Relationships among androgen receptor CAG repeat polymorphism, sex hormones and penile length in Han adult men from China: a cross-sectional study

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This study aimed to investigate the correlations among androgen receptor (AR) CAG repeat polymorphism, sex hormones and penile length in healthy Chinese young adult men. Two hundred and fifty-three healthy men (aged 22.8 ± 3.1 years) were enrolled. The individuals were grouped as CAG short (CAG≤20) or as CAG long (CAG>20) if their CAG repeat length was >20. Body height/weight, penile length and other parameters were examined and recorded by the specified physicians; CAG repeat polymorphism was determined by the polymerase chain reaction (PCR) method; and the serum levels of the sex hormones were detected by radioimmunoassay. Student’s t-test or linear regression analysis was used to assess the associations among AR CAG repeat polymorphism, sex hormones and penile length. This investigation showed that the serum total testosterone (T) level was positively associated with the AR CAG repeat length (P = 0.01); whereas, no significant correlation of T or AR CAG repeat polymorphism with the penile length was found (P = 0.593). Interestingly, an inverse association was observed between serum prolactin (PRL), and penile length by linear regression analyses (β = −0.024, 95% confidence interval (CI): −0.047, 0). Collectively, this study provides the first evidence that serum PRL, but not T or AR CAG repeat polymorphism, is correlated with penile length in the Han adult population from northwestern China.

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INTRODUCTION

Sex hormones, such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone (T), estrogen (E) and prolactin (PRL), have been identified as potential factors contributing to the formation of the penis during human pregnancy.1-2

Of all the sex hormones, T is prominent and has been well-studied.3-5

Hypogonadism, which is a medical term for the decreased functional activity of the gonads that results in lower levels of serum T, is frequently associated with a small penis or abnormal penile development during childhood and can be partially reversed by T treatment in clinic.5-6

However, it is still unknown why healthy adult men with normal T levels have different penile lengths and what factors are responsible for the fact that lower T levels are not always associated with a small penis size.

It has been reported that T executes its biological function through the androgen receptor (AR) signaling pathway. AR has a variable NH2-terminal domain that contains two functionally polymorphic microsatellites, one of which is a polyglutamine tract encoded by CAG repeats. A linear increase of CAG repeat length is associated with the gradual decrease of AR transactivation activity and transcriptional potential.6

Because some polymorphic variations of AR alter androgen sensitivity,7 one hypothesis to explain the variation in penile length in healthy adult men with similar circulating T levels is due to differences in the AR CAG repeat polymorphism. However, there are still no systemic investigations that have determined whether sex hormones or the AR CAG repeat polymorphism affect the final penile length in healthy adult men. Therefore, in the present study, we enrolled 253 healthy young adult men to examine the correlations among the AR CAG repeat polymorphism, sex hormones and penile length.

MATERIALS AND METHODS

Participants

A total of 253 healthy young adult men (age: 22.8 ± 3.1 years; height: 171.39 ± 5.86 cm; body mass: 64.43 ± 10.09 kg; mean ± standard deviation (s.d.)) were enrolled in this study. They were recruited among students from four universities or colleges in Xi’an, Shaanxi Province, People’s Republic of China. The physical condition of each participant was determined by his medical history and physical examination. Subjects who were receiving medication or had any chronic diseases were excluded. The study was approved by the Ethical Committee of the Xi’an Jiaotong University, and all of the volunteers provided their written consent before participation.
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RESULTS

AR CAG repeat polymorphism

In our study, the AR CAG repeat polymorphism ranged from 13 to 30, with an approximately normal distribution similar to those of previous studies in the Chinese population. The mean was 20.55 and the median was 20 (Figure 1).

Sex hormones and AR CAG repeat polymorphism

The correlation between AR CAG repeat polymorphism and sex hormones is still controversial. In our study, out of all sex hormones, the serum T concentration was 12.5% higher in the CAG group compared to the CAG group (25.6 ± 6.98 and 28.8 ± 7.72 nmol l⁻¹, respectively), and this difference reached statistical significance (P = 0.01). However, no significant correlation was found between AR CAG polymorphisms and the levels of FSH, LH, PRL, E₂ or progesterone (P = 0.88, 0.89, 0.62, 0.69 and 0.61, respectively) (Table 1).

AR CAG repeat polymorphism and penile length

Previous studies have shown that the AR CAG repeat length was negatively correlated with AR transactivation activity. Thus, we wanted to investigate whether AR CAG repeat polymorphism were able to affect the final penile length of adult men. After statistical analysis, despite the numerically longer penile length in the CAG group than in the CAG group, the difference did not reach statistical significance (10.2 ± 1.46 and 10.1 ± 1.28 cm, respectively, P = 0.59) (Table 1).

