

## No Association of the CAG Repeat Length in Exon 1 of the Androgen Receptor Gene with Idiopathic Infertility in Turkish Men: Implications and Literature Review

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TUFAN, A.C., SATIROGLU-TUFAN, N.L., AYDINURAZ, B., SATIROGLU, M.H., AYDOS, K. and HUSEYIN, B. *No Association of The CAG Repeat Length in Exon 1 of the Androgen Receptor Gene with Idiopathic Infertility in Turkish Men: Implications and Literature Review.* Tohoku J. Exp. Med., 2005, **206** (2), 105-115 — While the correlation between the CAG repeat length of the androgen receptor (AR) gene and idiopathic male infertility is still unclear, ethnic background of the population studied may play an important role in this association. The objective of this study was to determine whether changes in the CAG repeat length are associated with spermatogenic defects in Turkish infertile men. Reproductive hormone concentrations and the CAG repeat length in exon 1 of the AR gene in 47 idiopathic infertile men and 32 fertile controls were analyzed. The mean serum luteinising hormone (LH) and follicle stimulating hormone (FSH) levels were significantly higher in the infertile group than those of the control group ( $p < 0.0001$  for both comparisons), whereas the mean serum testosterone level in the infertile group did not differ significantly from that in the control group ( $p = 0.16$ ). The mean CAG repeat length of the AR gene in the infertile group did not differ significantly from that in the control group ( $22.28 \pm 0.37$  vs  $22.41 \pm 0.54$ , respectively;  $p = 0.84$ ). In addition, the frequency of having a CAG repeat number ( $\geq 24$ ) was also comparable between the infertile patients and fertile controls (31.9% vs 40.6%, respectively;  $p = 0.21$ ). In conclusion, reproductive hormones with elevated LH and FSH, and normal or low testosterone levels were suggestive of partial impairment of testicular function. However, no statistically significant relationship between the length of the CAG repeat and idiopathic impaired sperm production was observed in the Turkish population studied. These results support the findings of previously published European studies, but are contrary to the findings from Caucasian and North American population studies. Thus, ethnicity and genetic backgrounds seem to be important in this association, and studies from a variety of different ethnic and genetic backgrounds using

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comparable patient subgroups are valuable to further evaluate this association. ———  
 androgen receptor gene; CAG repeat; male infertility; defective spermatogenesis  
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Androgens are essential steroid hormones that determine the expression of the male phenotype (Yong et al. 2000). Their actions are mediated by a single androgen receptor (AR) translocating to the nucleus upon binding to its ligand in order to regulate the expression of androgen-responsive genes. Mutations that interfere with the AR function result in the complete feminization of 46 XY individuals and the complete androgen insensitivity syndrome. Studies have revealed that AR mutations that partially interfere with its activity can cause a wide spectrum of milder androgen insensitivity syndromes, from ambiguous genitalia in newborn infants to “idiopathic” male infertility. Since the definition of the sequence and structure of the AR gene and protein it codes, more than 300 mutations have been reported in the ligand binding and DNA binding domains, accounting probably for the most naturally mutated transcription factor known (Yaegashi et al. 1999).

Androgens acting via the AR are also required for normal spermatogenesis (van Rooijen et al. 1995; McLachlan et al. 1996). The AR gene located on the long arm of the X chromosome at Xq11-12 expands a length of more than 90 kb including 8 exons (Lubahn et al. 1988; Tilley et al. 1989; Marcelli et al. 1990) that code for a protein of approximately 110-114 kDa (910-919 amino

acids) with 4 functional domains (Fig. 1): exon 1 encodes the transactivation domain in the amino-terminal part of the protein (Lubahn et al. 1989; Jenster et al. 1995), exons 2 and 3 encode the DNA binding domain, exon 4 encodes the hinge domain and exons 5–8 encode the ligand binding domain (Lubahn et al. 1989; Quigley 1998). The transactivation domain makes up half the receptor (1,586 bp) and has two polymorphic repetitive DNA sequences consisting of the trinucleotide repeats, i.e., (CAG)<sub>n</sub> and (GGC)<sub>n</sub> (Fig. 1).

