No Association between FTO or HHEX and Endometrial Cancer Risk

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Introduction: Obesity and diabetes are known risk factors for endometrial cancer; thus, the genetic risk factors of these phenotypes might also be associated with endometrial cancer risk. To evaluate this hypothesis, we genotyped tag-single nucleotide polymorphisms (SNP) and candidate SNPs in FTO and HHEX in a primary set of 417 endometrial cancer cases and 406 population-based controls, and validated significant findings in a replication set of approximately 2,347 cases and 3,140 controls from three additional studies.

Methods: We genotyped 189 tagSNPs in FTO (including rs8050136) and five tagSNPs in HHEX (including rs1111875) in the primary set and one SNP each in FTO (rs12927155) and HHEX (rs1111875) in the validation set. Per allele odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the association between the genotypes of each SNPs (as an ordinal variable) and endometrial cancer risk using unconditional logistic regression models, controlling for age and site.

Results: In the primary study, the most significant finding in FTO was rs12927155 (OR, 1.56; 95% CI, 1.21-2.01; P = 5.8 × 10⁻⁵), and in HHEX, it was rs1111875 (OR, 0.80; 95% CI, 0.66-0.97; P = 0.026). In the validation studies, the pooled per allele OR, adjusted for age and study for FTO, was rs12927155 (OR, 0.94; 95% CI, 0.83-1.06; P = 0.29), whereas for HHEX, it was rs1111875 (OR, 1.00; 95% CI, 0.92-1.10; P = 0.96).

Conclusion: Our data indicate that common genetic variants in two genes previously related to obesity (FTO) and diabetes (HHEX) by genome-wide association scans were not associated with endometrial cancer risk.

Impact: Polymorphisms in FTO and HHEX are unlikely to have large effects on endometrial cancer risk but may have weaker effects.

Abstract

Introduction: Obesity and type II diabetes are major risk factors for endometrial cancer. Genetic variation that is associated with obesity and diabetes might provide clues to the molecular pathways mediating endometrial carcinogenesis. A genome-wide association study of body mass index (BMI) identified a 47 kb region on chromosome 16 encompassing the FTO gene intron 1-exon 2-intron 2 that is associated with BMI. To examine whether genetic variants associated with BMI (HHEX–) are associated with endometrial cancer risk, we genotyped tagSNPs and candidate SNPs in the FTO region on chromosome 10 marked by rs1111875 that is also associated with BMI. Another genome-wide association study of type II diabetes (HHEX) identified the HHEX gene region on chromosome 16 that is also associated with BMI. To examine whether genetic variants of FTO and HHEX are associated with endometrial cancer risk, we genotyped tagSNPs and candidate SNPs in the Polish Endometrial Case-Control Study (PECS) of 417 endometrial cancer cases and 406 population-based controls. Significant findings in this set were then selected for replication.

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validation in a replication set of approximately 2,347 cases and 3,140 controls from three additional studies.

**PECS Methods**

Genotyping of 189 tagSNPs in FTO (including rs8050136) and 5 tagSNPs in HHEX (including rs1111875) were done as part of an Illumina Infinium custom iSelect chip using a SNP selection strategy described previously (4). For FTO, four SNPs were excluded due to violations of quality control measures: concordance of 1% replicates, completion proportions, and departure from Hardy-Weinberg proportions ($P < 0.05$). An additional 14 SNPs were excluded due to minor allele frequencies (MAF) of <0.05 among controls. For HHEX, all five SNPs passed quality control filters. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the association between SNPs and endometrial cancer risk using unconditional logistic regression models, controlling for matching factors, age, and study site. We also conducted analyses of haplotypes, including the sequential haplotype scan (5) and the variable-sized sliding window–regularized regression association analysis (6) to localize a set of adjacent markers associated with risk. Due to the size of the FTO gene, the sequential haplotype scan was performed in three overlapping sections [section 1 (SNPs 1-65), rs8055834-rs17820875; section 2 (SNPs 60-115), rs10521303-rs8056199; and section 3 (SNPs 110-171), rs16952730-rs16953089].

