Singapore Journal of Obstetrics and Gynaecology
2009; 40(1): 9-12

Androgen Receptor Gene Trinucleotide (CAG) Repeat Polymorphisms in Infertile Male Patients

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ABSTRACT

CAG repeat expansion in exon 1 of the androgen receptor (AR) gene has been reported to be associated with male infertility in some populations. We have analyzed the CAG repeat motif in the AR gene in 25 of our male patients with infertility and in 51 men with proven fertility. The mean number of CAG repeats in the AR gene of men with non-obstructive azoospermia was 22.6 and 26.7 in men with severe oligozoospermia (defined as mean sperm density less than 5 x 10^6/ml). The mean CAG repeat length in combined non-obstructive azoospermia and severely oligospermic men was 25.9 +/- 3.3. The mean age of infertile male was 34.6. Among fertile-control men, the mean number of CAG repeats was 22.1 +/- 4.7. Each unit of increase in CAG length was associated with a 26.8% (CI 9.6- 46.6%) increase in odds of having severe idiopathic infertility. The odds ratio for severely impaired spermatogenesis was 2.08-fold higher for patients with ≥ 26 CAG repeats than in those with <26 CAG repeats.

Keywords: Azoospermia, oligozoospermia, CAG repeats

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INTRODUCTION

About 15% of couples have fertility issues, and in these couple, male infertility accounts for about 50% of causes [(1)]. Y chromosome microdeletion has been considered an important genetic factor in male infertility [(2)]. Androgens and a normal functioning androgen receptor (AR) are important for spermatogenesis. The androgen receptor gene has been mapped to the long arm (Xq11-12) of the X chromosome [(3, 10)].

The gene has two polymorphic sites in exon 1, characterized by different numbers of CAG and GGC repeats resulting in variable lengths of polyglutamine and polyglycine stretches. This seems to modulate the AR function.

Extremes of the CAG repeats can lead to different pathologies, and several studies have demonstrated that expansion of CAG repeats in the AR gene is associated with azoospermia [(4)], oligozoospermia [(5)], testicular atrophy and spinal bulbar muscular atrophy [(6)]. However, previous studies examining CAG repeat numbers in infertile men have reported conflicting results, with some showing no expansion [(4,7,8,9)]; whilst others reporting increased length of CAG repeats [(11,12,13,14)].

Studies involving different ethnicity reflected differences in findings. Singaporean, Australian, North American, and Japanese subject studies found an association between CAG length and male infertility, whereas this was not evident in studies from Europe. Therefore we undertook an analysis of CAG repeat in the AR gene among fertile and infertile male to assess its association with infertility in our local population.

MATERIALS AND METHODS

Method

Patients and Control Subjects

A total of 25 men with impaired spermatogenesis ranging from severe idiopathic oligozoospermia (mean sperm density < 5 x 10^6/mL) to idiopathic non-obstructive azoospermia as assessed by standard criteria, were recruited from the KKIV Centre and Andrology clinic. All except one patient (due to chemotherapy) had idiopathic impaired spermatogenesis. The mean sperm density was 1.6 X 10^6/mL and the median was 0.8 X 10^6/mL. Controls were 51 men with proven fertility recruited from the antenatal clinic and they had no previous infertility history or treatment.

DNA was extracted from peripheral blood through standard techniques and amplified with polymerase chain reaction. Sequencing was performed by agarose (metaphor) gel electrophoresis. The mean number of CAG repeats from infertile men with defective sperm production was compared with fertile controls. Statistical analyses were performed using SPSS software. Two- sample independent t test and logistic regression analysis were used as appropriate. Multiple comparisons were performed comparing the mean number of CAG repeats in azoospermic and oligospermic patients with that in fertile patients by using analysis of variance and the Dunnett test.

Odds ratio were also calculated for patients with more than or equal to 26 repeats and those with < 26 repeats. Statistical significance was defined as a 2-sided P value of less than 0.5. Data are reported as means (+/- SE).