The effect of the variation in the length of the GGC tract on AR activity is less clear compared to that of CAG tract. A few epidemiological investigations of the association between the number of GGC repeats and AR gene function have produced inconsistent results (Chen et al. 2002). On the other hand, the observation that the elongation of CAG repeat in exon 1 of the AR gene causes pathogenesis of X-linked spinal and bulbar muscular atrophy (SBMA, or Kennedy’s disease) strongly suggested that this region may play an important role in AR function (La Spada et al. 1991; Belsham et al. 1992). This hypothesis has extensively been investigated and a link between idiopathic male infertility and expansion of this polymorphic CAG tract in the AR gene has been suggested by several studies within the last

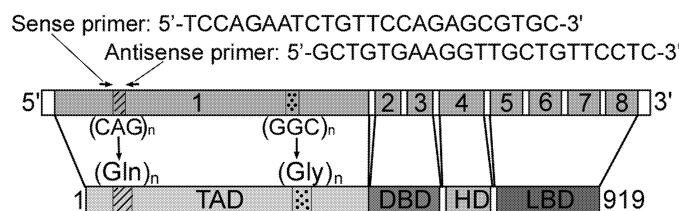


Fig. 1. Schematic representation (not to scale) of the structure of the androgen receptor gene and protein including the primer pair sequences used in this study. The gene consists of eight exons that when translated result in a protein with four main functional domains: the transactivation domain (TAD), the DNA-binding domain (DBD), the hinge domain (HD), and the ligand-binding domain (LBD). The TAD makes up half the receptor and has two polymorphic regions (shaded and dated boxes) consisting of the trinucleotide repeats, (CAG)<sub>n</sub> and (GGC)<sub>n</sub>, respectively. Gln, Glutamine; Gly, Glycine.

decade (Tut et al. 1997; Yoshida et al. 1999; Dowsing et al. 1999; Mifsud et al. 2001; Patrizio et al. 2001; Wallerand et al. 2001; Madgar et al. 2002; Pan et al. 2002; Asatiani et al. 2003; Casella et al. 2003; Mengual et al. 2003), whereas several others were unable to demonstrate an association (Giwercman et al. 1998; Hiort et al. 1999; Legius et al. 1999; Dadze et al. 2000; Sasagawa et al. 2000, 2001; Von Eckardstein et al. 2001; Yu and Handelsman 2001; Rajpert-De Meyts et al. 2002; Van Golde et al. 2002; Thangaraj et al. 2002; Kukuvtis et al. 2002; Erasmuson et al. 2003; Dhillon and Husain 2003; Lund et al. 2003; Tse et al. 2003; Ferlin et al. 2004). Thus, the ethnic background of the population studied may play an important role in the correlation between CAG repeat length and male infertility.

Recent studies in this field aimed to investigate the suggested association in diverse populations to further expand the information about the ethnicity and genetic backgrounds. Thus, the objective of this study was to determine for the first time whether changes in CAG repeat length of the AR gene are associated with spermatogenic defects in Turkish patients with idiopathic male infertility. A comprehensive review and summary of the previously published data concerning the correlation between CAG repeat length and male infertility is also provided in this study.

## MATERIALS AND METHODS

### *Subjects*

This study on human subjects or materials was approved by the Medical Ethics Committee of the Pamukkale University, Denizli, Turkey.

A total of 47 patients of Turkish ethnic origin were recruited from Gen-Art, IVF and Reproductive Biotechnology Center, Ankara, Turkey. All of these patients had non-obstructive impairment of spermatogenesis. Moreover, none of these patients showed any clinical indication of SBMA. Control subjects were 32 males of Turkish ethnic origin and of proven fertility, who have fathered at least one child by natural conception. Semen parameters were evaluated according to published recommendations (World Health Organization 1996). Semen analysis of the patients was performed at least twice at 4-8 week intervals after at least 2 days of abstinence, and the mean result recorded. The 47 patients were

divided into subgroups according to the severity of the defects in spermatogenesis: 18 had no spermatozoa in ejaculate (azoospermia), 7 had a sperm concentration of  $< 3 \times 10^6$ /ml ejaculate (severe oligozoospermia), 5 had a sperm concentration of  $3-19 \times 10^6$ /ml ejaculate (oligozoospermia) and 17 had a sperm concentration of  $\geq 20 \times 10^6$ /ml ejaculate with poor progressive motility within 1 h of ejaculation ( $\leq 25\%$  class a, or  $\leq 50\%$  class a and b) and/or sperm morphology ( $\leq 30\%$ ). Ages of the fertile controls ranged from 28 to 60 (mean  $37.3 \pm 8.4$ ) and that of infertile patients ranged from 26 to 54 (mean  $33.7 \pm 5.7$ ).