**PECS Results**

Among PECS controls, carrying an increased number of copies of the minor A allele of rs8050136 in FTO was associated with increased mean BMI (Kruskal-Wallis $P = 0.015$) but not with the prevalence of diabetes ($\chi^2$, $P = 0.26$). rs1111875 in HHEX was not associated with BMI ($P = 0.16$) or diabetes ($P = 0.56$). In the PECS case-control analyses, the minor A allele of rs8050136 was not associated with endometrial cancer risk (per allele OR, 1.05; 95% CI, 0.86-1.28; $P = 0.64$). However, 20 of the remaining 171 FTO tagSNPs were significantly associated with endometrial cancer risk (per allele $P$ values ranged from 0.00068 to 0.027) and represented independent SNPs ($n = 4$) or clustered into three linkage disequilibrium blocks. Haplotype analyses identified strong signals (haplotype $P < 10^{-3}$) in two of these regions (Figs. 1 and 2). The first region resides in intron 4 and is marked by SNP rs8063241 (OR, 0.71; 95% CI, 0.56-0.88, $P = 1.7 \times 10^{-3}$; Fig. 1). The second region in intron 8 is marked by three correlated tagSNPs that also had the lowest $P$ values in the single locus analysis [rs2689264 (MAF = 0.17): OR, 1.57; 95% CI, 1.22-2.02; $P = 4.5 \times 10^{-3}$; rs12927155 (MAF = 0.17): OR, 1.56; 95% CI, 1.21-2.01; $P = 5.8 \times 10^{-4}$; rs2540776 (MAF = 0.17): OR, 1.54; 95% CI, 1.19-1.99; $P = 8.7 \times 10^{-4}$; Figure 1. FTO sequential haplotype scan analysis of tagSNPs rs8055834 to rs17820875, Polish Endometrial Cancer Study (417 cases and 406 controls).
Fig. 2. The candidate SNP in HHEX, rs1111875, was associated with a 20% lower risk of endometrial cancer for each minor T allele (per allele OR, 0.80; 95% CI, 0.66-0.97; \( P = 0.026 \)). No other HHEX loci were associated with risk.

### Replication Studies

In an attempt to replicate our findings for FTO SNP rs12927155 and HHEX SNP rs1111875, we approached three independent case-control studies of women of European ancestry (5,522 subjects; Table 1), including the Study of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) with 1,494 endometrial cancer cases and 1,600 community controls, the Australian National Endometrial Cancer Study (ANECS) with 1,048 endometrial cancer cases and 1,010 population-based controls (7), and the Leuven Endometrial Cancer Study (LES; ref. 8) with 206 endometrial cancer cases and 649 hospital-based controls. The

### Table 1. Age-adjusted ORs and 95% CIs for the association between candidate polymorphisms and endometrial cancer risk in four independent case-control studies

<table>
<thead>
<tr>
<th>Study</th>
<th>FTO rs12927155</th>
<th></th>
<th></th>
<th>HHEX rs1111875</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>Per allele</td>
<td>Cases</td>
<td>Controls</td>
<td>Per allele</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>N</td>
<td>MAF</td>
<td>OR (95% CI)</td>
<td>P</td>
<td>N</td>
</tr>
<tr>
<td>PECS</td>
<td>417</td>
<td>406</td>
<td>0.17</td>
<td>1.53 (1.19-1.97)</td>
<td>0.001</td>
<td>417</td>
</tr>
<tr>
<td>SEARCH</td>
<td>1,121</td>
<td>1,596</td>
<td>0.16</td>
<td>1.01 (0.85-1.21)</td>
<td>0.88</td>
<td>1,119</td>
</tr>
<tr>
<td>ANECS</td>
<td>1,022</td>
<td>896</td>
<td>0.15</td>
<td>0.89 (0.74-1.08)</td>
<td>0.24</td>
<td>992</td>
</tr>
<tr>
<td>LES</td>
<td>204</td>
<td>648</td>
<td>0.16</td>
<td>1.08 (0.70-1.68)</td>
<td>0.72</td>
<td>0</td>
</tr>
<tr>
<td>Pooled1</td>
<td>2,347</td>
<td>3,140</td>
<td>0.15</td>
<td>0.94 (0.83-1.06)</td>
<td>0.29</td>
<td>2,111</td>
</tr>
</tbody>
</table>

*rs1111875 was not genotyped in the LES.
1Pooled estimates were calculated for the three replication studies (SEARCH, ANECS, and LES); therefore, models were also adjusted for study. Numbers do not sum to the total samples available because of missing genotype data.
distribution of age and BMI were similar for the three studies [age range (median): SEARCH, 26-71 (56); ANECS, 26-80 (62); LES, 20-80 (48); and BMI range (median): SEARCH, not reported; ANECS, 15.1-67.3 (28.0); LES, 16.4-89.0 (24.9)] and with the PECS. We excluded controls with a history of hystectomy (including 249 for SEARCH, 95 for ANECS, and 1 for LES). The SEARCH samples were genotyped using TaqMan assays, ANECS and LES samples were genotyped using the Sequenom iPLEX platform.

Among these three studies, the pooled per allele ORs, adjusted for continuous age and study for FTO was rs1292755 (OR, 0.94; 95% CI, 0.83-1.06; \(P = 0.29\)) and for HHEX, it was rs1111875 (OR, 1.00; 95% CI, 0.92-1.10; \(P = 0.96\)); Table 1). Between-study heterogeneity was not evident among these studies \((P = 0.23\) and 0.74, respectively), and the CIs for both SNPs excluded the ORs from the PECS.

**Conclusion**

Our data indicate that common genetic variants in two genes previously related to obesity (FTO) and diabetes (HHEX) by genome-wide association scans were not associated with endometrial cancer risk.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

**Acknowledgments**

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