Assessing the role of selenium in endometrial cancer risk: a Mendelian randomization study

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Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest

Author contribution statement

Conception or design of the work; the acquisition, analysis and interpretation of data for the work (PFK, DMG, DJT, ABS, TAO'M). Drafting the manuscript (PFK, DMG, DJT, ABS, TAO'M). Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved (PFK, DMG, DJT, ABS, TAO'M). All authors were involved in revision of the manuscript and provide final approval of the version to be published.

Keywords

genome- wide association studies, Mendelian randomisation, Blood selenium, Toenail selenium, endometrial cancer

Abstract

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Endometrial cancer is the most commonly diagnosed gynecological cancer in developed countries. Based on evidence from observational studies which suggest selenium inhibits the development of several cancers (including lung and prostate cancer), selenium supplementation has been touted as a potential cancer preventative agent. However, randomized controlled trials have not reported benefit for selenium supplementation in reducing cancer risk. For endometrial cancer, limited observational studies have been conducted assessing whether selenium intake, or blood selenium levels, associated with reduced risk, and no randomized controlled trials have been conducted. We performed a two-sample Mendelian randomization analysis to examine the relationship between selenium levels (using a composite measure of blood and toenail selenium) and endometrial cancer risk, using summary statistics for four genetic variants associated with selenium levels at genome-wide significance levels ($P < 5 \times 10^{-8}$), from a study of 12,906 endometrial cancer cases and 108,979 controls, all of European ancestry. Inverse variance weighted (IVW) analysis indicated no evidence of a causal role for selenium levels in endometrial cancer development (OR per unit increase in selenium levels Z-score = 0.99, 95% CI = 0.87-1.14). Similar results were observed for sensitivity analyses robust to the presence of unknown pleiotropy (OR per unit increase in selenium levels Z-score = 0.98, 95% CI 0.89-1.08 for weighted median; OR per unit increase in selenium levels Z-score = 0.90, 95% CI = 0.53-1.50 for MR-Egger). In conclusion, these results do not support the use of selenium supplementation to prevent endometrial cancer.

Ethics statements

(Authors are required to state the ethical considerations of their study in the manuscript, including for cases where the study was exempt from ethical approval procedures)

Does the study presented in the manuscript involve human or animal subjects: No

Data availability statement

Generated Statement: The datasets for this manuscript are not publicly available because Data are available from the authors of the original papers on request. Requests to access the datasets should be directed to Tracy O'Mara, tracy.omara@qimrberghofer.edu.au.

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Abstract
Endometrial cancer is the most commonly diagnosed gynecological cancer in developed countries. Based on evidence from observational studies which suggest selenium inhibits the development of several cancers (including lung and prostate cancer), selenium supplementation has been touted as a potential cancer preventative agent. However, randomized controlled trials have not reported benefit for selenium supplementation in reducing cancer risk. For endometrial cancer, limited observational studies have been conducted assessing whether selenium intake, or blood selenium levels, associated with reduced risk, and no randomized controlled trials have been conducted. We performed a two-sample Mendelian randomization analysis to examine the relationship between selenium levels (using a composite measure of blood and toenail selenium) and endometrial cancer risk, using summary statistics for four genetic variants associated with selenium levels at genome-wide significance levels ($P < 5 \times 10^{-8}$), from a study of 12,906 endometrial cancer cases and 108,979 controls, all of European ancestry. Inverse variance weighted (IVW) analysis indicated no evidence of a causal role for selenium levels in endometrial cancer development (OR per unit increase in selenium levels Z-score = 0.99, 95% CI = 0.87-1.14). Similar results were observed for sensitivity analyses robust to the presence of unknown pleiotropy (OR per unit increase in selenium levels Z-score = 0.98, 95% CI 0.89-1.08 for weighted median; OR per unit increase in selenium levels Z-score = 0.90, 95% CI = 0.53-1.50 for MR-Egger). In conclusion, these results do not support the use of selenium supplementation to prevent endometrial cancer.