Blood was taken for DNA extraction and for the measurement of reproductive hormone (LH, FSH, and testosterone) concentrations. DNA was isolated from 5-6 ml blood samples with QIAamp DNA Blood Mini Kit method (Qiagen, Hilden, Germany) according to the manufacturers "blood and body fluid protocol". The details of the reproductive hormone concentrations for fertile controls and infertile men are summarized in Table 1.

### *Polymerase chain reaction (PCR) of the AR gene and trinucleotide analysis*

The genomic DNA samples of all the subjects were blind-coded and the CAG repeat length of the AR gene was assessed by means of a PCR with primers specific for the repeat segment (sense primer: 5'-TCCAGAATCTGTTCCAGAGCGTGC, and antisense primer: 5'-GCTGTGAAGGTTGCTGTTCCTC; Mifsud et al. 2001; Fig. 1). Sense and antisense strands were directly sequenced with an Amersham DYEnamic ET Terminator Cycle Sequencing Kit (Amersham Biosciences Corp., Piscataway, NJ, USA) and an ABI PRISM<sup>®</sup> 310 (AME Bioscience Ltd., London, UK) DNA sequencer (performed at Iontek Ltd., Istanbul, Turkey).

### *Statistical analysis*

Results were statistically evaluated using the Mann-Whitney's U-test and the test for difference between two population proportions.  $p < 0.05$  was taken as statistically significant for both tests.

## RESULTS

The mean serum LH level in the infertile group was significantly higher than that of the control group ( $6.99 \pm 0.74$  IU/l [range 2.2-13.8] vs  $3.35 \pm 0.43$  IU/l [range 1.9-5.9], respectively;  $p < 0.0001$ ) (Table 1). The mean serum FSH level in the infertile group was also significantly higher

TABLE 1. *Hormonal features of fertile controls and infertile men with idiopathic spermatogenic disorder*

Study Groups	n	Age (years)	Hormone concentration (mean $\pm$ s.e. [range])		
			FSH (IU/l)	LH (IU/l)	Testosterone (nmol/l)
Fertile controls	32	28-74	3.54 $\pm$ 0.51 (1.1-5.43)	3.35 $\pm$ 0.43 (1.9-5.9)	21.79 $\pm$ 1.92 (10.3-27.5)
Infertile men**	47	26-54	13.53 $\pm$ 1.87* (3.4-36)	6.99 $\pm$ 0.74* (2.2-13.8)	18.45 $\pm$ 1.41 (3.8-27.8)
Severe oligozoospermia***	25	26-54	15.97 $\pm$ 1.88* (5.7-36)	7.76 $\pm$ 1.05* (4.2-13.8)	17.52 $\pm$ 2.2 (3.8-27.8)

Normal hormone ranges: follicle stimulating hormone (FSH), 1.5-8.0 IU/l; luteinising hormone (LH), 1.5-6.0 IU/l; and testosterone, 9.0-34.0 nmol/l.

\* Statistically different from fertile controls by Mann-Whitney's U-test at  $p < 0.05$ ; \*\* Cumulative;

\*\*\* These patients were selected from the cumulative infertile group according to the criteria given in materials and methods section.

than that in the control group (13.53  $\pm$  1.87 IU/l [range 3.44-36] vs 3.54  $\pm$  0.51 IU/l [range 1.1-5.43], respectively;  $p < 0.0001$ ) (Table 1). In contrast, the mean serum testosterone level in the infertile group did not differ significantly from that in the control group (18.45  $\pm$  1.41 nmol/l [range 3.8-27.8] vs 21.79  $\pm$  1.92 nmol/l [range 10.3-27.5], respectively;  $p = 0.16$ ) (Table 1). The same trend with more significant results was also

observed when severe oligozoospermic patients were tested in comparison to the fertile controls (Table 1).