Table 1: Age, penile length and sex hormones in men with AR CAG, and CAG polymorphisms

| Age (year) | Penile length (cm) | FSH (IU l⁻¹) | LH (IU l⁻¹) | PRL (μg l⁻¹) | Total T (nmol l⁻¹) | Total E₂ (pmol l⁻¹) | Progesterone (nmol l⁻¹) | CAGₗ (n=126, mean±s.d.) | CAGₘ (n=122, mean±s.d.) | P value |
|------------|------------------|-------------|------------|-------------|------------------|---------------------|----------------------|-----------------------|------------------------|--------|
| Age (year) | 22.9±3.6         | 10.2±1.5    | 7.3±2.0    | 9.4±2.5     | 25.6±7.0         | 390.7±93.6          | 1.14±0.5             | 0.59                   | 0.59                   | 0.52   |
| Penile length (cm) | 22.7±2.6         | 10.1±1.3    | 7.4±2.4    | 9.3±2.5     | 28.8±7.7         | 397.2±101.9         | 1.18±0.6             | 0.59                   | 0.62                   | 0.61   |

E₂: estrogen; FSH: follicle-stimulating hormone; LH: luteinizing hormone; PRL: prolactin; T: testosterone; s.d.: standard deviation

Figure 1: Percent distribution of the number of AR CAG repeats.
Linear regression analysis

The penile length was set as the dependent variable, and the AR CAG repeat polymorphism, sex hormones, body mass index and waist/hip ratios were set as the independent variables. As shown in Table 2, PRL was the only factor found to be significant after statistical analysis ($\beta = -0.024, P = 0.039$, 95% CI: $-0.047, 0$).

Considering that 42 out of the 248 participants had serum PRL levels exceeding the maximum value of the normal reference, individuals were grouped as PRL$_{N}$ (normal) if they harbored a concentration of $<20$ μg l$^{-1}$ and as PRL$_{H}$ (high) if they harbored a concentration of $\geq 20$ μg l$^{-1}$. As shown in Figure 2, a statistically significant decrease in the penile length was observed in the PRL$_{H}$ group compared with the PRL$_{N}$ group after the $t$-test analysis (PRL$_{N}$ vs PRL$_{H}$: $10.3 \pm 1.4$ vs $9.8 \pm 1.1$ cm, $P = 0.025$).

DISCUSSION

The goal of this study was to investigate the correlations among AR CAG repeat polymorphism, sex hormones and penile length in healthy young adult men from China. In total, we made two major new discoveries: (i) AR CAG repeat polymorphism have no relationship with human penile length and (ii) human serum PRL levels negatively correlate with penile length.

Our study first showed that the AR CAG repeat length was dramatically correlated with the total levels of serum T, but not with the levels of serum FSH, LH, PRL, E$_2$ or P. Similar results have been reported in several previous publications,$^{15-19}$ although some of the results are controversial.$^{20-23}$ For example, Stanworth et al.$^{24-27}$ showed that longer AR CAG repeats correlated with higher T and LH levels in men with type 2 diabetes and explained that less active AR with longer AR CAG produced less suppression of the LH release. Thereby, LH levels were increased, and higher T levels were stimulated.$^{26,27}$ However, Huhtaniemi et al.$^{17}$ conducted a multinational prospective cohort observational study and showed that CAG repeats were obviously correlated with total T but not with LH. One of their explanations was that the concomitant increase of circulating T levels in men with longer repeats could adequately compensate for the lower AR activity in order to prevent the apparent deficiency of androgen action. Skragic et al.$^{18}$ also reported a positive correlation between the CAG repeats and total T levels in polycystic ovary syndrome. However, Andersen et al.$^{29}$ showed that there was no significant correlation between the AR CAG repeat length and the total T, E$_2$, FSH or LH levels.

As previously described, the AR gene contains a great number of CAG repeats, which could result in various polyglutamine tracts. Longer polyglutamine tracts could cause a reduced transcriptional activation of AR.$^{7,24,25}$ Previous reports described ambiguous results regarding the association between expanded CAG repeat polymorphism and microgenia in the Japanese.$^{24,25}$ However, our results indicated that the genotype of CAG repeat polymorphism has no discernible effect on penile length. It is likely that the expansion of CAG repeats can be detected as a positive modifying factor in one population but not in another. However, individuals with longer AR CAG repeat length usually exhibit higher serum T levels. To a certain extent, the reduced effect of AR transactivation could be relieved by higher T concentrations. This is a likely explanation for why CAG repeat polymorphism had no discernible effect on the ultimate human penile length in our study.

