2020-03-18

Association between levels of sex hormones and risk of esophageal adenocarcinoma and Barrett's esophagus

Xie, Shao-Hua; Fang, Rui; Huang, Mingtao; Dai, Juncheng; Thrift, Aaron P; Anderson, Lesley A; Chow, Wong-Ho; Bernstein, Leslie; Gammon, Marilie D; Risch, Harvey A; Shaheen, Nicholas J; Reid, Brian J; Wu, Anna H; Iyer, Prasad G; Liu, Geoffrey; Corley, Douglas A; Whiteman, David C; Caldas, Carlos; Pharoah, Paul; Hardie, Laura J; Fitzgerald, Rebecca C; Shen, Hongbing; Vaughan, Thomas L; Lagergren, Jesper

Clin Gastroenterol Hepatol. 2020 Nov;18(12):2701-2709.e3.
http://doi.org/10.1016/j.cgh.2019.11.030
http://hdl.handle.net/10616/47080

Title: Association Between Levels of Sex Hormones and Risk of Esophageal Adenocarcinoma and Barrett's Esophagus

Short title: Sex hormones and risk of EAC/BE

Authors: Shao-Hua Xie,[1*] Rui Fang,[2*] Mingtao Huang,[2] Juncheng Dai,[2, 3] Aaron P. Thrift,[4,5] Lesley A. Anderson,[6] Wong-Ho Chow,[7] Leslie Bernstein,[8] Marilie D. Gammon,[9] Harvey A. Risch,[10] Nicholas J. Shaheen,[11] Brian J. Reid,[12] Anna H. Wu,[13] Prasad G. Iyer,[14] Geoffrey Liu,[15] Douglas A. Corley,[16] David C. Whiteman,[17] Carlos Caldas,[18] Paul D. Pharoah,[19,20] Laura J. Hardie,[21] Rebecca C. Fitzgerald,[22] Hongbing Shen,[2,3] Thomas L. Vaughan[12] and Jesper Lagergren[1, 23]

Affiliations: [1] Upper Gastrointestinal Surgery, Department of Molecular medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, Sweden; [2] Department of Epidemiology and Biostatistics, International Joint Research Center on Environment and Human Health, Center for Global Health, School of Public Health, Nanjing Medical University, Nanjing, China; [3] Jiangsu Key Lab of Cancer Biomarkers, Prevention, and Treatment, Collaborative Innovation Center for Cancer Medicine, Nanjing Medical University, Nanjing, China; [4] Dan L Duncan Comprehensive Cancer Center, Baylor College of Medicine, Houston, Texas; [5] Section of Epidemiology and Population Sciences, Department of Medicine, Baylor College of Medicine, Houston, Texas; [6] Centre for Public Health, Queen's University Belfast, Belfast, United Kingdom; [7] Department of Epidemiology, MD Anderson Cancer Center, Houston, Texas; [8] Department of Population Sciences, Beckman Research Institute and City of Hope Comprehensive Cancer Center, Duarte, California; [9] Department of Epidemiology, University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina; [10] Department of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, Connecticut; [11] Division of

Gastroenterology and Hepatology, University of North Carolina School of Medicine, Chapel Hill, North Carolina; [12] Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington; [13] Department of Preventive Medicine, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, California; [14] Division of Gastroenterology and Hepatology, Department of Internal Medicine, Mayo Clinic, Rochester, Minnesota; [15] Pharmacogenomic Epidemiology, Ontario Cancer Institute, Toronto, Ontario, Canada; [16] Division of Research, Kaiser Permanente Northern California, Oakland, California; [17] Cancer Control, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia; [18] Cancer Research UK, Cambridge Institute, Cambridge, United Kingdom; [19] Department of Oncology, University of Cambridge, Cambridge, United Kingdom; [20] Department of Public Health and Primary Care, University of Cambridge, Cambridge, United Kingdom; [21] Division of Epidemiology, Leeds Institute of Cardiovascular and Metabolic Medicine, School of Medicine, University of Leeds, Leeds, United Kingdom; [22] Medical Research Council Cancer Unit, Hutchison-Medical Research Council Research Centre, University of Cambridge, Cambridge, United Kingdom; [23] School of Cancer and Pharmaceutical Sciences, King's College London, United Kingdom

[*] These authors contributed equally to this work.