The mean of the AR gene CAG repeat length in the infertile group was 22.28  $\pm$  0.37 (range 18-29), while that of fertile controls was 22.41  $\pm$  0.54 (range 16-29), and the difference was statistically not significant ( $p = 0.84$ , Table 2). The mean length of the AR gene CAG tract in individ-

TABLE 2. *(CAG) repeat numbers of fertile controls and infertile men with idiopathic spermatogenic disorder*

Study groups	n	Age (years)	CAG repeat number (mean $\pm$ s.e. [range])
Fertile controls	32	28-60	22.41 $\pm$ 0.54 (16-29)
Infertile men (cumulative)	47	26-54	22.28 $\pm$ 0.34 (18-29)
Oligozoospermia**	30	26-54	21.6 $\pm$ 0.39 (18-25)
Poor motility and/or morphology only**	17	31-46	23.47 $\pm$ 0.68 (19-29)*
Oligozoospermia/severe oligozoospermia with poor motility and/or morphology**	11	31-49	21.18 $\pm$ 0.71 (18-25)
Oligozoospermia**	5	32-36	21.0 $\pm$ 1.14 (18-25)
Severe oligozoospermia**	7	30-49	21.85 $\pm$ 0.98 (18-25)
Azoospermia**	18	26-54	21.67 $\pm$ 0.46 (19-25)
Severe oligozoospermia (combined)**	25	26-54	21.72 $\pm$ 0.42 (18-25)

\* Statistically different from Oligozoospermia group by Mann Whitney's U-test at  $p < 0.05$ .

\*\* Patients in these subgroups were selected from the cumulative infertile group according to the criteria given in materials and methods section.

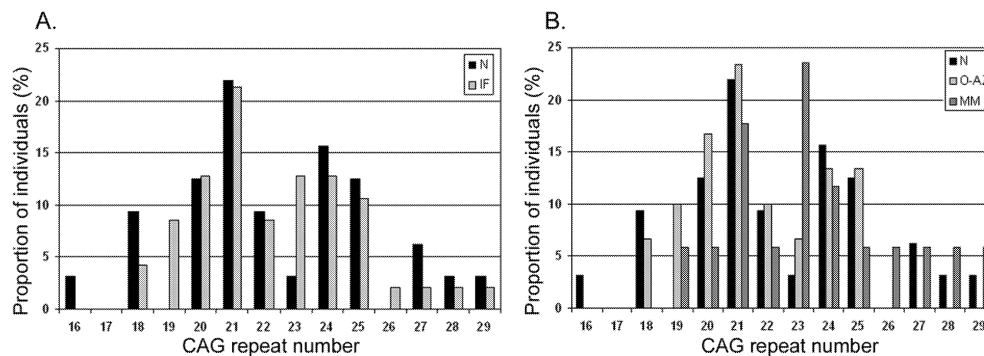


Fig. 2. Distribution of the CAG repeat sizes in exon 1 of the AR gene of fertile controls and infertile men in general (A), fertile controls, oligo-azoospermia group, and poor motility/morphology only group (B). F, fertile controls; IF, infertile men in general; O-AZ, oligo200-azoospermia group; MM, poor motility/morphology only group.

ual specific patient subgroups, i.e., oligozoo-azoospermia ( $n = 30$ ), poor motility/morphology only ( $n = 17$ ), oligozoo-severe oligozoospermia with poor motility/morphology ( $n = 11$ ), oligozoospermia only ( $n = 5$ ), severe oligozoospermia only ( $n = 7$ ), azoospermia only ( $n = 18$ ), severe oligozoo-azoospermia ( $n = 25$ ), were also comparable to that of fertile controls (Table 2). The highest mean CAG repeat number was observed in the poor motility/morphology only group (mean  $23.47 \pm 0.68$ , range 19-29), however, this was only significantly higher than the CAG repeat number of the oligozoo-azoospermic group (mean  $21.6 \pm 0.39$ , range 18-25) but not from the fertile controls (Table 2). In addition, the frequency of having a CAG repeat number ( $\geq 24$ ) was 31.9% in infertile men versus 40.6% in fertile controls ( $p = 0.21$ ), whereas the frequency of having a CAG repeat number ( $\geq 26$ ) was 8.5% in infertile men versus 12.5% in fertile controls ( $p = 0.28$ ) while non of which was statistically significant (Fig. 2 and Table 3).

