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Genetic polymorphisms in *AURKA*, *BRCA1*, *CCNE1* and *CDK2* are associated with ovarian cancer susceptibility among Chinese Han women

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ABSTRACT

Introduction: Centrosome aberrations and cell-cycle deregulation have important implications for ovarian cancer development. The *AURKA*, *BRCA1*, *CCNE1* and *CDK2* genes play pivotal roles in centrosome duplication and cell-cycle regulation.
Methods: Using a haplotype-based analysis, this study aimed to investigate whether genetic polymorphisms in these four genes may contribute to ovarian cancer susceptibility. A total of 22 single nucleotide polymorphisms (SNPs) in these four genes were genotyped in 287 cases of ovarian serous cystadenocarcinomas and 618 age-matched cancer-free controls from the Chinese Han population, and then haplotype blocks were reconstructed according to our genotyping data and linkage disequilibrium (LD) status of these SNPs.
Results: For *AURKA*, we found that haplotype GA [rs6064391 (T→G) + rs911162 (G→A)] was strongly associated with decreased ovarian cancer risk (adjusted OR = 0.31, 95% CI = 0.15–0.63, $P = 0.0012$). For *BRCA1*, we found that haplotype CGTAG was associated with decreased ovarian cancer risk (adjusted OR = 0.64, 95% CI = 0.41–0.98, $P = 0.0417$). Moreover, women harboring homozygous GA/CGTAG haplotypes showed the lowest risk (OR = 0.12, 95% CI = 0.02–0.94, $P = 0.0438$). In *CCNE1*, the SNPs rs3218035 and rs3218042 were significantly associated with increased ovarian cancer risk (rs3218035: adjusted OR = 5.20, 95% CI = 1.85–14.52, $P = 0.0017$; rs3218042: adjusted OR = 4.98, 95% CI = 1.75–14.19, $P = 0.0027$). For *CDK2*, no significant association was found.
Conclusions: This study indicates that genetic polymorphisms of *AURKA*, *BRCA1* and *CCNE1* may affect ovarian cancer susceptibility in Chinese Han women.

1. Introduction

Ovarian cancer is one of the most deadly diseases worldwide [1,2]. Because of the lack of specific early symptoms or effective tumor biomarkers, most of the patients with ovarian cancer are diagnosed at the advanced stages, with only 30–40% achieving a 5-year survival rate [3]. The etiology of ovarian cancer is associated not only with hormonal, reproductive and environmental factors but also with genetic factors. Several low-frequency, high-penetrance ovarian cancer susceptibility genes have been identified over the past two decades, the most important being germline mutations in *p53*, *PTEN*, *BRCA1* and *BRCA2* genes [4]. However, most cases of ovarian cancer cannot be explained by the above genes. Ovarian cancer, as a common and complex disease, may be interpreted by high-frequency, low-penetrance genetic variations according to the popular "common disease–common variants (CDCV) hypothesis" [5].

So far, single nucleotide polymorphisms (SNPs), of which there are approximately 15 million in the human genome [6], have become the most frequently used genetic markers in studying complex diseases. It would be costly to genotype all the known SNPs in the target gene. Fortunately, a set of closely linked SNP alleles in a region of a chromosome tends to be inherited together

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(being not easily separable by recombination) and forms a "haplotype"; a pair of haplotypes forms a diplotype. Based on linkage disequilibrium (LD), applying a much smaller subset of informative SNPs called haplotype-tagging SNPs (htSNPs) can capture gene-wide common variations [7,8]. Haplotype-based analysis is a cost-effective strategy for investigating associations between candidate genes and complex traits in population-association studies [7,8].

Centrosome aberration is a common phenomenon in various human malignancies. Numerical, structural and functional centrosome aberrations can cause spindle abnormalities, resulting in genomic instability and tumor formation [9,10]. Quite a few oncogenic and tumor suppressor proteins are localized in the centrosomes, and deregulation of these proteins may evoke centrosome abnormalities [11]. We have been conducting a series of studies to determine whether common genetic variants in centrosomal genes contribute to breast or ovarian cancer development and progression. In our previous studies we found that genetic variants in *BRCA1*, *AURKA* and *CCNE1* contributed to breast cancer susceptibility among the Chinese Han population [12,13]. We wondered whether the genetic polymorphisms in these genes were associated with susceptibility to ovarian cancer in the Chinese Han population.

Aurora-A kinase, a centrosome-associated serine/threonine kinase, regulates centrosome maturation, entry into mitosis, formation of the bipolar spindle, and cytokinesis [14,15]. Its genetic amplification, mRNA and protein over-expression are common in many types of solid tumors, including ovarian cancer, and are associated with aneuploidy, supernumerary centrosomes, defective mitotic spindles, and resistance to apoptosis [16]. It has been reported that there are strong functional interactions between Aurora-A and BRCA1. BRCA1, a breast- and ovarian-specific tumor suppressor, associates with BARD1, and acts as a powerful E3 ubiquitin ligase [17]. BRCA1 function is most critical during the S and G_2 phases of the cell cycle, blocking centrosome reduplication. The inhibition of BRCA1 causes rapid centrosome over-duplication and stimulates centrosome microtubule nucleation function [18]. Aurora-A localizes to the centrosome during G_2, and phosphorylates BRCA1, thus inhibiting its inhibitory activity during mitosis and enabling the establishment of mitotic spindles [19]. Aurora-A over-expression or BRCA1 knockdown lead to similar phenotypes, such as absence of the G_2–M checkpoint and centrosome amplification, suggesting that aberrant expressions or gene mutations of *AURKA* and *BRCA1* cooperate during tumor development [20,21].

