Acta Scientific Medical Sciences

Volume 1 Issue 2 July 2017

Research Article

Impression of CAG Trinucleotide Repeat Expansion in the Androgen Receptor Gene among the Endometriosis Patients in South of Iran

Zohreh Salehi[1], Leila Kohan[1] and Majid Yavarian[2]*

[1]*Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran*

[2]*Shiraz Nephro-Urology Research Center, Shiraz University of Medical Sciences, Iran*

***Corresponding Author:** Majid Yavarian Shiraz, Nephro-Urology Research Center, Shiraz University of Medical Sciences, Iran.

Received: June 02, 2017; **Published:** July 06, 2017

Abstract

Endometriosis with an estimated frequency of 5 - 10% among women at the reproductive age, is a common gynecologic disorder. The gynecological characteristic is presence of endometrial glandular and stromal cells existing in the extra-uterine environment. A genetic variation in the androgen receptor (AR) has been associated with the risk of developing endometriosis.

Aim: The aim of study is to evaluate the tri-nucleotide (CAG)n repeats in AR gene.

Material and Methods: In a period of six month (2016), 55 females with clinically diagnosed endometriosis and 55 healthy females age-matched randomly selected as control group. Molecular analysis of AR gene for (CAG)n repeats was performed by using Nested-PCR amplification followed by fragment analysis on ABI sequencer.

Results: The (CAG)n repeat length ranged in endometriosis patients from 15 to 33 (mode = 19) and for controls from 18 to 31 (mode = 27). Concerning to the $n < 21$, the difference in the number of (CAG)n repeats between endometriosis cases and controls was found statistically significant ($p = 0.037$) and odds ratio is 2.006 (95% CI = 1.043 - 3.857), as well as $n > 28$ ($p = 0.0001$, OR = 62.471, %95CI = 14.683 - 265.790).

Conclusion: It is concluded that (CAG)n repeats length in the exon 1 of AR gene in about 21% of is lower than controls. This finding shows a predisposing genetic factor in about 1 in fifth patient for the endometriosis in the region.

Keywords: Endometriosis; Androgen Receptor; CAG Repeats

Introduction

Endometriosis is an estrogen-dependent disorder that affects women during the reproductive ages. It occurs when tissue similar to the uterine lining (endometrium) attaches to organs in the pelvis and begins to grow. This displaced endometrial tissue causes irritation in the pelvis that may lead to pain and infertility [1]. Although the proliferation and differentiation of both eutopic and ectopic endometrium are mediated mainly by receptors for estrogens and progesterone. Androgen receptor (AR) is also expressed in these tissues and might contribute to the events that lead to the establishment of endometriosis [2,3].

The AR protein contains a polyglutamine tract of variable size in the N-terminal transactivation domain that can modulate its ability to enhance transcriptional events. This tract is encoded by a polymorphic cytosine, adenine, and guanine (CAG) microsatellite repeat within exon 1 of the AR gene, which is located on the X chromosome at Xq12-13 [4]. An in vitro study showed inverse relationship between the number of (CAG)n repeats and the AR activity. The (CAG)n repeat alleles with low number of repeats showed higher activity while high repeat number progressively decreased the transactivation activity [5]. These variations in the length of the (CAG)n repeat size altered AR transcriptional activity and have been associated with several human diseases such as ovarian, breast and endometrial cancers that committed with short alleles [6-10]. Increased (CAG)n repeat size is reported in the spinal and bulbar muscular atrophy (Kennedy disease) as well as male infertility [11,12].

The other findings are pharmacologic effects like a link between cyclosporine (CsA) induces gingival overgrowth in renal

transplanted patients and a smaller size of (CAG)n repeat in the AR gene [13].

To further evaluation, the length of (CAG)n repeats at the AR gene were studied among the endometriosis patients in compare to the distribution pattern of (CAG)n repeat without endometriosis in a cohort of Iranian women in the Fars province. In these study, the allele patterns were defined as the length of the (CAG)n repeat region.

