were analyzed by electrophoresis on 1.5% agarose gels and were gel excised according to the Qiaquick gel extraction kit (Qiagen, Hilden, Germany) protocol. These PCR fragments were subsequently sequenced using an ABI prism sequencing kit (Perkin-Elmer, Foster City, Calif) on an ABI PRISM 3700 sequencer, according to the manufacturer's protocol.

Statistical Analysis

The mean number of CAG repeats of fertile controls and patients with azoospermia were compared with 2 sample independent Student *t* tests. The CAG repeat number cutoff for subsequent analysis was selected from a receiver operating characteristic (ROC) curve by taking the value of the CAG length that maximizes the value of sensibility and specificity. A chi-square test was used to compare the number of men who had a CAG length <23 vs ≥ 23, and the relative risk was calculated with a confidence interval (CI) of 95%. We performed a binary logistic regression to determine whether the risk of infertility was constant among men with 23 or more CAG repeats. Statistical analyses were performed with SPSS software version 10.0 (SPSS Corp, Chicago, Ill) and statistical tests were evaluated with a significance level of 0.05.

In addition to the data analysis reported in this paper, we used the same methodology to analyze the data reported in other studies in patients with azoospermia for whom the length of the CAG repeat was described.

Results

We examined the distribution of the CAG repeat length by directly sequencing the PCR fragments in a sample of 102 ICSI candidates with azoospermia and in 96 fertile controls with the same ethnic origin. The mean AR gene CAG repeat was determined to be 23.25 ± 2.7 (range 18 to 32) in subjects with azoospermia, and 22.42 ± 2.8 (range 15 to 34) in control fertile men. The length of the CAG repeat tract was significantly longer in men with azoospermia than it was in fertile controls ($P = .033$). When we examined the distribution of the CAG repeat length in the control group we found 3 subjects with less

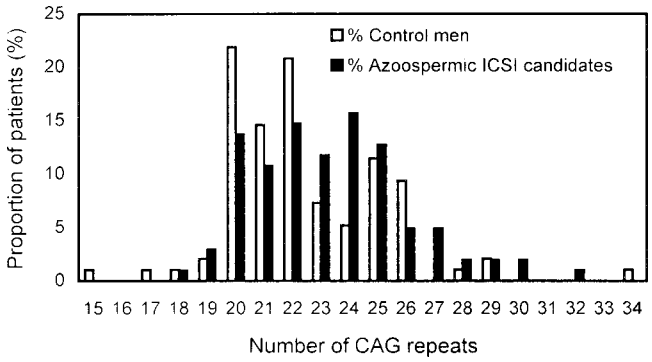

Figure 1. Distribution of CAG repeat sizes within the AR gene of ICSI candidates with azoospermia and control men.

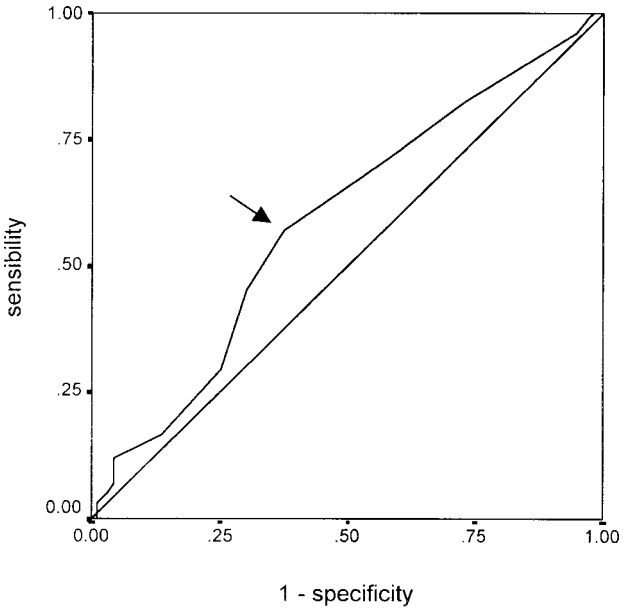

Figure 2. ROC curve analysis of the CAG repeat length of the AR gene in the sample. The arrow shows the cutoff point (22/23) that maximizes the sensibility and the specificity (sensibility = 0.57; specificity = 0.63). The area below the curve is 0.59 (P = .028).

than 19 CAG repeats and 1 subject with 34 CAG repeats. The remaining 92 fertile controls all had repeat lengths of 19 to 29. In patients with azoospermia we detected only 1 patient with less than 19 CAG repeats; all other infertile subjects had repeat lengths of 19 to 32 (Figure 1). Thus, the range was similar for men with azoospermia and controls.

