Associations between androgen receptor CAG repeat length and sperm morphology*

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BACKGROUND: The number of (trinucleotide) CAG repeats within the androgen receptor (AR) gene is inversely correlated with transcriptional activity of testosterone-target genes. Although abnormally long CAG repeats are strongly associated with male infertility, it is unclear whether CAG repeat length polymorphism can affect androgen receptor activity and sperm parameters. To explore the previously suggested association between CAG repeats and male fertility, we conducted this prospective cohort study.

METHODS: We enrolled 172 men attending the IVF unit in Shaare-Zedek Medical Center. Sperm concentration, motility and morphology and the number of CAG repeats in the AR gene were measured.

RESULTS: Mean CAG repeat length was greater in teratozoospermia (<14% normal forms, strict criteria) than in the normal morphology group [mean 66 95% confidence interval (CI) 22.19 66 0.38 versus 21.25 66 0.28, \( P = 0.02 \)]. Logistic regression models showed that longer CAG repeats were associated with abnormal sperm morphology [odds ratio for percentage of normal forms per unit increase in CAG repeat length 1.14 (95% CI 1.01–1.28), \( P = 0.04 \)]. No association was found between CAG repeat length and sperm concentration or motility.

CONCLUSIONS: We found a positive correlation between CAG repeat length and teratozoospermia. This finding validates the concept that AR function is inversely regulated by length of its CAG repeat tract.

Key words: androgen receptor gene/CAG repeat length/male subfertility/sperm morphology/teratozoospermia

Introduction

The androgen receptor (AR) mediates androgen action determining male sexual differentiation, initiation and promotion of spermatogenesis and growth of accessory sex organs. Mutations in the AR cause various degrees of androgen resistance, resulting in a range of androgen insensitivity syndromes and ambiguous genitalia (Loy and Yong, 2001). Minimal AR dysfunction can manifest solely as depressed spermatogenesis and ‘idiopathic’ male infertility in otherwise healthy males (Aiman et al., 1979).

An eight exon single copy gene in chromosome Xq11-12 encodes the AR (Tsai and O’Malley, 1994). The first exon, which encodes the transactivation domain, contains a segment of CAG repeats, translated to polyglutamine. This glutamine-repeat tract is polymorphic and its size varies from nine to 36 in normal individuals (Andrew et al., 1997).

The phenomenon of polyglutamine tracts, often of extensive length, is widespread. Expansion of CAG repeats in genes has been implicated in the pathogenesis of certain progressive neurodegenerative diseases (Lieberman and Fischbeck, 2000). CAG repeat tract expansion in the AR is associated with spinal bulbar muscular atrophy (SBMA, Kennedy disease) (La Spada et al., 1991). This fatal, X-linked, adult onset neuromuscular disease is significantly linked with expansion of CAG tract of >40 repeats and characterized by hypovirilization and testicular atrophy resulting in marked oligozoospermia or azoospermia.

In vitro studies have demonstrated a negative correlation between CAG repeat size and AR function (Chamberlain et al., 1994). Moreover, while short alleles were found to be associated with prostate cancer (Giovannucci et al., 1997; Stanford et al., 1997; Hsing et al., 2000) and polycystic ovary syndrome (Mifsud et al., 2000; Ibanez et al., 2003), longer AR CAG repeats were associated with moderate-to-severe undermasculinization (Lim et al., 2000) and with breast cancer (Levine and Boyd, 2001; Haiman et al., 2002).
The data regarding male subfertility is less consistent. Groups from China, Singapore, Japan, Australia, Greece, Germany and North America have shown that longer CAG repeats are associated with defective spermatogenesis (Tut et al., 1997; Dowsing et al., 1999; Yoshida et al., 1999; Mifsud et al., 2001; Patrizio et al., 2001; Kukuvitis et al., 2002; Pan et al., 2002; Asatiani et al., 2003; Casella 2003). However, other studies conducted in Swedish, Finnish, German, Indian and Japanese populations have failed to show a significant relationship between idiopathic defective spermatogenesis and the length of CAG repeats (Giwercman et al., 1998; Hiort et al., 1999; Dadze et al., 2000; Sasagawa et al., 2001; Van Golde et al., 2002; Rajpert-De Meyts et al., 2002; Thangaraj et al., 2002; Lund et al., 2003). One European study showed inverse correlation between sperm concentration and CAG repeat length in fertile men but no association with infertility (von Eckardstein et al., 2001). Two additional European studies have demonstrated an association between CAG repeat length and male subfertility, but found no correlation with sperm analysis variables (Legius et al., 1999; Wallerand et al., 2001). Recently some authors have suggested that differences in population distribution of CAG repeats and local environmental conditions are responsible for these disparities (Dadze et al., 2000; Patrizio et al., 2001).

Concern about the possible transmission of a premutation for neuromuscular disease and male subfertility makes interest in the clinical significance of CAG repeat expansion more than academic. The aim of this study was to assess the distribution of CAG repeat expansion in Israeli men and to determine its association with sperm parameters.