Keywords
Mendelian randomization, endometrial cancer, toenail selenium, circulating selenium, genome-wide association study

49

Introduction

50 **Introduction**
51 Endometrial cancer is the most commonly diagnosed cancer of the female reproductive
52 system in developed countries (1). Unlike breast and cervical cancers where a screening
53 program is available to the general population, there is currently no available screening test
54 for endometrial cancer and diagnosis relies on biopsy in symptomatic patients (2).
55 Furthermore, the incidence of endometrial cancer is rising (3), highlighting the need for
56 preventative measures. Selenium has received considerable attention as a possible cancer
57 preventive agent (reviewed in (4)). While randomized controlled trials have shown no benefit
58 for selenium supplementation in reducing cancer risk over a period of up to eight years (5),
59 some observational longitudinal studies assessing selenium intake or selenium levels, over a
60 period up to 25 years, have shown an inverse association between selenium and cancer risk
61 (reviewed in (4)). Thus, although findings from the longitudinal studies have been
62 inconsistent (4), they may provide insight into the longer term effects of selenium exposure.
63 A recent meta-analysis examining the association between selenium intake (dietary and
64 supplemental) and overall cancer risk, has suggested that there was a reduction in cancer
65 incidence among people consuming more than the recommended daily allowance of selenium
66 (55 μg/day; RR = 0.96, 95% CI = 0.92-0.99)(6).

67

68 Very few studies have assessed the effects of selenium on endometrial cancer. In terms of
69 cellular studies, it has been shown that a selenium metabolite can inhibit endometrial cancer
70 cell proliferation, potentially through disruption of estrogen signaling (7). Findings from
71 human studies, however, have been more equivocal. A population-based, case-control
72 observational study of 417 endometrial cancer cases and 395 controls specifically assessed
73 the role of dietary and supplemental selenium intake (as measured by questionnaire in the six
74 months prior to diagnosis or enrolment as a control) in endometrial cancer development (8).
75 In a comparison of the highest (≥103.2 μg) and lowest (<72.4 μg) selenium quartiles, this
76 study did not support an association between selenium intake and endometrial cancer risk
77 (OR = 0.74, 95% CI = 0.47-1.17) (8). Two small case-control studies (n < 100) have assessed
78 serum selenium levels in endometrial cancer cases and controls. Sundstrom et al (9) reported
79 lower blood selenium levels in 64 cases as compared to 61 non-cancer controls, with an
80 average of 1.01±0.05 v 1.40±0.08 μmol/L blood selenium in cases and controls respectively
81 (P<0.001). A subsequent study of 35 endometrial cancer cases and 32 non-cancer controls
82 reported a similar finding (average of 1.14±0.04 v 1.26±0.03 μmol/L blood selenium in cases
83 and controls respectively, P<0.01)(10). Inconsistent results from these observational studies
84 may be due to small sample sizes (8-10), reverse causation bias (9, 10), recall bias and
85 measurement error in the dietary assessment (8). No prospective studies have examined the
86 association of pre-diagnostic selenium levels with endometrial cancer risk. Thus, the role of
87 selenium in endometrial cancer development remains inconclusive.

88

89 As no intervention study has yet been performed to explore the role of selenium in
90 endometrial cancer risk, we employed a two-sample Mendelian randomization approach
91 which uses germline genetic variants associated with selenium levels to proxy for selenium
92 exposure (11). These germline genetic variants are largely independent from environment or
93 lifestyle factors, and are established prior to disease onset, thus analyses using these genetic
94 variants as instrumental variables are less susceptible to biases from confounding and reverse
95 causation. Further, genetic effects on exposure of interest are lifelong, and hence it is
96 comparable to a lifelong randomized controlled trial.