Testosterone levels in our patients were normal (mean testosterone = 499 ± 185 ng/dL; normal range is 275–850 ng/dL). LH was almost within the normal range (mean LH = 7.57 ± 5.8 U/L; normal range 1.5–7.5 U/L) and FSH concentrations were abnormally high (mean FSH = 15.65 ± 12.1 U/L; normal range 1.7–8 U/L). Most men with azoospermia have normal serum testosterone concentrations, together with elevated serum gonadotropin concentrations (Uchujima and Yoshida, 1995).

With the aim to identify a cutoff point for the number of CAG repeats in men with an increased risk of azoospermia, we performed the ROC curve (Figure 2). The cutoff point of between 22 and 23 CAG repeats was selected because it is the point that maximizes the sensibility (0.57) and the specificity (0.63) of the values. The area below the ROC curve is 0.59 (P = 0.028).

A chi-square test was performed to check the prediction power of the CAG repeat length in men with azoospermia. The odds ratio of 2.20 (95% CI, 1.24–3.88) indicates that a CAG repeat longer than 22 is associated with an increased risk of azoospermia. Finally, in order to determine whether the risk of azoospermia was constant along men with 23 or more CAG repeats, we performed a binary logistic regression. Table 1 shows the percentage of fertile and azoospermic men in each category. The logistic regression analysis shows that men with 23–26 CAG repeat have an odds ratio for azoospermia of 1.96 (95% CI 1.08–3.56) but men with more than 26 CAG repeats have an odds ratio for azoospermia of 4.09 (95% CI 1.24–13.53).

In order to confirm these data we also analyzed patients for whom we could obtain data from 5 independent studies (Yoshida et al, 1999; Dadze et al, 2000; Mifsud et al, 2001; Sasagawa et al, 2001; our study). Men were divided into 2 groups according their race (Asians and Caucasians). We found statistically significant differences ($P <$ .001) in the mean of the length of the CAG repeat in Asians (controls, n = 186, mean = 22.94 ± 2.89; azoospermic men, n = 103, mean = 24.77 ± 3.49), and we found a cutoff point of 23/24 in the ROC curve (area below the curve 0.640, $P <$ .001). Binary logistic regression analysis shows that Asian men with 24–26 CAG repeats have an odds ratio for azoospermia of 1.79 (95% CI 1.03–3.11), whereas Asian men with more than 26 CAG repeats have a risk of 3.4 (95% CI 1.71–6.71). Caucasians also exhibited a significant difference in the mean length of CAG repeats (controls, n = 213, mean = 21.44 ± 3.53; azoospermic men, n = 143, mean = 22.96 ± 2.88) ($P <$ .001). The ROC curve also allowed us to define a cutoff point of 22/23 (area below the curve = 0.616, $P <$ .001). Binary logistic regression analysis showed that Caucasian men with 23–26 CAG repeats have an odds ratio for azoospermia of 1.58 (95% CI 1.01–

Table 1. *Distribution of individuals in the fertile and azoospermic groups stratified according to the number of CAG repeats*

CAG Repeats	Fertile Controls	Azoospermic ICSI Candidates	n	OR for Azoospermia*
14–22	47.7%	42.3%	104	1
23–26	41.0%	59.0%	78	1.96 (95% CI 1.08–3.56)
>26	25%	75%	16	4.09 (95% CI 1.24–13.54)

Table 2. *Comparison between mean CAG repeats in exon 1 of the AR gene in infertile patients and fertile controls*