## DISCUSSION

Infertility affects 10-15% of all couples, and impaired sperm production accounts for about half of these cases. The cause of defective spermatogenesis, however, remains largely obscure. Androgens are required for normal spermatogenesis, i.e., they play an important role in round spermatid development, attachment to Sertoli cells

and elongation of spermatids (van Roijen et al. 1995; McLachlan et al. 1996). However, most males with impaired spermatogenesis have normal serum androgen levels. Therefore, attention was focused on the AR as a possible cause of the impaired spermatogenesis in subjects with idiopathic male infertility. It has long been postulated that defects in the AR gene can lead to partial or complete spermatogenic failure (Aiman et al. 1979; Aiman and Griffin 1982) and more recently it has been shown that impaired function of the AR induces abnormalities in androgen action resulting in abnormal conditions such as androgen insensitivity syndrome (AIS) and infertility (Yong et al. 2000).

As mentioned earlier the AR gene contains two polymorphic trinucleotide repeat loci:  $(CAG)_n$  and  $(GGC)_n$ , which code for polyglutamine and polyglycine tracts, respectively. Both loci are located in exon 1, which encodes the transactivation domain of the receptor protein (Fig. 1). Expansion of the polyglutamine segment in the male leads to SBMA, a fatal neuromuscular disease associated with low virilization, severe oligozoo-azoospermia, testicular atrophy, and reduced fertility (La Spada et al. 1991; Belsham et al. 1992). Relatively short polyglutamine tracts with or without associated polyglycine tract changes have been linked to an increased risk of prostate cancer, an androgen-dependent tumor (Irvine et al. 1994).

TABLE 3. Summary of previously published data on CAG repeat numbers of AR gene among infertile men and controls

Study (year)	Ethnicity	Infertile men				Controls			Statistically significant differences?	
		Number of men (number azoo-spermic)	Mean (range) (CAG) <sub>n</sub>	Number of men with (CAG) <sub>n</sub> ≥ 26	Number of men	Mean (range) (CAG) <sub>n</sub>	Number of men with (CAG) <sub>n</sub> ≥ 26	Mean (CAG) <sub>n</sub> <sup>a</sup>	Proportion with (CAG) <sub>n</sub> ≥ 26 <sup>b</sup>	
									Infertile	Azoospermic
Giwercman et al. (1998)	Swedish	33 (6)	21.9 (16-27)	3 (9.1%)	294 <sup>δ</sup>	23.2 (8-30)	51 (17.3%)	No	No	...
Legius et al. (1999)	Belgian	223 (22)	21 <sup>α</sup> (15-30)	18 (8.1%)	181 <sup>*δ</sup>	21 <sup>α</sup> (14-29)	4 (2.2%)	No	Yes (p < 0.01)	...
Hjort et al. (1999)	German	180 (13)	23 (13-30)	20 (11.1%)	53 <sup>δ</sup>	24 (17-39)	9 (17%)	No	No	...
Komori et al. (1999)	Japanese	59 <sup>η</sup>	21.2 (14-32)	7 (11.9%)	36	21.4 (16-31)	3 (8.3%)	No	No <sup>£</sup>	...
Dadze et al. (2000)	German	119 (18)	22 (16-34)	19 (16%)	22 <sup>δ</sup>	20.8 (15-26)	1 (4.5%)	No	No	No
Sasagawa et al. (2000)	Japanese	48 <sup>η</sup>	23.4 (16-32)	...	100	23.5 (15-35)	...	No	...	...
Sasagawa et al. (2001)	Japanese	30 (30)	23.4 (19-30)	9 (30%)	51	23.7 (17-28)	9 (17.6%)	No	...	No
Von Eckardstein et al. (2001)	German	43 <sup>η</sup>	...	...	131	...	...	No	...	...
Yu and Handelsman (2001)	Australian	54 (54)	19.8 (8-24)	...	106	19 (5-28)	...	No	...	...
Rajpert-De Meyts et al. (2002)	Danish	119 (30)	21.5 (15-29)	12 (10.1%)	110	21.8 (14-33)	12 (10.9%)	No	No	No
Von Golde et al. (2002)	Dutch	75 (75)	22.2 (13-36)	9 (12%)	70 <sup>β</sup>	21.7 (15-31)	5 (7.1%)	No	...	No
Thangaraj et al. (2002)	Indian	280 (280)	21.7 (12-32)	24 (8.6%)	201	22.4 (12-32)	24 (11.9%)	No	...	No
Kukuvitis et al. (2002)	Greek	109 (42)	21 <sup>α</sup> (15-33)	6 <sup>+</sup> (5.5%)	64	21 <sup>α</sup> (14-31)	1 (1.5%)	No	No	No
Erasmuson et al. (2003)	Caucasian	105 <sup>η</sup>	21.5 (12-30)	5 <sup>&amp;</sup> (4.8%)	93 <sup>*</sup>	20.99 (9-26)	0 <sup>&amp;</sup> (0%)	No	Yes (p < 0.02)	No
Lund et al. (2003)	Finish	192 <sup>η</sup>	22.2 (16-30)	27 (14.1%)	149	22.4 (15-29)	18 (12.1%)	No	No	No