97

98 **Materials and Methods**

99   Summary statistics for twelve genetic variants associated with selenium levels at genome-
100 wide significance ($P < 5\times10^{-8}$) were extracted from a genome-wide association study
101 (GWAS) meta-analysis of circulating selenium levels (n = 5,477; (12)) and toenail selenium
102 levels (n = 4,162; (13)) in European-ancestry individuals. These variants were at two separate
103 genetic loci; 5q14 (9 variants) and 21q22 (3 variants). To analyze the effect of selenium
104 exposure on endometrial cancer risk, we used summary statistics from the Endometrial
105 Cancer Association Consortium (ECAC) GWAS of 12,906 endometrial cancer cases and
106 108,979 controls of European descent (14). One of the 5q14 selenium-associated genetic
107 variants, rs558133, was excluded because it was not assessed by the ECAC GWAS (it does
108 not appear on the 1000 Genomes v3 reference panel) and no proxy with $r^2 > 0.8$ could be
109 found. These potential instrumental variables were pruned for linkage disequilibrium (LD; r^2
110 < 0.05) and four selenium-associated genetic variants (two independent variants per locus)
111 remained as instrumental variables. We used PhenoScanner v2 (15) to explore the possibility
112 of horizontal pleiotropy among the instrumental variables and their highly correlated variants
113 ($r^2 > 0.8$). Specifically, we examined traits associated with known risk factors of endometrial
114 cancer (i.e. body mass index, age at menarche, age at menopause, postmenopausal serum
115 estradiol levels, nulliparity, infertility and insulin levels) in the published literature at $P <$
116 7.14×10^{-3} (i.e. 0.05/number of known risk factors explored, n=7); none of these instrumental
117 variables were associated with these traits.
118
119 The reported effect for circulating and toenail selenium instrumental variables was expressed
120 in Z-score units per effect allele. For the purpose of Mendelian randomization analysis, Z-
121 scores were converted to beta and standard error values using the following equations, as per
122 Taylor et al (16), where N is the sample size, eaf is the effect allele frequency and SE is the
123 standard error of converted beta:
124

$$Beta = \frac{Z-score}{\sqrt{N}} \times \frac{1}{\sqrt{eaf(1-eaf)}}$$

125

$$SE = \frac{Beta}{Z-score}$$

126
127 Converted selenium level summary statistics for these instrumental variables and their
128 association with endometrial cancer risk are shown in Table 1. Because summary statistics
129 were expressed in Z-scores, neither the converted beta values for associations of genetic
130 variants with selenium levels nor the effect sizes from the Mendelian randomization analysis
131 have interpretable units, however they do provide the direction and statistical strength of
132 associations.
133
134 Individual Wald-type ratios for each of the instrumental variables were determined as a ratio
135 of instrumental variable-endometrial cancer regression over the instrumental variable-
136 selenium levels regression (17). Individual Wald-type ratios were meta-analyzed using the
137 inverse variance weighted (IVW) approach. A random effect model was used to account for
138 heterogeneity. The IVW approach assumes that instrumental variables do not exhibit
139 horizontal pleiotropy (where a single genetic variant has simultaneous effects on other
140 phenotype~~(s) independently of the exposure of interest~~that affect the outcome) or, if this is
141 violated, that the horizontal pleiotropy is "balanced" across all instrumental variables. Thus,
142 we implemented sensitivity analyses that are more robust to pleiotropy when it is
143 "unbalanced" (i.e. exhibiting directional pleiotropy): (i) weighted median analysis, which
144 provides valid causal estimate even when up to 50% of the weight comes from instrumental

