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Long Polyglutamine Tracts in the Androgen Receptor Are Associated with Reduced *Trans*-Activation, Impaired Sperm Production, and Male Infertility*

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ABSTRACT

The X-linked androgen receptor (AR) gene contains two polymorphic trinucleotide repeat segments that code for polyglutamine and polyglycine tracts in the N-terminal *trans*-activation domain of the AR protein. Changes in the lengths of these polymorphic repeat segments have been associated with increased risk of prostate cancer, an androgen-dependent tumor. Expansion of the polyglutamine tract causes a rare neuromuscular disease, spinal bulbar muscular atrophy, that is associated with low virilization, reduced sperm production, testicular atrophy, and infertility. As spermatogenesis is exquisitely androgen dependent, it is plausible that changes in these two repeat segments could have a role in some cases of male infertility associated with impaired spermatogenesis. To test this hypothesis, we examined the lengths of the polyglutamine and polyglycine repeats in 153 patients with defective sperm production and compared them to 72 normal controls of proven fertility. There was no significant association between the polyglycine tract and infertility. However, patients with 28 or more glutamines (Gln) in their AR had more than 4-fold (95% confidence interval, 4.9–3.2) increased risk of impaired spermatogenesis, and the more severe the spermatogenic defect, the higher the proportion of patients with a longer Gln repeat. Concordantly, the risk of defective spermatogenesis was halved when the polyglutamine tract was short (≤23 Gln). Whole cell transfection experiments using AR constructs harboring 15, 20, and 31 Gln repeats and a luciferase reporter gene with an androgen response element promoter confirmed an inverse relationship between Gln number and *trans*-regulatory activity. Immunoblot analyses indicated that the reduced androgenicity of the AR was unlikely to be due to a change in AR protein content. The data indicate a direct relation between length of the AR polyglutamine tract and the risk of defective spermatogenesis that is attributable to the decreased functional competence of AR with longer glutamine tracts. (*J Clin Endocrinol Metab* **82:** 3777–3782, 1997)

INFERTILITY affects 10–15% of all couples, and impaired sperm production accounts for about half of these cases. The cause of defective spermatogenesis remains largely obscure (1). Androgens are required for normal spermatogenesis; however, most males with impaired spermatogenesis have normal serum androgen levels. Therefore, attention was focused on the androgen receptor (AR) as a possible cause of the impaired spermatogenesis in subjects with idiopathic male infertility. The AR protein, when activated by androgen binding, translocates to the nucleus and binds to androgen response elements (ARE) in the promoter regions of androgen-responsive genes, causing specific gene transcription (2). The AR gene contains two polymorphic trinucleotide repeat loci: $[CAG]_n$, which codes for a polyglutamine tract, and $[GGC]_n$, which coded for a polyglycine tract. Both loci are located in exon 1, which encodes the *trans*-activation domain of the receptor protein (3). Expansion of the polyglutamine segment in the male leads to spinal bulbar muscular atrophy (SBMA) (4), a fatal neuromuscular disease associated with low virilization, oligospermia (reduced sperm production) or azoospermia (no sperm production), testicular atrophy, and reduced fertility (5, 6). Relatively short polyglutamine tracts with or without associated polyglycine tract changes have been linked to an increased risk (7) or an earlier age of onset (8) of prostate cancer, an androgen-dependent tumor. It is, therefore, plausible that spermatogenesis, an exquisitely androgen-dependent process, could be affected by changes in the lengths of these two microsatellite tracts. To explore the validity of this hypothesis we determined the lengths of the two AR polymorphic tracts in subjects with defective spermatogenesis and compared them to normal fertile controls. A significant relationship was found between the length of the polyglutamine tract and defective sperm production, and functional studies were performed to examine the *trans*-activation capacity of these AR variants *in vitro*.

