Genetic risk factors for ovarian cancer and their role for endometriosis risk

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HIGHLIGHTS
- A common genetic variant in HNF1B (rs11651755) was associated with endometriosis risk.
- rs11651755 has been previously described as risk factor for clear cell ovarian cancer.
- Endometriosis and clear cell ovarian cancer might share a common genetic etiology.

ABSTRACT

Objective. Several genetic variants have been validated as risk factors for ovarian cancer. Endometriosis has also been described as a risk factor for ovarian cancer. Identifying genetic risk factors that are common to the two diseases might help improve our understanding of the molecular pathogenesis potentially linking the two conditions.

Methods. In a hospital-based case–control analysis, 12 single nucleotide polymorphisms (SNPs), validated by the Ovarian Cancer Association Consortium (OCAC) and the Collaborative Oncological Gene-environment Study (COGS) project, were genotyped using TaqMan® OpenArray™ analysis. The cases consisted of patients with endometriosis, and the controls were healthy individuals without endometriosis. A total of 385 cases and 484 controls were analyzed. Odds ratios and P values were obtained using simple logistic regression models, as well as from multiple logistic regression models with adjustment for clinical predictors.

Results. rs11651755 in HNF1B was found to be associated with endometriosis in this case–control study. The OR was 0.66 (95% CI, 0.51 to 0.84) and the P value after correction for multiple testing was 0.01. None of the other genotypes was associated with a risk for endometriosis.

Conclusions. As rs11651755 in HNF1B modified both the ovarian cancer risk and also the risk for endometriosis, HNF1B may be causally involved in the pathogenetic pathway leading from endometriosis to ovarian cancer.

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1. Introduction

Endometriosis affects approximately 10% of all women of reproductive age [1]. The pathogenesis of the condition is largely unknown. A familial risk has been reported, and this supports the view that the disease may have a genetic background [2]. Recent genome-wide association studies have identified several genetic variants as risk factors for endometriosis [3–5]. In clinical practice, endometriosis is usually a concern...
when patients present with pelvic pain syndromes and also subfertility [6]. However, increasing evidence has recently been emerging to suggest that endometriosis is a risk factor for ovarian cancer [7,8]. The genetic background of ovarian cancer has been quite extensively investigated, and several validated genetic risk factors have been described [9–15]. Endometriosis is also one of the clinical and epidemiological risk factors that has been included in a risk prediction model for ovarian cancer [16]. However, these considerations have not taken into account the possibility that some genetic risk factors may cause endometriosis first, which then later develops into an ovarian cancer.

Identifying overlapping genetic risk factors for endometriosis and ovarian cancer might be able to provide evidence of which molecular pathways are involved not only in the etiology, but also in the pathogenetic pathway leading from endometriosis to ovarian cancer. The aim of the present study was therefore to test, in an endometriosis case–control study, whether validated genetic risk variants for ovarian cancer are also predictive for endometriosis risk.

2. Material and methods

2.1. Study population

From 2002 to January 2014, endometriosis patients and healthy control individuals were recruited for a case–control study at Erlangen University Hospital. The cases included in the study consisted of patients with histologically or clinically confirmed current or former endometriosis. The corresponding control individuals were recruited using local newspaper advertisements inviting participation by self-reported healthy women who were attending for a regular annual gynecological examination, without history of endometriosis and with no previous abdominal surgery and no pelvic pain syndrome, like dysmenorrhea, lower abdominal pain in general, dyspareunia, dysuria and dyschezia. All of the participants provided written informed consent and the medical faculty’s ethics committee approved the study.

2.2. Data acquisition

A standardized questionnaire including modules on pregnancy history, previous use of hormonal contraceptives, medical history, family history, and lifestyle was filled out by the patients and healthy control individuals, and was completed in a structured interview with trained medical personnel if any questions had not been fully answered. This questionnaire included a dedicated set of questions with regard to endometriosis history (information about previous surgeries, therapies and symptoms). Additional information for patients was obtained from the patient charts, such as information about medical procedures, tumor histology, and concomitant medication. Although ethnicity was not assessed, the population was predominantly Caucasian with an estimated non-Caucasian fraction of clearly under 5%.

2.3. Selection of SNPs

Thirteen validated single nucleotide polymorphisms (SNPs) from case–control studies conducted by the Ovarian Cancer Association Consortium (OCAC) and the Collaborative Oncological Gene-environment Study (COGS) project were originally planned for inclusion, which were well known at the time when the study was being planned (Table 1).

