Cheng, TH; Thompson, DJ; O’Mara, TA; Painter, JN; Glubb, DM; Flach, S; Lewis, A; French, JD; Freeman-Mills, L; Church, D; +82 more... Gorman, M; Martin, L; National Study of Endometrial Cancer Genetics Group (NSECG); Hodgson, S; Webb, PM; Australian National Endometrial Cancer Study Group (ANECS); Attia, J; Hollliday, EG; McEvoy, M; Scott, RJ; Henders, AK; Martin, NG; Montgomery, GW; Nyholt, DR; Ahmed, S; Healey, CS; Shah, M; Dennis, J; Fasching, PA; Beckmann, MW; Hein, A; Ekici, AB; Hall, P; Czene, K; Darabi, H; Li, J; Dörk, T; Dürst, M; Hillemanns, P; Runnebaum, I; Amant, F; Schrauwen, S; Zhao, H; Lambrechts, D; Depreeuw, J; Dowdy, SC; Goode, EL; Fridley, BL; Winham, SJ; Njølstad, TS; Salvesen, HB; Trovik, J; Werner, HM; Ashton, K; Otton, G; Proietto, T; Liu, T; Mints, M; Tham, E; RENDOCAS; CHIBCHA Consortium; Li, MJ; Yip, SH; Wang, J; Bolla, MK; Michailidou, K; Wang, Q; Tyrer, JP; Dunlop, M; Houlston, R; Palles, C; Hopper, JL; AOCS Group; Peto, J; Swerdlow, AJ; Burwinkel, B; Brenner, H; Meindl, A; Brauch, H; Lindblom, A; Chang-Claude, J; Couch, FJ; Giles, GG; Kristensen, VN; Cox, A; Cunningham, JM; Pharoah, PD; Dunning, AM; Edwards, SL; Easton, DF; Tomlinson, I; Spurdle, AB; (2016) Five endometrial cancer risk loci identified through genome-wide association analysis. Nature genetics, 48 (6). pp. 667-74. ISSN 1061-4036 DOI: https://doi.org/10.1038/ng.3562

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Five endometrial cancer risk loci identified through genome-wide association analysis

We conducted a meta-analysis of three endometrial cancer genome-wide association studies (GWAS) and two follow-up studies totaling 7,737 endometrial cancer cases and 37,144 controls of European ancestry. Genome-wide imputation and meta-analysis identified five new risk loci of genome-wide significance at likely regulatory regions on chromosomes 13q22.1 (rs11841589, near KLF5), 6q22.31 (rs13328298, in LOC643623 and near HEY2 and NCOA7), 8q24.21 (rs4733613, telomeric to MYC), 15q15.1 (rs937213, in EIF2AK4, near BMF) and 14q22.33 (rs2498796, in AKT1, near SIVA1). We also found a second independent 8q24.21 signal (rs17232730). Functional studies of the 13q22.1 locus showed that rs9600103 (pairwise $r^2 = 0.98$ with rs11841589) is located in a region of active chromatin that interacts with the KLF5 promoter region. The rs9600103[T] allele that is protective in endometrial cancer suppressed gene expression in vitro, suggesting that regulation of the expression of KLF5, a gene linked to uterine development, is implicated in tumorigenesis. These findings provide enhanced insight into the genetic and biological basis of endometrial cancer.

Endometrial cancer is the fourth most common cancer in women in the United States and Europe and the most common cancer of the female reproductive system. The familial relative risk is $\sim 2$ (refs. 3,4), but highly penetrant germline mutations in mismatch-repair genes 5 account for only a small proportion of familial aggregation. Our previous GWAS and subsequent fine-mapping identified the only two reported genome-wide significant risk loci for endometrial cancer, tagged by rs11263763 in HNF1B (intron 1)$^8$ and rs727479 in CYP19A1 (intron 4)$^9$.

To identify additional endometrial cancer risk loci, we reanalyzed data from our previous GWAS (the Australian National Endometrial Cancer Study (ANECs) and Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH) data sets$^{10}$) and conducted a meta-analysis with two further studies (Supplementary Fig. 1). The first study was an independent GWAS, the National Study of Endometrial Cancer (NSECG), including 925 endometrial cancer cases and 751 controls using competitive allele-specific PCR (KASPar, KBiosciences) and the Fluidigm BioMark System (Supplementary Table 3). Duplicate samples displayed concordance of $>98.5\%$ between different genotyping platforms (Supplementary Table 4). All five SNPs were associated with endometrial cancer at genome-wide significance ($P < 5 \times 10^{-8}$; Figs. 2 and 3, and Table 1), and these associations remained highly significant when analysis was restricted to cases with the endometrioid subtype only. Endometrioid-only analysis did not identify any additional risk loci. Expression quantitative trait locus (eQTL) analysis (Online Methods) in normal uterine tissue$^{20}$ and endometrial cancer tumor and adjacent normal tissue$^{21}$ did not yield any SNPs robustly associated with the expression of nearby genes at the endometrial cancer risk loci (Supplementary Table 5). However, for each risk locus, bioinformatic analysis including cell-type-specific expression and histone modification data identified correlated SNPs within 500 kb of the lead SNP in likely regulatory regions (Supplementary Table 6). The most compelling candidates for future functional analysis are described below.

Gene-environment Study (COGS) initiative$^{14–17}$ (Supplementary Table 1 and Supplementary Note).

