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The Relationship Between LOH Severity and Androgen Receptor Gene CAG Repeat Length of Korean Men Affected with LOH

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Abstract

Context: *There have been no investigations on the severity of late-onset hypogonadism (LOH) of Korean male according to their androgen receptor gene CAG repeat length.* ***Aim:*** *To investigate the relationship between late-onset hypogonadism (LOH) and androgen receptor gene CAG repeat length in Korean men. A total of 106 patients with LOH from Pyongyang City participated in this study. LOH was diagnosed in individuals who had a serum testosterone level below 3.5 ng/mL, along with a positive score on the AMS questionnaire. Genomic DNA was extracted from the plasma of patients and then sequenced for analyzing CAG and CGC repeat, which were then compared with the AMS score of each participant.* ***Results:*** *The average length of CAG repeat of the participants was 21.8 ± 2.8 (15~26). Men with CAG repeat length of more than 21 manifested significantly more severe LOH symptoms than those with shorter length ($p < 005$). The sensitivity of this cutoff for a diagnosis of LOH was 94.6% and the specificity was 37.5%.* ***Conclusions:*** *Longer CAG repeat is associated with severe LOH symptoms and Korean men with CAG repeat length greater than 21 are more likely to be affected by LOH.*

Keywords: Late-onset hypogonadism, androgen receptor, androgen receptor gene CAG repeat

INTRODUCTION

AR (androgen receptor) is a member of steroid-binding activated transcription factor which plays an important role in male sexual function. The action of testosterone is mediated by androgen receptors which are present in the cytosol and nucleus of the target cell. The AR gene is located on the X chromosome (q11-12) and consists of 8 exons that encode 919 amino acids [1]. Two known polymorphic repeats of this androgen receptor gene are the CAG and GGC repeats. The AR protein is made up of three distinct domains: the N-terminal transactivation domain (NTD), the DNA-binding domain (DBD), and the ligand-binding domain (LBD). There are polyglutamine and polyglycine tracts in NTD which are coded by CAG and GGC repeat, respectively [2]. After binding with the ligand, the receptor forms a complex with chaperone proteins in the cytoplasm, which triggers a conformational change in the receptor. This change causes the receptor to transform and move to the nucleus [3]. Once in the nucleus, the receptor binds to specific DNA sequences, where it dimerizes with another molecule, and the homodimer recruits additional proteins through specific interaction motifs [4]. This results in transcriptional activation of the AR gene [5].

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Earlier studies have found that the length of the AR CAG repeat is shortest in African Americans, intermediate in Caucasians, and longest in Asians, which aligns with the varying disease phenotypes, such as prostate cancer [6].

In one cross-sectional study, 822 elderly men were enrolled and their serum testosterone levels tended to decline 0.71 ± 0.32% per one CAG. This suggests that total testosterone and free testosterone levels were likely to decrease when the patient's CAG repeat length was shorter [7].

Crabbe and colleagues conducted cross-sectional analyses of serum hormone levels and AR CAG repeat lengths in healthy men across two separate studies, one with 2, 322 participants and another with 358. Their findings revealed a positive correlation between the CAG repeat length and levels of total testosterone and free testosterone. The variation in free testosterone levels among healthy men was partly attributed to differences in androgen sensitivity and feedback mechanisms linked to AR polymorphism [8].

In another cross-sectional study, they suggested that the compensatory increase in testosterone level might be due to the decreased sensitivity of androgen receptors, but this association may vary with the individual [9].

The CAG repeat length is significantly different depending on ethnicity or region. In addition, the relationship between CAG repeats length and other symptoms related to sexual function or other lab tests has not been consensual.

SUBJECTS AND METHODS

Patients and Design

This study was carried out at the training hospital of Pyongyang University of Medical Sciences between 2016 and 2018. After approval from the local ethics committee, 106 men in Pyongyang City were enrolled in the study. The average age of the participants was 54.0 ± 11.4 years, with ages ranging from 27 to 79. LOH was diagnosed when the serum testosterone level was below 3.5 ng/mL, along with a positive score on the AMS questionnaire [7, 10].

Exclusion criteria were the presence of pituitary or testimony, previous medical use affecting testicular function or steroid clearance rate, testosterone replacement therapy, severe liver function, nephropathy, and malnutrition.

METHODS

Korean (Translated) Version of the AMS Questionnaire and Serum Testosterone Level Measurement

The AMS questionnaire was translated into Korean under the expert guidance of psychologists, urologists, and endocrinologists. This Korean version of the AMS questionnaire was given to every participant, who was asked to complete it.