Materials and methods

Patients

One hundred and seventy-two patients attending the IVF Unit at Shaare-Zedek Medical Center in Jerusalem were prospectively enrolled in this study. Ethnic origin (Arab, Ashkenazi or Sepharadi Jew), demographic and clinical data were collected from patient files. All patients had at least two semen analyses, seminal culture, scrotal ultrasound and hormonal analysis (FSH, LH and testosterone serum levels). Patients with severe oligozoospermia (<106 sperm/ml) had chromosomal analysis. Patients with hypogonadotrophic hypogonadism, abnormal karyotype, hyperprolactinaemia, obstructive syndromes of the genital tract, gynaecomastia, exposure to radiation or chemicals, systemic illness and those under treatment with spermatogenesis impairing medication were excluded from the study. The study group included 114 men with idiopathic male infertility (see below) and the control group included 58 men with normal spermogram and female infertility (such as mechanical factor, endometriosis, polycystic ovary syndrome, low ovarian reserve etc. (n = 40) or unexplained infertility (n = 18). Patients with normal sperm parameters but no fertilization using ordinary IVF were excluded. Shaare-Zedek Medical Center’s institutional ethics committee approved the study. All participants gave their written informed consent to participate in the study.

Semen analysis

Values for semen parameters were calculated as means of at least two analyses taken at least 1 month apart. Normal semen analysis was defined according to World Health Organization (WHO, 1999) recommendations: sperm concentration >20×106/ml, motility >50% and >14% normal forms (strict criteria, Kruger et al., 1987). Patients with at least one abnormal semen parameter were included in the study group. Only those patients who had all three semen parameters within the normal range were considered as controls. Additional data were collected on specific morphological abnormalities, including abnormality location (head or non-head) and type of head abnormality (round/small or amorphous).

DNA analysis

Peripheral blood from each patient was collected in EDTA-containing tubes. Leukocytes were digested by proteinase K and purified by phenol chloroform. The DNA was precipitated by ethanol. The primers were labelled with [γ-32P]ATP using T4-polynucleotide kinase from MPI Company. Ten nanograms of genomic DNA were subjected to 35 cycles of PCR amplification. The primers were 5’-TCCAGAATCTGTGTTCCTCAT-3’ and 5’-GCTGTGGAAGTTGTCTGTTCAT-3’. The PCR amplification was performed under the following conditions: 95°C for 30 s, 58°C for 30 s and 72°C for 1 min. PCR products were separated by electrophoresis in denaturing 6% urea–polyacrylamide formamide gel, followed by autoradiography. PCR products, for which their CAG repeat lengths were identified by sequencing, were used as reference size.

Statistical analysis

Comparisons of mean CAG repeat lengths across dichotomized semen variables (normal versus abnormal) were carried out using Student’s t-test and Mann–Whitney U-test. Comparisons of mean CAG repeat lengths across quartile groups and of frequency distributions were conducted using the Kruskal–Wallis test for mean ranks. Binary logistic regression models were fitted to analyse the effects of CAG repeat length (independent variable) on sperm quality variables (dependent variables). Finally, analyses of correlations between CAG repeat length and severity of morphological abnormalities was carried out using Kendall’s tau-b correlation coefficients. All statistical analyses were performed using SPSS software (version 10.0.5, SPSS Inc., USA). Results are expressed as mean ± 95% CI.

Results

Clinical and demographic data of all participants are shown in Table I. There were no differences in age, semen parameters (concentration, motility and percentage of normal forms) and CAG length distribution between study subgroups (Arabs,
Ashkenazi and Sepharadi Jews). The median CAG repeat length was 22 repeats, the range was 13–32, the 25th percentile was 20 and the 75th percentile was 23 repeats. There was no correlation between age and any semen parameters.

Comparisons of mean CAG repeat length and of the frequency distribution of CAG repeat length for normal and abnormal semen parameters are shown in Table II and Figure 1. Mean CAG repeat length did not differ significantly for low versus normal concentration (21.61 ± 0.38 versus 21.46 ± 0.25 repeats, P = 0.4) or low versus normal motility (21.47 ± 0.28 versus 21.57 ± 0.34 repeats, P = 0.9). Mean CAG repeat length was, however, slightly greater in the abnormal morphology group than in the normal morphology group (22.19 ± 0.38 versus 21.25 ± 0.28 repeats, P = 0.02). Similar results were achieved when mean CAG repeat lengths were compared across semen variables grouped by quartiles rather than dichotomized: no significant differences in mean CAG repeat length were found across the quartile groups for concentration or motility (P = 0.75 and 0.83 respectively), whereas for morphology the CAG repeat length was greater in quartiles with poorer morphology than in those with better morphology (P = 0.01). The frequency distributions differed significantly (P < 0.05) among teratozoospermia and normal sperm morphology (Figure 1c), but not among normal and abnormal sperm concentration or motility (Figure 1a and b).