Authors	Ethnic Origin	Infertile Patients (n)			Fertile Men (n)	Mean ± SD of CAG Repeats (range)		Signif. diff. between Two Groups (*P*)	CAG Repeat Cutoff Point
		Azoo.	Oligo.	Total		Infertile	Fertile		
Tut *et al,* 1997	Chinese	. . .	. . .	153	72	. . .	. . .	Yes	≥27
Giwercman et al, 1998	Swedish	6	17	33	294	. . .	. . .	No	. . .
Dowsing et al, 1999	Australian	10	20	30	32	23.2 ± 0.7 (15–34)	20.5 ± 0.3 (17–25)	Yes (.0001)	≥26
Komori et al, 1999	Japanese	. . .	59	59	36	21.2 ± 4.2 (14–32)	21.4 ± 3.5 (16–31)	No	<16
Yoshida et al, 1999	Japanese	41	. . .	41	48	26.5 ± 3.5 (20–34)	23.9 ± 2.9 (17–30)	Yes (.001)	31–40
Dadze et al, 2000	German	18	101	119	22	22.0 ± 3.2 (16–34)	20.8 ± 3.3 (15–26)	No	. . .
Sasagawa et al, 2000	Japanese	. . .	. . .	48	100	23.4 ± 0.5 (16–32)	23.5 ± 0.3 (15–35)	No	. . .
Mifsud et al, 2001	USA	23	72	95	55	21.9 ± 0.31 (14–31)	20.7 ± 0.52 (8–27)	Yes (.034)	≥26
	Singapore	33	87	120	87	23.8 ± 0.4 (14–33)	22.4 ± 0.32 (11–29)	Yes (.043)	≥26
Patrizio et al, 2001	USA	16	53	69	45	23.5 ± 3.4 (18–39)	22.0 ± 2.8 (12–30)	Yes (.03)	. . .
Sasagawa et al, 2001	Japanese	30	. . .	30	51	23.4 ± 2.9 (19–30)	23.7 ± 3.2 (17–28)	No	. . .
von Eckardstein et al, 2001	German	. . .	. . .	43	131	. . .	. . .	No	. . .
Yu and Handelsman, 2001	Australian	54	. . .	. . .	106	19.8 (8–24)	19 (5–28)	No	. . .
Wallerand et al, 2001	French	37	. . .	37	50	23.9 ± 0.5 (13–28)	22.2 ± 0.4 (17–27)	Yes (.008)	. . .
Present study, 2002	Spanish	102	. . .	102	96	23.2 ± 2.8 (14–30)	22.4 ± 2.8 (15–34)	Yes (.033)	≥23/≥27

2.49), whereas Caucasian men with more than 26 CAG repeats have a risk of 4.80 (95% CI 1.80–13.05).

Discussion

In this paper we report a direct correlation between the length of the CAG repeat in exon 1 of the AR gene and the odds ratio for azoospermia. We were able to determine a cutoff point of between 22 and 23 CAG repeats, from which subjects have a 2.2 times greater probability of exhibiting azoospermia compared with subjects who have fewer than 23 CAG repeats. Furthermore, we also found that the risk of being azoospermic is not constant in subjects with 23 or more CAG repeats, but that it is further increased up to 4.09 times in men with more than 26 CAG repeats.

Several authors have found an association between the expansion of CAG repeats and male infertility (Tut et al, 1997; Dowsing et al, 1999; Yoshida et al, 1999; Mifsud et al, 2001; Patrizio et al, 2001; Wallerand et al, 2001), although other studies have not (Giwercman et al, 1998; Dadze et al, 2000; Sasagawa et al, 2000, 2001; von Eckardstein et al, 2001; Yu and Handelsman, 2001). Of interest, one group has suggested that CAG repeat lengths of <16 are associated with oligozoospermia (Komori et al, 1999) (Table 2).

Ethnic differences in the CAG repeat length are well known (Irvine et al, 1995), thus variation between the results of different studies could be due to ethnic differences. However, Mifsud et al (2001), through the study of 2 different ethnic populations (in the United States and Singapore), demonstrated that the differences in the length of the CAG tract are significantly associated with infertility independent of ethnicity.