RESULTS

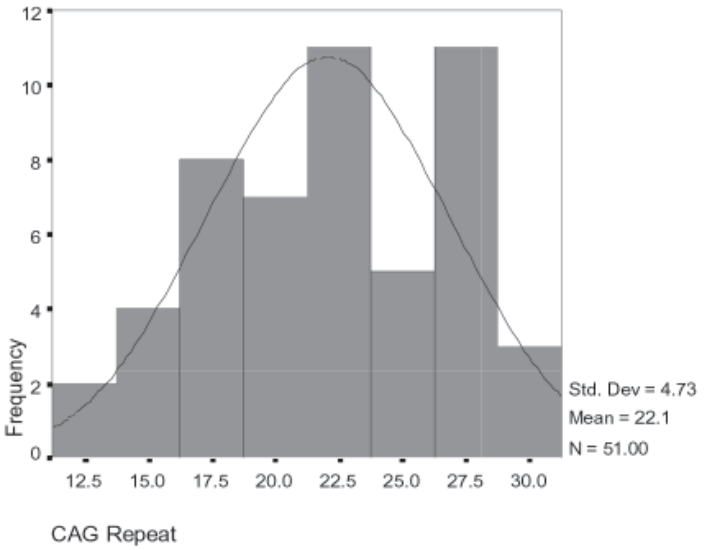
In our study, the mean androgen receptor CAG length in infertile men with severely impaired sperm production was significantly longer than in fertile controls (25.9 +/- 3.3 vs. 22.1 +/- 4.7, p<0.01).

Logistic regression showed that each unit of increase in CAG length was associated with a 26.8% (CI 9.6- 46.6%) increase in odds of having severe idiopathic infertility. The odds ratio for severely impaired spermatogenesis was 2.08-fold higher for patients with ≥ 26 CAG repeats than in those with <26 CAG repeats.

Table 1. Mean CAG Lengths

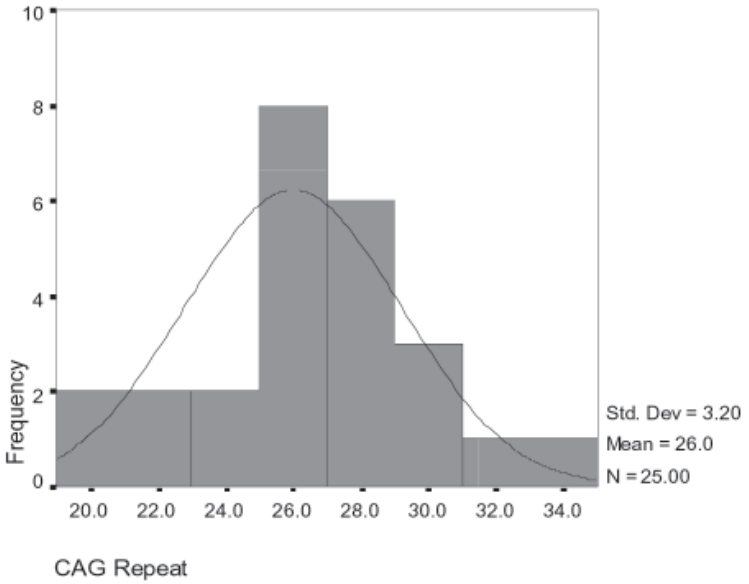
KKIVF Centre	No of Patients	Mean (+/- SE) CAG Length	Range
Fertile control	51	22.1 +/-4.7	12.5-30.0
Oligospermic men	20	26.7 +/-2.9	20-33
Azoospermic men	5	22.6 +/-2.8	20-27
Combined severe oligo and azoospermic men	25	25.9 +/-3.3	20-33

Fig 1. GROUP: Control



Range of CAG repeat: 12.5-30.0

Fig 2. GROUP: Infertile



Range of CAG repeat: 20.0-34.0

CONCLUSION

Our results indicate a relation between CAG repeat length in the androgen receptor gene and the risk of defective spermatogenesis. With assisted reproductive techniques like intracytoplasmic sperm injection (ICSI), the mutation could be inherited, and possibly leading to increase in male infertility in future generations. Further elongation of the CAG repeat in future generations may increase the severity of male infertility.

Screening for androgen receptor CAG repeat polymorphisms may therefore be important for males with infertility and impaired spermatogenesis.

Our study does have limitations. The number of subjects studied was small, and may not be reflective of our infertile male population in Singapore. The study has also not taken into account the contribution made by different ethnicity. Nonetheless, the findings concurred with that reported by Dowsing et al, 1999; Yoshida et al, 1999; Mifsud et al, 2001; Patrizio et al, 2001).

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