**CHEK2, MGMT, SULT1E1 and SULT1A1 Polymorphisms and Endometrial Cancer Risk**

Tracy A. O'Mara,1,2 Kaltin Ferguson,2 Paul Fahey,3 Louise Marquart,3 Hannah P. Yang,4 Jolanta Lissowska,5 Stephen Chanock,4Montserrat Garcia-Closas,4 Deborah J. Thompson,6 Catherine S. Healey,7 Alison M. Dunning,7 Douglas F. Easton,6 ANECS, Penelope M. Webb,2 and Amanda B. Spurdle 2

1 Hormone Dependent Cancer Program, Institute of Health and Biomedical Innovation, Queensland University of Technology, Australia
2 Genetics and Population Health Division, Queensland Institute of Medical Research, Brisbane, Australia
3 Statistics Unit, Queensland Institute of Medical Research, Brisbane, Australia
4 Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Department of Health and Human Services, Bethesda, United States of America
5 Department of Cancer Epidemiology and Prevention, M. Sklodowska-Curie Memorial Cancer Center and Institute of Oncology, Warsaw, Poland
6 Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom
7 Department of Oncology, University of Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom

Several single nucleotide polymorphisms (SNPs) in candidate genes of DNA repair and hormone pathways have been reported to be associated with endometrial cancer risk. We sought to confirm these associations in two endometrial cancer case-control sample sets and used additional data from an existing genome-wide association study to prioritize an additional SNP for further study. Five SNPs from the CHEK2, MGMT, SULT1E1 and SULT1A1 genes, genotyped in a total of 1597 cases and 1507 controls from two case-control studies, the Australian National Endometrial Cancer Study and the Polish Endometrial Cancer Study, were assessed for association with endometrial cancer risk using logistic regression analysis. Imputed data was drawn for CHEK2 rs8135424 for 666 cases from the Study of Epidemiology and Risk factors in Cancer Heredity study and 5190 controls from the Wellcome Trust Case Control Consortium. We observed no association between SNPs in the MGMT, SULT1E1 and SULT1A1 genes and endometrial cancer risk. The A allele of the rs8135424 CHEK2 SNP was associated with decreased risk of endometrial cancer (adjusted per-allele OR 0.83; 95%CI 0.70-0.98; p = .03) however this finding was opposite to that previously published. Imputed data for CHEK2 rs8135424 supported the direction of effect reported in this study (OR 0.85; 95% CI 0.65–1.10). Previously reported endometrial cancer risk associations with SNPs from in genes involved in estrogen metabolism and DNA repair were not replicated in our larger study population. This study highlights the need for replication of candidate gene SNP studies using large sample groups, to confirm risk associations and better prioritize downstream studies to assess the causal relationship between genetic variants and cancer risk. Our findings suggest that the CHEK2 SNP rs8135424 be prioritized for further study as a genetic factor associated with risk of endometrial cancer.

**Keywords:** endometrial cancer, single nucleotide polymorphism, CHEK2, MGMT, SULT1E1, SULT1A1

---

**Background**

History of a first-degree relative with endometrial cancer has been associated with a two-fold increased risk of endometrial cancer (Hemminki et al., 2005), and low-risk genetic factors are likely to be involved in the development of this disease, as has now been demonstrated for several other cancers (http://www.genome.gov/gwastudies/). Single nucleotide polymorphisms (SNPs) in genes involved in estrogen and DNA repair processes have been the focus of many candidate gene association studies for endometrial cancer, since unopposed exposure to endogenous or exogenous estrogen is a well-established risk factor.
factor for endometrial cancer development and estrogen metabolites have also been reported to cause DNA lesions. However, results from single studies of SNPs in candidate genes are known for being unreliable and chance remains a likely explanation for many reported statistically significant associations, with results from individual studies unfortunately rarely confirmed by subsequent studies. Very large studies with little margin for error and/or validation of results in other populations is thus an essential pre-requisite before reported associations can be accepted as real. In an attempt to validate associations between 5 SNPs in DNA repair and estrogen sulfation genes (CHEK2, Einarsdottir et al., 2007; MGMT, Han et al., 2006; SULT1E1 and SULT1A1, Rebbeck et al., 2006) and endometrial cancer risk, previously estimated from studies including at least 421–683 cases (Table 1), we genotyped these SNPs in a pooled sample of more than 1500 cases and 1600 controls from the Australian National Endometrial Cancer Study (ANECS) and the Polish Endometrial Case-Cancer Study (PECS). To clarify the results for the CHEK2 SNP, rs8135424 imputed genotype dosages were used for 666 endometrial cancer patients from the Studies of Epidemiology and Risk factors in Cancer Heredity (SEARCH) cohort and 5190 control subjects from the Wellcome Trust Case Control Consortium, using data drawn from an existing genome-wide association study of endometrial cancer.