Materials and Methods

Subjects

Totally, 55 clinically diagnosed endometriosis patients and 55 healthy individual age matched (33.2 ± 8.5 years) were randomly selected. The patients and controls were informed that blood would be used for research purposes and gave written consent. All studied individuals were white and of Iranian (Fars) origin and attention was paid to the selection of controls, who were from the same geographic area and of similar age as patients. All of them underwent complete pre-surgery clinical examination before the diagnostic operative laparoscopy. Indications for laparoscopy included chronic pelvic pain, infertility, ovarian cysts, or myoma. Endometriosis was diagnosed during the laparoscopic intervention diagnosis of ovarian endometriotic cysts and peritoneal lesions has always been confirmed histologically and direct visualization. Endometriosis was documented in 55 women.

As the diagnosis of endometriosis essentially can be proven by laparoscopy and because the disease is often asymptomatic, controls consisted of women in whom the disease was ruled out laparoscopically.

Determination of (CAG)n repeats in the exon 1 of the AR gen

Genomic DNA was isolated from whole blood cell and extracted using the salting-out method. Quantified by Nano drop, and stored at the -20°C until test assay. Molecular analysis was performed in two steps (Nested PCR) amplification which followed by fragment analysis by using ABI machine. Two sets of primers, used in two successive runs of polymerase chain reaction, the second set intended to amplify a secondary target within the first run product. Sets of primers in step1; AR-1 forward (5'-TAGGGCTGGGAAGGGTCTAC-3') and AR-1 reverse (5'-GCTGTGAAGGTTGCTGTTCC-3'). PCR was performed in a total volume of 20μL containing 25 ng genomic DNA, 0.25pmole of each primer. A 25-cycle amplification protocol performed in which the annealing temperature was 53°C. Sets of primers in step2; AR-2 forward (5'-TCCAGAATCTGTTCCAGAGC-3') and AF-2 reverse (6-Fam-TGGGGAGAACCATCCTCACC-3').

For the number of (CAG)n repeats fragment analysis was performed by ABI and analysed by using the *Gene Marker* software.

Statistical analysis was done using the SPSS version 23 statistical package Student t test or analysis of variance were used as appropriate. Distribution of cases according to the (CAG)n length was also tested using the Logistic Regression for all tests; significance was set at 5% and 1%.

Results

The range of age in case and control groups was 18 - 53 years. The bioinformatics data of patients and control is tabulated in the (Table 1).

Risk Factor	Endometriosis (mean ± SD)	Control (mean ± SD)	The significance level
BMI	25.38 ± 6.68	24.64 ± 4.75	P = 0.053
age of first sex	22.7 ± 6.4	18.3 ± 8.6	P = 0.01
age of first period	13.0 ± 1.8	13.7 ± 1.7	P > 0.05
Duration of period	7.1 ± 1.8	5.9 ± 1.7	P = 0.06
Age at first pregnancy	24.1 ± 6.2	17.5 ± 9.0	P = 0.005

Table 1: Clinical and Characteristics in Women with Endometriosis and with Women Healthy Controls.

Based on the data analysis, minimal and mild stage of the disease (stage I, II) was found in 20 (36.4%), moderate and severe (stage III, IV) in 35 (63.6%) cases. The sites of involvement in order were pelvic endometriosis (83.6%), ectopic pelvic (5.5%) whom had involved in both places, and followed by ectopic endometriosis (3.6%). About half of cases (49.1%) and 12.3% of control had family history of endometriosis (p < 0.001).

The average age of first sex among the patients and control group was 18.3 ± 8.6 and 22.7 ± 6.4 respectively. According to the result, age of first sex had a significant impact on the developing of disease (P < 0.01).

While the average age of first pregnancy, was 24.1 ± 6.2 in patients, in the control group significantly low (17.5 ± 9.4) and endometriosis had a crucial impact on pregnancy (P < 0.005).

The CAG repeat length ranged in endometriosis patients varies from 16 to 33 and in the control group from 17 to 28. The allele frequency with cut-off point of n ≤ 21, between two groups (control and patient) were significant (p = 0.037). The odds ratio was 2.006 (95% CI = 1.043-3.857). Moreover, the number of (CAG)n repeats upper than 28 (n ≥ 28) was statistically higher in patients in comparison with controls (p = 0.0001, OR = 62.471, %95CI = 14.683 - 265.790). The distribution of the allele frequency in patients and control subjects is shown in (Figure 1).