Grant support: Supported by Bengt Ihres Foundation (grant number SLS-78016); the Ruth and Richard Julin Foundation (grant number 2018-00137); the Swedish Research Council (grant numbers 521-2014-2536 and 2015-06275); the Swedish Cancer Society (grant number CAN 2015/460); and the National Natural Science Foundation of China (grant number 8151101160).

Abbreviations: BE, Barrett's esophagus; BMI, body mass index; CI, confidence interval; EAC, esophageal adenocarcinoma; FSH, follicle stimulating hormone; GRS, genetic risk

score; GWAS, genome-wide association studies; LH, luteinizing hormone; OR, odds ratio; SNP, single nucleotide polymorphism.

Corresponding author: Shao-Hua Xie, MD, PhD, Upper Gastrointestinal Surgery, Department of Molecular medicine and Surgery, Karolinska Institutet, Karolinska University Hospital, NS 67, SE 171 76 Stockholm, Sweden (email: shaohua.xie@ki.se).

Conflict of interests: No relevant conflicts of interest exist.

Author contributions: SHX, JD, APT and JL jointly conceived and designed the study. SHX, RF, MH and JD performed data analysis. SHX prepared the first draft of the paper. LAA, WHC, LB, MDG, HAR, NJS, BJR, AHW, PGI, GL, DAC, DCW, CC, PDP, LJH, RCF, TLV and JL contributed to the collection and acquisition of data. RF, MH, JD, APT, LAA, WHC, LB, MDG, HAR, NJS, BJR, AHW, PGI, GL, DAC, DCW, CC, PDP, LJH, RCF, HS, TLV and JL critically reviewed the manuscript for important intellectual content. All authors read and approved the final version for submission.

What You Need to Know

Background: Esophageal adenocarcinoma (EAC) occurs most frequently in men. Studies are needed to determine whether levels of sex hormones are associated with risk of EAC or Barrett's esophagus (BE).

Findings: In a Mendelian randomization analysis of data from patients with EAC or BE, we found an association between genetically predicted levels of follicle stimulating and luteinizing hormones and risk of BE and EAC.

Implications for patient care: Monitoring levels of follicle stimulating and luteinizing hormones might identify patients at risk for BE and EAC.

Abstract

Background & Aims: Esophageal adenocarcinoma (EAC) occurs most frequently in men. We performed a Mendelian randomization analysis to investigate whether genetic factors that regulate levels of sex hormones associated with risk of EAC or Barrett's esophagus (BE).

Methods: We conducted a Mendelian randomization analysis using data from patients with EAC (n=2488) or BE (n=3247) and control participants (n=2127), included in international consortia of genome-wide association studies in Australia, Europe, and North America. Genetic risk scores or single nucleotide variants were used as instrumental variables for 9 specific sex hormones. Logistic regression provided odds ratios (ORs) with 95% CIs.

Results: Higher genetically predicted levels of follicle stimulating hormones were associated with increased risks of EAC and/or BE in men (OR, 1.14 per allele increase; 95% CI, 1.01-1.27) and in women (OR, 1.28; 95% CI, 1.03-1.59). Higher predicted levels of luteinizing hormone were associated with a decreased risk of EAC in men (OR, 0.92 per standard deviation increase; 95% CI, 0.87-0.99) and in women (OR, 0.93; 95% CI, 0.79-1.09), and decreased risks of BE (OR, 0.88; 95% CI, 0.77-0.99) and EAC and/or BE (OR, 0.89; 95% CI, 0.79-1.00) in women. We found no clear associations for other hormones studied, including sex hormone-binding globulin, dehydroepiandrosterone sulphate, testosterone, dihydrotestosterone, estradiol, progesterone, or free androgen index.

Conclusions: In a Mendelian randomization analysis of data from patients with EAC or BE, we found an association between genetically predicted levels of follicle stimulating and luteinizing hormones and risk of BE and EAC.