We first performed genome-wide imputation using 1000 Genomes Project data, allowing us to assess up to 8.6 million variants with allele frequency $\geq 1\%$ across the different studies. Per-allele odds ratios and $P$ values for all SNPs in the GWAS and iCOGS analyses were obtained using a logistic regression model. There was little evidence of systematic overdispersion of the test statistic ($\hat{\lambda}_{GC} = 1.002–1.038$; Supplementary Fig. 2). A fixed-effects meta-analysis was conducted for all 2.3 million typed and well-imputed (info score $> 0.90$) SNPs in a total of 6,542 endometrial cancer cases and 36,393 controls. The strongest associations were with SNPs in linkage disequilibrium (LD) with previously identified risk SNPs for endometrial cancer in HNF1B$^{8,10,18}$ and CYP19A1 (refs. 9,19) (Fig. 1 and Table 1). For fourteen 1.5-Mb regions containing at least one new SNP with $P_{meta} < 1 \times 10^{-7}$, we performed regional imputation using an additional reference panel that comprised high-coverage whole-genome sequencing data for 196 UK individuals (Supplementary Table 2).

We identified five new regions containing at least one endometrial cancer risk SNP with $P_{meta} < 1 \times 10^{-7}$ and genotyped the most strongly associated SNP in each region in an additional 1,195 NSECG endometrial cancer cases and 751 controls using competitive allele-specific PCR (KASPar, KBiosciences) and the Fluidigm BioMark System (Supplementary Table 3). Duplicate samples displayed concordance of $>98.5\%$ between different genotyping platforms (Supplementary Table 4). All five SNPs were associated with endometrial cancer at genome-wide significance ($P < 5 \times 10^{-8}$; Figs. 2 and 3, and Table 1), and these associations remained highly significant when analysis was restricted to cases with the endometrioid subtype only. Endometrioid-only analysis did not identify any additional risk loci. Expression quantitative trait locus (eQTL) analysis (Online Methods) in normal uterine tissue$^{20}$ and endometrial cancer tumor and adjacent normal tissue$^{21}$ did not yield any SNPs robustly associated with the expression of nearby genes at the endometrial cancer risk loci (Supplementary Table 5). However, for each risk locus, bioinformatic analysis including cell-type-specific expression and histone modification data identified correlated SNPs within 500 kb of the lead SNP in likely enhancers and multiple potential regulatory targets (Supplementary Fig. 3 and Supplementary Table 6). The most compelling candidates for future functional analysis are described below.

rs13328298 (odds ratio (OR) = 1.13, 95% confidence interval (CI) = 1.09–1.18; $P = 3.73 \times 10^{-10}$) at 6q22.31 lies in the long noncoding RNA LOC643623, 54 kb upstream of HEY2 and 86 kb upstream of NCOA7.

A full list of authors and affiliations appears at the end of the paper.

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HEY2 encodes a helix-loop-helix transcriptional repressor in the Notch pathway, which maintains stem cells, and dysregulation has been associated with different cancers.22

The NCOA7 protein modulates the activity of the estrogen receptor via direct binding.22

The second locus (rs4733613: OR = 0.89, 95% CI = 0.85–0.93; \( P = 3.09 \times 10^{-9} \)) is at 8q24.21. Stepwise conditional logistic regression identified another independent signal in this region, rs17232730 (pairwise \( r^2 = 0.02 \), \( P_{\text{cond}} = 1.29 \times 10^{-5} \); Table 2). Both SNPs associated with endometrial cancer lie further from MYC (784–846 kb telomeric) than most of the other cancer-associated SNPs in the region, including those for cancers of the bladder,24 25 breast,15 26 colorectum,12 27 and prostate.29 30 rs17232730 is in moderate LD with the rs10088218 SNP for ovarian cancer \( (r^2 = 0.43) \), with both cancers having the same risk allele, but rs4733613 is not in LD \( (r^2 \leq 0.02) \) with any other cancer-associated SNP in the region (Supplementary Fig. 3). A role in tumorigenesis is implicated for several microRNAs (miRNAs) in the region.31 Of these, mir-1207-5p is reported to repress proliferation35 and is activated in endometrial tumors, especially in endometrial tissue36. Several SNPs in LD with rs2498796 are bioinformatically linked with regulation of expression in the endometrium.34

Bioinformatic analysis suggested that the causal variant in the intergenic 13q22.1 locus may affect a regulatory element that modifies the expression of AKT1.36 The final newly identified risk SNP for endometrial cancer was rs1841589 (OR = 1.15, 95% CI = 1.11–1.21; \( P = 4.83 \times 10^{-11} \)) at 13q22.1, 163 kb and 445 kb downstream of the Krüppel-like factors KLF5 and KLF12, respectively. KLF5 is a transcription factor associated with cell cycle regulation, and it has a role in uterine development, homoeostasis and tumorigenesis. Elevated KLF5 levels are strongly correlated with activating KRAS mutations,38 and KLF5 is targeted for degradation by the tumor suppressor FBXW7. Both FBXW7 and KRAS are commonly mutated in endometrial cancer.39 rs1841589 was one of five highly correlated SNPs \( (r^2 \geq 0.98) \) surpassing genome-wide significance in a 3-kb LD block bounded by rs9600103 \( (P = 8.70 \times 10^{-11}) \) and rs1841589 (Fig. 4a). There was no residual association signal at this locus \( (P_{\text{cond}} > 0.05) \) after conditioning on rs1841589.

