Common Variation in Nemo-Like Kinase Is Associated with Risk of Ovarian Cancer

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Abstract

Background: Overexpression of mitotic kinases has been associated with prognosis, histologic grade, and clinical stage in ovarian cancer, but the relationship between inherited variation in these genes and ovarian cancer risk has not been well defined.

Methods: We measured associations between 397 single nucleotide polymorphisms (SNPs) from 67 mitotic kinases and invasive epithelial ovarian cancer risk in two case–control studies (n = 671 cases; n = 939 controls). Thirty-six candidate SNPs (P < 0.05) were assessed in a replication analysis consisting of three additional studies (n = 1,094 cases; n = 829 controls).

Results: In initial analysis, thirty-six SNPs were suggestive of association with risk of serous ovarian cancer, all subtypes of ovarian cancer, or both (P < 0.05). Replication analyses suggested an association between rs2125846 in the Nemo-like kinase (NLK) gene and ovarian cancer (serous OR = 1.36, 95% CI: 1.11–1.67, P = 1.77 x 10⁻⁵; all subtypes OR = 1.30, 95% CI: 1.08–1.56, P = 2.97 x 10⁻⁴). Furthermore, rs2125846 was associated with risk in the combined discovery and replication sets (serous OR = 1.33, 95% CI: 1.15–1.54; all subtypes OR = 1.27, 95% CI: 1.12–1.45).

Conclusions: Variation in NLK may be associated with risk of invasive epithelial ovarian cancer. Further studies are needed to confirm and understand the biologic relationship between this mitotic kinase and ovarian cancer risk.

Impact: An association between SNPs in NLK and ovarian cancer may provide biologic insight into the development of this disease. Cancer Epidemiol Biomarkers Prev; 21(3); 523–8. ©2012 AACR.

Introduction

Ovarian cancer has the highest mortality of gynecologic malignancies. Factors associated with ovarian cancer risk include age, family history, fertility drug use, postmenopausal hormone therapy (1), and inherited factors (2–4). Whereas inherited mutations in BRCA1 and BRCA2 account for 50% of ovarian cancer cases in families with 2 or more confirmed cases (5), the remaining unexplained familial and sporadic ovarian cancer risk is likely, in part, attributable to common, low-penetrance alleles (6). Efforts to identify low-penetrance alleles by genome-wide association studies (GWAS) have identified variants in the chromosome 9p22 BNC2 locus (3), a 19p13 locus containing the MERIT40-encoding gene (7), and in the 2q31 and 8q24 loci (4).

Mitotic kinases are essential components in the regulation of mitosis and cytokinesis, acting upon various structures involved in mitotic entry, progression, and exit. These kinases phosphorylate proteins involved in centrosome duplication and separation, chromosome condensation, spindle assembly and fidelity, chromosome segregation, and cytokinesis, as well as have the ability to behave as oncogenes, providing a compelling link between errors in mitosis and oncogenesis (8). Indeed, errors in the choreography of the processes controlled by mitotic kinases disrupt successful division of mammalian cells and can lead to aneuploidy, genetic instability, and cancer. More specifically, alterations in these genes and dysregulation of protein products have been implicated in cancer development in mouse models (9) and in multiple human tumor types (8).
Mitotic kinases include members of the Aurora, Polo-like, and Nek families, as well as individual kinases involved in mitotic checkpoints, mitotic exit, and cytokinesis. Within the Aurora kinase family, the overexpression of both AURKA and AURKB has been associated with poor prognosis in epithelial ovarian cancers (10–13). Similarly, overexpression of polo-like kinases, such as PLK1 and PLK2, have also been shown to correlate with prognosis, histologic grade, and clinical stage in ovarian cancer (14–16). Although disregulation of these mitotic kinases has been associated with ovarian cancer prognosis, and one study of polymorphisms found evidence of association with risk and polymorphisms in Cdk1, a key mitotic kinase required for entry into mitosis (17), the relationship between alterations in these genes and ovarian cancer risk has not been well studied.