Results of logistic regression models are presented in Table III. When entered into the model as a continuous variable, CAG repeat length was not associated with sperm motility or concentration, whereas longer CAG repeats were negatively associated with normal sperm morphology [odds ratio for lower proportion of normal forms per unit increase in CAG repeat length 1.14 (95% CI 1.01–1.28), P = 0.04].

Mean CAG repeat lengths were compared for specific types of morphological semen abnormalities. No difference in mean CAG length was detected in the overall presence or absence of any type of head abnormality (21.72 ± 0.50 versus 21.31 ± 0.5 repeats, P = 0.34). Similarly, mean CAG length did not differ in the overall presence or absence of any non-head abnormality (21.89 ± 0.49 versus 21.22 ± 0.38 repeats, P = 0.11). When the analysis was stratified by specific types of head abnormalities (amorphous morphology and round/small head abnormalities) ranked by severity from 0 (absent) to very severe 4), no correlations were identified between mean CAG repeat length and the severity level of the abnormality (Kendall’s tau-b correlation coefficient = 0.08 for amorphous type, P = 0.17, and –0.02 for round/small type, P = 0.71).

Discussion
To date, several mutations in the AR gene have been reported with accompanying defects in the spermatogenesis of affected
patients. These cases, however, are quite rare (Akin et al., 1991; Tsukada et al., 1994; Yong et al., 1994; Knoke et al., 1999). Several recent studies have questioned the significance of polymorphism of the trinucleotide (CAG) repeat in exon 1 of the AR gene in infertile males (Loy and Yong, 2001). We investigated the association between expansion of the CAG repeat of the AR gene and defective sperm production in infertile Israeli men. The main finding of our study is a significantly positive correlation between CAG repeat length and teratozoospermia (Table II and III and Figure 1c).

In accordance with the findings of some authors (Giwercman et al., 1998; Dadze et al., 2000; Sasagawa et al., 2001; Rajpert-De Meyts et al., 2002; Van Golde et al., 2002; Asatiani et al., 2003) and in contrast to those of others (Tut et al., 1997; Dowsing et al., 1999; Yoshida et al., 1999; Mifsud et al., 2001; Patrizio et al., 2001), we found no association between CAG repeat elongation and semen concentration or motility. A possible explanation for these conflicting data is ethnic differences in the study populations, which may have led to different results in the European and Asian research. The CAG repeat length is highly polymorphic between ethnic groups (Sartor et al., 1999). African Americans generally have shorter CAG repeats (La Spada et al., 1991; Giovannucci et al., 1997), which are less prevalent in Asian populations (Irvine et al., 1995). This explanation is supported by the relative homogeneity of the European research results (Rajpert-De Meyts et al., 2002), while studies in Asian populations are less consistent. Especially confusing are the results of studies in the Japanese population (Komori et al., 1999; Yoshida et al., 1999; Sasagawa et al., 2001). Most participants in our study are of Semitic origin. Interestingly, there was no difference in CAG length distribution between men of different ethnic subgroups in our study (Arabs, Ashkenazi and Sepharadi Jews, Table I).

An attempt to categorize the semen analysis variables according to WHO and Kruger’s criteria and compare the groups for CAG repeat prevalence failed to yield significance. Recently, in a large multi-centre study, it was found that the accepted WHO criteria for normal semen analysis were poor predictors for fertility (Guzick et al., 2001). Even though sperm morphology appeared to be the most discriminating predictor of infertility. The sensitivity of sperm morphology as a predictor of infertility was corroborated by numerous studies, particularly since Kruger championed it as the main predictor of fertility (Kruger et al., 1987; Vawda et al., 1996; Menkveld et al., 2001). In our study the only variable significantly associated with CAG repeat length was morphology, expressed as percentage of normal forms.

Our findings validate the concept that polymorphism of AR CAG length may contribute to spermatogenesis efficiency through a subtle modulatory effect on AR function. CAG repeats most likely do not have a major independent effect on reproduction, but rather modify or fine-tune endocrine feedback systems and hormone action. If this were not so, the specific polymorphism would be expected to disappear from the reproductive pool. The overall fertility status of an individual depends not only on AR sequence alterations but rather on interactions between other genomic and environmental parameters. Probably comparison of groups according to any categorised sperm analysis variables is a crude instrument for such fine AR activity modifier as CAG repeat.

In conclusion, we found a positive correlation between AR CAG repeat length and teratozoospermia. This finding validates the concept that the length of AR CAG repeats tract is negatively associated with spermatogenesis. However, we do not conclude that mild expansion of the CAG repeat tract directly cause teratospermia, but assume that men with longer CAG repeats might be more prone to spermatogenesis defects in response to any pathogen/epigenetic factors. Genetic screening for AR CAG length maybe offered to couples with male factor infertility, at least in those populations where an association with reproductive variables could be found.

Acknowledgement

We thank the nursing and secretary staff of the IVF Unit in Shaare-Zedek Medical Center for their assistance.

References


Submitted on September 8, 2003; accepted on February 18, 2004