Dhillon and Husain (2003)	Indian	183 (103) <sup>Δ</sup>	22.2 (13-33)	...	59	21.5 (12-33)	...	No	No	No
Tse et al. (2003)	Hong Kong Chinese	85 (53)	23.1 (14-36)	19 (22.3%)	45	23 (16-30)	9 (20%)	No	No	No
Ferlin et al. (2004)	Italian	163 <sup>η</sup>	...	...	115	...	...	No	...	...
Tufan et al. (This Study)	Turkish	47 (25)	22.3 (18-29)	4 (8.5%)	32	22.4 (16-29)	4 (12.5%)	No	No	No
Tut et al. (1997)	Chinese	153 (28)	...	75 (49%)	72	...	29 (40.2%)	...	No	Yes (p < 0.02)
Yoshida et al. (1999)	Japanese	41 (41)	26.5 (20-34)	20 (48.8%)	48	23.5 (17-30)	15 (31.3%)	Yes (p = 0.0013)	...	Yes (p < 0.05)
Dowsing et al. (1999)	Australian	30 (10)	23.2 (15-34)	7 (23.3%)	32	20.5 (17-25)	0 (0%)	Yes (p = 0.0001)	Yes (p < 0.002)	Yes (p < 0.02)
Mifsud et al. (2001)	U.S.A.	95 (23)	21.9 (14-31)	7 (29.4%) <sup>θ</sup>	55	20.7 (8-27)	3 (5.4%)	Yes (p = 0.034)	...	Yes (p < 0.002)
	Singapore	120 (33)	23.1 (14-33)	8 (24.2%) <sup>θ</sup>	87	22.4 (11-29)	12 (13.8%)	Yes <sup>θ</sup> (p = 0.043)	...	No
Wallerand et al. (2001)	France	37 <sup>η</sup>	23.9 (13-28)	16 (43.2%)	50	22.2 (17-27)	7 (14%)	Yes (p = 0.008)	Yes (p < 0.002)	...
Patrizio et al. (2001)	U.S.A.	69 (16)	23.5 (18-39)	10 (14.5%)	45	22 (12-30)	4 (8.9%)	Yes (p = 0.03)	No	Yes (p < 0.03)
Madgar et al. (2002)	Israeli	61 (46)	18.6 (13-24)	0 (0%)	50	16.6 (11-22)	0 (0%)	Yes (p = 0.003)	No	No
Pan et al. (2002)	Taiwanese	48 (48)	23 <sup>α</sup> (10-41)	11 (22.9%)	47	21 <sup>α</sup> (14-27)	4 (8.5%)	Yes (p < 0.001)	...	Yes (p < 0.03)
Casella et al. (2003)	U.S.A.	70 <sup>η</sup>	22 <sup>α</sup> (17-33)	...	55	21 <sup>α</sup> (8-27)	...	Yes (p = 0.009)	...	...
Asatiani et al. (2003)	Caucasian	217 <sup>η</sup>	21.4 (13-30)	24 (11%)	62	19.4 (12-28)	1 (1.6%)	Yes (p < 0.001)	Yes (p < 0.02)	...
Mengual et al. (2003)	Spanish	102 (102)	23.3 (18-32)	17 (16.7%)	96	22.4 (15-34)	13 (13.5%)	Yes (p = 0.033)	...	No

(CAG)<sub>n</sub> = CAG repeat number; <sup>a</sup> According to the regarding reference; <sup>b</sup> By the test for the difference between two population proportions; <sup>c</sup> Fertility unknown; <sup>d</sup> Sperm counts unknown; <sup>e</sup> Median; <sup>f</sup> Predominantly had bladder cancer; <sup>η</sup> Men with poor semen quality in general; <sup>Δ</sup> Includes 33 men with obstructive azoospermia; <sup>θ</sup> Includes only azoospermic men; <sup>+</sup> (CAG) ≥ 30; <sup>α</sup> (CAG) ≥ 28; <sup>ε</sup> Yes for proportion with (CAG)<sub>n</sub> < 16 (p < 0.05).