Key words: esophageal neoplasms; sex difference; gonadal steroid hormones; causality

Esophageal adenocarcinoma (EAC) and its precursor lesion Barrett's esophagus (BE) are characterized by a strong male predominance, with the male-to-female ratios of EAC incidence of 6-to-1 on average in Western countries and up to 8-to-1 in the United States.[1-3] The reasons for this striking sex difference are not known, and seem not to be explained by the two major risk factors of EAC and BE, i.e. gastroesophageal reflux disease and obesity, given the similar exposure prevalence and strengths of associations with EAC and BE risk between the sexes.[1] Abdominal obesity, which is more common in men than in women, may contribute to the male predominance in EAC and BE.[1, 4] However, in a nationwide Swedish study, the male predominance in EAC was no weaker among lean individuals compared with the overweight, arguing against obesity as a factor completely explaining the excess male risk.[5] The male predominance in EAC may be attributable to certain biological differences between the sexes. Particularly, it has been hypothesized that sex hormonal and reproductive factors may play a role in the etiology of EAC and BE, i.e. that estrogenic exposures may prevent EAC development, whereas androgens may increase EAC risk. Such hypothesis is supported by a 16-year delayed onset of EAC in women than in men.[6] Recently, it has been proposed that a more rapid age-related immune system decline in males may explain the generally higher cancer risk in males than in females,[7] which may be driven by sex hormones.[8] However, the existing epidemiologic evidence regarding the role of sex hormone in the development of EAC or BE remains not conclusive.[1, 2] Recent observational studies have suggested associations between circulating sex hormone levels and risk of EAC or BE,[9-12] but due to possible confounding and other biases inherent in observational studies, no causal relation has been established.

Mendelian randomization analysis provides a useful tool for exploring causal effects of endogenous exposures on disease risk without adding any intervention.[13] Inheriting a genetic variant, determined by the random assortment of genes at conception, associated with life-

long changes in endogenous sex hormone levels can confer altered risk for EAC or BE which are not confounded by the known risk factors for these diseases. Therefore, the use of genetically predicted sex hormone levels as instrumental variables, based on established sex hormone-associated genetic variants, can facilitate causal inferences about the relation between sex hormone levels and the risk of EAC or BE.

To test the hypothesis that genetically determined endogenous sex hormone levels influence the risk of EAC and BE, we performed a Mendelian randomization analysis based on merged data from several large genome-wide association studies (GWAS) conducted in Australia, Europe, and North America.

Methods

Study participants

We analyzed GWAS data from participants in studies included in three consortia:

1) The Barrett's and Esophageal Adenocarcinoma Genetic Susceptibility Study (BEAGESS) within the Barrett's and Esophageal Adenocarcinoma Consortium (BEACON; http://beacon.tlvnet.net/), which included 1516 patients with histologically confirmed EAC, 2416 patients with BE, and 2187 control participants from 14 population-based case-control and cohort studies conducted in Australia, Europe, and North America;[14]

2) The Barrett's Oesophagus Gene Study in the United Kingdom, which included 882 BE patients who were identified at endoscopy and confirmed with histopathology;[15]

3) The Stomach and Oesophageal Cancer Study in the United Kingdom, which included 1003 EAC patients with International Classification of Disease coding of esophageal cancer (C15) and a pathological diagnosis of adenocarcinoma (M8140-8575).[15]

After GWAS data cleaning, quality control, and imputation procedures, the current study included 2488 EAC patients, 3247 BE patients, and 2127 control participants. The distribution of participants by study is shown in Supplementary Table 1. The individual studies included in this analysis were approved by institutional review boards or research ethics committees. Informed consent was obtained from each participant.

Genotyping and imputation

Genotyping of DNA from buffy coat or whole blood samples was performed using the Illumina Omni1M Quad platform (San Diego, CA) in accordance with standard quality-control procedures.[15, 16] The annotations were based on version H of the Illumina product files and corresponded to the Genome Reference Consortium GRCh37 release. For quality

control, genotyped single nucleotide polymorphisms (SNPs) or samples with call rate <95% were excluded. Based on control participants, SNPs with Hardy-Weinberg Equilibrium *P* value $<10^{-4}$ or minor allele frequency <0.01 were also excluded. Imputation was conducted at the study level, based on SHAPEIT2/IMPUTE2 using 1000 Genomes Phase 3 integrated variant set release in NCBI build 37 (hg19) coordinates.[17] Post-imputation quality control excluded SNPs with IMPUTE2 info score <0.8, call rate <95%, Hardy-Weinberg Equilibrium *P* value $<10^{-4}$ based on control participants, or minor allele frequency <0.01 in control participants.

