

·Letters to the Editor·

Analysis of the CAG repeat number in exon 1 of the androgen receptor gene in Slovene men with idiopathic azoospermia and oligoasthenoteratozoospermia

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Dear Sir,

I am Borut Peterlin, from Division of Medical Genetics, Department of Obstetrics and Gynaecology, University Medical Center, Ljubljana, Slovenia. We write to you to discuss if the number of CAG repeats in the androgen receptor (*AR*) gene is associated with male infertility in a group of 190 Slovene infertile men compared to 137 men with proven fertility.

Pathophysiology of subfertility involves a complex network of genetic and environmental factors. Stimulation and maintenance of spermatogenesis require androgens and functional AR. The first exon of the *AR* gene contains a polymorphic CAG repeat coding for a polyglutamine tract. Expansion of the CAG repeats to 40 or more causes spinal and bulbar muscular atrophy (SBMA) [1]. Besides progressive muscle weakness and atrophy, patients also show signs of mild androgen insensitivity: endocrine disturbances (including gynecomastia), testicular atrophy, oligozoospermia, azoospermia, impairment of sperm quality and infertility.

Both *in vivo* and *in vitro* studies have demonstrated reduced transactivation potential of the AR as the polyglutamine tract increased in length [2]. Inverse cor-

relation between sperm concentration and the number of androgen receptor CAG repeats was found within the normal fertile population; shorter CAG repeats were associated with higher sperm output [3].

The concept of the potential pathogenic effect of longer CAG repeats in the range of 24–40 (CAG)_n is important not only for better understanding of the etiology of male infertility, but also for the treatment of infertile men. Ten studies to date have shown the existence of the correlation between CAG repeat number and male infertility (in Chinese, Japanese, North American, French, Israeli, Taiwan [China] and Spanish populations), but 20 other studies did not confirm the association (in Swedish, Belgian, German, Japanese, Danish, Dutch, Indian, Greek, New Zealander, Finish, Hong Kong [China] and Italian populations) [4]. No data are available for Slavic populations.

In this letter, we would like to provide such data gained from a group of 190 Slovene infertile men compared to 137 men with proven fertility.

Patients with obstructive azoospermia as a result of epididymal stenosis or congenital bilateral absence of deferens and those with Klinefelter and Kallman syndrome, cytogenetic abnormalities and Y chromosome microdeletions were excluded. The study group con-

sisted of 74 men with idiopathic non-obstructive azoospermia (NOA), 116 men with oligoasthenoteratozoospermia (OAT) and a control group of 137 men with proven fertility (at least one offspring). All patients were Slovene or of Slavic origin. Informed consent was obtained from each patient. The study was approved by the National Ethics' Committee.

The number of CAG repeats of the *AR* gene was analyzed using the polymerase chain reaction (PCR) method and electrophoresis on Spreadex EL 1200 gels (Elchrom Scientific AG, Cham, Switzerland) [1, 5]. Statistical analysis was performed using the statistical package SPSS (version 11.0, SPSS Inc., Chicago, IL, USA). The difference in the number of CAG repeats between the infertile and the control group was tested by the Mann–Whitney *U*-test. The CAG repeat number in infertile patients was correlated to clinical characteristics using Spearman's correlation coefficient.

Statistical analysis showed no significant difference of the CAG repeat number between the infertile and control groups ($P = 0.425$; Figure 1). The mean CAG repeat number was 19.65 ± 2.79 (range 11–27; median 20) in infertile males and 19.35 ± 2.79 (range 12–26; median 19) in fertile men.

The number of CAG repeats did not correlate with any clinical characteristics of patients: testicular volume ($P = 0.121$), sperm concentration ($P = 0.726$), motility "a" ($P = 0.101$) and normal morphology ($P = 0.886$), FSH levels ($P = 0.201$) and Johnsen's histological score

($P = 0.505$). The mean CAG repeat number was 19.23 ± 3.0 (range 13–27; median 19.5) in the group of the NOA patients and 19.91 ± 2.64 in the OAT group (range 11–27; median 20). There was no significant difference in CAG repeat number between the groups of NOA and OAT ($P = 0.089$).