Here we tested the hypothesis that inherited variation in genes encoding mitotic kinases increases the risk of invasive epithelial ovarian cancer. Single-nucleotide polymorphisms (SNPs) from 67 mitotic kinase genes were genotyped in 2 ovarian cancer case-control studies followed by replication in 3 additional studies.

Materials and Methods

Study populations

The current hypothesis was tested in a collaborative effort that combined studies of invasive epithelial ovarian cancer from Mayo Clinic (MAY) and the North Carolina Ovarian Cancer Study (NCO). Details of the study protocols have been published previously (refs. 18, 19; Supplementary Table S1). Questionnaire data obtained from all subjects included established risk factors such as demographics, reproductive history, family history of cancer, medical and surgical history, and lifestyle habits. Candidate SNPs (P < 0.05) identified in this discovery population were assessed in a replication study comprised of 3 additional ovarian cancer studies: a case-control study from Brigham and Women’s Hospital (BWH), the Tampa Bay Ovarian Cancer Study (TBO), and the Familial Ovarian Tumour Study (TOR; Supplementary Table S2). Details of these studies have also been previously described (4). We restricted all analyses to unique study participants. We excluded 44 samples with more than 10% missing data, leaving 1,981 samples. The sample call rate was 99.74% and the concordance for the 129 duplicate samples was 100%. Eleven SNPs with significant deviations from Hardy–Weinberg equilibrium in controls (P < 0.001), assessed using Pearson goodness of fit or Fisher exact tests, were excluded from analyses.

The replication analyses utilized genotype data from a published GWAS of ovarian cancer (4, 21). TBO and TOR samples were genotyped using the Illumina 610K platform, and BWH samples were genotyped using the Illumina 317K platform. Imputation of approximately 2.5 million SNPs using HapMap data as the reference was carried out for each replication site using MACH, and imputed genotypes were used where observed genotype data were missing.

Statistical analysis

SNP-specific analyses were done to evaluate the association of genotypes at each SNP and serous ovarian cancer risk and risk of all histologic types of ovarian cancer combined. Associations were estimated as ORs with associated 95% CIs using unconditional logistic regression under log-additive genetic models. We also carried out haplotype analyses using Haplostat, for genes with multiple SNPs at P < 0.05, estimating haplotype frequencies for each gene using all SNPs within the gene and then testing the global significance (P < 0.05) for haplotype association with risk using a likelihood ratio test. Individual haplotype associations were evaluated using a log-additive model. All discovery single SNP and haplotype models were adjusted for age, geographic location, body mass index (BMI), oral contraceptive use, hormone replacement therapy (HRT), and parity and age at first birth. Single SNP P < 0.05 was used to select SNPs for replication. All replication analyses were adjusted for age and study center (additional covariate data were not available). Assessment of the most promising SNPs in combined analysis by histologic subtype was done using polytomous logistic regression using control status as the reference outcome. Heterogeneity of SNP associations by histologic subtype was measured by applying polytomous logistic regression to cases only.

As a conservative approach to adjusting for the large number of statistical tests, we used the following method. First, we sought replication of any SNP with single SNP P value <0.05 in discovery analyses. Second, we used a modified correlation-adjusted Bonferroni adjustment in replication analyses, to account for multiple testing. Recognizing that some correlation due to LD exists between the replication SNPs, we first determined the effective number of independent tests using a principal components based method (22), which indicated that our
The goal of this analysis was to assess whether common genetic variation in mitotic kinases is associated with risk of invasive epithelial ovarian cancer. To achieve this, we genotyped 397 tagSNPs in 67 genes (Supplementary Table S3) encoding mitotic kinases in 2 case–control studies (Supplementary Table S1). We first restricted our analysis to serous invasive ovarian cancer cases and controls (n = 407 cases, n = 939 controls); this selection was based on recent findings from the Ovarian Cancer Association Consortium (OCAC) showing that GWAS associations with serous ovarian cancer were generally stronger than for all histologic subtypes combined, possibly because of refinement of the phenotype under study (4). Twenty SNPs tested were suggestive of association with risk of serous ovarian cancer in a log-additive model (P < 0.05; Table 1, Supplementary Table S4). These 20 SNPs were located in 13 different genes: CDC7, CDK6, CSNK2A, SIK3, MAST2, NEK2, NEK4, NEK8, NLK, PRKG2, STK4, TEX14, and TRIB3.