Subjects and Methods

Patients were recruited from the subfertility clinic after giving informed consent. Patients who had hypopituitarism, hyperprolactinemia, or infective or obstructive syndromes of the genital tract were excluded. Sperm parameters were assessed according to standard criteria (9) and were the mean of at least two analyses performed 3 months apart. Control subjects, recruited from the contraceptive clinic, were men of proven fertility with no previous infertility history or treatment and without any genetic disease.

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Trinucleotide repeat allele analysis

DNA was extracted from the peripheral blood of patients and control subjects using standard techniques (10). The CAG repeat segment was amplified using primers ALS and A2 (Fig. 1) (4). One microcurie of [α-^{33}P]deoxy-ATP was added to make a final reaction volume of 25 μL. We used a 2-step 30-cycle amplification protocol in which the denaturing temperature was 95 C for 45 s, and the combined annealing and extension temperature was 68 C for 1.5 min. In the first cycle the sample was denatured for 5 min. The GGC repeat segment was amplified using 70% deaza-GTP, thermostable vent DNA polymerase, and the primers A7.1 (CTCATCCTGGCACACTCTCTTCACAGC) and A8 (GGACTGGGATAGGGCACTCTGCTCACC; Fig. 1). Denaturing temperatures of 98 C for 1 min and annealing/extension at 70 C for 5 min were used for 40 cycles to amplify this GC-rich region. The reactions were terminated with 20 μL of a solution containing 95% formamide in 20 mmol/L ethylenediamine tetraacetate and 0.05% of bromophenol blue and xylene cyanol. The mixture was heat denatured for 3 min at 95 C before being electrophoresed on an 8% polyacrylamide-7 mol/L urea sequencing gel at 70 watts for 6 h. Gels were dried and exposed to Kodak X-Omat film (Eastman Kodak, Rochester, NY) overnight. The size of the most prominent (usually upper doublet) band was determined by comparison with PCR products of known lengths and also with dideoxy sequencing ladders. Each allele was examined on at least two separate occasions using different PCR reactions, and alleles of the same size were examined together to eliminate any discrepancies in length. Some repeats were also directly sequenced to confirm the accuracy of the length assignments, and these were subsequently used as reference samples. Sequencing was performed using automated fluorescent sequencing (Perkin-Elmer, Foster City, CA) in both the forward and reverse directions.

Single strand conformation polymorphism (SSCP) analyses

The rest of the coding sequence of AR (exons 2–8) was examined by PCR-SSCP and silver staining (10) to screen for other associated subtle mutations in our group of patients. This was to ensure that our data were not confounded by exonic mutations that were linked to any particular trinucleotide repeat allelle. Mutations so uncovered were not included in this report.

Construction of CAG expression plasmids

Two AR alleles with polyglutamine sizes at the extremes of our range were selected, 1 with 31 glutamines (Gln) from an infertile patient, and the other with 15 Gln from a normal control. The segments containing either 15 or 31 polyglutamine repeats were amplified from genomic DNA using the primer pairs A1 and A2 (3) and high fidelity Pfu DNA polymerase (Stratagene, La Jolla, CA). The amplified fragments containing the CAG trinucleotide tract were gel purified and double digested with *Eag*I and *Bfr*I, and the central fragment with overhanging ends was ligated to an AR expression plasmid (BHEXE-pAR) that had the corresponding segment excised. The resulting vectors, encoding ARs with 31 and 15 Gln, were used along with the original BHEXE-pAR (20 Gln) for cotransfection experiments. The spliced portions were sequenced to confirm the lengths of polyglutamine tracts as well as to exclude any inadvertent mutations.