The average CAG repeat length in exon 1 of the AR gene found in other white populations is similar to ours. For instance, among white men with normal fertility in the United States, the mean for control fertile men is 22 ± 2.8 (range 12–30) (Patrizio et al, 2001) and in a population in France, a mean of 22.2 ± 0.4 (range 17–27) has been reported (Wallerand et al, 2001). We found a similar variable range of CAG repeats in normal control men (15–34) and a mean (22.4 ± 2.8) that was similar to the above data. We found no subjects with more than 40 CAG repeats, which is typical of men with SBMA. However, we found a statistically significant difference ($P = .033$) in the mean of the CAG repeat length between fertile men and those with azoospermia.

In this paper we introduced a novel methodology, the ROC curve, for the analysis of this type of data. It has allowed us to identify and to define a cutoff point between 22 and 23 CAG repeats from which the risk of azoospermia increases 2.2 times. It is interesting that a cutoff of 22 CAG (<22) repeats was associated with an increased risk of prostate cancer (Irvine et al, 1995). Our data are also in agreement with reports in which a CAG repeats length ≤22 is associated with a reduced risk of male infertility (Mifsud et al, 2001; Tut et al, 1997).

Binary logistic regression of the data allows us to support our hypothesis. The risk of infertility is not constant among men with 23 or more CAG repeats. This risk is duplicated when men with more than 26 CAG repeats are considered. Several authors have also found cutoffs points of 26 or 27 CAG repeats in men in whom the risk of infertility became very high (Tut et al, 1997; Dowsing et

al, 1999; Mifsud et al, 2001). Thus, their data agree with our results. Remarkably, Tut et al (1997) found an odds ratio of 4.02 (95% CI, 4.02–0.77) for infertility in men with more than 26 CAG repeats.

As a confirmation of the findings reported in this paper, we used the same methodology to analyze published CAG length data in men with azoospermia. Because the ethnicity of the studied populations may influence the length of CAG repeats, patients with azoospermia for whom we could obtain data from 5 independent studies (Yoshida et al, 1999; Dadze et al, 2000; Mifsud et al, 2001; Sasagawa et al, 2001; our study) were divided into 2 groups according to their race. Thus, we found high statistically significant differences ($P < .001$) in the mean of the CAG length repeat in a group of Asians as well as in a group of Caucasians ($P < .001$). We also found similar cutoff points in both groups (23/24 and 22/23, respectively) and the binary logistic regression analysis shows similar odds for azoospermia in both groups regardless of race. Thus, although we are analyzing data from 2 different types of populations and although the length of CAG repeats in the AR gene may be influenced by race (in fact Asians have longer CAG repeats than European populations) it seems that the increment in the length of CAG repeats in exon 1 of the AR gene is directly related to the risk of being azoospermic.

It is estimated that 10%–20% of patients with male infertility could have reduced androgen receptor function as a result of long polyglutamine tracts (Yong et al, 1998) and some hypotheses have been proposed. Hsiao et al (1999) proposed that the change in the CAG length can contribute to the different activation capacity of the receptor. Transactivation experiments show that the relationship between polyglutamine tract and AR transactivation was inverse and linear from 0 to 50 glutamines (Kazemi-Esfarjani et al, 1995). An inverse relationship between CAG repeat length and AR messenger RNA and protein levels has also been described (Choong et al, 1996). Another group has identified that the expansion of glutamine repeats in the AR results in a structurally altered protein with reduced transcriptional capacity (Chamberlain et al, 1994). Our findings are consistent with all these cited potential mechanisms because we found a greater risk of azoospermia with a greater number of CAG repeats. Thus, our results provide a clinical complement and confirmation of the basic predictions of the above experiments. A polyglutamine tract of about 23 (corresponding to CAG repeats length of 22) would represent the baseline activation status of the AR. A higher number of glutamine residues would increase the repression of the receptor, leading to a reduction in its transactivation function and, consequently, to a lower activation of the androgen-regulated genes and, as a consequence, to infertility.