**Material and Methods**

The ANECS and PECS study populations and selection criteria have been described elsewhere (Gaudet et al., 2008; O’Mara et al., 2011). Genotyping for ANECS samples was performed using the Sequenom® MassARRAY platform (Sequenom, San Diego CA, USA), while the genotyping for PECS samples was performed using the Illumina iSelect Custom BeadChip (Illumina INC., San Diego CA, USA). All SNPs passed quality control filters that included Hardy-Weinberg Equilibrium, minimum duplicate concordance and, minimum sample and assay success rates. Pooled odds ratios (OR) and 95% confidence intervals (CI) were calculated to estimate the association between SNPs and endometrial cancer risk using logistic regression models, adjusting for age and...
study. Additional analyses included adjustment for body-mass index (BMI) (World Health Organization categories: < 25, 25 to < 30, 30 to < 35 and ≥ 35 kg/m²) and stratification by histological subtype (endometrioid vs other) and ethnicity (Caucasian vs other). To assess possible interaction with smoking for rs2308321, the significance of multiplicative interaction was assessed by the change in the likelihood ratio estimate after inclusion of smoking*genotype to a simpler model without this term. All statistical analyses were performed using the Statistical Packages of Social Sciences for Window, version 17 (SPSS Inc., Chicago, IL).

Imputed genotype dosages for rs8135424 were obtained for 666 SEARCH cases with endometrioid histology genotyped as part of a genome-wide association study of endometrial cancer using an Illumina Infinium 610K array (Spurdle et al., 2011), and for 5190 UK control subjects who had been genotyped using an Illumina Infinium 1.2M array as part of the Wellcome Trust Case Control Consortium (WTCCC, 2007). Non-genotyped SNPs were imputed using 1000 Genomes data as a reference panel (August 2010 Release http://www.1000genomes.org). Imputed genotype dosages were compared between cases and controls, adjusting for the first 3 principal components of the genomic kinship matrix to take into account any difference in population structure between cases and controls. This part of the analysis was performed using GenABEL (Aulchenko et al., 2007), ProbABEL (Aulchenko et al., 2010) and MACH (Li et al., 2009).

**Results**

Results are shown in Table 2. The SULT1A1 SNP (rs1801030) was found to be exceedingly rare in the ANECS study group, and monomorphic in PECS, and was thus excluded from further analysis. Contrary to the previous studies, we found no evidence of association between the individual SNPs from the SULT1E1 (rs3736599) and MGMT (rs2308321 and rs12917) genes and endometrial cancer risk. The results were unchanged when we adjusted for BMI, or excluded non-endometrioid cancers from the analysis (data not shown). Since the previous report suggested a trend for decreased risk of endometrial cancer with increased exposure to smoking for rs2308321-G carriers (p trend = .01), but not for rs2308321-G non-carriers (p trend = .7; p interaction = .04) (Han et al., 2006), we also assessed the interaction of rs2308321 with smoking. There was no evidence for similar interaction of rs2308321 with smoking in our dataset (p = .3).

Our results did show an association between the CHEK2 SNP rs8135424 and decreased endometrial cancer risk (per A allele adjusted OR 0.83; 95% CI 0.70–0.99; p = .03). Again the results were not markedly altered by exclusion of non-endometrioid cases from the analysis (OR 0.82; 95% CI 0.69–0.98, p = .03), or with additional adjustment for BMI (OR 0.85; 95% CI 0.70–1.01). There