Bioinformatic analysis suggested that the causal variant in the intergenic 13q22.1 locus may affect a regulatory element that modifies KLF5 expression (Supplementary Fig. 3): rs9600103 overlaps a vertebrate conservation peak and a DNAse I hypersensitivity site (DHS) in estrogen- and tamoxifen-treated Ishikawa cells in the Encyclopedia of DNA Elements (ENCODE)30 (Fig. 4a). In addition, in a Hi-C chromatin capture experiment in HeLa S3 cells,31 a chromatin interaction loop was observed between a segment containing the KLF5 promoter and the rs1841589–rs9600103 locus \( (P = 0.004); \) 4 Supplementary Fig. 4).

We further investigated the epigenetic landscape of a 16-kb region around rs1841589 and rs9600103 that contained the SNPs most strongly associated with endometrial cancer, by analysis of three endometrial cancer cell lines: Ishikawa ( homozygous for the rs9600103[A] and rs1841589[G] high-risk alleles; providing a

Table 1 Risk loci associated with endometrial cancer at \( P < 5 \times 10^{-8} \) in the meta-analysis

<table>
<thead>
<tr>
<th>Locus</th>
<th>SNP</th>
<th>Position (bp)</th>
<th>Nearby gene(s)</th>
<th>EAFa</th>
<th>Allelic OR (95% CI)</th>
<th>( P )</th>
<th>Allelic OR (95% CI)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly identified loci</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>13q22.1</td>
<td>rs11841589</td>
<td>73,814,891</td>
<td>KLF5, KLF12</td>
<td>G</td>
<td>0.74</td>
<td>1.15(1.11–1.21)</td>
<td>4.83 \times 10^{-11}</td>
<td>0.19</td>
</tr>
<tr>
<td>6q22.31</td>
<td>rs13328298</td>
<td>126,016,580</td>
<td>HEY2, NCOA7</td>
<td>G</td>
<td>0.58</td>
<td>1.13(1.09–1.18)</td>
<td>3.73 \times 10^{-10}</td>
<td>0.00</td>
</tr>
<tr>
<td>8q24.21</td>
<td>rs4733613</td>
<td>129,599,278</td>
<td>MYC</td>
<td>G</td>
<td>0.87</td>
<td>0.84(0.80–0.89)</td>
<td>3.09 \times 10^{-9}</td>
<td>0.00</td>
</tr>
<tr>
<td>15q15.1</td>
<td>rs937213</td>
<td>40,322,124</td>
<td>E2F2AK4, BMF</td>
<td>C</td>
<td>0.58</td>
<td>0.90(0.86–0.93)</td>
<td>1.77 \times 10^{-8}</td>
<td>0.36</td>
</tr>
<tr>
<td>14q32.33</td>
<td>rs2498796</td>
<td>105,243,220</td>
<td>AKT1, SIVA1</td>
<td>G</td>
<td>0.70</td>
<td>0.89(0.85–0.93)</td>
<td>3.55 \times 10^{-8}</td>
<td>0.00</td>
</tr>
<tr>
<td>Previously reported loci</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>17q12</td>
<td>rs11263763</td>
<td>36,103,565</td>
<td>HNF1B</td>
<td>A</td>
<td>0.54</td>
<td>1.10(1.15–1.25)</td>
<td>2.78 \times 10^{-19}</td>
<td>0.37</td>
</tr>
<tr>
<td>15q21</td>
<td>rs244098</td>
<td>51,537,806</td>
<td>CYP19A1</td>
<td>C</td>
<td>0.62</td>
<td>1.17(1.13–1.23)</td>
<td>4.51 \times 10^{-13}</td>
<td>0.00</td>
</tr>
</tbody>
</table>

For all newly identified loci, the lead SNP was either directly genotyped or imputed with an information score of greater than 0.9. HNF1B and CYP19A1 have been previously reported by Painter et al. and Thompson et al.35

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of transcription factor binding52) and pan acetylation of histone H4 and chromatin immunoprecipitation (ChIP) using antibodies

Fig. 5 rs4733613). (a) 13q22 rs11841589 (13q22). (b) 8q24 rs4733613 (8q24). (c) 6q22 rs13328298 (6q22). (d) 14q32 rs2498796 (14q32).

Figure 2 Forest plots for new endometrial cancer risk loci. The odds ratio and 95% confidence interval (L, lower; U, upper) for the loci in each study of the meta-analysis are listed and shown in the adjacent plot. The $I^2$ heterogeneity scores (all <0.4) suggest that there is no marked difference in effects between studies. (a) rs11841589 (13q22). (b) rs13328298 (6q22). (c) rs4733613 (8q24). (d) rs17232730 (8q24; pairwise $I^2 = 0.02$ with rs4733613). (e) rs937213 (15q15). (f) rs2498796 (14q32).