Because 4 of the 13 genes identified in the single SNP serous analysis contained multiple candidate SNPs, we carried out haplotype analyses to better understand the patterns of risk in these genes. CDK6 haplotypes of the 4 SNPs (rs2282990, rs3731348, rs17690388, and rs2282983) were suggestive of association with risk of serous invasive ovarian cancer (global haplotype association P = 0.0034; Supplementary Table S5a). The first CDK6 risk haplotype was perfectly tagged by the minor allele (A) of rs17690388 and was associated with a decrease in risk of serous ovarian cancer (OR = 0.63, 95% CI: 0.40–0.99; P = 0.044). The second CDK6 risk haplotype captured the minor alleles of rs2282990 (T) and rs2282983 (C) and the major allele at rs3731348 (G) and was associated with an increase in serous ovarian cancer risk (OR = 2.42, 95% CI: 1.30–4.50; P = 0.0054). SIK3 and TEX14 each contained haplotypes associated with risk of serous ovarian cancer that were captured by variation at single SNPs (Supplementary Table S5b,c). Thus, associations between variation in SIK3 and TEX14 and serous ovarian cancer were best described by the single SNPs rs7928320 and rs12944693. NLK haplotypes were not associated with risk of serous ovarian cancer (Supplementary Table S5d).

Having observed possible associations with serous ovarian cancer, we evaluated whether variation in mitotic kinases were also associated with risk of all histologic subtypes of invasive ovarian cancer. Specifically, we tested all 397 SNPs using a larger group of cases (n = 671), comprised of 407 serous (60.8%), 28 mucinous (4.2%), 115 endometroid (17.2%), 50 clear cell (7.5%), and 69 other (10.3%) epithelial ovarian cancers. Twenty-three SNPs were suggestive of association with invasive ovarian cancer in 15 different genes (Table 1, Supplementary Table S4). SNPs in only 6 of these genes were also possible candidates in the serous-only analysis: CDK6, SIK3, NEK4, NLK, STK4, and TEX14.

We next evaluated the 36 SNPs identified as candidates in the discovery phase (unadjusted P < 0.05 in serous or all subtypes) in a replication study of 1,094 invasive ovarian cancer cases and 829 controls (Supplementary Table S3) using data from a published ovarian cancer GWAS. After adjustment for multiple testing none of these 36 SNPs were statistically significantly associated (P < 1.41 × 10⁻³)

### Table 1. Discovery single SNP associations with risk of invasive ovarian cancer in a log-additive model (P < 0.01)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>MAF</th>
<th>SNP</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Serous</td>
<td></td>
<td></td>
<td>All subtypes</td>
<td></td>
</tr>
<tr>
<td>CDK6</td>
<td>7</td>
<td>0.07</td>
<td>rs2282990</td>
<td>1.71 (1.25–2.34)</td>
<td>8.2 × 10⁻⁴</td>
<td>1.61 (1.22–2.12)</td>
<td>6.7 × 10⁻⁴</td>
</tr>
<tr>
<td>NLK</td>
<td>17</td>
<td>0.19</td>
<td>rs2125846</td>
<td>1.35 (1.09–1.66)</td>
<td>6.0 × 10⁻³</td>
<td>1.28 (1.07–1.54)</td>
<td>7.2 × 10⁻³</td>
</tr>
<tr>
<td>PLK1</td>
<td>5</td>
<td>0.11</td>
<td>rs12513877</td>
<td>1.16 (0.88–1.53)</td>
<td>0.285</td>
<td>1.34 (1.08–1.66)</td>
<td>8.6 × 10⁻³</td>
</tr>
<tr>
<td>SIK3</td>
<td>11</td>
<td>0.22</td>
<td>rs7928320</td>
<td>0.76 (0.61–0.93)</td>
<td>8.4 × 10⁻³</td>
<td>0.80 (0.67–0.95)</td>
<td>0.0117</td>
</tr>
<tr>
<td>TEX14</td>
<td>17</td>
<td>0.06</td>
<td>rs12944693</td>
<td>1.49 (1.07–2.08)</td>
<td>0.020</td>
<td>1.52 (1.14–2.04)</td>
<td>4.3 × 10⁻³</td>
</tr>
</tbody>
</table>

