CAG REPEAT NUMBER IN ANDROGEN RECEPTOR GENE AND MALE INFERTILITY

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ABSTRACT

Androgens are essential for male sexual development and for fertility. They exert their action through the androgen receptor (AR), a ligand-activated transcription factor. The 5' end of exon 1 of the AR gene includes a polymorphic CAG triplet repeat that varies in number between 10 to 36 in the normal population. There is controversy over an association between high CAG repeat numbers in the AR gene and male infertility. We have evaluated the possible effect of long CAG repeats in the AR on infertility in men from the Republic of Macedonia (R. Macedonia). A group of 222 infertile/subfertile males with different sperm counts and a control group of 152 proven fathers were studied. The CAG repeat number was determined by fluorescent polymerase chain reaction (PCR) amplification of exon 1 of the AR gene analyzed by capillary electrophoresis. Mean CAG length did not differ significantly between males with azoospermia (22.0 ± 3.1), mild oligozoospermia (22.4 ± 2.6), severe oligozoospermia (23.0 ± 4.2), normozoospermia (21.8 ± 2.4), or known causes of infertility (22.1 ± 2.9) and fertile controls (22.3 ± 2.9). However, we found a significantly higher percentage of CAG repeats >26 ($p = 0.022$), >27

($p = 0.018$) and >28 ($p = 0.009$) in males with mild oligozoospermia. These results indicate a possible association between CAG repeat length and mild oligozoospermia. Further studies on a larger number of patients with mild oligozoospermia are warranted to confirm this association.

Key words: Androgen receptor (AR); CAG repeats; Male infertility; Oligozoospermia

INTRODUCTION

Infertility occurs in 10-15% of couples, male factor infertility representing about 50% of the cases [1]. It is now evident that a significant proportion of male infertility is associated with a variety of genetic abnormalities. Genetic defects associated with male infertility include chromosomal aberrations, Y chromosome microdeletions involving one or more azoospermia factor (AZF) regions, mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, and mutations in the androgen receptor (AR) gene.

Androgens are essential for male sexual development and for fertility. They act through the AR, which is a transcriptional factor that contains functional domains for DNA binding, ligand binding and transcriptional regulation. The 5' end of exon 1 of the AR gene includes a polymorphic CAG triplet repeat that codes for a polyglutamine tract. The number of CAG repeats in the normal population varies between 10 and 36. Expansion of the polyglutamine tract to >38 repeats in males leads to Kennedy disease [spinal bulbar muscular atrophy

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(SBMA)] [2]. In addition to neurological symptoms, SBMA patients show signs of hypogonadism, such as gynecomastia, impotence, testicular atrophy and reduced fertility.

In vitro studies have demonstrated a negative correlation between CAG repeat size and AR function [3]. Short AR CAG repeat tracts have been associated with prostate cancer [4] and polycystic ovary syndrome [5], while long CAG repeat tracts have been associated with moderate-to-severe undermasculinization [6], breast cancer [7] and male subfertility [8]. The possible association of a long CAG repeat with male infertility in Asian populations was suggested because of a four-fold increase in the risk of impaired spermatogenesis in males who had >28 CAG repeats [8]. Since then, the association of the long CAG repeat number in the AR gene and male infertility has been controversial. This study aims to evaluate the possible effect of long CAG repeat tracts in the AR on infertility among Macedonian men.

MATERIALS AND METHODS

Materials. A total of 222 patients (representing 231 X chromosomes) attending the Andrological Outpatient Unit at the Clinic for Endocrinology and Metabolic Disorders, Faculty of Medicine, Skopje, R. Macedonia, were enrolled in the study. All patients gave their informed consent to participate in the study. Semen analysis was performed in accordance with the World Health Organization (WHO) guidelines. All patients were routinely screened for the presence of chromosomal aneuploidies of the X and Y chromosomes by quantitative fluorescent polymerase chain reaction (QF-PCR) analysis and for the presence of Y microdeletions by multiplex analysis of six sequence tagged sites (STS) in the three AZF regions. The studied group included 27 patients with a known cause of infertility (Y microdeletions, XXY, XX males and obstructive azoospermia representing 36 X chromosomes). The other 195 patients were grouped according to the results of semen analysis (patients with azoospermia, $n = 73$; severe oligozoospermia or sperm counts of $<5 \times 10^6/\text{mL}$, $n = 51$; mild oligozoospermia or sperm counts of $>5 \times 10^6/\text{mL}$, $n = 31$; and normozoospermia with unexplained couple infertility, $n = 40$). In addition, 152 men who have fathered at least one

child by natural conception and whose paternity was proved by DNA analysis served as controls.

Methods. Genomic DNA was isolated from leukocytes using the Proteinase K/SDS digestion-phenol/chloroform extraction-ethanol precipitation method (9). The CAG repeat number was determined by fluorescent PCR amplification of exon 1 of the AR gene using the following primers: direct 5'-(HEX) TCC AGA ATC TGT TCC AGA GCG TGC-3' and reverse 5'-GCT GTG AAG GTT GCT GTT CCT CA-3'. The size of the PCR product was determined by capillary electrophoresis on an ABI PRISM™ 310 Genetic Analyzer (Applied BioSystems, Foster City, CA, USA). The number of CAG repeats predicted by the Genescan software (Applied BioSystems) was compared with the actual CAG repeats determined by direct dideoxy terminator cycle sequencing using the Big Dye Terminator Sequencing Kit v1.0 (Applied BioSystems) in male DNA samples with 14, 19, 21, 25 and 29 CAG repeats. Figure 1 shows the Genescan profiles of DNAs with 19, 21, 23 and 26 CAG repeats, while Figure 2 shows part of the DNA sequence of AR gene exon 1 in a DNA sample with 19 CAG repeats.