comparison with the ENCODE data), ARK-2 (homozygous for the low-risk T alleles at both SNPs) and AN3CA (a non-KLF5-expressing line that is homozygous for the high-risk alleles) (Supplementary Fig. 5). We conducted formaldehyde-assisted identification of regulatory elements (FAIRE; to identify regions of open chromatin) and chromatin immunoprecipitation (ChIP) using antibodies against dimethylation of histone H3 a lysine 4 (H3K4me2; a marker of transcription factor binding52) and pan acetylation of histone H4 (pan H4ac; a marker of active chromatin). Although ChIP analysis with antibody against H4ac did not yield a consistent signal in the region, peaks in signals from FAIRE and ChIP with antibody against H3K4me2 were specifically present in the KLF5-expressing lines and were colocalized with the conservation peak and DHS from the ENCODE data at rs9600103, providing strong evidence for open chromatin conformation capture (3C) experiments for the KLF5 region and found an interaction between the NcoI restriction fragment containing the rs11841589–rs9600103 risk locus SNPs and the promoter region of KLF5 (Fig. 4b).
We investigated the regulatory nature of the region around rs11841589–rs9600103 using allele-specific luciferase reporter assays in Ishikawa cells (Fig. 4c). We used paired t-tests to compare the activities of fragments containing the rs11841589 and rs9600103 alleles with that of the pGL3-promoter reporter vector (no-insert) control (Supplementary Table 7). Fragments containing the rs9600103[T], rs11841589[T] and rs11841589[G] alleles had activity significantly lower than that of the pGL3-promoter control (P = 0.014). In contrast, the construct containing the rs9600103[A] risk allele had luciferase activity similar to that of the pGL3-promoter control (P = 0.23) and significantly higher than that of the construct with the corresponding rs9600103[T] protective allele (P = 0.02). These results suggest that the endometrial cancer risk tagged by rs11841589 is at least partly due to a regulatory element containing rs9600103 that interacts with the KLF5 promoter region, with the rs9600103[A] risk allele likely associated with increased gene expression.

In summary, this meta-analysis identified five new endometrial cancer risk loci at genome-wide significance, bringing the total number of common risk loci for endometrial cancer identified by GWAS to seven (Fig. 1). Together with other risk-associated SNPs reaching study-wide significance\(^{32,53,54}\), these loci explain ~5.1% of the familial relative risk for endometrial cancer. These new endometrial cancer risk SNPs lie in likely enhancers predicted to regulate gene expression for proteins or miRNAs with known or suspected roles in tumorigenesis, and we specifically showed that a functional SNP at 13q22.1 may lie within a transcriptional repressor of KLF5. Our findings further clarify the genetic etiology of endometrial cancer, provide

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**Table 2** Conditional analysis of the 8q24 locus showing two independent association signals

<table>
<thead>
<tr>
<th>Allelic OR (95% CI)</th>
<th>Pairwise ( r^2 ) with</th>
<th>All-histology meta-analysis</th>
<th>Conditioning on rs4733613</th>
<th>Conditioning on rs17232730</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs4733613</td>
<td>129,599,278</td>
<td>G</td>
<td>0.87</td>
<td>0.02</td>
</tr>
<tr>
<td>rs17232730</td>
<td>129,537,746</td>
<td>G</td>
<td>0.88</td>
<td>0.02</td>
</tr>
<tr>
<td>rs10088218*</td>
<td>129,543,949</td>
<td>G</td>
<td>0.87</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*Position with respect to Build 37 of the reference genome. \( \dagger \)Effect allele. \( \ddagger \)Other allele. \( \dagger \)Effect allele frequency. rs10088218 is associated with ovarian cancer (all subtypes), with the association being more significant for cancers of serous histology. rs10088218[G] is the risk allele for both endometrial cancer and ovarian cancer.
regions for functional follow-up and add key information for future risk stratification models.


METHODS

Methods and any associated references are available in the online version of the paper.

Accession codes. Data access was granted by the respective management groups of the participating studies, that is, the Australian National Endometrial Cancer Study (ANECS), the Queensland Institute of Medical Research Controls, the Hunter Community Study (HCS), the Studies of Epidemiology and Risk Factors in Cancer Heredity (SEARCH), the Wellcome Trust Case Control Study (HCS), the Studies of Epidemiology and Risk Factors in Ovarian Cancer (NOVA), the Breast Cancer Family Registry (BCFR), the Breast Cancer Association Consortium (BCAC) and the Ovarian Cancer Association Consortium (OCAC). Genotype data are not freely accessible but can be obtained by submitting an application to the respective management committees, institutions or data owners.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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We thank the many individuals who participated in this study and the numerous institutions and their staff that supported recruitment, detailed in full in the Supplementary Note.

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**Figure 4** The 13q22.1 endometrial cancer susceptibility locus. (a) Diagram showing the 16-kb region around rs11841589, rs9600103 and correlated SNPs rs7981863, rs7988505 and rs7989799 (vertical black bars). DHS density signal in estrogen- and tamoxifen-treated Ishikawa cells in ENCODE (Supplementary Note) and conservation in 100 vertebrates. FAIRE and ChIP assays for H3K4me2 and H4ac in endometrial cancer cell lines ARK-2 (rs9600103 genotype TT), Ishikawa (rs9600103 genotype AA) and AN3CA (rs9600103 genotype AA) show evidence for enrichment of histone modifications. The vertical dotted line represents the position of rs9600103. (b) 3C experiment for KLF5-expressing Ishikawa cells. Relative interaction frequencies are shown for an NcoI restriction fragment containing risk SNPs rs9600103 and rs11841589 (bait fragment) and NcoI fragments across the KLF5 promoter region, plotted against fragment position on chromosome 13. NcoI restriction sites (black vertical bars) are displayed below the schematic of the KLF5 transcripts. The profile for trimethylation of histone H3 at lysine 4 (H3K4me3), indicative of promoters, from multiple ENCODE cell lines is also shown. The graph represents three biological replicates. Error bars, s.d. An interaction was seen with the fragment containing a KLF5 transcriptional start site (fragment shaded in gray). (c) Luciferase reporter assays to analyze the activity of 3-kb fragments containing either rs9600103 or rs11841589 (KLF5 expression)