We are not at all proposing that an increase of CAG repeats greater than 22 is the cause of azoospermia in our patients. Rather, our proposal is that the greater number of CAG repeats is only a risk factor for azoospermia. Our results agree with those of others in that many of the cases of azoospermia or male idiopathic infertility could have a multifactorial basis. Thus, the combination of greater CAG repeat length with other known or still-unknown risk factors could lead to infertility. The stability of the CAG repeats is still unknown. It has been shown that about 5% of daughters conceived through ICSI have an inherited AR allele with either contraction or expansion up to 8 unit base pairs (Cram et al, 2000). Because expansions of CAG repeats can be deleterious (not only because they could lead infertility but because they can also lead to SMBA) perhaps it should be further considered if the study of this polymorphism in azoospermic ICSI candidates could have potential implications for genetic counseling.

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References

Arbizu T, Santamaria J, Gomez JM, Quilez A, Serra JP. A family with adult spinal and bulbar muscular atrophy, X-linked inheritance and associated testicular failure. *J Neurol Sci.* 1983;59:371–382.

Chamberlain NL, Driver ED, Miesfeld RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res.* 1994;22: 3181–3186.

Chang CS, Kokontis J, Liao ST. Molecular cloning of human and rat complementary DNA encoding androgen receptors. *Science.* 1988; 240:324–326.

Choong CS, Kemppainen JA, Zhou ZX, Wilson EM. Reduced androgen receptor gene expression with first exon CAG repeat expansion. *Mol Endocrinol.* 1996;10:1527–1535.

Cram DS, Song B, McLachlan RI, Trounson AO. CAG trinucleotide repeats in the androgen receptor gene of infertile men exhibit stable inheritance in female offspring conceived after ICSI. *Mol Hum Reprod.* 2000;6:861–866.

Dadze S, Wieland C, Jakubiczka S, Funke K, Schroder E, Royer-Pokora B, Willers R, Wieacker PF. The size of the CAG repeat in exon 1 of the androgen receptor gene shows no significant relationship to impaired spermatogenesis in an infertile Caucasoid sample of German origin. *Mol Hum Reprod.* 2000;6:207–214.

Dowsing AT, Yong EL, Clark M, McLachlan RI, De Kretser DM, Trounson AO. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. *Lancet.* 1999;354:640–643.

Giwercman YL, Xu C, Arver S, Pousette A, Reneland R. No association between the androgen receptor gene CAG repeat and impaired sperm production in Swedish men. *Clin Genet.* 1998;54:435–436.

Gottlieb B, Trifiro M, Lumbroso R, Pinsky L. The androgen receptor gene mutations database. *Nucleic Acids Res.* 1997;25:158–162.

Hardy DO, Scher HI, Bogenreider T, Sabbatini P, Zhang ZF, Nanus DM, Catterall JF. Androgen receptor CAG repeat lengths in prostate cancer:

correlation with age of onset. *J Clin Endocrinol Metab.* 1996;81: 4400–4405.

Hsiao PW, Lin DL, Nakao R, Chang C. The linkage of Kennedy's neuron disease to ARA24, the first identified androgen receptor polyglutamine region-associated coactivator. *J Biol Chem.* 1999;274:20229–20234.

Irvine RA, Yu MC, Ross RK, Coetzee GA. The CAG and GGC microsatellites of the androgen receptor gene are in linkage disequilibrium in men with prostate cancer. *Cancer Res.* 1995;55:1937–1940.

Kazemi-Esfarjani P, Trifiro MA, Pinsky L. Evidence for a repressive function of the long polyglutamine tract in the human androgen receptor: possible pathogenetic relevance for the (CAG)n-expanded neuronopathies. *Hum Mol Genet.* 1995;4:523–527.

Komori S, Kasumi H, Kanazawa R, Sakata K, Nakata Y, Kato H, Koyama K. CAG repeat length in the androgen receptor gene of infertile Japanese males with oligozoospermia. *Mol Hum Reprod.* 1999;5:14–16.

