

The relation between isolated micropenis in childhood with CAG and GGN repeat polymorphisms in the androgen receptor gene

Esra TUĞ^{1*}, Sezen GÜNTEKİN ERGÜN¹, Mehmet Ali ERGÜN¹, Fatma Nihal DİLEK², Emriye Ferda PERÇİN¹

¹Department of Medical Genetics, School of Medicine, Gazi University, Ankara, Turkey

²Dr. Sami Ulus Obstetrics Child Health and Illness Training and Research Hospital, Ankara, Turkey

Received: 26.10.2017 • Accepted/Published Online: 08.04.2018 • Final Version: 30.04.2018

Background/aim: In micropenis cases accompanied by external genital abnormalities such as hypospadias and cryptorchidism, infertility and spermatogenic failures have been reported to correlate with androgen receptor (*AR*) gene CAG and GGN repeat polymorphisms. While there is one study on isolated micropenis and CAG repeats, no study related to GGN repeats has been reported. We investigated the relation between CAG and GGN repeats in the *AR* gene with development of penis length in boys with isolated micropenis.

Materials and methods: A total of 24 Turkish boys with isolated micropenis (<-2.5 SD) and 64 healthy controls who had normal basal serum gonadotropin levels were examined. Genotyping was performed by DNA sequencing of the patients and controls.

Results: The distribution of CAG and GGN repeat lengths in our patients and controls was within the normal range and did not significantly differ between the patients and the controls.

Conclusion: CAG repeat length in the *AR* constitutes one of multiple genetic factors relevant to the development of isolated micropenis, and the expansion of this repeat can be detected as a likely modifying factor. Moreover, the interactions of other genes that may be involved in the etiology of isolated micropenis with CAG and GGN repeats have to be taken into consideration.

Key words: Micropenis, androgen receptor (*AR*) gene, CAG repeat, GGN repeat

1. Introduction

Penis formation and growth are androgen dependent, and androgen-dependent penile growth, which is important for adult penis length, takes place in three different periods: in late gestation, in the first 4 years after birth, and at puberty (1). Defective androgen effect during the critical period frequently results in micropenis with hypospadias or genital ambiguity, whereas impaired androgen effect after the critical period usually leads to isolated micropenis (2,3). If the measured penile length is more than 2.5 standard deviations (SDs) below the mean for the age, it is termed micropenis. Any defect in the hypothalamo-pituitary-gonadal axis can result in micropenis. Micropenis has a common occurrence and may present in isolated form or may accompany male sex development abnormalities, or occurs as a part of a number of syndromes (3,4).

The human androgen receptor (*AR*) gene, located on chromosome Xq11-12 (5), is a transcription factor regulating the development of male reproductive organs in the fetus and secondary sex characteristics in response to testosterone and 5 α -dihydrotestosterone (DHT)

at puberty (6). The *AR* gene contains polymorphic (CAG)*n*CAA repeat encoding polyglutamine, and (GGT)3GGG(GGT)2(GGC)*n* repeat encoding polyglycine in exon 1, referred to as the CAG and GGN repeats, respectively. Variation in the CAG repeat length (9–33 glutamine residues) is observed in the normal population and is known to be associated with regulation of androgen receptor activity (6). Regarding abnormal expansion of the CAG repeats, 44 repeats are associated with reduced androgen receptor function both in vivo and in vitro (7). It is known that defective androgen receptors are directly involved in androgen insensitivity syndrome (AIS), spinal bulbar muscular atrophy (SBMA), and prostate cancer (8). *AR* gene mutations in patients with AIS are known to be associated with variable development of the Wolffian duct, micropenis, hypospadias, and cryptorchidism (8,9).

GGN repeats in the *AR* gene vary in length from 19 to 23 repeats, the most common length being 23 GGN (6). There are conflicting results regarding GGN repeats and *AR* gene activity (10,11). Moreover, epidemiological investigations of the association between the number

* Correspondence: esratug@hotmail.com

of GGN repeats and male infertility have produced inconsistent results (6,12,13). However, there is a study about the relation between the length of the GGN repeat of the *AR* gene and the most common male congenital abnormalities including hypospadias and cryptorchidism (14).