Measurement of AR function

Plasmid constructs were transfected into COS-7 cells, a heterologous mammalian cell line that does not express endogenous AR. The reporter gene was pMAMneo-LUC (Clontech, Palo Alto CA), containing the luciferase gene coupled to the mouse mammary tumor virus long terminal repeat. The mouse mammary tumor virus long terminal repeat has several ARE that make it a strong promoter when activated by ligand-bound AR. COS-7 cells were transiently transfected using the lipofection technique. A DNA mix containing expression vector (1 μg), pMAMneo-LUC (1 μg) and pβGal (0.5 μg) was preincubated for 45 min at room temperature with 10 μL Lipofectamine (Promega, Madison, WI) in 400 μL serum-free medium. The DNA-liposome complexes were overlayed onto 80–95% confluent COS-7 cells in a total volume of 2.4 mL, and transfection was continued for 16–24 h before the addition of growth medium containing 10% charcoal-stripped FCS, penicillin-streptomycin, a 5α-reductase inhibitor (finasteride, 10^{-7} mol/L), and the indicated amounts of androgens. After 40–48 h of incubation, the cells were rinsed twice with phosphate-buffered saline and lysed with 400 μL reporter lysis buffer (Promega). Cells were scraped from the petri dishes, and after one freeze-thaw cycle, the cell lysates were cleared by centrifugation at 12,000 $\times$ *g* for 10 min. Cell lysates (20 μL) were added to 100 μL luciferase substrate, and luciferase activity measured with a luminometer. Transfection efficiency was assessed by β-galactosidase activity and luciferase activity normalized by the protein content of the cell lysates. Total protein in the supernatant was quantified using the method of Lowry *et al.* (11), with BSA as the standard.

Western analysis

Immunoblot analyses were used to study the effect of polyglutamine length on levels of AR protein. Equal volumes of 2 $\times$ SDS-PAGE gel loading buffer (100 mmol/L Tris Cl, pH 6.8; 200 mmol/L dithiothreitol; 4% SDS; 0.2% bromophenol blue; and 20% glycerol) were added to the cell extracts (30 μg total protein), and the samples of cleared cell lysates were heated to 100 C for 5 min before loading onto a 7% SDS-PAGE gel. Electrophoresis was carried out in SDS-PAGE running buffer (25 mmol/L Tris, 250 mmol/L glycine, and 0.1% SDS) at 80 V for 1.5 h. The gel was preequilibrated for 15 min in transfer buffer (48 mmol/L Tris base, 39 mmol/L glycine, and 20% methanol) before electroblotting (100 V, 1 h) onto nitrocellulose membrane (Hybond ECL, Amersham, Singapore) using the Mini Trans-Blot Cell (Bio-Rad, Hercules, CA). We used the rabbit monoclonal antibody, PG-21 (a gift from Dr. G. Prins), to recognize the first 21 N-terminal amino acids of the human AR. AR-antibody complexes were subsequently visualized by enhanced chemiluminescence following the manufacturer's protocol (ECL System, Amersham).

Statistical analyses

The computed odds ratio (OR) was used as an estimate of the relative risk. Confidence intervals (CIs) on the OR were constructed based on the logarithmic transformation method of Katz (12). Student's *t* test was used to evaluate differences in *trans*-activation experiments. Values of $P < 0.05$ were considered significant.

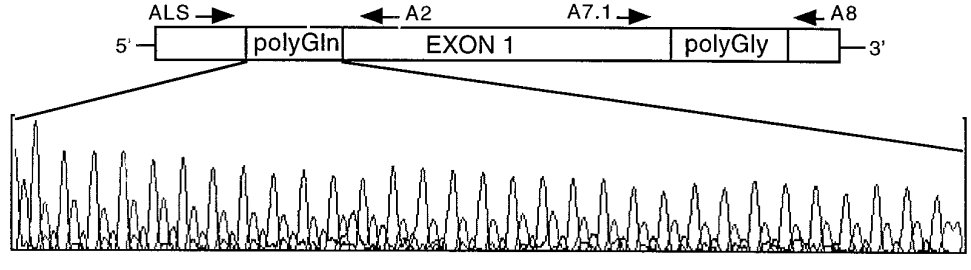

FIG. 1. Schematic representation of exon 1 from the AR gene and sequencing electropherogram of an expanded trinucleotide tract coding for polyglutamine from a patient with defective spermatogenesis. The primer pairs ALS, A2 and A7.1, A8 were used to amplify segments coding for the polyglutamine and polyglycine tracts, respectively. The tract shown consists of 30 CAG repeats and a terminal CAA codon resulting in 31 Gln.