Siz	patient	control
15	0	0
16	1	0
17	1	2
18	8	2
19	7	2
20	7	2
21	7	10
22	5	18
23	3	12
24	6	30
25	2	19
26	3	8
27	3	3
28	18	2
29	15	0
30	12	0
31	9	0
32	2	0
33	1	0

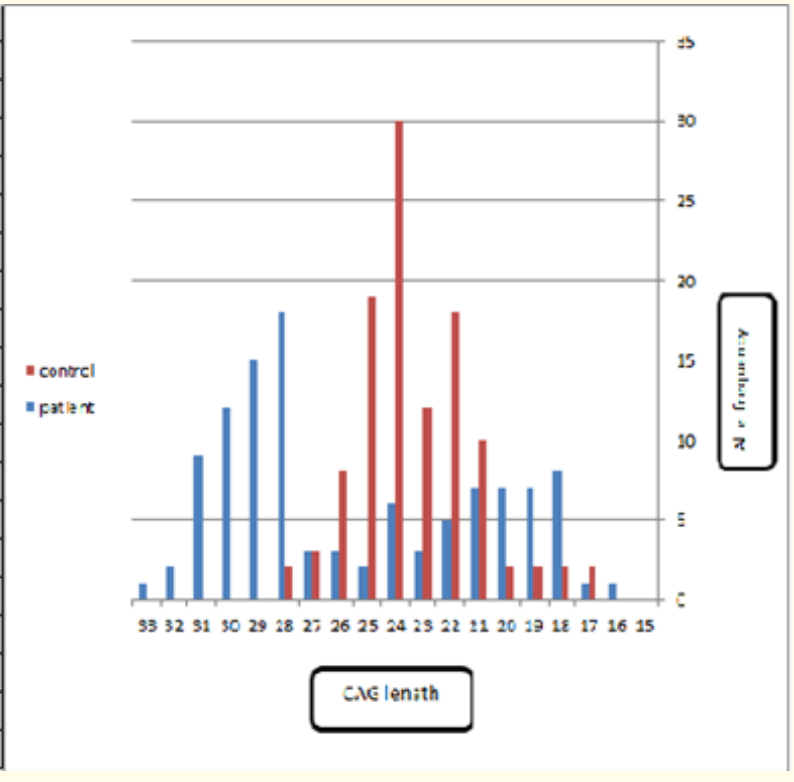

Figure 1: Distribution of the CAG Allele between the Cases and Control.

Discussion

Endometriosis classified based on the number and character of peritoneal lesions, ovarian and other organ involvement, and presence, type, and extent of adhesions into four stages (I to IV) [14]. In the pathogenesis of several stage of endometriosis at least 8 theories are concerned. These theories are based on retrograde menstruation, Metaplasia, Hormones, Oxidative stress and inflammation, Immune dysfunction, Apoptosis suppression, Genetic, and stem cells [15]. Different signalling pathways through gene regulation is postulated [16] and may introduce different stage of disease.

Family study indicates 5 to 7 percent of first-degree relatives have tendency to present endometriosis in polygenic and multifactorial manner [17].

The regulation of endometrial tissue is regulated by estrogen and progesterone and expression of several genes affected during of menstruation cycles.

The AR gene, a member of the steroid/nuclear receptor super family, on the X-chromosome comprises of eight exons and in female one X and corresponded genes is inactive. This gene encodes the AR protein that containing three domains: 1-N-terminal transactivation domain, 2- central DNA-binding domain, and 3-C-terminal ligand- binding domain. The gene also comprises two variable number of thri-nucleotide repeats, one toward the 5' end (CAG repeats) and the other is GGC repeats that located in the 3' portion of exon 1 [18,19].

The number of the CAG varies in different ethnic group from 8 to 35 repeats [20]. Its repeat in this study was 13 to 27 and one report from the north of Iran, it was 13 - 26 [21]. The length of a polymorphic (CAG)n repeat sequence is inversely correlated with transcriptional activity by the androgen receptor. Shorter (CAG)n alleles, by increasing transactivation, may result in increased AR-mediated sensitivity of the endometriosis.

According to the population study, the relative AR (CAG)n repeat length has significant variation in different race groups. It is documented that Afro-Caribbean has shortest repeat lengths therefore have greatest AR activity). The Caucasian and Hispanic have mid-size but the Thai people present longest repeat length and lowest AR activity [22].

