Kathryn L. Terry1,2*, Shelley S. Tworoger2,3, Margaret A. Gates2,3, Daniel W. Cramer1,2 and Susan E. Hankinson2,3

1Obstetrics and Gynecology Epidemiology Center, Department of Obstetrics and Gynecology, Brigham and Women’s Hospital, 221 Longwood Avenue, Boston, MA 02115, USA, 2Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, USA and 3Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, MA 02115, USA

*To whom correspondence should be addressed. Tel: +617 732 4895; Fax: +617 732 4899; Email: kerryt@partners.org

Insulin-like growth factor (IGF) 1 and its binding proteins foster cellular proliferation and inhibit apoptosis. In vitro studies show that IGF1 increases ovarian cell growth and invasive potential, suggesting a role for the IGF1 pathway in ovarian cancer etiology. We evaluated genetic variation in the IGF1, IGFBP1 and IGFBP3 genes in relation to ovarian cancer risk by genotyping 29 haplotype-tagging single nucleotide polymorphisms in 1173 cases and 1201 controls from the New England Case-Control (NECC) study and 296 cases and 854 controls from the Nurses’ Health Study (NHS). The association of haplotypes and single nucleotide polymorphisms (SNPs) with ovarian cancer was estimated using unconditional (NECC) and conditional (NHS) logistic regression. Additionally, we evaluated the association of SNPs with IGF1, IGF-binding protein (IGFBP) 3 and IGFBP2 plasma levels (n = 380 NHS controls). Our data suggest a decreased risk for women carrying haplotype 2C of the IGF1 gene [odds ratios (ORs) = 0.82, 95% confidence intervals (CIs) = 0.69–0.98] and an increased risk for women carrying haplotype 1D (OR = 1.41, 95% CI = 1.03–1.94) or 2D (OR = 1.20, 95% CI = 1.01–1.41) in the binding proteins. When evaluated individually, three SNPs in the IGFBPs (rs10228265, rs4985815 and rs2270628) were associated with increased ovarian cancer risk, and several IGF1 (rs11111285, rs1996656 and rs1019731) and IGFBP3 (rs2270628, rs2854746 and rs2854744) SNPs were significantly associated with IGF1, IGFBP3 and IGFBP2 plasma levels. Some haplotypes and SNPs in the IGF pathway genes may be associated with ovarian cancer risk; however, these results need to be confirmed. Of particular interest was the IGFBP3 SNP rs2270628, which was associated with both increased IGF1 plasma levels and higher ovarian cancer risk.

Introduction

Insulin-like growth factor (IGF) 1 and its binding proteins foster cellular proliferation and inhibit apoptosis. Biologic evidence from in vitro studies shows that IGF1 increases ovarian cell growth and invasive potential, suggesting a role for the IGF1 pathway in ovarian cancer etiology (1–4). Epidemiologic data regarding plasma IGF1 levels and ovarian cancer risk are conflicting. Two prospective studies observed that plasma IGF1 levels are associated with an increased risk of ovarian cancer among younger women (5,6), whereas data from the Nurses’ Health Study showed no clear association of IGFBP3 with ovarian cancer risk; however, these results need to be confirmed. Of particular interest was the IGFBP3 SNP rs2270628, which was associated with both increased IGF1 plasma levels and higher ovarian cancer risk.

Abbreviations: CIs, confidence intervals; FPPR, false-positive report probability; htSNPs, haplotype-tagging single nucleotide polymorphisms; HWE, Hardy–Weinberg Equilibrium; IGF, insulin-like growth factor; IGFBP, IGF-binding protein; NECC, New England Case–Control; NHS, Nurses’ Health Study; ORs, odds ratios; SNPs, single nucleotide polymorphisms.

Given that genetic variation in the IGF1, IGFBP1 and IGFBP3 genes are likely to influence plasma levels of IGF1 (9) and its binding proteins and that genetic variation may be a better measure of exposure over a lifetime than a single plasma measurement, we examined whether genetic variation in IGF1, IGFBP1 and IGFBP3 was associated with ovarian cancer risk in two large population-based studies [New England Case–Control (NECC) study and NHS].