Results

Patients (n = 153) with varying degrees and types of impaired spermatogenesis were recruited from the infertility clinic. Their mean serum FSH and LH levels were slightly raised at 6.4 IU/L (normal, 0.8–4.7) and 7.53 IU/L (normal, 2.0–6.9) respectively. Serum PRL and testosterone levels were 213 IU/mL and 6.58 ng/mL, respectively, not different from those in the normal population. Based on the average semen quality on at least two occasions, the patients were classified into the following categories: azoospermia (no spermatozoa detected), severe oligospermia (<5 million sperm/mL), moderate oligospermia (5–20 million sperm/mL), and those with normal sperm concentrations (>20 million/mL) but with abnormalities of motility or morphology (Table 1). Subjects (n = 72) of proven fertility and with no history of infertility served as controls. The racial compositions of the patient and control groups were similar: ethnic Chinese formed 64% and 59% of the patient and control groups, respectively.

PCR-SSCP was used to screen DNA from the peripheral blood of the patients and control subjects for mutations in exons 2–8 of the AR gene. Several point mutations were uncovered (13, 14), and these patients were excluded from the present report. DNA fragments coding for the polymorphic polyglutamine and polyglycine tracts of AR (Fig. 1) were amplified in the presence of [α-^{33}P]deoxy-ATP, and their sizes were determined by comparison with sequencing ladders on denaturing polyacrylamide gels (Fig. 2). Several alleles with longer and shorter trinucleotide fragments were directly sequenced to confirm the accuracy of size assignments (Fig. 1). The polyglycine tract was less polymorphic and consisted of 7 alleles (Table 1). The most common allele, forming about 62–77% of the cases in each category, coded for 23 glycines. There were no significant differences in the size of the polyglycine tract between patients and controls. In contrast, the distribution of alleles encoding the polyglutamine tract suggested that patients with reduced sperm counts differed significantly, with respect to the prevalence of Gln number beyond 27, compared to normal fertile controls (Table 2). Patients with 28 or more Gln in their AR had more than 4-fold (95% CI, 4.9–3.2) increased risk of reduced spermatogenesis. There was also a trend whereby the more severe the spermatogenic defect, the greater the proportion of patients with the longer polyglutamine alleles. Thus, in azoospermic patients 17.8% had 29 or more Gln in their AR compared to 15.7%, 15.3%, and 3.4% for severe oligospermia, moderate oligospermia, and abnormal motility cases, respectively. Interestingly, the risk of reduced spermatogenesis was halved (OR, 0.57; 95% CI, 0.42–0.77) with short repeat lengths of 23 or fewer Gln (Table 2). For Gln repeats of 21 or fewer, the OR was 0.44, suggesting that the shorter the length the less the chance of defective spermatogenesis. The data indicate an inverse relationship between the length of the polyglutamine tract in the AR and the risk of reduced spermatogenesis.

To investigate whether the differences in polyglutamine lengths encountered in our group of patients and controls can lead to varying AR function *in vitro*, AR complementary DNA encoding 31 and 15 Gln were constructed from patient and control genomic DNA, respectively. These AR sizes were at the extreme range encountered in our subjects. AR variants were expressed in COS-7 cells, and their ability to *trans*-activate a reporter gene was examined. There was an inverse relationship between AR polyglutamine tract length and its *trans*activation function (Fig. 3A). Thus, the AR with 31 Gln had only 64% of the *trans*-activation capacity of the AR with