In the cell cycle, cyclins are prime regulators which play a critical role in the control of cell proliferation by forming a complex with different cyclin-dependent kinases (CDKs). The cyclin E–CDK2 complex binds to pRb, thus activating the E2F family of transcription factors which are necessary for the transcription of genes required for DNA replication and hence for progression of the cell into the S phase [22]. CDK2–cyclin E also triggers initiation of centrosome duplication, and activation of CDK2–cyclin E is thought to link DNA replication and centrosome duplication [23]. Over-expression of cyclin E and high activity of cyclin E–CDK2 has been reported in several human cancers [22]. The expression of cyclin E gradually progresses from benign to borderline to malignant ovarian tumors [24]. Cyclin E over-expression has been shown to be an independent poor prognostic factor for patients with advanced ovarian cancer, and it was associated with amplification of the cyclin E gene named *CCNE1* [25].

In this study, we comprehensively analyzed the associations between htSNPs and haplotypes in *AURKA*, *BRCA1*, *CCNE1* and *CDK2* and ovarian cancer susceptibility in the Chinese Han population.

2. Materials and methods

2.1. Study population

A total of 287 cases with clinically confirmed ovarian serous cystadenocarcinoma were recruited from Peking University Third Hospital, Beijing Cancer Hospital and Beijing Hospital between 1999 and 2010. All subjects were genetically unrelated ethnic Chinese Han women. Patients' epidemiological information was collected from their medical records, including age at diagnosis, height, weight, age at menarche and/or menopause, age at the first full-term pregnancy (FFTP), smoking history, and family history of ovarian cancer or other cancers in first-degree relatives. The 618 cancer-free controls were selected from individuals who participated in a community-based screening program for non-infectious diseases conducted in Beijing. The selection criteria included no history of cancer, Chinese Han ethnic background, and frequency-matched to the cases by 5-year age groups. All controls provided the same epidemiological information as we collected from the patients. This study was approved by the Ethics Committee of Peking University Health Science Center.

2.2. SNPs selection

Since individual SNPs may fail to capture the whole contribution of a locus to a particular trait, haplotype-based association analyses are believed to provide higher resolution and potentially greater power for identifying modest etiological effects of genes. To better understand the contributions of these candidate genes *AURKA*, *BRCA1*, *CCNE1* and *CDK2* to ovarian cancer, we selected haplotype-tagging SNPs (htSNPs) in each LD block which detect haplotypes above 5% by the Haploview v.4.2 software program on the basis of the information of these candidate genes in the HapMap database [HapMap Data Release #27; Chinese Beijing population (CHB)]. For *AURKA* gene, we identified six htSNPs (rs6064391, rs911162, rs2298016, rs8117896, rs10485805, rs6024836) in the *AURKA* locus – spanning from 2 kb upstream to 10 kb downstream of *AURKA* gene, minor allele frequency (MAF) $>$ 5% in CHB – and also analyzed a missense SNP rs2273535 (T→A, located in exon 4, resulting in Phe31Ile) [12] and a probable-risk SNP rs2064863 [26]. For the *BRCA1* gene, we selected six htSNPs to distinguish all common haplotypes, these being rs8176323, rs8176303, rs8176199, rs3737559 and rs8067269 (spanning from 10 kb upstream to 10 kb downstream of the *BRCA1* gene; MAF $>$ 5% in CHB). For the *CCNE1* gene, we identified six htSNPs in the *CCNE1* locus, these being rs8102137, rs3218035, rs3218038, rs3218042, rs1406 and rs3218076 (spanning from 10 kb upstream to 10 kb downstream of the *CCNE1* gene; MAF $>$ 5% in CHB) [13]. For the *CDK2* gene, we identified only two common SNPs in the CHB population according to the HapMap database.

2.3. DNA isolation, genotyping assay and quality control

For the control group, genomic DNA was extracted from blood leukocytes by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. However, patient's genomic DNA was extracted from formalin-fixed paraffin-embedded (FFPE) non-tumor tissues. Genotyping was done by the AB StepOne® Real-Time PCR System (Applied Biosystems, Foster City, CA) using the TaqMan® Assay. Primers and probes were supplied directly by Applied Biosystems as Assays-by-Design and Assays-on-Demand products, and the polymerase chain reaction (PCR) conditions were as described previously [12]. Each genotyping plate contained positive and negative controls. As a quality control, we repeated the genotyping on 3% of the samples, and all genotypes were checked independently by two fellow