Genetic risk scores or genetic variants of sex hormones

SNPs associated with sex hormones at the GWAS significance level ($P < 5\times10^{-8}$) in populations of European descent were identified from published GWAS indexed in the NHGRI-EBI GWAS Catalog (http://www.ebi.ac.uk/gwas). SNPs predicting the levels of the following nine sex hormones were found: sex hormone-binding globulin,[18-20] dehydroepiandrosterone sulphate,[20, 21] testosterone,[22, 23] dihydrotestosterone,[23] estradiol.[20] follicle stimulating hormone (FSH),[20] luteinizing hormone (LH),[20, 24] progesterone,[20] and free androgen index.[20] Multiple SNPs were identified for each of the following six hormone measures: sex hormone-binding globulin, dehydroepiandrosterone sulphate, testosterone, dihydrotestosterone, LH, and progesterone. For these six hormones, sex hormone-specific genetic risk scores (GRSs) were calculated by summing the number of risk alleles (0 for none, 1 for heterozygous, and 2 for homozygous) weighted by the per allele change in the sex hormone level for each participant. For example, we constructed a GRS of sex hormone-binding globulin for each male participant based on five SNPs as follows:

$GRS_{\text{sex hormone-binding globulin}}$ in men = rs12150660-T × 0.110 + rs2411984-A × 0.034 – rs7910927-T × 0.050 – rs293428-A × 0.029 – rs1042522-G × 0.127.[18, 20]

Sex-specific GRSs were constructed for sex hormone-binding globulin, whereas GRSs for testosterone and dihydrotestosterone were constructed in men only because the availability of identified SNPs predicting levels of these sex hormone measures limited to men. More detailed information about the included genetic variants is presented in Table 1.

Covariates

We assumed that genetically predicted hormone levels were not associated with any risk factor for EAC or BE, and thus act as confounders. Yet, in the analyses (see Statistical Analysis below) we still considered the potential influence of the main risk factors recurrent gastroesophageal reflux symptoms,[25] body mass index (BMI),[26] and tobacco smoking.[27] Information on these four covariates was retrieved from written questionnaires or personal interviews. Data were harmonized across studies and merged into a single dataset. Recurrent reflux symptoms were defined as symptoms of heartburn or regurgitation occurring at least weekly. BMI was calculated as the body weight divided by square of height (kg/m^2). Adult weight before any disease-related weight loss was used when available. Otherwise, we used weight at 1 year, 5 years, or 20 years before the data collection, depending on the varying data collection in the individual studies. Participants who had ever smoked at least 100 cigarettes or smoked regularly were defined as ‘ever smokers’. In BEAGESS, the missing data on covariates was low, but among the 1885 patients with EAC or BE from the United Kingdom, information regarding reflux symptoms was missing in 776 (41%) participants and BMI data were missing in 1209 (64%) participants.

Statistical analysis

Logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (CI) for the associations between sex hormone-specific GRSs or single SNPs and the risk of EAC, BE, as well as a combined outcome of EAC or BE (hereafter 'EAC/BE'), and separately in men and women. The ORs were adjusted for age (continuous) and the first four principal components that reflected the population structure to control for population stratification. The GRSs and single SNPs were included in the models as continuous variables and the ORs and 95% CIs were calculated for per standard deviation increase in GRS and for per allele increase when single SNPs were used.

To ensure the 'exclusion restriction' assumption of an instrumental variable analysis that the instrumental variables (GRSs or single SNPs) were independent of the four covariates, we assessed the associations between the instrumental variables and these covariates, i.e. recurrent reflux symptoms (yes or no), BMI (continuous), and tobacco smoking (yes or no) among the control participants, using analysis of variance or chi square test, whichever was appropriate. Participants with missing data were excluded in each of these analyses.

We used the MR-Egger method, which was adapted from the Egger regression used in meta-analysis, to assess the possible pleiotropic effects (in which a SNP might affect more than 1 phenotypic characteristics) of the SNPs included in the GRSs. In the MR-Egger regression, an intercept differing from zero suggests existence of directional horizontal pleiotropy. The MR-Egger regression was performed for the GRSs based on three or more SNPs only, i.e. those for sex hormone-binding globulin, dehydroepiandrosterone sulphate, and testosterone. Some SNPs were included in two or more GRSs of different sex hormones, i.e. rs12150660 and rs727428 predicting both sex hormone-binding globulin and testosterone. Thus, we re-

estimated the associations between the corresponding GRSs and the risk of EAC and BE after excluding these SNPs, to assess the robustness of the estimates.

