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Research paper

Association of androgen receptor CAG repeat polymorphism and risk of epithelial ovarian cancer

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ABSTRACT

Background: Biological and epidemiologic evidence suggested that androgen and its receptor may play an important role in ovarian carcinogenesis. However, results of previous association studies about ovarian cancer and AR CAG repeat polymorphism were inconsistent. Furthermore, none of these studies were conducted in Asians.
Methods: We evaluated the relationship between AR CAG repeat length and epithelial ovarian cancer (EOC) risk among a Chinese population including 1800 pathologically confirmed EOC patients and 1800 frequency matched controls.
Results: Women with longer AR CAG repeats had a decreased EOC risk (OR = 0.87 for per CAG_A increase, 95% CI: 0.81–0.95). Compared to those with shorter (<22) CAG_A repeat length, women with of longer (≥22) CAG_A repeats had a 34% decreased EOC risk (OR = 0.66, 95% CI: 0.57–0.75). For CAG_S and CAG_L, the results remained consistent.
Conclusions: Our findings suggest that androgen signaling contributes to the development of ovarian cancer.

1. Introduction

Ovarian cancer remains the leading cause of death from gynecological malignancies, and the second most important gynecological malignancies among women worldwide (Sankaranarayanan and Ferlay, 2006; Jemal et al., 2010). Although shown to be associated with family history, genetic factors, diet, inflammation, reproductive factors such as null-parity and oral contraceptive use, and obesity, the etiology of ovarian cancer remains poorly understood (Holschneider and Berek, 2000; McLaughlin et al., 2007; Shu et al., 2009; Kurman and Shih Ie, 2010; Zheng et al., 2010; Ma et al., 2013).

The androgen receptor (AR) is a nuclear transcription factor which mediates the actions of testosterone and dihydrotestosterone. AR signaling is critical for ovarian growth and maintenance (Terry et al., 2005a). Androgen is produced by ovarian theca lutein cells and ARs exist in the normal surface epithelium of the ovaries. Epidemiologic studies also supported an important role of androgen and their receptors in ovarian cancer (Schildkraut et al., 2007). In exon 1 of the *AR* gene, a cytosine, adenine, guanine (CAG) trinucleotide repeat codes for a polyglutamine tract which normally ranges from 6 to 39 repeats. It has been explored in relation to cancer risk in many studies (Sleddens et al., 1992; Sleddens et al., 1993; Davies et al., 1995). Molecular analyses showed that the transactivational capacity of the AR decreased with increasing number of glutamines encoded by the CAG repeat tract (Schildkraut et al., 2007).

Several studies that have addressed the association between CAG repeat length and risk of ovarian cancer (Terry et al., 2005a; Terry et al., 2005b; Schildkraut et al., 2007; Ludwig et al., 2009). However, the results were non-consistent, and all the studies were conducted in non-Asians. In view of the conflicting results about the relationship between AR repeat polymorphism and ovarian cancer risk, as well as dichotomizing repeat length of AR CAG repeat in different ethnic groups, we aim to examine this relationship in a large population-based, case–control study of Epithelial Ovarian Cancer (EOC) among Chinese women. This is the first study to conducted in a relatively large group of Asian women.

2. Materials and methods

2.1. Subjects

Subjects participating in this study were ethnically homogenous Han Chinese. Cases were 1800 pathologically confirmed EOC patients, while controls were 1800 healthy subjects which were frequency-matched to the cases by age group (within 5 years old), and residential

Abbreviations: EOC, epithelial ovarian cancer; AR, androgen receptor; CAG, cytosine, adenine, guanine; ICD-O, International Classification of Diseases for Oncology; ORs, odds ratios; average allele, CAG_A; shorter allele, CAG-S; longer allele, CAG-L.

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areas (urban or countryside). Histological subtypes of ovarian cancer were classified according to International Classification of Diseases for Oncology (ICD-O) codes. The controls had no known history of cancers, or benign ovary diseases. Each subject was interviewed face-to-face by trained personnel, using a formatted questionnaire to obtain the demographic data, and health characteristics. To avoid the possible influence of preclinical diseases on exposure status, cases were asked about exposures that occurred at least 1 year before the diagnosis date, and controls were asked about exposures that occurred >1 year before the interview date. After the interview, each subject provided 3–5 mL of venous blood. Approval for this study was obtained from the institutional review board of General Hospital of Jinan Military Command, Jinan, China. Written informed consent was obtained from all participants or from the patients' representatives.

2.2. Genotyping

DNA was extracted using a QIAamp 96 DNA blood kit (Qiagen, Valencia, CA). Genotyping was conducted at the General Hospital of Jinan Military Command. Genomic DNA was PCR-amplified using the fluorescently labeled primers (F: 5′-FAM-TCC-AGA-ATCTGT-TCC-AGA-GCG-TGC-3′; R: 5′-GCT-GTG-AAG-GTTGCT-GTT-CCT-CAT-3′). The length of these fragments varied by the number of CAG repeats. Fragments were run on denaturing polyacrylamide gels on the Applied Biosystems Prism 3700XL and analyzed by Applied Biosystems Prism Genescan automated fluorescence detection (Applied Biosystems, Foster City, CA). Fragment lengths were determined from a series of sequenced PCR samples..