To account for diurnal fluctuations in testosterone levels, total testosterone was measured between 8:00 and 11:00 a.m. using an immunological measurement kit.

Androgen Gene CAG Repeat Sequencing

Genomic DNA was isolated from the plasma of the patients, and PCR was carried out to amplify the target region. The PCR products, which were identified by means of 2% agarose gel electrophoresis, were then sequenced for analyzing CAG and CGC repeat length (China, Sangon Company). The primer sequences were 5′-GCCTGTTGAACTCTTCTGAGC-3′ and 5′-CGATGGGCTTGGGGAGAACCA TCCTCA-3′.

Statistical Analysis

Receiver-operating characteristic (ROC) curve analysis was used to identify potential criteria for predicting the presence of LOH based on CAG repeat length, and the area under the curve (AUC) was calculated. The optimal cutoff value for maximizing the Youden index was determined, and sensitivity and specificity were calculated using this cutoff. All statistical analyses were conducted with SPSS 22.0, and a P value of less than 0.05 was considered statistically significant.

RESULTS

CAG Repeat Length in Participants

Average CAG repeat length, minimum and maximum lengths in Korean men are shown in Table 1.

Table 1. Average CAG repeat length ($\overline{X} \pm$ SD).

n	Average	Min	Max
106	21.8 ± 2.8	15	26

The average CAG repeat length was 21.8 ± 2.8. The minimum length was 15 and the maximum was 26.

The Relationship Between CAG Repeat Length and LOH

Relationship Between CAG Repeat Length and Severity of LOH

To explore the relationship between CAG repeat length and LOH, we compared the CAG repeat length with the severity of LOH, as assessed by the AMS questionnaire (Table 2).

Table 2. Comparison between severity of LOH and CAG repeat length ($\overline{X} \pm$ SD).

Severity (n)	Normal (36)	Mild (26)	Moderate (18)	Severe (26)
Repeat length	20.3 ± 2.56	20.5 ± 2.41	22.0 ± 2.07	22.9 ± 2.79$^{*, \#}$

Note: *Compared with normal, $^{\#}$: Compared with mild.

The CAG repeat length was longer when LOH symptoms were severe while the CAG repeat length was shorter in mild LOH patients of normal and mild LOH were significantly shorter than ones of severe LOH ($p < 0.05$).

The Relationship Between CAG Repeats Length, Serum Testosterone Level, and the AMS Total Score

Correlation analysis was conducted to assess the relationship between LOH symptoms, CAG repeat length, and testosterone levels. As LOH symptoms were greatly affected by the age of patients, we performed correlation analysis between CAG repeat length and severity of LOH symptoms before and after adjustment of age but also after adjustment of age to see whether there was a difference between them (Table 3).

Table 3. The relation between CAG repeat length and testosterone level/ AMS total score.

	Before Adjustment of Age			After Adjustment of Age		
	CAG	*Testosterone*	*AMS Score*	*CAG*	*Testosterone*	*AMS Score*
CAG	1.000	–0.169	0.329**	1.000	–0.170	0.444**
Testosterone	–0.169	1.000	0.065	–0.170	1.000	0.103
AMS score	0.329**	0.065	1.000	0.444**	0.103	1.000

Note: n = 106.

The correlation coefficient between CAG repeat length and the AMS total score was 0.329 before adjustment of age and 0.444 after adjustment of age ($p < 0.01$).

The Cutoff Value of CAG Repeat for Predicting the Presence of LOH

Since there was a correlation between CAG repeat length and LOH symptom severity, we performed the ROC curve analysis to obtain the cutoff value of CAG repeat for the prediction of LOH (Figure 1).

The AUC of ROC of CAG repeat length in LOH diagnosis was 0.739 ± 0.086 and the 95% CI was 0.571~0.906 (Table 4). The maximum Youden index was 0.321 and the cutoff value was 20.50 with a specificity of 37.5% and sensitivity of 94.6%. As the CAG repeat length should be an integer, the cutoff value of the CAG repeat is 21.