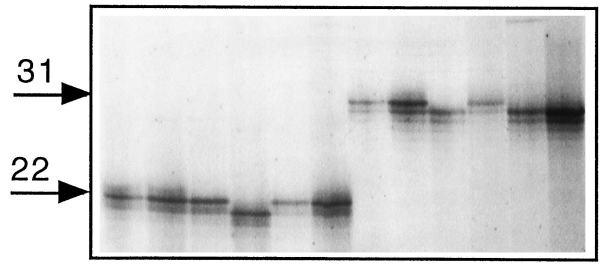

FIG. 2. Representative autoradiogram showing differences in (CAG)n repeat lengths between patients and controls. The CAG repeat segment of the AR gene was amplified in the presence of [α-^{33}P]deoxy-ATP before separation in a denaturing polyacrylamide gel. The first six lanes were from normal fertile controls, and the last six were from patients with azoospermia and severe oligospermia. Numbers on the *left* represent the length of the polyglutamine tracts encoded by each fragment. Stutter bands can be seen *below* the most prominent doublet.

TABLE 1. Size of the polyglycine tract in patients with defective spermatogenesis and in fertile controls

Glycine no.	Azoospermia (n = 26)[a]	Severe oligospermia (n = 64)[b]	Moderate oligospermia (n = 26)[c]	Abn. motility morphology (n = 26)[d]	Controls (n = 72)[e]
20	0	4.6	7.6	3.8	1.3
21	7.6	4.6	0	3.8	2.7
22	3.8	0	0	3.8	4.1
23	76.9	62.5	76.9	76.9	63.8
24	7.6	20.3	11.5	3.8	22.2
25	13.8	6.2	3.8	7.6	2.7
26	0	1.5	0	3.4	1.3

Values are percentages of the number of cases (n) in each category of subjects. Minor differences in numbers (n) compared to Table 2 were due to insufficient DNA for positive size assignment in some cases.

[a] Azoospermia, no sperm detectable.

[b] Severe oligospermia, less than 5 million sperm/mL.

[c] Moderate oligospermia, 5–20 million sperm/mL.

[d] Normal sperm concentrations (>20 million sperm/mL), but minor defects in motility and morphology.

[e] Normal fertile controls.

TABLE 2. Relationship between size of polyglutamine tract and severity of sperm defects

Glutamine no.	Azoospermia (n = 28)	Severe oligospermia (n = 70)	Moderate oligospermia (n = 26)	Abnormal motility, morphology (n = 29)	Controls (n = 72)	OR[a]	95% CI
≤21	0	7.1	7.6	17.2	9.7	0.44	0.24–0.81
≤23	10.7	27.1	23.0	31.0	34.7	0.57	0.42–0.77
24–25	25	24.2	26.9	34.4	25	NS	
26–27	32	24.2	23.0	27.5	30.5	NS	
≥28	32.1	24.2	26.9	6.8	9.7	4.02	4.9–3.2
≥29	17.8	15.7	15.3	3.4	5.5	3.69	4.6–2.9
≥30	10.7	5.7	3.8	0	0		
≥31	3.57	4.28	0	0	0		

Values are percentages of the total (n) in each subject category. Subject categories are explained as in Table 1: CI, Confidence intervals (12); NS, no significant differences between controls and patients.

[a] Comparing cases with reduced sperm counts (azoospermia, severe and moderate oligospermia) to controls and those with abnormal sperm motility or morphology only.

15 (by *t* test, $P = 0.005$). This pattern was observed with 100 nmol/L of both of the physiological androgens, testosterone and dihydrotestosterone (DHT). These differences in *trans*-activation function, whereby the shorter the glutamine tract, the greater the *trans*-activation function, were also observed when a dose-response experiment was performed (Fig. 3B). Thus, at 10 or 30 nmol/L DHT, the greatest *trans*-activation activity was seen with the smallest AR (15 Gln), whereas the longest (31 Gln) had a significantly lower *trans*-activation capacity ($P < 0.01$). Concurrent Western analyses (Fig. 3C) showed that the amounts of immunoreactive AR protein present at any specific hormone dose for the three AR variants were similar (comparing lanes 1, 4, and 7; 2, 5, and 8; and 3, 6, and 9), indicating that the *trans*-activation differences observed were unlikely to be due simply to changes in the AR protein content. The amounts of AR protein in this experiment were within the linear range of the immunoblot assay as shown in Fig. 3D. For each AR variant, we observed slight increases in AR protein at the higher DHT concentrations (comparing lanes 1–3, 4–6, and 7–9), probably reflecting increased receptor stability in the presence of higher doses of androgen (15).