The *AR* gene has been examined in patients with defective male sex differentiation. Although *AR* gene CAG and GGN repeat polymorphisms in micropenis cases accompanied by hypospadias or cryptorchidism, in infertility, and in spermatogenic failures have been reported (6,15–21), there is only one study involving isolated micropenis and CAG repeat polymorphisms (2). In addition, no study has been reported regarding isolated micropenis and GGN repeat polymorphisms. Thus, the aim of the present study was to determine the relation between isolated micropenis and *AR* gene CAG and GGN repeat polymorphisms in children whose basal serum gonadotropin levels were normal.

2. Materials and methods

2.1. Subjects

Twenty-four Turkish boys with isolated micropenis (age, 1–15 years; 9.83 ± 3.30) and 64 healthy boys as controls (age, 5–15 years; 10.14 ± 2.64) were studied. All patients were referred by a pediatric endocrinologist to our department for evaluation of genetic etiology. Written informed consents were obtained from the patients' parents/guardians for genetic analysis, which had been approved by the Institutional Ethics Board Committee.

The selection criteria included the following: (i) stretched penile length that was obtained by the standard method as described previously (2,3) below -2.5 SD of the mean age matched normal; (ii) lack of associated other genital abnormality such as hypospadias or cryptorchidism; (iii) no demonstrable malformation syndromes known to be associated with genital abnormalities; (iv) normal growth and development; (v) 46,XY karyotype; (vi) to be in the normal range by age of basal serum gonadotropin levels in patients. Basal serum luteinizing hormone (LH), follicle-stimulating hormone (FSH), and testosterone (T) levels were within age-matched reference data in all cases (3,22). Penile length was measured when the penis was fully stretched, not flaccid; the glans penis was held with the thumb and forefinger, and the measurement was taken from the pubic ramus to the distal tip of the glans penis over the dorsal side. The suprapubic fat pad was pressed inwards as much as possible, and, if present, the foreskin was retracted during the measurement (2,3). Patients with excess weight/obesity were excluded from the study.

The controls were 64 Turkish boys referred to our department with normal external genitalia and penile length, and laboratory findings revealing normal basal

serum gonadotropin levels. All of the subjects (patients and controls) had 46,XY karyotypes.

2.2. Methods

Genomic DNA samples of all the subjects were extracted by the salting out method from peripheral blood leukocytes (23). CAG/GGN repeat motifs of exon 1 in the *AR* gene were screened for determining as the size of a polymerase chain reaction (PCR) (GeneAmp PCR System 9700, Applied Biosystems, Waltham, MA, USA) product containing the polymorphic microsatellites. The *AR* gene exon 1 was amplified from genomic DNA in 2 different PCR reactions, using primers: first primer forward 5'-TTCCAGAATCTGTTCCAGAGC-3' reverse: 5'-CTCTGGGACGCAACCTCTCT-3' and second primer forward 5'-CCTGGCACACTCTCTTCACA-3' reverse 5'-CCAGGGTACCACACATCAGG-3' for CAG and GGN repeats, respectively. The reaction components for each PCR consisted of a total volume of 25 μ L of reaction mixture containing 2 μ L of DNA, 2.5 μ L of 10x buffer, 2.5 mM MgCl₂, 0.5 μ L of 10 mM deoxynucleotide triphosphate (dNTP) mix, 0.5 μ L of 10 pmol each primer, and 0.3 μ L of 1.25 u/50 μ L Taq Polymerase (Fermentas, USA). PCR protocols were applied as follows: after 94 °C for 5 min denaturation, 94 °C for 30 s, 64 °C for 30 s, 72 °C for 30 s repeated 35 times, and following 72 °C for 5 min extension for first primers. The reaction components for each PCR consisted of a total volume of 25 μ L of reaction mixture containing 1 μ L of DNA, 2.5 μ L of 10x buffer, 1.5 mM MgCl₂, 0.5 μ L of 10 mM dNTP mix, 0.5 μ L of 10 pmol each primer, and 0.2 μ L of 1.25 u/50 μ L Taq Polymerase (Fermentas, USA). PCR protocols were applied as follows: after 94 °C for 5 min denaturation, 94 °C for 15 s, 59 °C for 30 s, 72 °C for 30 s repeated 35 times, and following 72 °C for 5 min extension for second primers. Amplification was confirmed by electrophoresing 3 μ L of the PCR product on a 1.5% agarose gel containing ethidium bromide and subsequent visualization under ultraviolet illumination. Sequence analysis was carried out automated DNA sequencer and protocols provided by the manufacturer by MWG Eurofins, Germany.