The study included three HNF1B SNPs (rs7405776, rs757210 and rs11651755). This gene encodes a member of the homeodomain-containing superfamily of transcription factors. Expression of this gene is altered in some types of cancer. The SNP rs8170 localizes to C19orf62, also known as BABAM1, and appears to regulate the retention of BRCA1 at double-strand DNA breaks and maintain stability of this complex at sites of DNA damage. Also activated during tumor development is ANKLE1 with SNP rs2363956. TIPARP encodes a member of the poly(ADP-ribose) polymerase superfamily. rs2665390 at 3q25 is intrinsic to TIPARP and results in a transcript variant. BRCA1/2-deficient cells survive by using PARP1 as an alternative DNA repair mechanism. rs11782652 associated in the first intron of CHMP4C. CHMP4C is involved in the final steps of cell division, coordinating midbody resolution with the abscission checkpoint, and is frequently overexpressed in ovarian tumor tissues. The risk-associated SNP rs2072590 lies in HAGLR. The protein encoded by this gene may play a role in the regulation of cell adhesion processes. The minor allele of rs10088218 has been found to be associated with a decreased risk of ovarian cancer and is a noncoding RNA of LINC00824. MLTL10 encodes a transcription factor and has been identified as a partner gene involved in several chromosomal rearrangements. Multiple transcript variants encoding different isoforms have been found for this gene, such as SNP rs1243180. 17q21.31 contains rs9303542, which is located in the intron of SKAPI. SKAPI regulates mitotic progression and expression and increases with neoplastic development. rs10069690 in TERT influences reverse transcriptase activity. It plays a role in cellular senescence and deregulation and may be involved in oncogenesis.

2.4. DNA extraction and genotyping

Blood samples were collected in citrate-phosphate-dextrose-adeno nine (CPDA) tubes (Sarstedt AG, Numbrecht, Germany). Germline

<table>
<thead>
<tr>
<th>Table 1</th>
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<tbody>
<tr>
<td>SNP</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>rs2072590</td>
</tr>
<tr>
<td>rs2665390</td>
</tr>
<tr>
<td>rs10089690</td>
</tr>
<tr>
<td>rs11782652</td>
</tr>
<tr>
<td>rs100896218</td>
</tr>
<tr>
<td>rs3814113α</td>
</tr>
<tr>
<td>rs1243180</td>
</tr>
<tr>
<td>rs7405776</td>
</tr>
<tr>
<td>rs757210</td>
</tr>
<tr>
<td>rs11651755</td>
</tr>
<tr>
<td>rs9303542</td>
</tr>
<tr>
<td>rs8170</td>
</tr>
<tr>
<td>rs2363956</td>
</tr>
</tbody>
</table>

MAF, minor allele frequency.
* According to assembly GRCh38.p2.
α Failed genotyping.
* Genome-wide significance was only reached for clear cell ovarian cancer.

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DNA was extracted up to 2006 using a standard salting-out procedure as previously described [17], and since 2006 using an automated chemagic MSL I system (Perkin Elmer, Baesweiler, Germany). Genotyping was performed using TaqMan Open Array Genotyping Plates (as part of one 32-plex panel) in accordance with the manufacturer’s instructions.

2.5. Statistical methods

Primary study aim was to explore the associations between selected SNPs and endometriosis. For each SNP, a simple logistic regression model was fitted using case–control status as the outcome and the genotype of each SNP (ordinal; 0, 1, or 2 minor alleles) as predictor. The odds ratio (OR) per minor allele with confidence interval was calculated and the corresponding Wald test was performed. The P-values (one per SNP) were corrected for multiple testing using the Bonferroni method.

For each SNP, a multiple logistic regression model was fitted to investigate the effect of the SNP on endometriosis in addition to several well-known clinical predictors (age, body mass index, menarche, cycle length, bleeding time, number of pregnancies, number of births: each continuous and ordinal, respectively; use of oral contraceptives and smoking: both yes versus no). Odds ratios per minor allele and confidence intervals were calculated, and the P-values were corrected for multiple testing using the Bonferroni–Holm method. Patients for whom clinical predictor variables were missing were excluded.

All of the tests were two-sided, and a P value of <0.05 was regarded as statistically significant. Calculations were carried out using the R system for statistical computing (version 3.0.1; R Development Core Team, Vienna, Austria, 2013).