The number of the (CAG)*n* repeats in the *AR* gene in infertile men was analyzed in 30 studies with differences in the results in several populations [4]. It was reported that the odds ratio for azoospermia is 7-fold higher in patients with $26 \geq$ CAG repeats. Our study revealed that 4.4% of patients had $26 \geq$ CAG repeats compared to 1.5% of fertile controls. In other studies, the number of men with $26 \geq$ CAG repeats varied from 0% in the Israeli population to 49% in the Chinese population, with the average 18.2% [2, 4]. Two studies showed no difference in the mean (CAG)*n* repeat number, but a statistically significant proportion of men with $26 \geq$ CAG repeats [4]. The Italian study showed an association of the (CAG)*n* repeat number only with joint distribution with another trinucleotide (GGC)*n* repeat in exon 1 of the *AR* gene.

The variability of the results by various research groups might be due to different ethnic origins and, hence, different genetic modifiers of the populations studied. The diagnostic criteria used in different studies could also have affected the results. All studies determined that patients with genetic causes of infertility (karyotype mutations, Kallman syndrome, Y chromosome microdeletions and CF mutations) should be ex-

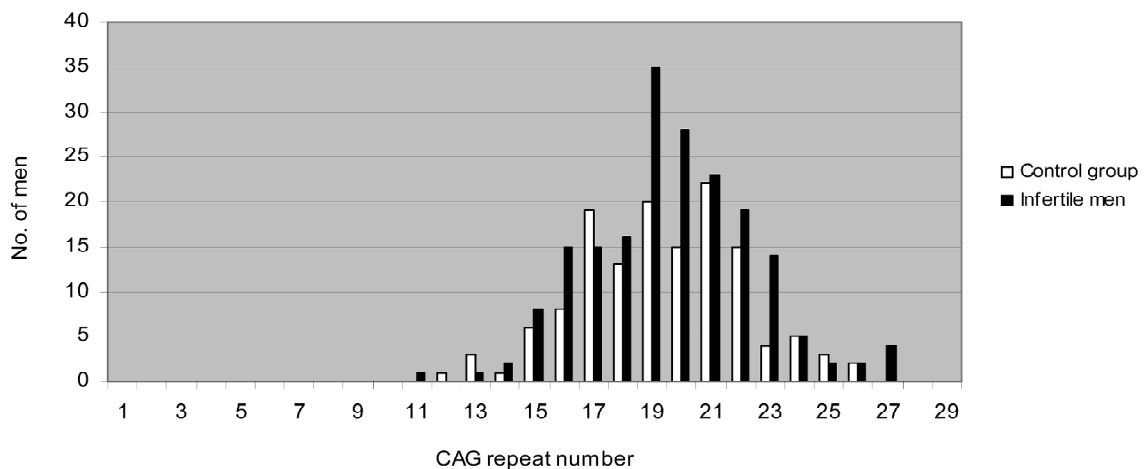


Figure 1. The distribution of CAG repeats within the androgen receptor (*AR*) gene of 190 men with idiopathic non-obstructive azoospermia (NOA) and oligoasthenoteratozoospermia (OAT) (infertile men), and 137 men with proven fertility (control group).

cluded from the analysis, however not all known genetic causes for male infertility were determined to be excluded in all studies. Additionally, in our study, after excluding men with obstructive azoospermia, only men with impaired spermatogenesis were taken into account. The size of the study groups is also likely to have contributed to the conflicting findings. We analyzed 190 patients, and in previous studies the size of the study group varied from 33 to 280, with an average of 102 patients being analyzed.

In conclusion, our analysis on the Slovene population indicates that when spermiologic diagnostic categories, NOA and OAT, are taken into consideration and analyzed together they do not correlate with the CAG repeat number in the *AR* gene. The number of CAG repeats in the *AR* gene within the normal range is not a clinically relevant genetic risk factor for the development of NOA and OAT in the Slovene population of men entering the ICSI procedure.

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