This variation in (CAG)n repeat length not only associate with various disease and malignancy like prostate cancer [23], it also has effect on attitude of individuals as well. In accordance with a review (Minkov and Bond, 2015), more CAG repeats are associated with androgen insensitivity, while fewer repeats are supposed to be linked to more sexual partners and violent and impulsive behaviour [24]. A study on Barcelona-Spanish girls suggested that shorter (CAG)n repeats increase androgen sensitivity and subsequent ovarian hyper-androgenism, a key feature of PCOS [25].

A possible molecular mechanism regarding how a change in poly-glutamine length can affect the activity of the receptor might involve a nuclear G-protein, a Ras-related nuclear protein/ARA24 that acts as a co-activator with the AR and can bind differentially with different lengths of poly-glutamines within AR. AR-CAG/ARA24 interactions become stronger as the number of glutamines decreases, thereby increasing co-activation capability [26]. Although the likely increase in AR intrinsic activity with each reduction in AR-CAG length is relatively small, these effects are genetically determined and, therefore, exert their effects over the entire lifetime of the individual. Small changes, can, over time, have significant pathologic effects (4). Although the precise molecular mechanisms involved in its pathogenesis are largely unknown. High estrogen levels can induce uncontrolled endometrial cellular proliferation and hence, potentially, the development of endometriosis [27]. Endometriosis, as an estrogen-dependent disorder that tends to be modulated by sex steroids, may actually represent a pathologic condition potentially associated with AR polymorphisms. In this study, distribution of CAG repeats in compare to the control clearly shows two extremities (Figure 1), a high repeat (n ≥ 28) that may explain those cases that referred due to infertility (P = 0.0001, OR = 62.471, %95CI = 14.683-265.790) and other theories like immune dysfunction and other spectrum a short repeat which could explain this genetic variation. The cut off (CAG)n repeats with n= or < 21, which covers about 21% of cases, statistically, difference in the endometriosis cases and controls (p = 0.037) and odds ratio is 2.006 (95% CI = 1.043 - 3.857).

Indeed, androgens appear to contrast the proliferative effect exerted by estrogens in normal endometrium. Somatic expansions of the AR-CAG repeat may decrease the transactivation power of the receptor and thus diminish the anti-proliferative signal inferred by the hormone [28].

These findings are similar with those from a previous study in which a Chinese group significant differences in the distribution of (CAG)n repeats is was found in women with and without endometriosis [29].

In summary, the results of the present study argue the possibility constitutes a genetic predisposition for the endometriosis in about 20% of cases.

Bibliography

1. Worley MJ., *et al.* "Endometriosis-associated ovarian cancer: a review of pathogenesis". *International Journal of Molecular Sciences* 14.3 (2013): 5367-5379.

2. Ferro P., *et al.* "The androgen receptor CAG repeat: a modifier of carcinogenesis?". *Molecular and Cellular Endocrinology* 193 (2002): 109-120.

3. Fujimoto J., *et al.* "Expression of size-polymorphic androgen receptor (AR) gene in ovarian endometriosis according to the number of cytosine, adenine, and guanine (CAG) repeats in AR alleles". *Steroids* 64.8 (1999): 526-529.

4. Lattuada D., *et al.* "Androgen receptor gene cytosine, adenine, and guanine trinucleotide repeats in patients with endometriosis". *Journal of the Society for Gynecologic Investigation* 11.4 (2004): 237-240.

5. Chamberlain NL., *et al.* "The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function". *Nucleic Acids Research* 22.15 (1994): 3181-3186.

6. Terry KL., *et al.* "Androgen receptor cytosine, adenine, guanine repeats, and haplotypes in relation to ovarian cancer risk". *Cancer Research* 65.13 (2005): 5974-5981.

7. Chen HT., *et al.* "Androgen receptor CAG repeats non-random X chromosome inactivation, and loss of heterozygosity at Xq25 in relation to breast cancer risk". *BMC Cancer* 14 (2014): 144.

8. Li K., *et al.* "Association of androgen receptor exon 1 CAG repeat length with risk of hepatocellular carcinoma: a case-control study". *Tumour Biology* 35.12 (2014): 12519-12523.