Materials and methods

Study population

New England Case–Control study. Data and specimens from this NECC study of ovarian cancer come from two enrollment phases (phase 1: 1992–1997 and phase 2: 1998–2002) corresponding to two funding periods. Details regarding case and control enrollment are described elsewhere (10). Briefly, 2347 women residing in eastern Massachusetts or New Hampshire with a diagnosis of incident ovarian cancer were identified through hospital tumor boards and statewide cancer registries. Of these women, 1845 were eligible and 1306 (71% of the eligible cases, 1231 epithelial cases) agreed to participate. Controls were identified through a combination of random digit dialing, drivers’ license lists and town resident lists. In the first phase, 421 (72%) of the eligible women identified through random digit dialing agreed to participate and 102 (51%) of the eligible women identified through town resident lists agreed to participate. In the second phase, 1843 potential controls were identified, 1267 were eligible, 546 declined to participate by phone or by mail via an ‘opt-out’ postcard and 721 (57%) were enrolled. Controls were frequency matched to cases on age and state of residence.

All study participants were interviewed at the time of enrollment about known and suspected ovarian cancer risk factors. To avoid the possible impact of preclinical disease on exposure status, cases were asked about exposures that occurred at least 1 year before diagnosis and controls were asked about exposures that occurred >1 year before the interview date. More than 95% of the participants provided a blood specimen that was separated into plasma, red blood cell and buffy coat components and stored in −80°C freezers.

Nurses’ Health Study. The NHS cohort was established in 1976 among 121 700 US female registered nurses aged 30–55 years. Women completed an initial questionnaire and have been followed biennially by questionnaire to update exposure status and disease diagnoses (11). In 1989–1990, 32 826 participants submitted a blood sample; details of the collection are described elsewhere (11). All samples have been stored in liquid nitrogen freezers since collection. Follow-up of the NHS blood study cohort was 96.1% in 2006. In 2001–2004, 33 040 additional women provided a buccal cell specimen using a mouthwash protocol; follow-up was 99% through 2006. We extracted DNA from each buccal cell specimen within 1 week of receipt using Qiagen DNA Extraction Kit (Qiagen, Valencia, CA) and stored the DNA at −80°C. These studies were approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women’s Hospital.

NHS nested case–control study. We collected information on new diagnoses of ovarian cancer and confirmed each diagnosis using methods described previously (12). For this analysis, we included all cases with a DNA specimen submitted prior to diagnosis (incident cases) plus cases who submitted a DNA specimen within 4 years after diagnosis (prevalent cases). The incident and prevalent cases were similar with respect to stage, histology and survival time (13). All cases were diagnosed prior to 1 June 2004 and had no history of a prior cancer, other than non-melanoma skin cancer.

We randomly selected three controls per case from the study participants with DNA available, no prior bilateral oophorectomy and no history of cancer, other than non-melanoma skin cancer, at the time of case diagnosis. We excluded 27 controls from the analysis due to unavailability of genotyping data (n = 25) or because the participant was later diagnosed with ovarian cancer and was included in the analysis as a case (n = 2). Cases and controls were matched on age, menopausal status at baseline and diagnosis, month of blood collection, time of day of blood draw, fasting status and postmenopausal hormone use at blood draw.

Genotyping methods

We selected IGF1, IGFBP1 and IGFBP2 haplotype-tagging single nucleotide polymorphisms (htSNPs) identified by the Breast and Prostate Cancer Cohort Consortium (http://ccnt.hsc.usc.edu/MECGenetics/) (9,12,14–16). Their method of htSNP selection has been described elsewhere (17). Briefly, single
nucleotide polymorphisms (SNPs) were identified through previous report, public databases or resequencing in a multietnic sample of advanced prostate cancer. Database 27 contained SNPs identified for IGF1 and 56 SNPs identified for the 35 kb region that includes both IGFBP1 and IGFBP3. Here, we present results adjusted for the matching factors only. Analyses were performed separately for each study (NECC and NHS), estimates were tested for heterogeneity using the Q statistic and the two result-

...markers. In Caucasians, 14 htSNPs were measured in 477 NHS controls (19). Of the controls with plasma measure-