3. Results

3.1. Patient characteristics

A total of 1375 subjects (749 patients with endometriosis and 626 controls) were recruited; 390 individuals had to be excluded because sufficient germline DNA was not available. There seemed to be no differences between all recruited patients and the study population (Supplementary Tables 1 and 2). Genotyping was carried out in a total of 960 individuals overall. Clinical data were available for 899 of the 960 individuals overall. Clinical data were available for 899 of the 960 individuals overall. Clinical characteristics are listed in Table 2. Genotyping was carried out in a total of 960 individuals, and the percentage of missing values in each variable was complete genotyping. The percentage of missing values in each variable was below 5% except for cycle length (16.3%) and bleeding time (8.5%).

The participants’ average age was 35.9 years and their mean body mass index was 23.4 kg/m². There was a nominal difference in age between the cases and controls. While the cases had an average age of 37.7, the controls were on average 34.5 years old. Additional patient characteristics are listed in Table 2.

3.2. Genotyping results

Genotyping results are shown in Table 3. Lowest minor allele frequencies were seen for rs2665390 (TIPARP), rs11782652 (CHM4PC), rs10088218 (LINCO00824), and rs8170 (BABAM1), at 7.61%, 6.4%, 12.94%, and 17.34%, respectively. One SNP, rs3814113, was excluded because genotyping failed. All minor alleles were the same as in the ovarian cancer studies, and the odds ratios are thus comparable with regard to the direction of effect. All genotyped SNPs were within the Hardy–Weinberg equilibrium (Table 3).

3.3. Association with endometriosis case–control status

Odds ratios and P values for successfully genotyped SNPs are shown in Table 4. None of the SNPs showed statistical significance after multiple testing, with the exception of one in HNF1B – namely rs11651755, with an adjusted OR of 0.66 (95% CI, 0.51 to 0.84). Two further SNPs in HNF1B were genotyped (rs7405776 and rs757210). Although neither of these SNPs achieved statistical significance after correction for multiple testing, both SNPs together with rs11651755 were among the three SNPs with the lowest P values in the multiple logistic regression models.

4. Discussion

In this case–control study, validated ovarian cancer SNPs were tested in patients with endometriosis. The validated ovarian cancer risk SNP rs11651755 in HNF1B was identified as a susceptibility locus for endometriosis. This could be an indication that HNF1B plays a role in the pathogenesis and possibly in the progression of endometriosis to ovarian cancer.

Until now, the common molecular pathway for the pathogenesis of endometriosis and ovarian cancer has been poorly understood [18]. Recent molecular studies have sought to link the two conditions via pathways related to oxidative stress, inflammation, and estrogen exposure. As a result of repetitive hemorrhage, with an accumulation of heme and free iron in endometriotic lesions, reactive oxygen species are produced and might play a role in the development of ovarian carcinoma [19]. Activation of oncogenic KRAS and PI3K pathways and inactivation of tumor suppressor genes PTEN and ARID1A are suggested as major pathogenic mechanisms for endometriosis associated clear-cell and endometrioid ovarian cancer [20]. Similarly, cytokines and mediators are responsible for the microenvironment of endometriosis and endometriosis-associated ovarian carcinoma. The current finding might help develop current hypotheses in a different direction.

HNF1B has been discussed in connection with a variety of diseases and molecular processes. It has been described as a member of the homeodomain-containing superfamily of transcription factors [21]. Clinical phenotypes that have been reported, with either genetic changes or altered tissue expression, include renal malformations, early-onset diabetes, malformations of female and male genitalia, and other dysfunctions involving the pancreas, liver, and genitourinary system [22]. Genetic variants have been described as altering the risk for serous and clear cell ovarian cancer, endometrial cancer, and prostate cancer [23–26].
As endometriosis is associated with both a risk for endometrial cancer [27] and more clearly with a risk for clear cell ovarian cancer [7,8], molecular associations are of special interest in this context. Different SNPs within HNF1B have been shown to differentially influence HNF1B expression [25]. HNF1B appears to be overexpressed in clear cell tumors and silenced in serous ovarian cancers, underlining the different hypothesized pathogenesis of these two subtypes of ovarian cancer—in which serous ovarian cancer most likely originating from fallopian tube cells [28] and clear cell ovarian cancer originating from uterine cells and being linked with endometriosis [7]. Our results are in line with these findings. For both diseases the C-allele was associated with a lower risk for the disease (OR for endometriosis: 0.66 in our study and OR for clear cell ovarian cancer: 0.77 in OCAC findings). This supports a common genetic etiology.