3

145   variables with horizontal pleiotropic effects (18); and (ii) random effect MR-Egger analysis,
146   which provides valid pleiotropy-corrected causal estimates even if all instrumental variables
147   are invalid (19). MR-Egger analysis corrects for the directional pleiotropy by introducing an
148   intercept which captures the average pleiotropic effects of all included variants on the
149   outcome. An exponentiated MR-Egger intercept that deviates from 1 is an indicator of
150   directional pleiotropy. It should also be noted that the validity of IVW and MR-Egger
151   regression estimates rely on satisfaction of the InSIDE (instrument strength independent of
152   direct effect) assumption where the instrument strength does not correlate with the horizontal
153   pleiotropic effects on the outcome (19).
154
155   To assess the strength of the instruments, F statistics and the proportion of variance (R^2) in
156   circulating and toenail selenium explained by instrumental variables were calculated as per
157   Rees et al (20) and Yarmolinsky et al (21). We used the I^2_{GX} (22) statistic to assess weak
158   instrument bias for MR-Egger analysis using the "MendelianRandomization" package in R
159   (23). This statistic quantifies the regression dilution bias due to violation of the NO
160   Measurement Error (NOME; genetic associations with exposure of interest are measured
161   without error) assumption. An I^2_{GX} statistic approaching 1 indicates that violation of the
162   NOME assumption does not substantially dilute the effect estimates of MR-Egger analysis
163   towards a null association. Unless otherwise stated, Mendelian randomization analyses were
164   performed using the "TwoSampleMR" package in R (24).
165
166   **Results**
167   The combined multi-allelic instrument explained 2.9% of the variation in circulating and
168   toenail selenium levels. Individual Wald-type ratios and F statistics for instrumental variables
169   are presented in Table 2. F statistics for these instrumental variables were all greater than 10
170   (range 19.24 to 44.55) indicating instruments were unlikely to suffer from weak instrument
171   bias. Mendelian randomization analysis did not support an association between selenium
172   levels and endometrial cancer risk using the IVW method (OR per unit increase in selenium
173   levels Z-score = 0.99, 95% CI = 0.87-1.14, P = 0.93). We found limited evidence for
174   heterogeneity amongst the individual casual estimates for the included variants by Cochran's
175   Q statistic (25) (Cochrain's Q statistics = 7.22, P = 0.07). The exponentiated intercept of MR-
176   Egger regression was~~produced an intercept of~~ 1.03 (95% CI = 0.91-1.16, P = 0.72) and
177   therefore provided no evidence of directional pleiotropy across the multi-allelic instrument.
178   Further, the I^2_{GX} statistic, quantifying weak instrument bias in the context of MR-Egger, was
179   minimal (I^2_{GX}= 92%). This suggests that any potential bias towards a null association as a
180   result of NOME violation is ≤8%. Association estimates from sensitivity analyses (MR-Egger
181   regression and weighted median analysis) were consistent with that reported by IVW analysis
182   (OR per unit increase in selenium levels Z-score = 0.90, 95% CI = 0.53-1.50, P = 0.72 for
183   MR-Egger; OR per unit increase in selenium levels Z-score = 0.98, 95% CI = 0.89-1.08, P =
184   0.70 for weighted median).
185
186   **Discussion**
187   To our knowledge, this is the first Mendelian randomization study evaluating the effect of
188   selenium on endometrial cancer. This analysis does not support a causal relationship between
189   selenium levels and endometrial cancer risk. However, given the fact that the combined
190   multi-allelic instrument explains a small amount of the variance in circulating and toenail
191   selenium levels (<3%), the power to detect a causal association in Mendelian randomization
192   analysis may be limited and thus, we cannot rule out the possibility that genetically predicted
193   selenium levels have some effect on endometrial cancer risk. This analysis should be
194   revisited when more genome-wide significant selenium variants are identified from future,

larger GWAS studies. Further, statistical power for Mendelian randomization analyses may also be increased through the use of more precise effect estimates from larger GWAS of endometrial cancer.

The validity of Mendelian randomization analysis holds under the condition that three important assumptions are fulfilled. These assumptions require that genetic variants chosen as instrumental variables are:

1. Strongly associated with the exposure of interest
2. Not associated with any confounder(s) that affects the relationship between the exposure of interest and outcome
3. Not associated with outcome, independent of the exposure (i.e. no horizontal pleiotropy)

Our instrumental variables have high F-statistics (greater than 10), thus fulfilling assumption 1. Assumptions 2 and 3 are difficult to validate. We have attempted to minimize violation of assumption 2 by scanning associations of instrumental variables from the literature, finding none of the instrumental variables to be associated with known endometrial cancer risk factors. However, we are limited in exploring this assumption by the GWAS that have been conducted for these risk factors, and we cannot discount the possibility that associations between these variants and unknown endometrial cancer risk factors may exist. Sensitivity testing (by MR-Egger regression and weighted median analysis) has been used to address assumption 3 and we have not found evidence that this assumption has been violated. However, given the limitations of these tests (e.g. the low statistical power of the MR-Egger intercept test, discussed below), we cannot rule out this possibility.

The strengths of our study include incorporation of multiple selenium level-associated genetic variants as a multi-allelic instrument to maximize the variation in selenium levels explained; and use of the largest available GWAS datasets to provide the greatest statistical power possible. Limitations of this study include use of instrumental variables from mixed gender GWAS which were assessed in female-only endometrial cancer GWAS. Although both selenium GWASs controlled for the effect of sex, we cannot not exclude the possibility that there is a residual effect of this covariate which may violate the assumption that instrumental variables are strongly associated with the exposure. Another potential limitation of two-sample Mendelian randomization is that by using two different GWAS sample sets to obtain the instrumental variable-exposure and -outcome effect, population stratification may have confounded the observedGWAS associations despite all populations being of European descent. Weaknesses of the MR-Egger regression sensitivity analysis performed in our study include its relatively lower statistical power as compared to the IVW and weighted median analysis methods, and its vulnerability to weak instrument bias which may bias MR-Egger regression towards the null (19). However, we assessed the extent to which weak instrument bias may have affected our MR-Egger results using the I_{GX}^2 statistic, and found it to be negligible.