Plasma measurements
As part of a previous study, plasma IGFBP1, IGFBP3 and IGFBP2 levels were measured in 477 NHS controls (19). Of the controls with plasma measure-

Statistical analysis
We used chi square tests to check HWE; all SNPs included in this analysis had a HWE P value ≥0.05. Among controls, we observed significant differences in genotype frequencies by self-reported ethnicity for some SNPs. Since we lack adequate power to evaluate the association between IGF1, IGFBP1 and IGFBP3 SNPs as a part of this study. Details of the laboratory methods have been described previously (19). Briefly, total IGF1, IGFBP3 and IGFBP2 levels were measured by enzyme-linked immunosorbent assay after acid extraction with reagents from Diagnostic Systems Laboratory (Webster, TX). The intra-assay coefficients of variation from blinded quality control samples ranged from 2 to 10%.

Haplotype results
The four haplotype blocks in IGF1 and three haplotype blocks spanning IGFBP1 and IGFBP3 are described in Table I. Overall, we observed no global associations between common haplotype patterns in the four IGF1 haplotype blocks and ovarian cancer risk in either study (data not shown). However, one haplotype in block 2 (2C) was individually associated with decreased ovarian cancer risk in the NECC study and pooled analysis (OR = 0.82, 95% CI = 0.69–0.98) and haplotype 4C was associated with a non-significant decrease in risk of similar magnitude (Table II). For the binding proteins, we observed a global association between common haplotype patterns in the first (P = 0.02) and second (P = 0.005) blocks and ovarian cancer risk in the NHS study. In the pooled analysis, haplotypes 1D (OR = 1.41, 95% CI = 1.03–1.94) and 2D (OR = 1.20, 95% CI = 1.01–1.41) increased risk of ovarian cancer (Table III). We observed no significant heterogeneity in haplotype–ovarian cancer associations between the NECC and NHS studies.

SNP results
In the pooled analysis, we observed no significant associations between IGF1 htSNPs and ovarian cancer risk (Table IV).
SNPs in the IGFBP genes were significantly associated with ovarian cancer risk. In the first haplotype block of the binding proteins, rs10228265 was associated with a small but significant increased risk of ovarian cancer in the NECC study, which remained significant in the pooled analysis (OR = 1.16, 95% CI = 1.03–1.29). In the NHS study, rs35539615 was associated with a decreased risk and rs1065780 was associated with an increased risk but these associations were attenuated in the pooled analysis. The first SNP of the second haplotype block of the binding protein (rs4988515) was associated with an increased risk in both studies and was significantly associated with risk in the pooled analysis (OR = 1.42, 95% CI = 1.00–2.02). rs4619 was significantly associated with an increased risk in the NHS study but not overall. The only IGFBP3 SNP in the second haplotype block was significantly associated with risk in both studies (pooled OR = 1.22, 95% CI = 1.07–1.39). Although there were no significant associations in the final haplotype block, two SNPs (rs2854746 and rs2854744) were associated with a decreased risk of ovarian cancer that nearly reached significance. When we evaluated heterozygous and homozygous variant genotypes separately in relation to ovarian cancer risk, estimates were similar to the log-additive ORs, although less stable, particularly for SNPs with low minor allele frequency (supplementary Table II is available at Carcinogenesis Online). There was no significant heterogeneity between log-additive SNP association estimates from the two studies. Associations between IGF and SNP and ovarian cancer risk did not differ by age or menopausal status (data not shown).

We evaluated all significant SNP and haplotype associations for the IGF1, IGFBP1 and IGFBP3 genes using the FPRP method. Assuming a low prior probability of an association and an FPRP level of 0.2,
Genetic variation in IGF genes and ovarian cancer

Table IV. Association between SNPs in the IGF1, IGFBP1 and IGFBP3 genes and ovarian cancer risk in the NECC study and NHSa