In this context, it has been shown that HNF1B overexpression in immortalized endometriosis epithelial cells changed the morphology of the endometrial cells, with the formation of multinucleated cells [25], indicating that maintenance of HNF1B expression in endometriosis cells might play an essential role in the pathogenesis of endometriosis cells to clear cell ovarian cancer.

Ovarian endometriosis cysts have been shown to express HNF1B in 40% of cases. Histopathologically, these HNF1B overexpressing ovarian endometriosis cells have been described as displaying reactive atypia [29]. Additionally, clear cell ovarian cancer tumors are accompanied by ovarian endometriosis in more than half of the cases [30,31]. These data strengthen the hypothesis that endometriosis is part of the pathogenesis of clear cell ovarian cancer.

Although HNF1B has been discussed as a potential gene involved in the development of ovarian cancer from endometriosis, little is known about its role in the pathogenesis of endometriosis independently of an ovarian cancer history. The present study provides evidence that HNF1B SNP rs11651755 is not only involved in the pathogenesis of ovarian clear cell cancer, but is also an etiological factor for endometriosis itself. This would suggest the hypothesis that HNF1B is involved very early in the pathogenesis of clear cell ovarian cancer, even before the development of endometriosis.

There is also some additional information linking HNF1B to the pathogenesis of endometriosis. Osteopontin has been hypothesized as a direct target of HNF1B as a transcription factor, as osteopontin contains functional binding HNF1 sites in its promotor region [32]. The clinical relevance of this finding has been demonstrated in studies showing that endometriosis patients have higher plasma levels of osteopontin than patients without endometriosis [33,34]. The finding in the present study of a genetic variant that most likely maintains HNF1B expression in the pathogenesis of both endometriosis and clear cell ovarian cancer should encourage further research to explore the role of this molecular pathway in the early pathogenesis of this endometrium–endometriosis–ovarian cancer complex. Other genes (MERIT40, TIPARP, BNC2, TERT) have not shown any association with endometriosis. The involvement of these molecular pathways in the pathogenesis of both endometriosis and ovarian cancer thus appears less likely, and those ovarian cancers might be driven by a different molecular mechanism, independently of endometriosis. This is also reflected in the odds ratios that associate endometriosis with high-grade serous ovarian cancer (OR 1.13; 95% CI 1.02–1.22).

### Table 3
Genotyping results. Minor and major alleles and Hardy–Weinberg equilibrium calculations. MAF is measured in all subjects.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Alleles^a^</th>
<th>MAF (%)</th>
<th>Homozygous common^b^</th>
<th>Heterozygous^b^</th>
<th>Homozygous rare^b^</th>
<th>P-value HWE</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2072590</td>
<td>HAGLROS/HAGLR</td>
<td>C/A</td>
<td>31.9%</td>
<td>424 (45.6%)</td>
<td>418 (45.0%)</td>
<td>88 (9.5%)</td>
<td>0.68</td>
</tr>
<tr>
<td>rs2665390</td>
<td>TIPARP</td>
<td>T/C</td>
<td>7.6%</td>
<td>786 (84.9%)</td>
<td>139 (15.0%)</td>
<td>1 (0.1%)</td>
<td>0.92</td>
</tr>
<tr>
<td>rs10069690</td>
<td>TERT</td>
<td>C/T</td>
<td>25.6%</td>
<td>522 (56.6%)</td>
<td>328 (35.6%)</td>
<td>72 (7.8%)</td>
<td>0.74</td>
</tr>
<tr>
<td>rs11782652</td>
<td>CHMNP4C</td>
<td>A/G</td>
<td>6.4%</td>
<td>818 (87.2%)</td>
<td>120 (12.8%)</td>
<td>0 (0.0%)</td>
<td>0.94</td>
</tr>
<tr>
<td>rs10088218</td>
<td>LINCO00244</td>
<td>G/A</td>
<td>12.9%</td>
<td>691 (75.8%)</td>
<td>206 (22.6%)</td>
<td>15 (1.6%)</td>
<td>0.87</td>
</tr>
<tr>
<td>rs1243180</td>
<td>MLTI0</td>
<td>T/A</td>
<td>31.7%</td>
<td>453 (48.2%)</td>
<td>377 (40.2%)</td>
<td>109 (11.6%)</td>
<td>0.68</td>
</tr>
<tr>
<td>rs74005776</td>
<td>HNF1B</td>
<td>G/A</td>
<td>39.0%</td>
<td>316 (36.3%)</td>
<td>457 (49.6%)</td>
<td>133 (14.4%)</td>
<td>0.39</td>
</tr>
<tr>
<td>rs757210</td>
<td>HNF1B</td>
<td>C/T</td>
<td>40.7%</td>
<td>308 (34.9%)</td>
<td>432 (48.9%)</td>
<td>143 (16.2%)</td>
<td>0.59</td>
</tr>
<tr>
<td>rs11651755</td>
<td>HNF1B</td>
<td>T/C</td>
<td>48.6%</td>
<td>238 (25.7%)</td>
<td>477 (51.5%)</td>
<td>212 (22.9%)</td>
<td>0.51</td>
</tr>
<tr>
<td>rs9303542</td>
<td>SKAPI</td>
<td>A/G</td>
<td>26.2%</td>
<td>507 (54.2%)</td>
<td>367 (39.2%)</td>
<td>62 (6.6%)</td>
<td>0.74</td>
</tr>
<tr>
<td>rs8170</td>
<td>BABAM1</td>
<td>G/A</td>
<td>17.3%</td>
<td>636 (67.7%)</td>
<td>22 (2.3%)</td>
<td>22 (2.3%)</td>
<td>0.83</td>
</tr>
<tr>
<td>rs23693556</td>
<td>ANKLE1</td>
<td>C/T</td>
<td>48.4%</td>
<td>252 (27.2%)</td>
<td>449 (48.6%)</td>
<td>223 (24.1%)</td>
<td>0.52</td>
</tr>
</tbody>
</table>