Statistical Analysis. The frequencies of the CAG repeat alleles were compared between the various groups of infertile/subfertile males and fertile controls using the χ^2 and Fisher's exact tests. Differences in the mean of (CAG) n length between different groups of patients vs. controls were tested by the independent samples t-test. Statistical significance was defined as $p < 0.05$.

RESULTS

The distribution of CAG repeats in the subgroups of infertile/subfertile men and of fertile controls is shown in Figure 3. The range of CAG repeat number, mean CAG repeat length and median for all subgroups is given in Table 1. Mean CAG length did not differ between the subgroups of infertile/subfertile males and the fertile controls. The percentages of long CAG repeats (defined at the cut offs: >26, >27 and >28) are given in Table 2. The highest number of long CAG repeats was found in patients with mild oligozoospermia, the difference being statistically significant when compared to fertile controls.

Many studies have investigated the suggested link between increased CAG repeat length in exon 1 of the AR gene and depressed spermatogenesis and male infertility. Studies conducted in China [10], Singapore [11], Japan [12], Australia [13],

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Figure 3. Distribution of CAG repeats in exon 1 of the AR gene in infertile/subfertile males with different sperm counts (azoospermia, $n = 73$; severe oligozoospermia, $n = 51$; mild oligozoospermia, $n = 31$; normozoospermia, $n = 40$), infertile men with known cause of infertility ($n = 36$ X chromosomes) and in fertile males ($n = 152$).

Table 1. The CAG repeat numbers in different groups of infertile/subfertile patients and fertile controls

Group	Number of Chromosomes	CAG Repeat Range	Mean $\pm$ SD	Median
Azoospermia	73	13–32	22.0 $\pm$ 3.1	21.0
Severe oligozoospermia	51	17–28	22.4 $\pm$ 2.8	22.0
Mild oligozoospermia	31	13–34	23.0 $\pm$ 4.2	22.0
Normozoospermia	40	18–28	21.8 $\pm$ 2.4	21.0
Known cause of infertility	36	16–30	22.0 $\pm$ 2.9	22.5
Fertile controls	152	15–30	22.3 $\pm$ 3.9	22.0

Table 2. The percentage of long CAG repeats in different groups of infertile/subfertile patients and fertile controls

Group (number of chromosomes)	CAG Repeats					
	>28		>27		>26	
	<i>n</i> (%)	<i>p</i> Value	<i>n</i> (%)	<i>p</i> Value	<i>n</i> (%)	<i>p</i> Value
Azoospermia (<i>n</i> = 73)	2 (2.7)	0.449	3 (4.1)	0.866	7 (9.6)	0.424
Severe oligozoospermia (<i>n</i> = 51)	0 (0.0)	0.410	1 (2.0)	0.401	2 (3.9)	0.486
Mild oligozoospermia (<i>n</i> = 31)	3 (9.7)	0.009	5 (16.1)	0.018	6 (19.4)	0.022
Normozoospermia (<i>n</i> = 40)	0 (0.0)	0.266	1 (2.5)	0.553	1 (2.5)	0.323
Known cause of infertility (<i>n</i> = 36)	1 (2.8)	0.529	1 (2.8)	0.625	2 (5.6)	0.821
Fertile controls (<i>n</i> = 152)	2 (1.3)		7 (4.6)		10 (6.6)	

for the conflicting reports [20]. To clarify the relevance of CAG repeat size and male infertility, further studies with more homogeneous infertile/subfertile patients should be conducted.

We analyzed the number of CAG repeats among different subgroups of infertile/subfertile patients categorized according to their sperm counts (azoospermia, severe oligozoospermia, mild oligozoospermia and normozoospermia). All those with known causes of spermatogenic failure, such as Y microdeletions, XXY, XX males and obstructive azoospermia were analyzed as a separate subgroup consisting of patients with a known cause of infertility.

This is the first report of the distribution of AR CAG repeats among men from R. Macedonia and the first to correlate the length of AR CAG repeat tracts with the impaired spermatogenesis and male infertility. We failed to find an association between the mean number of CAG repeats among any of the infertile/subfertile subgroups and the fertile controls. However, we found a significantly higher percentage of CAG repeats >26 ($p = 0.022$), >27 ($p = 0.018$) and >28 ($p = 0.009$) in males with mild oligozoospermia. These results indicate a possible association between CAG repeat length and mild oligozoospermia. It is possible that long AR CAG repeat number acts as a low penetrance risk factor,

which slightly impairs spermatogenesis, and probably acts in a more complex manner together with other genetic and/or environmental factors. Further studies on a larger number of patients with mild oligozoospermia are warranted to confirm the association between impaired spermatogenesis and mild oligozoospermia.

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