2.3. Statistical Analyses

All statistical tests were 2-sided, and a *P* value threshold of 0.05 was set. All analyses were conducted using SAS, version 9.2 (SAS Institute, Cary, North Carolina). The Chi-square test was used to examine the differences in the frequency distributions of categorical variables. Differences in the means of the CAG repeat lengths (in terms of CAG units) in the AR gene between patients and controls were tested using Student's T-test. Odds ratios (ORs) and their 95% CI were calculated from unconditional logistic regression analyses. AR CAG repeats were first examined as a continuous variable for CAG_A (average allele), CAG-S (shorter allele), CAG-L (longer allele). This approach assumes that each one-unit increment in CAG repeat length is related to a constant proportional change in relative risk. We also analyzed repeat polymorphisms as a categorical variable. The median values of the CAG repeat variables for the control population were used as a cutoff point.

Table 1
Distribution of demographic characteristics for EOC cases and controls.

Category	Cases (N = 1800)	Controls (N = 1800)	P value
Age (year)	50.5 ± 7.6	50.2 ± 7.0	0.218
Education (less than middle school)	702 (39.0%)	670 (37.2%)	0.272
Ever smoker	266 (14.8%)	257 (14.3%)	0.670
Use of hormone replacement therapy	74 (4.10%)	50 (2.76%)	0.028
Body mass index (kg/m_2)	23.9 ± 3.4	23.7 ± 3.5	0.082
Histopathology			
Serous	1296 (72%)		
Mucinous	126 (7%)		
Clear cell	90 (5%)		
Endometrioid	162 (9%)		
Others	126 (7%)		

Continuous variables: mean values ± standard deviation, p-value from t-tests;
Categorical variables: numbers and percentages, P-values from x^2 test.

3. Results

Characteristics of the study population were shown in Table 1. Totally 1800 EOC cases and 1800 healthy controls were included in this study. There were no differences in distribution of age, gender, education, smoking, and body mass index (BMI), except for use of hormone replacement therapy. Generally speaking, cases were slightly elder, higher educated, more likely to be smokers, users of hormone replacement therapy, and had higher BMI. Among the 1800 EOC cases, 1296 (72%) were Serous, 126 (7%) were Mucinous, 90 (5%) were Clear cell, 162 (9%) were Endometrioid, while 126 (7%) were others. Table 2 showed the distribution of AR repeat numbers among the subtypes of EOC.

Table 3 presented the associations between AR CAG polymorphism and EOC risk. Women with longer AR CAG repeats had a decreased EOC risk (OR = 0.87 for per CAG_A increase, 95% CI: 0.81–0.95). Except for CAG_A, we also examined AR CAG repeats as a continuous variable for CAG-S and CAG-L. The results remained significant (OR = 0.89 for per CAG_S increase, 95% CI: 0.80–0.98; OR = 0.85 for per CAG_L increase, 95% CI: 0.80–0.93). Then we analyzed repeat polymorphism as a categorical variable, which used the median value of the CAG repeat variables for the control population as a cutoff point. Compared to those with shorter (<22) CAG_A repeat length, women with longer (≥22) had a 34% decreased EOC risk (OR = 0.66, 95% CI: 0.57–0.75). For CAG_S and CAG_L, the results were consistent (OR for CAG_S = 0.71, 95% CI: 0.62–0.81; OR for CAG_L = 0.64, 95% CI: 0.56–0.73). when limited to Serous subtype, the significant associations kept (Table 2).

4. Discussion

Ovarian cancer is one of the most important malignant tumors in females (Cramer, 2012). Its pathogenesis is closely related to androgen and AR (Edmondson and Monaghan, 2001; Haruta et al., 2011; Doufekas and Olaitan, 2014). The most remarkable characteristic of AR is that it contains one CAG with triad nucleotide repetitive sequences (Hsing et al., 2000; Clark et al., 2003). In current study, we examined the relationship between AR CAG repeat and EOC risk in a large population-based, case–control study in Chinese women. Women with longer AR CAG repeats had a decreased EOC risk (OR = 0.87 for per CAG_A increase, 95% CI: 0.81–0.95). Compared to those with shorter (<22) CAG_A repeat length, women with the longer (≥22) had 34% decreased EOC risk. To be best of our knowledge, this is the first study to examine the association between AR repeat length polymorphism and ovarian cancer risk in a relatively large population of Asian women.

Androgen is produced by ovarian theca lutein cells and AR exists in the normal surface epithelium of the ovaries (Mesiano et al., 2002). Most ovarian cancers express ARs (Edmondson and Monaghan, 2001; Bertone-Johnson, 2005). Studies showed that the gene polymorphisms of androgen receptor (AR), especially the polymorphism of repetitive sequence in CAG (the first exon), are closely related to the occurrence and development of ovarian cancer (Terry et al., 2005a; Terry et al., 2005b; Schildkraut et al., 2007; Ludwig et al., 2009). However, most of the studies focused on non-Asians, and results were inconsistent due to ethnic differences and sample size. Thus, we conducted this case–control study.

In our study, we found women with longer AR CAG repeat length had a decreased EOC risk. These results were consistent to the finding of Ludwig ea. al (Ludwig et al., 2009), which found that the risk of ovarian cancer was decreased by 11% with each additional CAG repeat (P = 0.006 for the longer allele). However, among African Americans, shorter allele caused increased ovarian cancer risk (Schildkraut et al., 2007). This results may be explained by confounding due to population stratification and the sample size. It is biologically plausible for the inversed association between CAG repeat length and risk of ovarian cancer, in view of the inversed relationship between CAG length and transactivation activity