### Table 2

<table>
<thead>
<tr>
<th>SNP</th>
<th>Gene</th>
<th>Genotype</th>
<th>Pooled Adj OR (95%CI) *</th>
<th>P-value</th>
<th>No. of cases (%)</th>
<th>No. of controls (%)</th>
<th>ANECS</th>
<th>PECS</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1801030</td>
<td>SULT1A1</td>
<td>AA</td>
<td>Not polymorphic</td>
<td></td>
<td>1165 (100)</td>
<td>1094 (100)</td>
<td>392</td>
<td>404</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1165 (100)</td>
<td>1094 (100)</td>
<td>392</td>
<td>404</td>
</tr>
<tr>
<td>RS3736599</td>
<td>SULT1E1</td>
<td>CC</td>
<td>1 (ref)</td>
<td>0.99</td>
<td>231 (19.7)</td>
<td>220 (20.2)</td>
<td>83 (21.0)</td>
<td>80 (19.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>1.00 (0.83-1.20)</td>
<td></td>
<td>15 (1.3)</td>
<td>10 (0.9)</td>
<td>8 (2.0)</td>
<td>7 (1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>1.03 (0.87-1.21)</td>
<td></td>
<td>1170</td>
<td>1091</td>
<td>396</td>
<td>404</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>924 (79.0)</td>
<td>861 (78.9)</td>
<td>305 (77.0)</td>
<td>317 (78.5)</td>
</tr>
<tr>
<td>rs2308321</td>
<td>MGMT</td>
<td>AA</td>
<td>1 (ref)</td>
<td>0.99</td>
<td>231 (19.7)</td>
<td>220 (20.2)</td>
<td>83 (21.0)</td>
<td>80 (19.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>1.28 (0.67-2.43)</td>
<td></td>
<td>15 (1.3)</td>
<td>10 (0.9)</td>
<td>8 (2.0)</td>
<td>7 (1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GG</td>
<td>1.03 (0.87-1.21)</td>
<td></td>
<td>1173</td>
<td>1099</td>
<td>397</td>
<td>406</td>
</tr>
<tr>
<td>RS12917</td>
<td>MGMT</td>
<td>CC</td>
<td>1 (ref)</td>
<td>0.99</td>
<td>231 (19.7)</td>
<td>220 (20.2)</td>
<td>83 (21.0)</td>
<td>80 (19.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CT</td>
<td>1.22 (0.72-2.10)</td>
<td></td>
<td>1170</td>
<td>1099</td>
<td>397</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TT</td>
<td>0.99 (0.85-1.14)</td>
<td></td>
<td>889 (75.8)</td>
<td>810 (73.7)</td>
<td>278 (70.0)</td>
<td>296 (72.9)</td>
</tr>
<tr>
<td>rs8135424</td>
<td>CHEK2</td>
<td>GG</td>
<td>1 (ref)</td>
<td>0.99</td>
<td>231 (19.7)</td>
<td>220 (20.2)</td>
<td>83 (21.0)</td>
<td>80 (19.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AG</td>
<td>0.83 (0.69-1.01)</td>
<td></td>
<td>15 (1.3)</td>
<td>10 (0.9)</td>
<td>8 (2.0)</td>
<td>7 (1.7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>1.03 (0.87-1.21)</td>
<td></td>
<td>1173</td>
<td>1099</td>
<td>397</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>889 (75.8)</td>
<td>810 (73.7)</td>
<td>278 (70.0)</td>
<td>296 (72.9)</td>
</tr>
</tbody>
</table>

Note: * Adjusted for age (continuous) and study (ANECS, PECS)

Abbreviations: ANECS — Australian National Endometrial Cancer Study; PECS — Polish Endometrial Cancer Study

Downloaded from https://www.cambridge.org/core. IP address: 54.70.40.11, on 29 Apr 2019 at 14:42:40, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
was also no difference in ORs when our analysis was restricted to only Caucasian samples (1288 cases and 1337 controls; data not shown). However, this finding is in the opposite direction to that previously observed in a Swedish population (cases \( n = 705 \), controls \( n = 1565 \); per A allele adjusted OR 1.26; 95% CI 1.06–1.51, \( p = .01 \)) (Einarsdottir et al., 2007). The direction of risk associated with rs8135424 was the same in both sample sets included in this study, despite a somewhat lower minor allele frequency in the Australian (0.19) controls compared to Polish controls (0.33). In an attempt to clarify the findings for rs8135424, we analyzed imputed data from an independent UK dataset. While the results were not significant, the direction of the association was similar to that observed in the Australian/Polish dataset (per A allele OR 0.85; 95% CI 0.65–1.10).

**Discussion**

We were not able to replicate previously reported endometrial cancer risk associations with SNPs from genes involved in estrogen metabolism and DNA repair in our larger study population. The rs8135424 SNP has not been investigated in other cancers and it is also not genotyped, or in strong linkage disequilibrium with SNPs that are genotyped by the Illumina Human 1M Duo BeadChip commonly used for genome-wide association studies. Although the imputed data accessed was less than optimal (Imputation \( R^2 = .53 \)), the conflicting results reported by the original Swedish study compared to the combined larger datasets from Australian, Polish and UK studies suggest that further research using independent sample sets will be important to clarify the association of rs8135424 with endometrial cancer risk.