Table 4. AUC of ROC curve and cutoff value of CAG repeat length.

n	AUC	95% CI	Max Youden Index	The Cutoff Value of CAG Repeat	Sensitivity	Specificity
106	0.739 ± 0.086*	0.571~0.906	0.321	20.50	0.946	0.375

*Note: *: P < 0.05.*

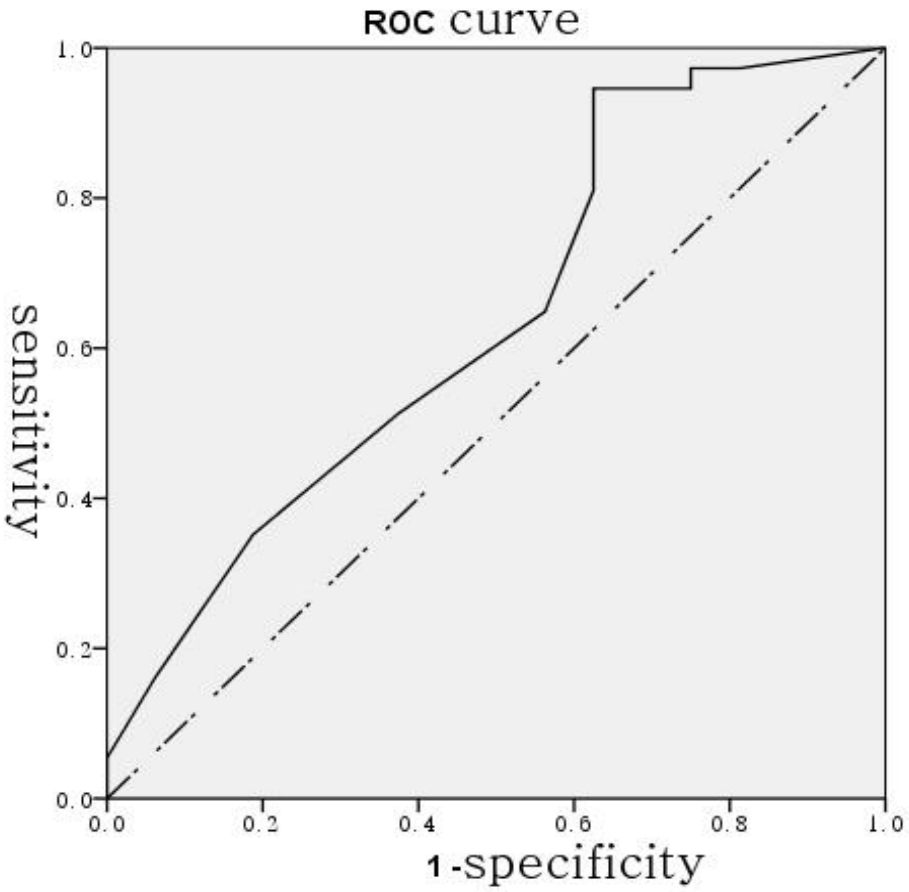

Figure 1. The curve of ROC of CAG repeat length for prediction of LOH.

DISCUSSION

The CAG repeat of the AR gene varied significantly depending on ethnicity [11, 12].

The CAG repeat sequence ranges from 9 to 36 repeats, with an average length of 21 repeats in Caucasian populations [12].

In an Asian country, 262 males were enrolled in the study which determined their average CAG repeat length was 22.1 ± 4.6 and average serum total testosterone was 5.6 ± 2.2 ng/mL [13].

According to another study, normal CAG repeat length is between 9 and 37. If serum testosterone level and AR sensitivity played an important role equally in androgen signaling, it would be necessary to measure the testosterone level and CAG repeat length simultaneously to evaluate the interaction between them [14].

In our study, the average CAG repeat length was 21.8±2.8 and the maximum length detected was 26 while the minimum was 15.

It has been known that the CAG repeat length of the AR gene is inversely associated with the transcriptional activity of the gene. The very example of this association could be seen in Kennedy syndrome where there is partial androgen resistance. The shorter the CAG repeat length, the stronger the transcription activity of the AR gene [15].

Huhtaniemi and colleagues conducted a multinational prospective study to examine the relationship between various reproductive hormones and AR CAG repeat length in the European male aging study. They found that men with longer AR CAG repeat had higher testosterone levels, which could effectively compensate for reduced AR activity [16].

According to our results, the CAG repeat length was likely to be longer when there were more severe symptoms of LOH while the CAG repeat length of normal or mild LOH patients was significantly shorter than that of severe LOH patients ($p < 0.05$). In addition, the correlation coefficient between CAG repeats and AMS total score was 0.329 without the adjustment of age, while the correlation coefficient was 0.444 after the adjustment of age ($p < 0.01$). This indicates that there is a close association between CAG repeat and severity of LOH symptoms.

Furthermore, the cutoff value of CAG repeat length for the prediction of LOH was 21 and the sensitivity and specificity of the cutoff value were 94.6%, and 37.5%, respectively.