The identification of preventative agents for cancer is an attractive avenue of research because unlike other approaches for disease prevention, such as lifestyle changes, taking a dietary supplement (e.g. selenium) should be considerably easier to implement. Candidate dietary supplements can be identified by observational studies; however, moving these candidates through to human use requires the establishment of expensive randomized controlled trials. For example, a recent prostate cancer prevention trial, examining the benefit

245 of selenium and/or vitamin E supplement on cancer risk, failed because of adverse effects and
246 lack of efficacy, at a cost of >US$110 million (26, 27); whereas, a subsequent Mendelian
247 randomization study was able to recapitulate the results of this trial using publicly available
248 GWAS data (21).
249
250 In conclusion, Mendelian randomization analysis provided no support for selenium
251 supplementation in the prevention of endometrial cancer. More generally, these findings
252 further highlight the value of Mendelian randomization for rapidly excluding proposed
253 interventions that are unlikely to be successful, prior to the initiation of expensive and
254 lengthy trials. This approach could allow resources to be targeted towards trials of alternative
255 interventions with more promising genetic evidence.
256

257 **Conflict of Interest Statement**
258 The authors declare no potential conflicts of interest.
259

260 **Author Contributions**
261 Conception or design of the work; the acquisition, analysis and interpretation of data for the
262 work (PFK, DMG, DJT, ABS, TAO'M). Drafting the manuscript (PFK, DMG, DJT, ABS,
263 TAO'M). Agreement to be accountable for all aspects of the work in ensuring that questions
264 related to the accuracy or integrity of any part of the work are appropriately investigated and
265 resolved (PFK, DMG, DJT, ABS, TAO'M). All authors were involved in revision of the
266 manuscript and provide final approval of the version to be published.
267

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363
364

Table 1. Genetic associations with selenium levels and endometrial cancer risk

Instrumental Variables	Chr:Pos*	R^2†	EA	OA	EAF_{Se}	Z-score	$Beta_{Se}$	SE_{Se}	P_{Se}	EAF_{EC}	$Beta_{EC}$	SE_{EC}	P_{EC}
rs1789953	chr21:44482936	0.04	T	C	0.14	5.52	0.16	0.03	3.4×10^{-8}	0.13	-0.04	0.02	0.12
rs6586282	chr21:44478497		T	C	0.17	-5.89	-0.16	0.03	3.96×10^{-9}	0.17	-0.04	0.02	0.04
rs6859667	chr5:78745042	0.03	T	C	0.96	-6.92	-0.36	0.05	4.4×10^{-12}	0.96	0.02	0.04	0.54
rs921943	chr5:78316476		T	C	0.29	13.14	0.29	0.02	1.9×10^{-39}	0.29	0.00	0.02	0.90

*from hg19; †pairwise LD in Europeans (1000 Genomes) provided for instrumental variables at the same locus; Se: Selenium; EC: Endometrial cancer; EA: Effect allele; OA: Other allele; EAF: Effect allele frequency from each GWAS; Beta: effect size; SE: Standard error; P: P-value. $Beta_{EC}$ and SE_{EC} are the natural log odds ratio of endometrial cancer risk and associated standard error, respectively. Estimates for Selenium levels have been taken from (13) and estimates for EC from (14).

Table 2. F statistics and Individual Wald-type ratios for all instrumental variables

Instrumental variables	F statistic	$Beta_{Se-EC}$	SE_{Se-EC}	P_{Se-EC}
rs1789953	34.07	-0.22	0.14	0.12
rs6586282	36.88	0.26	0.13	0.04
rs6859667	19.24	-0.07	0.11	0.54
rs921943	44.55	-0.01	0.06	0.89

Se: Selenium; EC: Endometrial cancer; Beta: effect size in standard deviation unit; SE: Standard error; P: P value

9

375

10