Discussion

The AR is critical for male sexual development, and sperm formation is extremely dependent on androgens (16). Suppression of endogenous androgen production has been used as a form of male contraception (17). Androgen levels in our patients (mean testosterone, 6.58; normal range, 3.2–9.5 ng/mL) were normal. We wondered whether defects of the androgen response pathway could be involved in the etiology of idiopathic male infertility. Trinucleotide repeat tract expansions are very common in receptor genes (18), and there is evidence that they may serve a regulatory function (19). Patients with SBMA have evidence of deficient virilization and defective sperm production (20) in association with expanded polyglutamine tracts in their AR. We, therefore, investigated whether the lengths of the two polyglutamine and polyglycine tracts in the AR were related to defective sperm production. As missense mutations of the AR gene can also lead to mild androgen insensitivity and defective sperm production (21), cases with exonic point mutations uncovered by PCR-SSCP screening (13, 14) were excluded from the present analysis. There was no significant relationship between the length of the polyglycine tract and male infertility. In contrast, moderate expansion of the polyglutamine tract (≥28 Gln), although still in the polymorphic range, was associated with a significantly increased risk of defective spermatogenesis. There was a trend whereby the greater the spermatogenic defect, the greater the proportion of patients with long polyglutamine tracts. In contrast, short polyglutamine tracts were significantly associated with reduced risk of infertility. These differences were not due to ethnic origins (22), as the racial composition of patients and controls were similar, the majority being of Southern Chinese descent.

The maximum polyglutamine length encountered in our patients was 31 Gln, well short of the 40 or more Gln found in SBMA patients. Although there is evidence that the pathologically expanded tracts found in SBMA patients can reduce AR *trans*-activation (23), our data suggest that the polymorphic expansions in Gln number encountered in our patients could also significantly reduce AR function *in vitro*. There was an inverse relationship between the length of the polyglutamine tract and the ability to *trans*-activate a reporter gene with AREs in its promoter. This reduced *trans*-activation was seen with both of the physiological androgens, testosterone and DHT, and at doses of DHT between 10–100 nmol/L. Relatively high doses of androgens were selected because androgen levels are 50- to 100-fold higher in the testes than those in plasma (24). High concentrations of androgens, although greater than their K_d *in vitro*, appear essential for sperm production, as spermatogenesis could be impaired when testosterone levels in seminiferous tubule fluid were below 45 nmol/L (16). Examination of messenger ribonucleic acid and protein expression of AR constructs harboring 0–66 Gln suggest that repeat expansion could be associated with reduced AR messenger ribonucleic acid and protein expression (25). Reduction of immunodetectable AR with increasing polyglutamine length was not observed in our study, although the range examined (15–31 Gln) could be too narrow for these differences to be evident.

Thus, both *in vivo* and *in vitro* data support the concept that the longer its Gln repeat, the less androgenic the AR. On the other hand, short Gln repeats are associated with increased risk of the androgen-dependent tumor, prostate cancer (26). Prostate cancer can be considered a manifestation of an ex-