The SNPs assessed in our study were chosen because of their reported associations with endometrial cancer risk. Our findings do not support those previously reported, although our large sample size from two independent studies provided sufficient power (> 80%) to detect the ORs reported in the previous studies. Our results also highlight the inconsistency of results from single candidate gene SNP association studies with relatively small numbers of cases and emphasize the value of replication in large sample groups and multi-center studies (Gaudet et al., 2010; Lurie et al., 2011; O'Mara et al., 2011; Setiawan et al., 2009; Spurdle et al., 2011).

**Competing Interests**

The authors have no competing interests to declare.

**Acknowledgments**


ANECS was supported by project grants from the National Health and Medical Research Council (NHMRC) of Australia (ID##39435), The Cancer Council Queensland (ID#196615) and Cancer Council Tasmania (ID#403031 and ID#457636). ABS and PMW are supported by NHMRC Senior Research Fellowships. TOM is supported by an Australian Postgraduate Award, an Institute of Health and Biomedical Innovation PhD Top-Up and a Smart State PhD Award. PECS was funded by the intramural research program at the US National Cancer Institute, Division of Cancer Epidemiology and Genetics in the Hormonal and Reproductive Epidemiology Branch. SEARCH was funded by Cancer Research UK grants [C490/A11021, C8197/A10123, C1287/A7497, C1287/A10112], BCC grant [2077NovPR17] and EU FP7 COGS [HEALTH-F2-2009-223175]. AMD was supported by Cancer Research Grant [C8197/A10865] and The Joseph Mitchell Trust.

ANECS would like to thank Felicity Lose, Jyotsna Batra, Xiaqing Chen and Jonathan Beesley from The Molecular Cancer Epidemiology and Cancer Genetic laboratories at QIMR for technical assistance. We would like to thank the patients that were involved in this study. We also thank the Australian Red Cross Blood Services (ARCBS) donors who participated as healthy controls in this study. We are grateful to the staff at ARCBS for their assistance with the collection of risk factor information and blood samples, and members of the Molecular Cancer Epidemiology Laboratory for their assistance with collection and processing of ARCBS blood samples. ANECS would also like to gratefully acknowledge the cooperation of the following institutions: NSW: John Hunter Hospital, Liverpool Hospital, Mater Misericordiae Hospital (Sydney), Mater Misericordiae Hospital (Newcastle), Newcastle Private Hospital, North Shore Private Hospital, Royal Hospital for Women, Royal Prince Alfred Hospital, Royal North Shore Hospital, Royal Prince Alfred Hospital, St George Hospital; Westmead Hospital, Westmead Private Hospital; Qld: Brisbane Private Hospital, Greenslopes Hospital, Mater Misericordiae Hospitals, Royal Brisbane and Women’s Hospital, Wesley Hospital, Queensland Cancer
Registry; SA: Adelaide Pathology Partners, Burnside Hospital, Calvary Hospital, Flinders Medical Centre, Queen Elizabeth Hospital, Royal Adelaide Hospital, South Australian Cancer Registry; Tas: Launceston Hospital, North West Regional Hospitals, Royal Hobart Hospital; Vic: Freemasons Hospital, Melbourne Pathology Services, Mercy Hospital for Women, Royal Women’s Hospital, Victorian Cancer Registry; WA: King Edward Memorial Hospital, St John of God Hospitals Subiaco & Murdoch, Western Australian Cancer Registry.

PECs would like to thank Neonila Szeszenia-Dabrowska of the Nofer Institute of Occupational Medicine ( Lodz, Poland) and Witold Zatonski of the M. Sklodowska-Curie Institute of Oncology and Cancer Center (Warsaw, Poland) for their contribution to the PECS; Pei Chao and Michael Stagner (IMS, Silver Spring, MD, USA) for their invaluable management of the PECS; Laurie Burdette of the Core Genotyping Facility for genotyping; and the physicians, nurses, interviewers and study participants for their dedicated efforts.

SEARCH would like to thank the women who took part in this research and grateful for input from Paul Pharoah, Shahana Ahmed, Catherine S. Healey, Mitul Shah, Jonathan Morrison, Kyriaki Michailidou, Caroline Baynes, Craig Luccarini, Don Conroy, Patricia Harrington, Rebecca Mayes, Hannah Munday, Bridget Curzon, Sue Irvine, Clare Jordan, Barbara Perkins, Radka Platte, Anabel Simpson, Anne Stafford and Judy West.

References