NOTE: Results based on 671 cases (407 serous) and 939 controls. Adjusted for age, geographic location, BMI, oral contraceptive (OC) use, HRT, and parity/age at first birth. MAF among controls.

*Based on a test for trend.
Table 2. *NLK* SNP associations in discovery, replication, and combined analyses

<table>
<thead>
<tr>
<th>SNP</th>
<th>Serous Cases</th>
<th>Control Cases</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2125846</td>
<td>Discovery</td>
<td>407</td>
<td>1.35 (1.10–1.66)</td>
<td>3.87 × 10⁻³</td>
<td>671</td>
<td>1.29 (1.08–1.54)</td>
</tr>
<tr>
<td></td>
<td>Replication</td>
<td>679</td>
<td>1.35 (1.11–1.67)</td>
<td>1.77 × 10⁻³</td>
<td>1,094</td>
<td>1.29 (1.07–1.55)</td>
</tr>
<tr>
<td></td>
<td>Combined</td>
<td>1,086</td>
<td>1.33 (1.15–1.54)</td>
<td>NA</td>
<td>1,765</td>
<td>1.27 (1.12–1.45)</td>
</tr>
</tbody>
</table>

NOTE: Discovery and replication analyses adjusted for age group and study site. Combined analyses adjusted for age group, study site, and replication phase. P values based on test for trend; P values were not estimated for combined analyses due to inability to correctly account for multiple testing across phases.

Discussion

In an analysis of genes encoding kinases required for normal cell division, we have identified a SNP, rs2125846, in the Nemo-like kinase (*NLK*) locus that is associated with risk of ovarian cancer. This SNP showed a very similar influence on risk of ovarian cancer in the discovery and replication studies (OR = 1.35) and was also associated with risk of the serous subtype (combined OR = 1.33) and all subtypes (combined OR = 1.27) of ovarian cancer.
Mitotic Kinases and Ovarian Cancer Risk

It is important to interpret this association with caution, as the rs2125846 association did not retain significance after adjustment for multiple testing in the replication phase. However, we have identified a biologically interesting candidate ovarian cancer SNP that warrants replication in larger studies of ovarian cancer.

NLK is a mitogen-activated protein kinase-like kinase belonging to the serine/threonine kinase superfamily. Studies in C. elegans have shown that NLK is involved in the cancer-related Wnt/beta-catenin signalling pathway (23, 24). Furthermore, NLK has been shown to inhibit several transcription factors such as NF-kB, Smads, API, and p53 (24, 25). Several functional studies have found a relationship between NLK expression and various cancer types. A prostate cancer study showed in cell lines that NLK expression is decreased in metastases compared with normal prostate epithelium and that overexpression of NLK induces apoptosis, particularly among androgen receptor–expressing cells (24). Similarly, overexpression of NLK was shown to induce apoptosis in colon cancer cell lines (26). Finally, NLK is upregulated in hepatocellular carcinomas, and disruption of NLK inhibits hepatocellular carcinoma cell growth (27). However, there are currently no functional data for the intrinsic NLK SNPs identified in this study. In addition, no associations for NLK variants have been identified in candidate gene studies or GWAS of cancer.

Overall, we report on the evaluation of the contribution of inherited variation in mitotic kinases to ovarian cancer risk. These results warrant further investigation in independent studies of ovarian cancer to understand the biological relationship between NLK and ovarian cancer risk.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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References


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