9. Rajender S., *et al.* "Phenotypic heterogeneity of mutations in androgen receptor gene". *Asian Journal of Andrology* 9.2 (2007): 147-179.

10. Giovannucci E., *et al.* "The CAG repeats within the androgen receptor gene and benign prostatic hyperplasia". *Urology* 53.1 (1999): 121-125.

11. La Spada AR., *et al.* "Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy". *Nature* 352.6330 (1991): 77-79.

12. Hiort O and Holterhus PM. "Androgen insensitivity and male infertility". *International Journal of Andrology* 26.1 (2003): 16-20.

13. Al Sayed AA., *et al.* "The role of androgen receptor gene in cyclosporine induced gingival overgrowth". *Journal of Periodontal Research* 49.5 (2014): 609-614.

14. Rock JA. "The revised American Fertility Society classification of endometriosis: reproducibility of scoring. ZOLADEX Endometriosis Study Group". *Fertility and Sterility* 63.5 (1995): 1108-1110.

15. Sourial S., *et al.* "Theories on the pathogenesis of endometriosis". *International Journal of Reproductive Medicine* (2014): 179515.

16. Aghajanova L and Giudice LC. "Molecular evidence for differences in endometrium in severe versus mild endometriosis". *Reproductive Science* 18.3 (2011): 229-251.

17. Bischoff F and Simpson JL. "Genetics of endometriosis: heritability and candidate genes". *Best Practice and Research Clinical Obstetrics and Gynaecology* 18.2 (2004): 219-232.

18. Vottero A., *et al.* "Androgen receptor-mediated hypersensitivity to androgens in women with nonhyperandrogenic hirsutism: skewing of X-chromosome inactivation". *The Journal of Clinical Endocrinology and Metabolism* 84.3 (1999): 1091-1095.

19. Lumbroso R., *et al.* "Codon-usage variants in the polymorphic (GGN)n trinucleotide repeat of the human androgen receptor gene". *Human Genetics* 101.1 (1997): 43-46.

20. Michael Minkov. "Michael Harris Bond Genetic polymorphisms predict national differences in life history strategy and time Orientation". *Personality and Individual Differences* 76 (2015): 204-215.

21. Ashtiani ZO., *et al.* "Are GSTM1, GSTT1 and CAG repeat length of androgen receptor gene polymorphisms associated with risk of prostate cancer in Iranian patients?". *Pathology and Oncology Research* 17.2 (2011): 269-275.

22. Ackerman CM., *et al.* "Hapo Study Cooperative Research Group. Ethnic variation in allele distribution of the androgen receptor (AR) (CAG)n repeat". *Journal of Andrology* 33.2 (2012): 210-215.

23. Poelaert F., *et al.* "Androgen Receptor Gene Copy Number and Protein Expression in Treatment-Naïve Prostate Cancer". *Urologia Internationalis* (2017).

24. Minkov M and Bond MH. "Genetic polymorphisms predict national differences in life history strategy and time orientation". *Personality and Individual Differences* 76 (2015): 204-215.

25. Ibáñez L., *et al.* "Androgen receptor gene CAG repeat polymorphism in the development of ovarian hyperandrogenism". *The Journal of Clinical Endocrinology and Metabolism* 88.7 (2003): 3333-3338.

26. Mifsud A., *et al.* "Androgen receptor gene CAG trinucleotide repeats in anovulatory infertility and polycystic ovaries". *The Journal of Clinical Endocrinology and Metabolism* 85.9 (2000): 3484-3488.

27. Terakawa N., *et al.* "Growth inhibition by progestins in a human endometrial cancer cell line with estrogen-independent progesterone receptors". *Cancer Research* 47.7 (1987): 1918-1923.

28. Kamal AM., *et al.* "Androgen receptors are acquired by healthy postmenopausal endometrial epithelium and their subsequent loss in endometrial cancer is associated with poor survival". *British Journal of Cancer* 114.6 (2016): 688-696.

29. Hsieh YY., *et al.* "Androgen receptor trinucleotide polymorphism in endometriosis". *Fertility and Sterility* 76.2 (2001): 412-413.

Volume 1 Issue 2 July 2017

© All rights are reserved by Majid Yavarian., *et al*.