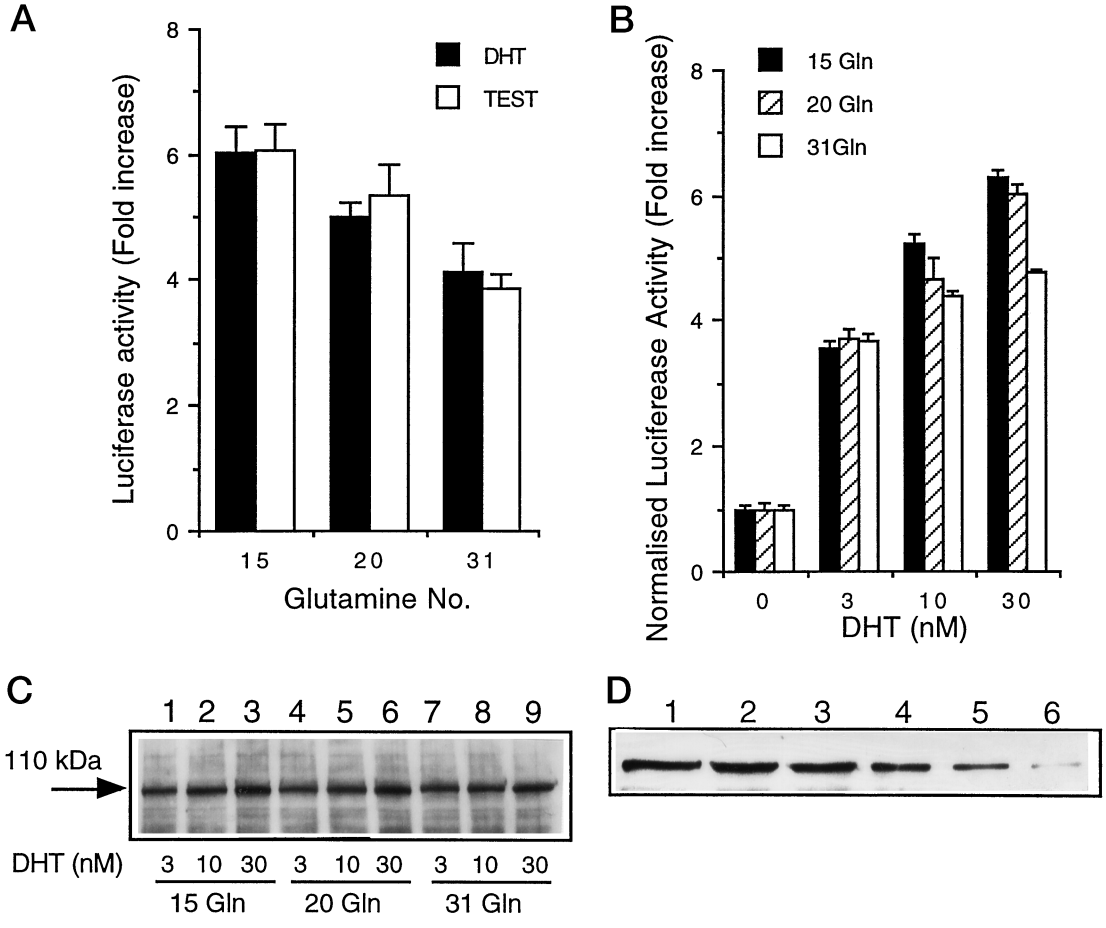

FIG. 3. *Trans*-activation capacity of AR variants with long and short polyglutamine tracts. ARs with polyglutamine tracts of three sizes (15, 20, and 31 Gln) were expressed in COS-7 cells and examined for their ability to *trans*-activate a luciferase reporter construct. *Bars* were the means ± SE of four replicates. A, In the presence of saturating doses (100 nmol/L) of the physiological androgens, DHT and testosterone (TEST), AR with 31 Gln had significantly less *trans*-activation capacity than AR with 15 Gln for both DHT ($P = 0.005$) and TEST ($P = 0.018$). B, Responses of AR variants to increasing doses of DHT. The longer AR (31 Gln) had significantly lower *trans*-activation capacity compared to the shorter AR (15 Gln) at doses of DHT above 10 nmol/L ($P < 0.01$). C, Immunoblot showing AR protein from the cell lysates of the experiment depicted in B. AR variants with 15 (lanes 1–3), 20 (lanes 4–6), and 31 (lanes 7–9) Gln were exposed to 3 nmol/L (lanes 1, 4, and 7), 10 nmol/L (lanes 2, 5, and 8), and 30 nmol/L (lanes 3, 6, and 9) DHT. Total protein (30 μg) from representative cell lysates were separated on an SDS-PAGE gel and blotted, and AR protein (*arrow*) was identified with a specific monoclonal antibody. D, Immunoblot to establish the linear range of the assay. Cell lysates from the aliquot (lane 3, C) with the strongest signal were separated on an SDS-PAGE gel and analyzed as described above. Lanes 1, 2, 3, 4, 5, and 6 contained 52.5, 45, 37.5, 30, 22.5, and 15 μg total protein, respectively. Lane 4 in this gel is equivalent to lane 3 in C.

cessive response to androgens, and androgen suppression or ablation therapy has been used to control the malignancy (27). A length less than 23 Gln was associated with a 2-fold increased risk of the cancer (7). Cases with short polyglutamine lengths had an earlier age of onset of prostate cancer (8), increased extraprostatic extension, and higher histological grade of tumor (28). Interestingly, ARs with polyglutamine lengths of 23 or fewer, the same size that was found to give an excessive risk of prostate cancer (7), were associated with a reduced risk of male infertility in the present study, suggesting that the greater androgenicity associated with the shorter Gln repeats could boost germ cell replication but at the long term risk of overstimulating the growth of prostatic tissue. Collectively, the evidence supports the hypothesis that the Gln repeat has a role in AR function by fine-tuning the balance between excess and deficient receptor function. The highly polymorphic nature of the Gln repeat would imply a subtle gradation of AR function among individuals, possibly allowing alleles with evolutionary advantages to be selected and transmitted to future generations.