Based on this information, in a study of exon 1 of the AR gene in infertile males of predominantly (60%) Chinese ethnic origin it was found that expansion of the trinucleotide (CAG) repeat and thus of the polyglutamine tract could be associated with increased risk of impaired spermatogenesis (Tut et al. 1997). This finding was then corroborated by several studies from a variety of different ethnic populations including Japanese, Caucasian, North American, French, Israeli, Taiwanese and Spanish (Yoshida et al. 1999; Dowsing et al. 1999; Mifsud et al. 2001; Wallerand et al. 2001; Patrizio et al. 2001; Madgar et al. 2002; Pan et al. 2002; Casella et al. 2003; Asatiani et al. 2003; Mengual et al. 2003). However, several others, who have investigated Swedish, Belgian, German, Japanese, Danish, Dutch, Indian, Greek, New Zealander, Finish, Hong Kong Chinese and Italian populations were unable to demonstrate an association (Giwerzman et al. 1998; Legius et al. 1999; Hiort et al. 1999; Dadze et al. 2000; Sasagawa et al. 2000, 2001; Von Eckardstein et al. 2001; Yu and Handelsman 2001; Kukuvitis et al. 2002; Rajpert-De Meyts et al. 2002; Van Golde et al. 2002; Thangaraj et al. 2002; Dhillon and Husain 2003; Erasmuson et al. 2003; Lund et al. 2003; Tse et al. 2003; Ferlin et al. 2004). Interestingly, a study by Komori et al. (1999) has suggested that reduction rather than expansion of the CAG repeat (< 16 repeats) is closely related to impaired sperm production in an infertile Japanese population.

This study was designed to investigate the hypothesis that there is association between the length of the CAG repeat of the AR gene and various degrees of impaired sperm production in infertile males of Turkish ethnic origin. To our knowledge this is the first study showing the CAG repeat length polymorphism in the Turkish population. In addition, an extensive literature search has been performed to demonstrate the hypothesized role of ethnicity and genetic background in this association (Table 3).

The results from analyses of reproductive hormones with elevated LH and FSH, and normal or low testosterone levels were suggestive of partial impairment of testicular function and were in

agreement with results from previously published studies (Dowsing et al. 1999; Yoshida et al. 1999). No statistically significant relationship between the length of the CAG repeat of the AR gene and idiopathic impaired sperm production was observed in the Turkish population studied ( $p = 0.84$ , Table 2). The frequency of having longer CAG repeat number was also not different in the infertile group compared with that of fertile controls (Fig. 2A, and Table 3). The range of the size of the CAG repeats in the infertile group was 18-29 and in the fertile control group 16-29 (Fig. 2A). Also within the subgroups of infertile males presenting with varying degrees of impaired sperm production (azoospermia, severe oligozoospermia, oligozoospermia and poor motility/morphology only group) we found no statistically significant association between the size of their respective CAG repeats or polyglutamine tracts and the severity of impaired sperm production (Table 2).

Tut et al. (1997) suggested that an enlargement of the CAG tract repeats ( $\geq 28$ ) in infertile males is associated with a 4-fold increased risk of infertility, whereas Mifsud et al. (2001) reported the odds ratio for azoospermia as 7-fold higher for patients with  $\geq 26$  CAG repeats than in those with < 26 CAG repeats. These studies suggested that the more severe the spermatogenic defect the higher the CAG repeat number should be. However, in this study the highest CAG repeat number observed in azoospermia group was 25 (in 4 men), whereas in the fertile control group 2 men with 27 and one man each with 28 or 29 CAG repeats were observed. Interestingly, all infertile men with CAG repeat numbers ( $\geq 26$ ) were in the poor motility/morphology only subgroup in this study (one man with 26, 27, 28, or 29 CAG repeats; Fig. 2B). The highest mean CAG repeat number was observed also in the poor motility/morphology only subgroup (mean  $23.47 \pm 0.68$ ), however, this was only significantly higher than the CAG repeat number of the oligozoo-azoospermic group (mean  $21.6 \pm 0.39$ ) but not from the fertile controls ( $22.41 \pm 0.54$ ) (Table 2). However, this significance was probably coincidental, since it disappeared when we selected the oligozoo/severe oligozoospermia patients, who at



the same time had poor sperm motility and/or morphology ( $n = 11$ ) and compared their mean CAG repeat number ( $21.18 \pm 0.71$ ) with that of fertile controls ( $p = 0.25$ ).