So far, we conclude that individuals who have CAG repeat length greater than 21 should be under strict monitoring of LOH from their mid or elder ages for prevention or taking measures if needed.

CONCLUSIONS

The average CAG repeat length of Korean males was 21.8 ± 2.8, with a minimum length of 15 and a maximum length of 26. The longer the CAG repeats, the more likely it is to be severe in LOH symptoms. The cutoff length of CAG repeat to predict LOH is 21, with the sensitivity and the specificity being 94.6%, and 37.5%, respectively.

REFERENCES

1. Lu J, der Steen TV, Tindall DJ. Are androgen receptor variants a substitute for the full-length receptor? Nat Rev Urol. 2015;12(3):137–144.
2. Tan MH, Li J, Xu HE, Melcher K, Yong EL. Androgen receptor: structure, role in prostate cancer, and drug discovery. Acta Pharmacol Sin. 2015;36(1):3–23.
3. McEwan IJ, Brinkmann AO. Androgen Physiology: Receptor and Metabolic Disorders. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Androgen Physiology: Receptor and Metabolic Disorders. Endotext. South Dartmouth (MA): MDText.com, Inc.; 2016.
4. Heemers HV, Tindall DJ. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. Endocr Rev. 2007;28(7):778–808.
5. Wang Q, Li W, Zhang Y, Yuan X, Xu K, Yu J, Chen Z, Beroukhim R, Wang H, Lupien M, Wu T. Androgen receptor regulates a distinct transcription program in androgen-independent prostate cancer. Cell. 2009;138(2):245–256.
6. Edwards AL, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics. 1992;12(2):241–253.

7. Krithivas K, Yurgalevitch SM, Mohr BA, Wilcox CJ, Batter SJ, Brown M, Longcope C, McKinlay JB, Kantoff PW. Evidence that the CAG repeat in the androgen receptor gene is associated with the age-related decline in serum androgen levels in men. J Endocrinol. 1992;162(1):137–142.
8. Crabbe P, Bogaert V, De Bacquer D, Goemaere ST, Zmierczak H, Kaufman JM. Part of the interindividual variation in serum testosterone levels in healthy men reflects differences in androgen sensitivity and feedback set point: contribution of the androgen receptor polyglutamine tract polymorphism. J Clin Endocrinol Metab. 2007;92(9):3604–3610.
9. Skjærpe PA, Giwercman YL, Giwercman A, Svartberg J. Androgen receptor gene polymorphism and the metabolic syndrome in 60-80 years old Norwegian men. Int J Androl. 2010;33(3):500–506.
10. Wang C, Nieschlag E, Swerdloff R, Behre HM, Hellstrom WJ, Gooren LJ, Kaufman JM, Legros JJ, Lunenfeld B, Morales A, Morley JE. Investigation, treatment, and monitoring of late-onset hypogonadism in males: ISA, ISSAM, EAU, EAA, and ASA recommendations. Eur J Endocrinol. 2008;159(5):507–514.
11. Ackerman CM, Lowe LP, Lee H, Hayes MG, Dyer AR, Metzger BE, Lowe WL, Urbanek M. Ethnic variation in allele distribution of the androgen receptor (AR) (CAG)n repeat. J Androl. 2012;33(2):210–215.
12. Casella R, Maduro MR, Lipshultz LI, Lamb DJ. Significance of the polyglutamine tract polymorphism in the androgen receptor. Urology. 2001;58(5):651–656.
13. Kim JW, Bae YD, Ahn ST, Kim JW, Kim JJ, Moon DG. Androgen receptor CAG repeat length as a risk factor of late-onset hypogonadism in male population. Sex Med. 2018;6(3):203–209.
14. Pastuszak AW, Rodriguez KM, Nguyen TM, Khera M. Testosterone therapy and prostate cancer. Transl Androl Urol. 2016;5(6):909–920.
15. Mhatre AN, Trifiro MA, Kaufman M, Kazemi-Esfarjani P, Figlewicz D, Rouleau G, Pinsky L. Reduced transcriptional regulatory competence of the androgen receptor in X-linked spinal and bulbar muscular atrophy. Nat Genet. 1993;5(2):184–188.
16. Huhtaniemi IT, Pye SR, Limer KL, Thomson W, O'Neill TW, Platt H, Payne D, John SL, Jiang M, Boonen S, Borghs H. Increased estrogen rather than decreased androgen action is associated with longer androgen receptor CAG repeat. J Clin Endocrinol Metab. 2009;94(1):277–284.