<table>
<thead>
<tr>
<th>Gene SNP</th>
<th>rs number</th>
<th>NECC</th>
<th>NHS</th>
<th>Pooled</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)b</td>
<td>OR (95% CI)b</td>
<td>OR (95% CI)b</td>
<td></td>
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<tr>
<td>IGFBP1/IGFBP3</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>igfbp1_1</td>
<td>rs10228265</td>
<td>1.16</td>
<td>1.03–1.32</td>
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<tr>
<td>igfbp1_2</td>
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<td>0.78–1.05</td>
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</tr>
<tr>
<td>igfbp1_4</td>
<td>rs2201638</td>
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<td>0.83–1.55</td>
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<tr>
<td>igfbp1_3</td>
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<td>1.10</td>
<td>0.95–1.20</td>
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<td>igfbp3_1</td>
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<tr>
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<tr>
<td>igfbp3_7</td>
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<tr>
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<td>1.00</td>
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<td>Additional SNPs</td>
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<td>igfbp3_5</td>
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<td>igfbp3_10</td>
<td>rs2132570</td>
<td>1.10</td>
<td>0.88–1.92</td>
<td>1.23</td>
</tr>
</tbody>
</table>

Restrictd to Caucasians.

aAdjusted for the matching factors, represents risk for a 1 unit increase in minor allele number.

Histology-specific results

In pooled analyses, we observed some significant associations between htSNPs and specific ovarian cancer histologies. The strongest associations were with serous invasive and mucinous ovarian cancers. One IGFBP3 SNP (rs2270628) was associated with an increased risk of serous invasive ovarian cancer (OR = 1.40, 95% CI = 1.04–1.87). The IGF1 SNP rs35766 was associated with a reduced risk of mucinous and clear cell ovarian cancers (OR = 0.63, 95% CI = 0.44–0.92 and OR = 0.68, 95% CI = 0.47–0.99, respectively). For clear cell ovarian cancer, the IGFBP1 SNP rs35539615 was associated with a decreased risk (OR = 0.68, 95% CI = 0.48–0.95). Although we observed differences in the association between IGF1, IGFBP1 and IGFBP3 SNPs and ovarian cancer by histologic category, sample sizes were small and estimates are probably unstable, particularly for less common subtypes.

Plasma results

In a subset of NHS controls, we observed that two htSNPs in IGF1 (rs11111285 and rs19966656) were associated with plasma IGF1 levels (P = 0.01 and 0.03, respectively) and two IGF1 htSNPs (rs11111285 and rs1019731) were associated with plasma IGFBP2 levels (P = 0.008 and 0.07, respectively). In IGFBP3, we observed an association between one htSNP (rs2270628) and plasma IGFB1 levels (P = 0.04) and two htSNPs (rs2854746 and rs2854744) with plasma IGFBP3 levels (P = 0.0003 and 0.0098, respectively) (supplementary Table III is available at Carcinogenesis Online).

Interestingly, the IGFBP3 SNP (rs2270628) associated with IGF1 plasma levels was also associated with an increased risk of ovarian cancer in the NHS population (OR = 1.36) as well as in the NECC study (OR = 1.18). In addition, two IGFBP3 SNPs (rs285744 and rs2854746) were associated with an increased IGFBP3 plasma levels and a nearly significant decreased risk of ovarian cancer in the pooled analysis. We observed no significant associations between haplotypes associated with ovarian cancer risk (IGF1 haplotype 1C and IGFBP1/IGFBP3 haplotypes 1D and 2D, data not shown).

In exploratory analyses, we evaluated the interaction between genotype and plasma levels for the nine SNPs that were significantly associated with either ovarian cancer or plasma levels in NHS. For most SNPs, we observed no interaction with IGF1, IGFBP1 or IGFBP2 plasma levels (P > 0.05) (supplementary Table IV is available at Carcinogenesis Online). However, we observed significant interactions for IGF1 SNP rs1996656 (Pinteraction = 0.02), IGFBP1 SNP rs10228265 (P = 0.03) and IGFBP1 SNP rs4988515 (Pinteraction = 0.002) with IGFBP2 plasma levels. For women who were wild-type for IGF1 rs1996656, IGFBP2 plasma levels had a non-significant inverse association with ovarian cancer risk, whereas women who carried the variant for IGF1 rs1996656 had a non-significant positive association between IGFBP2 plasma levels and ovarian cancer risk. For women wild-type for IGFBP1 SNPs rs10228265 or rs4988515, IGFBP2 plasma levels were positively associated with ovarian cancer risk, whereas among women who carried the variant for IGFBP1 rs1996656 had a non-significant positive association between IGFBP2 plasma levels and ovarian cancer risk.

