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ORIGINAL ARTICLE

Association between genetic polymorphisms of inflammatory response genes and the risk of ovarian cancer

Zhi-Hui Lu*, **Xi-Juan Gu, Ke-Zhi Shi, Xuan Li, Dian-Dian Chen, Li Chen**

Department of Oncology, The First Affiliated Hospital of Nanchang University, Nanchang City, Jiangxi Province, 330006, China

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Background/Purpose: Inflammation plays an important role in promoting ovarian tumorigenesis and cancer progression. However, the relationship between polymorphisms in inflammatory response genes and risk of ovarian cancer remains poorly understood. In this study, we investigated the association of *PPARG* Pro12Ala, *IL6*-174G/C, E-selectin S128R, *NFKB1*-94 ins/del, *NFKBIA*-826C/T, and *ICAM-1* K469E polymorphisms with ovarian cancer risk in a Chinese population.
Methods: Genotyping of the polymorphisms was performed on 687 cases and 687 controls employing the PCR-RFLP technique, and the logistic regression model was used to measure the risk association.
Results: A significantly increased risk association was observed for the heterozygous genotypes of *PPARG* [odds ratio (OR) = 1.52, 95% confidence interval (CI) = 1.01–2.29] and E-selectin (OR = 1.77, 95% CI = 1.07–2.93) polymorphisms, as well as the homozygous ins/ins genotype of *NFKB1* polymorphism (OR = 1.39, 95% CI = 1.00–1.92). By contrast, *ICAM-1* KE genotype was associated with a decreased ovarian cancer risk (OR = 0.77, 95% CI = 0.60–0.98). In addition, the *NFKB1* del/del + *NFKBIA* TT combination was also found to be associated with a decreased ovarian cancer risk, with OR = 0.12 (95% CI = 0.01–0.95). The associations of the *NFKB1* and *ICAM-1* polymorphisms replicated the findings of previous reports, assuring the reliability of the results obtained.
Conclusion: *NFKB1* and *ICAM-1* polymorphisms could serve as useful ovarian cancer risk prediction biomarkers for the Chinese population, while the utility of *PPARG* and E-selectin polymorphisms as biomarkers requires further confirmation in independent ovarian cancer cohorts.

Conflicts of interest: The authors have no conflicts of interest relevant to this article.

* Corresponding author. Department of Oncology, The First Affiliated Hospital of Nanchang University, Nanchang City, Jiangxi Province, 330006, China.
E-mail address: zhihui0992@gmail.com (Z.-H. Lu).

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Introduction

Ovarian cancer is one of the leading forms of cancer, both in China and worldwide.[1] Each year, there is an estimated 225,500 new incidences of ovarian cancer cases globally.[2] The lack of effective screening methods causes >70% of ovarian cancer patients to be diagnosed at late stages, which leads to the low 5-year survival rate and high mortality rate of the disease. Worldwide, ovarian cancer contributes to 114,000 deaths annually.[3] A method for the identification of individuals at a higher risk of the cancer is therefore necessary for improving ovarian cancer screening strategy.

The principal risk factors for the occurrence of ovarian cancer are germ line mutations in the *BRCA1* or *BRCA2* genes.[4] However, the carriers of such mutations account for only a small portion of total ovarian cancer cases, and a substantial proportion of ovarian cancer risk among sporadic cases remained unexplained. It has been suggested that common low penetrance genetic variations could confer moderate risk to ovarian cancer cases without a heritable basis.[5] Single nucleotide polymorphisms of genes involved in cancer-related pathways represent the candidates of such genetic variations.

Inflammation has been established as an underlying cause in the development of many cancers.[6] Several events which delay inflammation in the ovaries, such as childbirth and oral contraceptive use, have been associated with a reduced risk of ovarian cancer.[7] On the contrary, events which cause inflammation, such as endometriosis, have been suggested to increase the risk of ovarian cancer.[8] It has been thought that inflammation could induce and activate several oxidant-generating enzymes, which can lead to damages in the DNA. Given the important link between inflammation and ovarian carcinogenesis, inherited variation in inflammatory response genes could affect ovarian cancer susceptibility.