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.

^a Minor/major allele.

^b Values are rounded and need not add up exactly to 100%.

### Table 4
SNPs with P-values for the odds ratios (ORs) obtained using the simple logistic regression model. Unadjusted and adjusted ORs with 95% confidence intervals (in brackets) and uncorrected P-values are shown.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Unadjusted analysis</th>
<th>Analysis adjusted for clinical predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (CI)^a</td>
<td>Uncorrected P value^b</td>
<td>Corrected P value^c</td>
</tr>
<tr>
<td>rs2072590</td>
<td>0.99 (0.80–1.22)</td>
<td>0.94</td>
<td>1.00</td>
</tr>
<tr>
<td>rs2665390</td>
<td>0.87 (0.60–1.07)</td>
<td>0.46</td>
<td>1.00</td>
</tr>
<tr>
<td>rs10069690</td>
<td>1.32 (1.06–1.63)</td>
<td>0.01</td>
<td>1.00</td>
</tr>
<tr>
<td>rs11782652</td>
<td>0.93 (0.62–1.39)</td>
<td>0.72</td>
<td>1.00</td>
</tr>
<tr>
<td>rs10088218</td>
<td>1.01 (0.75–1.34)</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td>rs1243180</td>
<td>1.02 (0.83–1.24)</td>
<td>0.87</td>
<td>1.00</td>
</tr>
<tr>
<td>rs74005776</td>
<td>0.96 (0.78–1.17)</td>
<td>0.65</td>
<td>1.00</td>
</tr>
<tr>
<td>rs757210</td>
<td>0.93 (0.76–1.13)</td>
<td>0.45</td>
<td>1.00</td>
</tr>
<tr>
<td>rs11651755</td>
<td>0.84 (0.69–1.02)</td>
<td>0.08</td>
<td>1.00</td>
</tr>
<tr>
<td>rs9303542</td>
<td>1.19 (0.95–1.48)</td>
<td>0.13</td>
<td>1.00</td>
</tr>
<tr>
<td>rs8170</td>
<td>0.80 (0.62–1.03)</td>
<td>0.09</td>
<td>1.00</td>
</tr>
<tr>
<td>rs23693556</td>
<td>0.97 (0.80–1.17)</td>
<td>0.75</td>
<td>1.00</td>
</tr>
</tbody>
</table>

^a OR calculated with simple logistic regression model, one SNP per model.

^b P-value, uncorrected for multiple testing.

^c P-value, corrected for multiple testing (Bonferroni-Holm).

^d OR calculated with multiple logistic regression model.


I. Bach, M.G. Matti, S. Cerri, M., Yani, Two members of an HNF1 homoeoprotein family are expressed in human liver, Nucleic Acids Res. 19 (1991) 3553–3559.