2.3. Statistical analysis

Statistical calculations were performed using SPSS 16.0 (SPSS Inc, Chicago, IL, USA) for Windows. Data were expressed as mean \pm standard deviation. Differences among the groups were compared by the Mann–Whitney U test. P values less than 0.05 were considered statistically significant.

3. Results

The *AR* gene CAG repeat polymorphism was successfully genotyped in 24 patients and 64 controls. In the patients, mean serum FSH, LH, and testosterone levels were 1.77 ± 2.76 , 0.93 ± 1.18 , and 1.83 ± 4.33 , respectively. Mean body

mass index (BMI) for patients was 23.16 ± 4.41 . CAG and GGN repeat regions were successfully genotyped in both the patient and control groups. CAG repeat length ranged from 13 to 32 repeats (22.17 ± 3.43) in patients and 15 to 28 (21.63 ± 2.59) repeats in controls. Statistically significant differences were not observed in patients' and controls' CAG repeat sizes ($P = 0.681$). GGN repeat length ranged from 7 to 20 repeats (16.00 ± 3.38) in patients and 13 to 20 (17.12 ± 0.74) repeats in controls. Statistically significant differences were not observed in patients' and controls' GGN repeat sizes ($P = 0.484$).

4. Discussion

In fetal life, male external genitalia differentiation is associated with normal androgen production and successful response of target organs to androgen (9). Micropenis may be an independent abnormality or a part of many syndromes, can occur as a result of pituitary/hypothalamic insufficiency or primary testicular insufficiency, or can be idiopathic, which is a highly heterogeneous condition under the influence of various genetic and environmental factors (2). Thus, the cause of isolated micropenis may be hormonal (hypogonadotropic hypogonadism), testicular (hypergonadotropic hypogonadism) or due to end organ resistance, and also iatrogenic (drug use during the pregnancy) (3,4). Any mutation that plays a role in androgen production may result in isolated micropenis (2).

While the CAG and GGN repeat numbers vary by ethnicity, the length of the polymorphic trinucleotide CAG repeat region in exon 1 of the *AR* gene varies from 9 to 33 in the normal population (5,24). There is an inverse correlation between CAG repeat length and the level of expression or transactivation function of the *AR* gene (25). *AR* gene repeat variants with a high number of CAG have lower androgen receptor transcriptional activity (26). When CAG repeat length is over 38, this may lead to reduced virilization, defective spermatogenesis, and SBMA, known also as Kennedy's disease (7). Furthermore, *AR* gene CAG repeat length may contribute to the etiology of androgen-related genital abnormalities (27). While the

expansion of the CAG repeats in the *AR* gene is unlikely to constitute a major cause of cryptorchidism (27,28), the length of the GGN repeat of the *AR* gene has been found to be related to cryptorchidism and hypospadias in previous studies (14). The *AR* gene CAG repeat polymorphisms in the Turkish population in infertile patients have been studied and the range of CAG repeats was found to be 18–29 and 16–29 in infertile and fertile men, respectively (29). However, the relation between isolated micropenis and repeat polymorphisms has not been studied.

The polymorphic GGN region of the *AR* gene also plays a role in the receptor function (10), but there are a limited number of studies investigating the effects of this polymorphism in hypospadias or cryptorchidism (14). While only two studies have described CAG repeat polymorphisms in boys with micropenis, GGN polymorphisms in boys have not been studied. These studies do not reveal a significant impact of CAG repeat lengths on the development of isolated micropenis (2,30).

In the present study, the distribution of CAG and GGN repeat lengths in our patients and controls were within the normal range, and CAG and GGN repeat lengths in the boys with isolated micropenis did not significantly differ from those of the controls. This result is compatible with the previous studies revealing the CAG and GGN repeat lengths in Caucasian populations (31–35). The CAG repeat length in the *AR* gene constitutes one of multiple genetic factors relevant to the development of micropenis, and expansion of the CAG repeat length can be regarded as a likely modifying factor in some patient populations but not in other patient populations.

In conclusion, we did not find a correlation between *AR* gene CAG and GGN polymorphisms and micropenis. However, we selected a limited number of Turkish patient and controls. Further studies will guide us for better clarification on the relevance of *AR* gene abnormalities in the development of isolated micropenis.