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**Region (bp)**: Chr. 13 (q22.1)

**Position (bp)**:

73,595,000 73,615,000 73,635,000 73,655,000

**FAIRE**

73,806,000 73,808,000 73,810,000 73,812,000 73,814,000 73,816,000 73,818,000 73,820,000

**KLF5**

73,595,000 73,615,000 73,635,000 73,655,000

**H3K4me2**

73,806,000 73,808,000 73,810,000 73,812,000 73,814,000 73,816,000 73,818,000 73,820,000

**H4ac**

73,595,000 73,615,000 73,635,000 73,655,000

---

**Table 7**

**Accession codes**

rs11841589[T]

rs11841589[G]

rs9600103[T]

rs9600103[A]

pGL3-Promoter
National Institutes of Health (R01 CA128978, U19 CA148537, U19 CA148065 and U19 CA148112), the US Department of Defense (W81XWH-10-1-0341), the Canadian Institute of Health Research (CIHR) for the CIHR Team in Familial Risks of Breast Cancer, the Susan G. Komen Foundation for the Cure, the Breast Cancer Research Foundation and the Ovarian Cancer Research Fund.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Letters


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Online Methods

Study overview. Cases and controls were matched as summarized in Supplementary Table 1. Each sample set is described in the Supplementary Note. The overall study design is illustrated in Supplementary Figure 1.

Additional endometrial cancer GWAS. The National Study of Endometrial Cancer Genetics (NSEC) consisted of 925 histologically confirmed endometrial cancer cases from the UK (86% with endometrioid-only histology). Genotyping was performed using Illumina 660W Quad arrays.

These cases were matched with 1,286 cancer-free controls from the UK1/CORGI12 and SP1 (ref. 11) colorectal studies genotyped using Illumina Hap550, Hap300 and Hap240S arrays and 1958 Birth Cohort13 controls from the Wellcome Trust Case Control Consortium 2 (WTCCC2)13 genotyped using Illumina Infinium 1.2M arrays.

Original endometrial cancer GWAS. As described previously, cases with endometrioid histology were selected from two population studies; the UK Studies of Epidemiology and Risk factors in Cancer Heredity (SEARCH; n = 681) and the Australian National Endometrial Cancer Study (AN ECS; n = 606), and genotypes were generated using Illumina Infinium 610K arrays. In comparison with our previous study10, this meta-analysis analyzed ANECS and SEARCH as two groups and included additional controls8,56. SEARCH cases were compared with 2,501 controls from the National Blood Service (NBS), part of the WTCCC2 controls13. ANECS cases were compared to controls recruited as part of the Hunter Community Study56 or Brisbane Adolescent Twin Study57, genotyped using Illumina Infinium 610K arrays.

Phase 1 iCOGS genotyping. For the iCOGS genotyping stage, 4,330 women with a confirmed diagnosis of endometrial cancer and European ancestry were recruited via 11 studies in western Europe, North America and Australia, collectively called the Endometrial Cancer Association Consortium (ECAC).

Healthy female controls with European ancestry and known age at sampling were selected from controls genotyped by the Breast Cancer Association Consortium (BCAC)15 or Ovarian Cancer Association Consortium (OCAC)56 iCOGS projects. Eight case–control groups were matched on the basis of geographical location, and principal-components analysis (PCA) was conducted; individuals who clustered outside the main centroid in pairwise plots of the first four principal components were excluded (Supplementary Fig. 7).

Cases and controls were genotyped on a custom Illumina Infinium iSelect array with 211,155 SNPs, designed by the Collaborative Oncological Gene-environment Study (iCOGS), a collaborative project involving four consortia. SNPs were included on this array on the basis of promising regions of interest in previous breast, ovarian and prostate14 studies and also the 1,483 top SNPs from our previous endometrial cancer GWAS10 analysis. Cases and MoMaTEC controls were genotyped by the Génome Québec Innovation Center. BCAC and OCAC control samples were genotyped at four centers. Raw intensity data files for all consortia were sent to the COGS data coordination center at the University of Cambridge for centralized genotype calling and quality control, so that all case and control genotypes were called using the same procedure.

SNP genotyping array quality control. Genotype calling was performed using Illumina's proprietary Gencall algorithm and Illuminus58. Duplicate samples displayed >99% concordance. Standard quality control measures applied to genotyping arrays are described in our original GWAS10 and included genotypic call rate <0.95; deviation from Hardy–Weinberg equilibrium at P < 1 × 10−7; and visual inspection of cluster plots for most significant SNPs. For iCOGS, all endometrial cancer cases and MoMaTEC controls were genotyped by the Génome Québec Innovation Center. BCAC and OCAC control samples were genotyped at four centers. Raw intensity data files for all consortia were sent to the COGS data coordination center at the University of Cambridge for centralized genotype calling and quality control, so that all case and control genotypes were called using the same procedure. Duplicate samples for quality control showed a concordance of >99%. Samples were excluded on the basis of the following measures: missingness >5%, heterozygosity rates ((N-O)/N) >5 s.d. from the mean, X-chromosome heterozygosity rate (PLINK P score) >0.2 and pairwise identity by descent (IBD) >0.1875 (cutoff for second-degree relatives). PCA was conducted using EIGENSTRAT59 software. Analysis was conducted using PLINK60 and the R packages GenABEL and SNPMatrix61,62.