It is well documented in the literature that the size of the CAG repeats in the AR gene varies in a race-specific manner (Sartor et al. 1999). An extensive literature search supported this finding and the hypothesis that ethnic background plays a major role in terms of CAG repeat number and its association with idiopathic male infertility (Table 3). While it is possible to explain the differences in results observed in several different populations studied with this statement, it is interesting that 3 studies originated from the same population, i.e., Japanese men, reported opposing results to each other (Yoshida et al. 1999; Komori et al. 1999; Sasagawa et al. 2001). Yoshida et al. (1999) examined 41 azoospermic Japanese men and reported the mean CAG repeat length as  $26.5 \pm 3.5$  (range 20-34) in comparison to  $23.5 \pm 2.9$  (range 17-30) CAG repeats in fertile controls ( $p = 0.0013$ ). Komori et al. (1999) examined 59 Japanese infertile men with oligozoospermia in general and found that the mean CAG repeat length in infertile men did not differ significantly from that of fertile controls ( $21.2 \pm 4.2$ , range 14-32, vs  $21.4 \pm 3.5$ , range 16-31, respectively), however, they reported that the frequency of having CAG repeats ( $< 16$ ) was significantly higher in infertile men ( $p < 0.05$ ). Both of these studies suggested that either expansion (Yoshida et al. 1999) or reduction (Komori et al. 1999) in CAG repeat length is associated with male idiopathic infertility. Opposing to these findings Sasagawa et al. (2001), who examined 30 azoospermic Japanese men, found that the mean CAG repeat length in infertile group was  $23.4 \pm 2.9$  (range 19-30) and was not statistically different from that of fertile controls ( $23.7 \pm 3.2$ , range 17-28). A similar disagreement is also true for two Australian studies, which suggested significant (Dowsing et al. 1999) and no significant (Yu and Handelsman 2001) association between the CAG repeat length and idiopathic male infertility (Table 3). These opposing results from the same population suggest the possibility that the infertile males

included in these reports may represent a heterogeneous group with respect to the cause of defective spermatogenesis.

One of the main reasons to study the CAG repeat length in exon 1 of the AR gene has been to find a biomarker, which is associated with idiopathic male infertility. Another concern was the fact that with the increased use of intracytoplasmic sperm injection (ICSI) to treat severe male infertility, moderately expanded AR gene CAG repeats within the upper normal range would be vertically transmitted to female offspring and, therefore, perpetuate male infertility in second generation sons. Further, given that the AR CAG tract can expand during spermatogenesis (Zhang et al. 1994), it has been postulated that transmission of the AR gene by ICSI may in some cases lead to an increased risk of infertility and even SBMA in future generations. To that extend a study by Cram et al. (2000) examined the AR gene CAG repeat lengths in a large group of infertile men and those of their respective ICSI-conceived daughters. They found that in 95 out of 99 cases studied, the AR CAG tracts ranging in size from 15-28 repeats exhibited stable inheritance in female offspring. Only in 4 father-daughter pairs, there was a discordance in the expected inheritance pattern with evidence for both CAG expansion (from 20 to 24, and from 22 to 23) and contraction (from 26 to 18, and from 26 to 22) of the paternal AR allele. Thus, they concluded that these findings tend to alleviate concerns regarding ICSI may promote the transmission of AR alleles with expanded CAG tracts and suggested that the risk of SBMA in second generation sons would be extremely low.

In conclusion, the literature search suggested that the chance to find an association between the polyglutamine tract length and the male idiopathic infertility in a given population is approximately 50%, which indicates the major role and importance of the ethnicity and genetic backgrounds in this association. As it has been suggested in most European studies (Table 3) we were unable to show an association with the length of the CAG repeat tract of the AR gene and idiopathic male infertility in Turkish men. However, this associa-

tion seems to be important especially in Caucasian and North American populations. It is obvious that studies from a variety of different ethnic and genetic backgrounds using comparable patient subgroups are extremely valuable to further evaluate this association.

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