Acknowledgment

This study was funded by Projects of Scientific Investigation of Gazi University (01/2012-25).

References

1. Ma YM, Wu KJ, Dang Q, Shi Q, Gao Y, Guo P, Xu S, Wang XY, He DL, Gong YG. Testosterone regulates keratin 33B expression in rat penis growth through androgen receptor signaling. *Asian J Androl* 2014; 16: 817-823.
2. Ishii T, Sato S, Kosaki K, Sasaki G, Muroya K, Ogata T, Matsuo N. Micropenis and the *AR* Gene: mutation and CAG repeat-length analysis. *J Clin Endocrinol Metab* 2001; 86: 5372-5378.
3. Paris F, De Ferran K, Bhangoo A, Ten S, Lahlou N, Audran F, Servant N, Poulat F, Philibert P, Sultan C. Isolated 'idiopathic' micropenis: hidden genetic defects? *Int J Androl* 2011; 34: 518-525.
4. Massa GG, Langenhorst V, Oostdijk W, Wit JM. Micropenis in children: etiology, diagnosis and therapy. *Ned Tijdschr Geneesk* 1997; 141: 511-515.

5. Jaaskelainen J. Molecular biology of androgen insensitivity. *Mol Cell Endocrinol* 2012; 352: 4-12.
6. Lundin KB, Giwercman YL, Rylander L, Hagmar L, Giwercman A. Androgen receptor gene GGN repeat length and reproductive characteristics in young Swedish men. *Eur J Endocrinol* 2006; 155: 347-354.
7. Radpour R, Rezaee M, Tavasoly A, Solati S, Saleki A. Association of long polyglycine tracts (GGN repeats) in exon 1 of the androgen receptor gene with cryptorchidism and penile hypospadias in Iranian patients. *J Androl* 2007; 28: 164-169.
8. Brinkmann AO. Molecular basis of androgen insensitivity. *Mole Cell Endocrinol* 2001; 179: 105-109.
9. Sultan C, Paris F, Terouanne B, Balaguer P, Georget V, Poujol N, Jeandel C, Lumbroso S, Nicolas JC. Disorders linked to insufficient androgen action in male children. *Hum Reprod Update* 2001; 7: 314-322.
10. Gao T, Marcelli M, McPhaul MJ. Transcriptional activation and transient expression of the human androgen receptor. *J Steroid Biochem Mol Biol* 1996; 59: 9-20.
11. Ding D, Xu L, Menon M, Prem Veer Reddy G, Barrack ER. Effect of GGC (glycine) repeat length polymorphism in the human androgen receptor on androgen action. *Prostate* 2005; 62: 133-139.
12. Lundin KB, Giwercman A, Richthoff J, Abrahamsson PA, Giwercman YL. No association between mutations in the human androgen receptor GGN repeat and inter-sex conditions. *Mol Hum Reprod* 2003; 9: 375-379.
13. Ferlin A, Bartoloni L, Rizzo G, Roverato A, Garolla A, Foresta C. Androgen receptor gene CAG and GGC repeat lengths in idiopathic male infertility. *Mol Hum Reprod* 2004; 10: 417-421.
14. Aschim EL, Nordenskjöld A, Giwercman A, Lundin KB, Ruhayel Y, Haugen TB, Grotmol T, Giwercman YL. Linkage between cryptorchidism, hypospadias, and GGN repeat length in the androgen receptor gene. *J Clin Endocrinol Metab* 2004; 89: 5105-5109.
15. Allera A, Herbst MA, Griffin JE, Wilson JD, Schweikert HU, McPhaul MJ. Mutations of the androgen receptor coding sequence are infrequent in patients with isolated hypospadias. *J Clin Endocrinol Metab* 1995; 80: 2697-2699.
16. Batch JA, Evans BAJ, Hughes IA, Patterson MN. Mutations of the androgen receptor gene identified in perineal hypospadias. *J Med Genet* 1993; 30: 198-201.
17. Sutherland RW, Wiener JS, Hicks JP, Marcelli M, Gonzales ET Jr, Roth DR, Lamb DJ. Androgen receptor gene mutations are rarely associated with isolated penile hypospadias. *J Urol* 1996; 156: 828-831.
18. Griffin JE. Androgen resistance the clinical and molecular spectrum. *N Eng J Med* 1992, 326: 611-618.