Phase 2 NSEC genotyping. A second genotyping phase consisted of assaying five SNPs with P < 1 × 10−7 and IMPUTE info scores >0.94 from the NSEC–AN ECS–SEARCH–iCOGS meta-analysis: samples were NSEC cases and controls not previously used in the NSEC gen GWAS or NSEC iCOGS. Genotyping was conducted using competitive allele-specific PCR (KASPart, KBiosciences) and the Fluidigm BioMark HD System, using standard protocols. The genotyping call rate was >0.98, and there was a >0.985 concordance between different genotyping platforms (Supplementary Table 4). There was no significant deviation from Hardy–Weinberg equilibrium (β > 0.05). Sequences for genotyping primers are listed in Supplementary Table 8.

Genome-wide and regional imputation. Genome-wide imputation for all SNP-array-generated data was conducted using IMPUTE (v2)63 and the 1000 Genomes Project (2012 release) as reference panel. For the first-pass genome-wide analysis, we prephased chromosomes using SHAPEIT64 to improve the computational speed. Imputation was carried out separately for each of the three GWAS (for each GWAS, the cases and controls were imputed together and used as a single data set, using only SNPs that passed quality control in both cases and controls) and for the iCOGS study (all studies within iCOGS were imputed together). SNPs with MAF <0.1% were removed from all studies before imputation. Genome-wide imputation produced 9,594,066 SNPs with MAF ≥21% and info ≥0.4 in at least one of the three GWAS and eight iCOGS groups. Of these, 8,308,423 SNPs met these criteria in all studies. The iCOGS genotyping array (~200,000 SNPs) is aimed at capturing previously prioritized cancer-associated SNPs and not genome-wide coverage, but nonetheless 8,631,871 SNPs met the criteria of MAF ≥21% and info ≥0.4, of which 5,437,135 had info ≥0.7 and 2,333,040 had info ≥0.9.

Regional imputation of regions of interest (1.5-Mb region around SNPs with meta-analysis P < 1 × 10−5) used both 1000 Genomes Project 2012 release and 196 high-coverage, whole-genome-sequenced UK individuals as reference panels as a means to improve imputation accuracy65. All SNPs reported in this study had an info score ≥0.9 in all data sets.

Association testing. Association testing was carried out using SNPTEST (v2)66 employing frequentist tests with a logistic regression model for each of the 11 groups as matched in Supplementary Table 1. There was little evidence of systematic overdispersion of the test statistic from the quantile–quantile plots (Supplementary Fig. 2) and the genomic inflation λGC calculated using all genotyped SNPs passing quality control for the three GWAS. For iCOGS, 105,000 SNPs after LD pruning (r2 < 0.2) and >500 kb from the 1,483 endometrial cancer prioritized SNPs on the iCOGS were used. λGC was between 1.002 and 1.038 for each study. Conditional logistic regression analysis was conducted for each locus of genome-wide significance using SNPTEST to look for the presence of multiple independent association signals. This was carried out in a stepwise manner, first conditioning for the most significant SNP and subsequently for any SNPs that remained significant at Pcond < 1 × 10−4. Regional association plots (Fig. 1 and Supplementary Fig. 6) were created using LocusZoom67.

Meta-analysis. Inverse variance, fixed-effects meta-analysis of the 11 groups (3 GWAS and 8 iCOGS groups) was conducted using GWAMA68. The per-allele effect size of each SNP in a particular study is represented by β (the log-transformed odds ratio) and its standard error. Between-study differences are represented by the P heterogeneity score69,70. Forest plots of the genome-wide significant loci (Fig. 2) representing risk effects across different studies were generated using metareg. A random-effects meta-analysis was also performed for SNPs with β >0.3. The results of the second replication phase (NSEC gen replication) were subjected to meta-analysis in a 12-way meta-analysis for the top five SNPs yielding a total of 7,737 endometrial cancer cases and 37,144 controls. 6,635 (86%) of the endometrial cancer cases had endometrioid-only histology, and association testing and meta-analysis were also conducted with just these samples.
Bioinformatic analysis and functional annotation of genome-wide significant risk loci. The five new genome-wide significant loci and SNPs in LD (r² > 0.7 in the European 1000 Genomes Project population) were annotated using HaploRegV2 (ref. 71), RegulomeDB25 and data from ENCODE26 in Supplementary Table 6. These include information such as promoter and enhancer histone marks, DHSs, bound proteins, altered motifs, GENCODE and dbSNP annotations, RegulomeDB score and phastCons conservation scores.

The bioinformatic analysis in Supplementary Figure 3 used data sets described by Hnisz et al.72 and Corradin et al.74 to identify likely enhancers in a cell-type-specific context for the risk loci. Enhancer–gene interactions are predicted by identifying ‘super-enhancers’ (regions containing neighboring H3K27ac modifications) from 86 cell and tissue types, and the expressed transcript with the transcription start site closest to the center of the super-enhancer was then assigned as the target gene. PrexTIGE pairs cell-type-specific H3K4me1 and gene expression data from 13 cell types to identify likely enhancer–gene interactions.