La Spada AR, Wilson EM, Lubahn DB, Harding AE, Fischbeck KH. Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature.* 1991;352:77–79.

McPhaul MJ, Marcelli M, Zoppi S, Griffin JE, Wilson JD. Genetic basis of endocrine disease. 4. The spectrum of mutations in the androgen receptor gene that causes androgen resistance. *J Clin Endocrinol Metab.* 1993;76:17–23.

Mifsud A, Sim CK, Boettger-Tong H, Moreira S, Lamb DJ, Lipshultz LI, Yong EL. Trinucleotide (CAG) repeat polymorphisms in the androgen receptor gene: molecular markers of risk for male infertility. *Fertil Steril.* 2001;75:275–281.

Nagashima T, Seko K, Hirose K, Mannen T, Yoshimura S, Arima R, Nagashima K, Morimatsu Y. Familial bulbo-spinal muscular atrophy associated with testicular atrophy and sensory neuropathy (Kennedy-Alter-Sung syndrome). Autopsy case report of two brothers. *J Neurol Sci.* 1988;87:141–152.

Patrizio P, Leonard DG, Chen KL, Hernandez-Ayup S, Trounson AO. Larger trinucleotide repeat size in the androgen receptor gene of infertile men with extremely severe oligozoospermia. *J Androl.* 2001; 22:444–448.

Quigley CA, De Bellis A, Marschke KB, el-Awady MK, Wilson EM, French FS. Androgen receptor defects: historical, clinical, and molecular perspectives. *Endocr Rev.* 1995;16:271–321.

Sasagawa I, Suzuki Y, Ashida J, Nakada T, Muroya K, Ogata T. CAG repeat length analysis and mutation screening of the androgen receptor gene in Japanese men with idiopathic azoospermia. *J Androl.* 2001; 22:804–808.

Sasagawa I, Suzuki Y, Tateno T, Nakada T, Muroya K, Ogata T. CAG repeat length of the androgen receptor gene in Japanese males with cryptorchidism. *Mol Hum Reprod.* 2000;6:973–975.

Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. Long polyglutamine tracts in the androgen receptor are associated with reduced transactivation, impaired sperm production, and male infertility. *J Clin Endocrinol Metab.* 1997;82:3777–3782.

Uchujima Y, Yoshida K-I. Significance of testicular volume in infertile patients. *Jpn J Fertile Steril.* 1995;40:195–202.

von Eckardstein S, Syska A, Gromoll J, Kamischke A, Simoni M, Nieschlag E. Inverse correlation between sperm concentration and number of androgen receptor CAG repeats in normal men. *J Clin Endocrinol Metab.* 2001;86:2585–2590.

Wallerand H, Remy-Martin A, Chabannes E, Bermont L, Adessi G, Bittard H. Relationship between expansion of the CAG repeat in exon 1 of the androgen receptor gene and idiopathic male infertility. *Fertil Steril.* 2001;76:769–774.

World Health Organization. *WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction.* 4th ed. Cambridge, United Kingdom: Cambridge University Press; 1999.

Yong EL, Ghadessy F, Wang Q, Mifsud A, Ng SC. Androgen receptor transactivation domain and control of spermatogenesis. *Rev Reprod.* 1998;3:141–144.

Yong EL, Lim LS, Wang Q, Mifsud A, Lim J, Ong YC, Sim KS. Androgen receptor polymorphisms and mutations in male infertility. *J Endocrinol Invest.* 2000;23:573–577.

Yoshida KI, Yano M, Chiba K, Honda M, Kitahara S. CAG repeat length in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. *Urology.* 1999;54:1078–1081.

Yu B, Handelsman DJ. Pharmacogenetic polymorphisms of the AR and metabolism and susceptibility to hormone-induced azoospermia. *J Clin Endocrinol Metab* 2001;86:4406–4411.

Zirkin BR, Santulli R, Awoniyi CA, Ewing LL. Maintenance of advanced spermatogenic cells in the adult rat testis: quantitative relationship to testosterone concentration within the testis. *Endocrinology.* 1989;124: 3043–3049.