None of our patients with moderate expansion of the CAG repeat segment exhibit any sign of neuromuscular disease. The greatest CAG repeat number in our patients was 31, whereas all cases of SBMA have segment lengths above 40 (4). Besides SBMA, Gln repeat expansions have been implicated in several other neurodegenerative disorders (29), including, Huntington's disease, spinocerebellar ataxia type 1, dentatorubral-pallidoluysian atrophy, and Machado-Joseph's disease. In these neurodegenerative diseases, there is no overlap between disease-causing alleles and their normal counterparts. Moderate expansion of the polyglutamine tract (28–31 Gln) exerts a modulatory effect on the usual AR function, whereas expansion beyond a threshold (≥40 Gln)

is likely to trigger a separate process that is neurotoxic. The size distribution of polyglutamine alleles in our patients with defective spermatogenesis overlapped that of fertile controls. Polyglutamine expansion confers an increased risk, but is not an absolute index of male infertility. This is not surprising, as conception is still possible with reduced sperm counts, albeit the chance of success is less than normal. In some of our cases repeat expansion may be one of several factors (30) contributing to defective spermatogenesis.

In summary, this study suggests for the first time that subjects with long polyglutamine tracts in their AR have a significantly increased risk of defective spermatogenesis. Cases with 28 or more Gln in their AR protein have a 4-fold higher risk of male infertility compared to fertile controls. On the other hand, ARs with short Gln repeats are associated with a reduced risk of male infertility. *In vitro* expression of a range of AR variants encountered in our subjects showed an inverse relationship between the length of the Gln repeat and *trans*-activation function. Thus, both *in vivo* and *in vitro* data suggest that the length of the Gln repeat could have an etiological role in male infertility through its effect on AR *trans*-activation competence. Further study of the structural mechanisms of regulation by the AR Gln repeats and the genes so regulated could lead to a greater understanding of trinucleotide repeat tracts in general, and the design of rational hormonal therapy (13) for male infertility in particular.

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