Endometrial tissue expression quantitative trait loci analysis for associated SNPs using GTEx and TCGA data. Publicly available data generated by the Genotype-Tissue Expression (GTEx) Project25 and The Cancer Genome Atlas (TCGA) were accessed to examine tissue-specific eQTLs. For GTEx, expression and genotype data were generated from 70 normal uteri from post-mortem biopsies, using an Affymetrix Expression array and Illumina Omni 5 M SNP array. GTEx provided processed results, evaluating association between genotype and expression data. The expression levels are represented as a rank-normalized score. TCGA genotype and copy number variation (CNV) data were derived from Affymetrix 6.0 SNP arrays. Expression data were from RNA-seq arrays (Illumina HiSeq and Illumina Genome Analyzer) for 458 endometrial cancer tissues and 30 adjacent normal endometrial tissues. Association analyses for TCGA data sets were performed as follows. Genes within 500 kb flanking our SNPs of interest were selected for analysis. Because there may be significant variation in tumor tissue copy number, somatic CNVs were taken into account by regressing gene expression to average copy number spanning the gene. Residual unexplained variance in gene expression was then regressed on the genotype of the lead SNP at each locus, using genotyped or imputed data. Statistical comparisons were subject to Bonferroni correction for number of tests (number of sample sets and number of genes assessed).

DNA and RNA extraction from cell lines. Cell lines from the laboratory of D. Church, acquired as gifts from B. Weigelt (currently at the Memorial Sloan Kettering Cancer Center) and K. Dedes (University of Zurich), were routinely tested for mycoplasma contamination. Nucleic acids were quantified using Nanodrop 2000 (Thermo Scientific) spectrophotometry.

Quantification of KLF5 expression in endometrial cancer cell lines. Extracted RNA was treated with DNase I, and cDNA was reverse transcribed from RNA using the High-Capacity cDNA Reverse Transcription kit (Applied Biosystems). TaqMan Gene Expression Assays were used for KLF5 and GAPDH (details available upon request). The absolute expression of KLF5 was quantified using qRT–PCR on the ABI 7900HT cycler (Applied Biosystems), and the critical threshold was manually set at 0.2. Relative expression was calculated using the ∆∆Ct method described by Livak and Schmittgen75, with GAPDH as an endogenous control.

Formaldehyde-assisted identification of regulatory elements. FAIRE was conducted using a method adapted from Giretti et al.76. Briefly, cross-linking was performed on a rocker at room temperature. 1% formaldehyde was added to ~1 × 10⁶ cells for 5 min, and 115 mM glycine was added to inhibit cross-linking. For each cell line, a non-cross-linked control was prepared in parallel for all remaining steps. After two rinses with 4 °C PBS, cells were suspended in successive buffers: lysis buffer I (50 mM HEPES-KOH, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 0.5% NP-40 and 0.25% Triton X-100); lysis buffer II (10 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA and 0.5 mM EGTA); lysis buffer III (10 mM Tris-HCl, 2.1 M NaCl, 1 mM EDTA, 0.1% sodium deoxycholate and 0.5% N-lauroylsarcosine). Cells were incubated on a rocker at 4 °C for 10 min in each lysis buffer, then spun down at 1,300g for 5 min, and the supernatant was removed. The cells were then sonicated using the Bioruptor in 7 to 15 30-s cycles to generate fragments 100–1,000 bp in size, and gel electrophoresis in 1% agarose was used to confirm DNA fragment sizes. The DNA was extracted with a standard phenol–chloroform method and ethanol precipitated. 50 ng of DNA from paired cross-linked and non-cross-linked cells was analyzed in duplicate by SYBR Green qPCR using primers at ~1 kb intervals in the 13q22.1 region downstream of KLF5 (Supplementary Table 8). The ∆∆Ct method31 was used to normalize results to the input DNA from non-cross-linked cells and then expressed relative to the rhodospin promoter as negative control. For each experiment, there were two replicates for the cross-linked cells and non-cross-linked controls, each performed on two occasions. Cross-linked chromatin immunoprecipitation. About 1 × 10⁶ cells were cross-linked using 1% formaldehyde for 10 min. Glycine was used to stop the cross-linking, cells were then rinsed twice in PBS and cell scrapers were used to detach cells adhered to the Petri dish surface. Cells were then resuspended in lysis buffer (1% SDS, 10 mM EDTA (Ambion) and 50 mM Tris-HCl (Ambion)), incubated for 10 min and then sonicated using the Bioruptor (Diagenode) in 7 to 15 30-s cycles to generate fragments 1,000–1,500 bp in size. Gel electrophoresis in 1% agarose confirmed the size of the DNA fragments. The fragmented DNA was then diluted ten times to the immunoprecipitation dilution buffer (1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl and 150 mM NaCl), and each cell line was separated into four tubes: input chromatin, no-antibody control and one tube for each antibody. Five microliters of antibody to H3K4me2 (Millipore, 07-030) and acetylated histone H4 (Millipore, 06-866) was added to the antibody tubes and, along with the no-antibody control, incubated overnight at 4 °C for immunoprecipitation. The input chromatin was kept refrigerated at 4 °C until the reverse cross-linking on day 2. Phenylmethylsulfonyl fluoride and protease inhibitors were added to the lysis buffer and immunoprecipitation dilution buffer to deactivate proteases, and sodium butyrate was added to these solutions to inhibit histone deacetylases. Five microliters of protein A Dynabeads was added to each tube, and beads were incubated for 4 h. A series of washes were performed using Tris-sucrose–EDTA (TSE) (1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, 150 mM NaCl and 0.1% SDS), TSE II (1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, 500 mM NaCl and 0.1% SDS), Buffer III (0.25 M lithium chloride, 1 mM EDTA, 10 mM Tris-HCl, 1% tert-glycyl type NP-40 and 1% sodium deoxycholate) and Trits–EDTA (1×). Three hundred microliters of extraction solution (1% SDS 0.1 M sodium bicarbonate) was added, and Dynabeads were removed after a 30-min incubation. Then 0.7 M NaCl was added and reverse cross-linking occurred overnight at 65 °C. DNA was purified using the QiAquick PCR purification kit (Qiagen). One microliter of DNA was analyzed in duplicate or triplicate by SYBR Green qPCR as above, and the ∆∆Ct method was used to identify areas with enrichment. For each experiment, there were two replicates for each antibody along with the input and no-antibody control, each performed on two occasions. Sequences for the primers used are listed in Supplementary Table 8.