Discussion

This is the first study to comprehensively evaluate genetic variation across the IGF1, IGFBP1 and IGFBP3 genes and ovarian cancer risk. We observed suggestive associations between haplotypes in IGF1 block 2 (2C) as well as in IGFBP1/IGFBP3 blocks 1 (1D) and 2 (2D) and ovarian cancer risk. In addition, one IGFBP3 SNP (rs2270628) that was associated with ovarian cancer risk was also associated with IGF1 levels.

Two previous studies have evaluated SNPs in the IGF1 and IGFBP3 genes in relation to breast cancer risk and circulating IGF1 and IGFBP3 levels (9,25). We genotyped the same SNPs as Al-Zahrani et al. (25) who selected their SNPs based on resequencing data from the National Institute of Environmental Health Sciences Environmental Genome Project (http://egp.gs.washington.edu/). In both these studies as well as of our own, rs2854746 was associated with significantly increased IGFBP3 plasma levels. Al-Zahrani et al. observed an inverse association between this SNP and breast cancer risk but this was not replicated by the BPC3 study. We observed no association between this SNP and ovarian cancer risk in our data. Furthermore, the BPC3 study reported an association between IGFBP3 SNP rs2854746 and IGFBP3 levels, which we also observed in our data. In IGF1, BPC3 investigators observed a significant increase in IGF1 plasma levels with the IGF1 SNP variant for rs796539. We were unable to successfully genotype this SNP in our own study but observed a strong association between rs11111285, the tagging SNP we selected to replace rs796539 (P = 0.0003 and 0.0098, respectively) (supplementary Table III is available at Carcinogenesis Online).
(rs1996656) and IGFBP1 (rs10228265 and rs1553009) genes modified the association between IGFBP2 levels (but not IGF1 or IGFBP3 levels) and ovarian cancer risk. However, interactions between these SNPs and IGFBP2 levels should be interpreted with caution due to the small sample size in the plasma analyses.

Our analyses include data from both retrospective (NECC) and prospective (NHS) studies. NECC cases were identified and enrolled in the study in <10 months on average. If some of the SNPs we have studied are associated with better ovarian cancer survival, then they may be overrepresented in our case population, leading to a spurious association with ovarian cancer or attenuating a true protective association. Compared with NHS cases, variant alleles were more common in the NECC cases for two IGF1 SNPs (rs5742665 and rs2946834) and one IGFBP1 SNP (rs10228265). However, none of the SNPs we genotyped were significantly associated with ovarian cancer survival in our data (data not shown). Given the large number of statistically tested performed, the associations we observed may be due to chance, particularly those of borderline significance. As noted earlier, none of our significant SNP or haplotype associations with ovarian cancer met the noteworthiness criteria, suggesting that these observations need to be validated in another population.

Plasma analyses were limited by the collection of one sample that may not reflect exposure over a long period. However, in a reproducibility study, the intraclass correlation over 1–3 years was >0.80 for IGF1 and IGFBP2 and 0.6–0.85 for IGFBP3 (26), suggesting that one sample is fairly representative over several years.

Strengths of this analysis include its large sample size (1326 cases and 2002 controls), allowing additional analyses by histologic category, and prospective collection of blood samples in NHS. In addition, we have taken a comprehensive approach to evaluate genetic variation in the IGF1, IGFBP1 and IGFBP3 genes using hSNPs identified by the Breast and Prostate Cancer Cohort Consortium.

In short, this is the first assessment of genetic variation in the IGF1, IGFBP1 and IGFBP3 genes and ovarian cancer risk. We observed that some haplotypes and SNPs in the IGF pathway may influence ovarian risk, including the IGFBP3 SNP (rs2270628), which is associated with increased IGF1 plasma levels. These results warrant further research on the potential role of the IGF pathway on ovarian cancer risk.

Supplementary material

Supplementary Tables I–IV can be found at http://carcin.oxfordjournals.org/

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References


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