Polymorphisms in several inflammatory response genes, such as *PPARG* Pro12Ala polymorphism, *IL6* -174G/C polymorphism, E-selectin S128R (A561C) polymorphism, *NFKB1*-94 ins/del polymorphism, *NFKBIA* -826C/T polymorphism, and *ICAM-1* K469E polymorphism, have been found to be associated with various cancers.[9–16] However, the involvement of these polymorphisms in ovarian cancer in the Chinese population has not been well established. To address this matter, we investigated the association between the six inflammatory response genetic polymorphisms mentioned above and the risk of ovarian cancer in a Chinese population.

Materials and methods

Study participants

The study was approved by the Medical Research Ethics Board of the First Affiliated Hospital of Nanchang University, Nanchang City, China and all samples were collected from the above hospital from July 2010 to December 2013. A total of 687 females who were histopathologically confirmed ovarian cancer patients and 687 controls were recruited into the study. Controls were disease-free individuals without cancers and age-matched to the cases in terms of frequency. Blood samples were collected from the participants after obtaining written informed consent.

Genotyping

Genomic DNA was extracted from the blood samples obtained using EasyPure Blood Genomic DNA Kit (TransGen Biotech, Beijing, China) according to the manufacturer's protocol. The genomic DNA obtained was then used in polymerase chain reaction (PCR). The genetic polymorphisms were detected by the PCR-restriction fragment length polymorphism (RFLP) method. Researchers were blinded to the identity and the case–control status of the samples. For all polymorphisms, ~10% of the samples were chosen at random and sequenced to confirm the genotypes.

PPARG Pro12Ala polymorphism

The PCR primers of *PPARG* Pro12Ala polymorphism used were 5′-GCC AAT TCA AGC CCA GTC-3′ and 5′-GAT ATG TTT GCA GAC AGT GTA TCA GTG AAG GAA TCG CTT TCC-3′. The PCR condition was 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 1 minute at 94°C, 30 seconds at 60°C, 30 seconds at 72°C, and a final extension for 5 minutes at 72°C. The PCR amplification produced fragment of 270 bp in size. BstUI restriction enzyme was then used to digest the 270 bp fragment for determination of genotype. The CC genotype remained uncut after restriction digestion. The GG genotype gave a 227 bp and a 43 bp fragment, and heterozygous genotype (GC) genotype was detected by the presence of all the above bands in agarose gel.

IL6-174G/C polymorphism

The PCR condition used for *IL6* -174G/C polymorphism was 5 minutes of initial denaturation at 94°C, followed by 35 cycles of 45 seconds at 94°C, 30 seconds at 58°C, 30 seconds at 72°C, and a final extension for 5 minutes at 72°C. The primers used were 5′-TGA CTT CAG CTT TAC TCT TGT-3′ and 5′-CTG ATT GGA AAC CTT ATT AAG-3′. The PCR reaction gave an amplification product of 161 bp in size. The fragment was then digested by Hsp 92II restriction enzyme. The GG genotype remained 161 bp on agarose gel as it was uncut by the restriction enzyme. However, the restriction digestion produced two smaller fragments, which were 118 bp and 43 bp in size, for CC genotype. In addition, heterozygous genotype (GC) was found to have all the fragments detected in homozygous genotypes, which were fragments with sizes of 161 bp, 118 bp, and 43 bp.

E-selectin S128R (A561C) polymorphism

The E-selectin S128R (A561C) polymorphism was genotyped by first amplifying the region of interest under the following conditions: 10 minutes of initial denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 60.5°C, 30 seconds at 72°C, and a final extension for 10 minutes at 72°C. The PCR primers used were 5′-ATGGCACTCTGTAGGACTGCT-3′ and 5′-GTCTCAGCTCACGATCACCAT-3′. The