Chromatin conformation capture. Experiments were performed as described in Ghousain et al.77, using the KLF5-expressing Ishikawa endometrial cancer cell line from the American Type Culture Collection. The cell line was authenticated using short-tandem-repeat (STR) profiling, and routinely tested for mycoplasma contamination (QIMR Berghofer in-house Support Services).

Briefly, Ishikawa cells were cross-linked with 1% formaldehyde for 10 min, the reaction was quenched with 125 mM glycine, and cells were washed with PBS and collected by scraping. Cells were lysed for 30 min on ice in 10 mM Tris–HCl, pH 7.5, 10 mM NaCl and 0.2% Igepal with protease inhibitors and homogenized in a Dounce homogenizer. Nuclei were pelleted and resuspended in 1 ml of 1.2× restriction buffer (NEB 3.1) with 0.3% SDS for 1 h at 37 °C. 2% Triton X-100 was added, and 1,000 U NcoI was then added three times over 24 h at 37 °C with shaking. The enzyme was inactivated, and digested DNA was diluted 8× before ligation with 4,000 U of T4 DNA ligase overnight at 16 °C. Cross-links were reversed by proteinase K digestion.
at 65 °C overnight, and DNA was purified by phenol–chloroform extraction and ethanol precipitation. The final DNA pellet was dissolved in 10 mM Tris (pH 7.5) and purified through Amicon Ultra 0.5-ml columns (Millipore). 3C interactions were quantified by SYTO9 qPCR (performed on a RotorGene 6000) using primers designed to amplify across ligated NcoI restriction fragments with one constant primer within the risk fragment (including rs11841589 and rs9600103) and a series of test primers within NcoI fragments spanning 76 kb of the KLF5 promoter region. BAC clones (RP11-81D9 and RP11-179I20) covering the region were digested with NcoI, ligated with T4 ligase and used to determine PCR efficiency. 3C analyses were performed on three independent 3C libraries, with each data point in duplicate. Data were normalized to the signal from the BAC clone library and from a non-interacting chromosomal region using the ΔΔCt method with incorporated individual primer pair efficiencies.

**Luciferase reporter assays.** For luciferase reporter assays, the regions chr. 13 73,810,509–73,813,452 around rs9600103 and chr. 13: 73,813,268–73,816,290 around rs11841589 were cloned into the pGL3-Promoter vector (Promega) to test for regulatory effects in Ishikawa cells. Ishikawa cells were selected because they express KLF5, showed evidence of a DHS, FAIRE and H3K4me2 enrichment at rs9600103, and were readily transfectable. Site-directed mutagenesis was used so both the high- and low-risk alleles of rs9600103 and rs11841589 were tested. After sequencing to verify the correct insert sequences, cells were performed on three occasions (total of 11 assays). The sequences for the regulatory activity78. Luciferase activity experiments had 3 or 4 replicates, into the pGL3-Promoter vector previously used as a length of DNA with no putative enhancer regions was normalized to the ground from Lipofectamine-only controls firefly luciferase activity from the control for transfection efficiency. After 48 h, luciferase activity was measured (Dual-Glo Luciferase Assay System, Promega), and after subtracting background from Lipofectamine-only controls firefly luciferase activity from the putative enhancer regions was normalized to the Renilla luciferase values for each sample. Levels of firefly luciferase activity were compared with a control plasmid consisting of an empty pGL3 vector and also a noncoding 2.2-kb plasmid from the pENTRA plasmid (Invitrogen) cloned into the pGL3-Promoter vector previously used as a length of DNA with no regulatory activity79. Luciferase activity experiments had 3 or 4 replicates, each performed on three occasions (total of 11 assays). The sequences for the primers used are listed in Supplementary Table 8.

ANOVA found significant differences in luciferase levels (P < 0.0001, F:11.6) but no significant differences between replicates conducted on different days (P = 0.91, F:0.09). There were no significant differences between the pENTRA control and the empty pGL3-Promoter vector (P = 0.085); pGL3-Promoter vector was used as control. We conducted paired t tests for all comparisons using the average of biological repeats, between the pGL3 no-insert, rs9600103[A], rs9600103[T], rs11841589[G] and rs11841589[T] fragments (Supplementary Table 7, results unadjusted for multiple comparisons).