19. McPhaul MJ. Molecular defects of the androgen receptor. *J Steroid Biochem Mol Biol* 1999; 69: 315-322.
20. Dowsing AT, Yong EL, Clark M, McLachlan RI, De Kretser DM, Trounson AO. Linkage between male infertility and trinucleotide repeat expansion in the androgen-receptor gene. *Lancet* 1999; 354: 640-643.
21. Yoshida K, Yano M, Chiba K, Honda M, Kitahara S. CAG repeat length in the androgen receptor gene is enhanced in patients with idiopathic azoospermia. *Urology* 1999; 54: 1078-1081.
22. Neely EK, Hintz RL, Wilson DM, Lee PA, Gautier T, Argente J, Stene M. Normal ranges for immunochemiluminometric gonadotropin assays. *J Pediatr* 1995; 127: 40-46.
23. Aljanabi SM, Martinez I. Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. *Nucleic Acids Res* 1997; 25: 4692-4693.
24. Ackerman CM, Lowe LP, Lee H, Hayes MG, Dyer AR, Metzger BE, Lowe WL, Urbanek M, for The Hapo Study Cooperative Research Group. Ethnic variation in allele distribution of 1 the Androgen Receptor (*AR*) (CAG)*n* repeat. *J Androl* 2012; 33: 210-215.
25. Castro-Nallar E, Bacallao K, Parada-Bustamante A, Lardone MC, López PV, Madariaga M, Valdevenito R, Piottante A, Ebensperger M, Castro A. Androgen receptor gene CAG and GGN repeat polymorphisms in Chilean men with primary severe spermatogenic failure. *J Androl* 2010; 31: 552-559.
26. Chamberlain NL, Driver ED, Miesfeld R. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucleic Acids Res* 1994; 11: 3181-3186.
27. Lim HN, Nixon RM, Chen H, Hughes IA, Hawkins JR. Evidence that longer androgen receptor polyglutamine repeats are a causal factor for genital abnormalities. *J Clin Endocrinol Metab* 2001; 86: 3207-3210.
28. Sasagawa I, Suzuki Y, Tateno T, Nakada T, Muroya K, Ogata T. CAG repeat length of the androgen receptor gene in Japanese males with cryptorchidism. *Mol Hum Reprod* 2000; 6: 973-975.
29. Tufan AC, Şatıroğlu-Tufan NL, Aydınuraz B, Şatıroğlu MH, Aydos K, Bağcı H. 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: 105-115.
30. Sasagawa I, Suzuki Y, Muroya K, Ogata T. Androgen receptor gene and male genital anomaly. *Arch Androl* 2002; 48: 461-466.
31. Sasaki M, Kaneuchi M, Sakuragi N, Fujimoto S, Carroll PR, Dahiya R. The polyglycine and polyglutamine repeats in the androgen receptor gene in Japanese and Caucasian populations. *Biochem Biophys Res Commun* 2003; 312: 1244-1247.
32. Ruhayel Y, Lundin K, Giwercman Y, Hallden C, Willen M, Giwercman A. Androgen receptor gene GGN and CAG polymorphisms among severely oligozoospermic and azoospermic Swedish men. *Hum Reprod* 2004; 19: 2076-2083.

33. Sasagawa I, Suzuki Y, Ashida J, Nakada T, Muroya K, Ogata T. CAG repeat length analysis and mutation screening of the androgen receptor gene in Japanese men with idiopathic azoospermia. *J Androl* 2001; 22: 804-808.
34. Martinez-Garza SG, Gallegos-Rivas MC, Vargas-Maciel M, Rubio-Rubio JM, de Los Monteros-Rodríguez ME, González-Ortega C, Cancino-Villarreal P, de Lara LG, Gutiérrez-Gutiérrez AM. Genetic screening in infertile Mexican men: chromosomal abnormalities, Y chromosome deletions, and androgen receptor CAG repeat length. *J Androl* 2008; 29: 654-660.
35. Dadze S, Wieland C, Jakubiczka S, Funke K, Schröder E, Royer-Pokora B, Willers R, Wieacker PF. The size of the CAG repeat in exon 1 of the androgen receptor gene shows no significant relationship to impaired spermatogenesis in an infertile Caucasoid sample of German origin. *Mol Hum Reprod* 2000; 6: 207-214.