Interestingly, a negative correlation between the serum PRL levels and penile length was detected in our study. PRL is viewed as a classical endocrine hormone that is not just produced by pituitary lactotroph cells but is also secreted by several tissues, including the mammary glands, prostate, brain, some immune cells and others.$^{29}$ Various actions of PRL have been reported, including its effects on water and salt balance,$^{30}$ growth and development,$^{30}$ endocrinology and metabolism,$^{31-33}$ brain and behaviour,$^{33-35}$ reproduction$^{34,36}$ and immune regulation.$^{35,36}$ Pathological hyperprolactinemia has been reported to result in galactorrhea oligo/amenorrhea in women, impotence in men and loss of libido and infertility in both sexes.$^{19}$ PRL receptor-deficient animals have shown an almost complete failure to lactate, infertility in females and delayed fertility in males.$^{40}$ Furthermore, Bartke et al.$^{41}$ showed that PRL could modulate responses to the negative steroid feedback at the pituitary level and reported that both LH and T levels were reduced in hyperprolactinemic men. Similar results have reported that long-term hyperprolactinemia could cause the suppression of the hypothalamic-pituitary gonadal axis and a decrease in the levels of gonadotropin-releasing hormone, LH and T in males.$^{42}$ Recently, Roke et al.$^{44}$ reported that boys with antipsychotic-induced hyperprolactinemia had obviously lower T levels than male adolescents with normal PRL levels. Therefore, we supposed that higher levels of PRL suppressed the hypothalamic-pituitary-gonadal axis and decreased T levels, resulting in an inhibitory effect on penile growth.

In summary, this report presents evidence that men with longer AR CAG repeats had higher T levels that could compensate partly or totally for the weaker activity of AR. Furthermore, men with longer AR CAG repeats do not necessarily have shorter penile lengths. This may be due to the equilibrium between AR CAG repeats and T levels. A promising and important finding of this study was the presence of higher PRL levels in healthy adult men with shorter penile lengths; we are the first to report this discovery. These findings have potential

Table 2: Linear regression analysis for the associations between penile length and other variables. Regression coefficients ($\beta$), significance values ($P$) and 95% confidence intervals (CI) were calculated for the estimates$^a$

| Model          | Unstandardized coefficients ($\beta$) | $P$ value | 95% CI for $\beta$ |
|----------------|---------------------------------------|-----------|-------------------|
| (Constant)     | 12.373                                |           | (8.354, 16.392)   |
| FSH            | 0.007                                 | 0.911     | (-0.113, 0.127)   |
| LH             | 0.013                                 | 0.840     | (-0.113, 0.139)   |
| PRL            | -0.024                                | 0.039     | (-0.047, 0)       |
| T              | -0.016                                | 0.337     | (-0.050, 0.017)   |
| E$_2$          | -0.001                                | 0.318     | (-0.004, 0.001)   |
| Progesterone   | 0.066                                 | 0.767     | (-0.374, 0.506)   |
| CAG$_{N}$      | 0.010                                 | 0.785     | (-0.064, 0.084)   |
| BMI            | 0.052                                 | 0.352     | (-0.058, 0.162)   |
| Waist/hip ratio| -2.977                                | 0.223     | (-7.788, 1.833)   |

$^a$BMI: body mass index; CAG$_{N}$: CAG repeats; E$_2$: estrogen; FSH: follicle-stimulating hormone; LH: luteinizing hormone; PRL: prolactin; T: testosterone. *Dependent variable: penile length

Figure 2: Penile length in the PRL$_{N}$ and PRL$_{H}$ groups. The box plots show the average penile lengths for the PRL levels in the PRL$_{N}$ and PRL$_{H}$ groups. PRL$_{N}$ and PRL$_{H}$ (with *) indicate normal and high levels of PRL, respectively. PRL$_{N}$: prolactin (normal); PRL$_{H}$: prolactin (normal).
implications for the interpretation of epidemiological studies, the diagnosis of hypogonadism in borderline situations and possibly the individualization of microplexin therapies in men.

**AUTHOR CONTRIBUTIONS**

YM and KJW participated in the design of the trial, conducted the data acquisition, interpreted and statistically analyzed the data and drafted the manuscript. LN and JZ designed the study and offered the study materials. HJX and ZKM conducted the data acquisition, interpreted and statistically analyzed the data. BK and XYW conducted the data acquisition. YGG and DLH designed the study, interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

**COMPETING INTERESTS**

The authors declare no competing interests.

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