All statistical tests were two-sided. The statistical software packages R 3.4.2 (R Foundation for Statistical Computing, Vienna, Austria) and SAS 9.4 (SAS Institute, Cary, NC) were used for the analyses.

Power estimation

We estimated the statistical power using in the web tool mRnd for power calculations in Mendelian randomization analysis (http://cnsgenomics.com/shiny/mRnd).[28] Assuming the predicting SNPs explain 20% of the variance in levels of a sex hormone, with the given sample size at the significance level of 0.05, our study had 78% and 29% power to detect an OR of 1.2 in men and in women, respectively, for per standard deviation change in the sex hormone levels.

Results

Participants

Selected characteristics of the study participants are shown in Table 2. The mean age (standard deviation) was 65.1 (10.4) years in EAC patients, 63.1 (12.0) years in BE patients, and 61.7 (11.2) in control participants. There were more male than female participants in all groups. Compared with control participants, more patients with EAC and BE had recurrent reflux symptoms, higher BMI, and were ever smokers.

Genetically predicted sex hormone levels and EAC/BE risk

Table 3 presents the sex-specific ORs and 95% CIs for GRSs or single SNPs predicting sex hormone levels in relation to the risk of EAC, BE and EAC/BE. Higher genetically predicted FSH levels were associated with increased risks of EAC, BE, and EAC/BE in both men and women. The point estimates (ORs) for per allele increase in FSH level were numerically higher in women (OR 1.28, 95% CI 1.03 to 1.59 for EAC/BE) than in men (OR 1.14, 95% CI 1.01 to 1.27 for EAC/BE).

Higher genetically predicted LH levels were associated with reduced risk of EAC in men (OR for per standard deviation increase 0.92, 95% CI 0.87 to 0.99), and we observed a similar association in women (OR 0.93, 95% CI 0.79 to 1.09). Higher genetically predicted LH levels were also associated with reduced risks of BE (OR for per standard deviation increase 0.88, 95% CI 0.77 to 0.99) and EAC/BE (OR 0.89, 95% CI 0.79 to 1.00) in women, but no such associations were observed in men.

No statistically significant associations were found between single SNPs of estradiol or free androgen index and the risk of EAC, BE, or EAC/BE in any of the sexes. No associations

were observed between GRSs of sex hormone-binding globulin, dehydroepiandrosterone sulphate, or progesterone and the risk of EAC, BE, or EAC/BE in men or women. No associations were found for GRS of testosterone or dihydrotestosterone in men. All ORs for per standard deviation increase in GRSs of these sex hormones were close to one (range 0.95-1.15).

Assessment of pleiotropy

The MR-Egger regressions found no evidence for pleiotropy for GRSs of sex hormone-binding globulin (MR-Egger intercept 0.013, *P*=0.724 for EAC/BE in men; intercept -0.012, *P*=0.219 in women) or testosterone (intercept -0.028, *P*=0.352) (Supplementary Table 2). The ORs for sex hormone-binding globulin and testosterone remained unchanged after excluding the SNPs predicating levels of multiple sex hormones from the GRSs (Table 3). On the other hand, pleiotropy was indicated for GRS of testosterone (MR-Egger intercept 0.111, *P*=0.045 for EAC/BE in men; intercept 0.231, *P*=0.055 in women; Supplementary Table 2).

Independence of instrumental variables with covariates

As expected, the genetic variants for FSH and LH were not associated with any of the four covariates, i.e. recurrent reflux symptoms, BMI, or tobacco smoking in control participants (*P* <0.05 for all comparisons; Supplementary Tables 3-5).

Discussion

This Mendelian randomization analysis indicated that higher genetically predicted FSH levels increase the risk of EAC and BE and higher LH levels decrease the risk, in both sexes. No associations were found for the other seven sex hormones under study.

The strong male predominance in EAC and BE has prompted the hypothesis that sex hormonal and reproductive factors may be involved in the etiology of these conditions. But the existing evidence is limited and inconclusive.[1, 2] A few studies have directly investigated the associations between circulating sex hormone levels and the risk of EAC or BE.[9-12] However, these studies were all restricted to men because of the low incidence of EAC in women, and most had a cross-sectional design, i.e. the sex hormone levels were tested at the time of the cancer diagnosis for which why the temporal relation could not be established. A recent case-control study nested in prospective cohorts found inverse associations between higher circulating levels of dehydroepiandrosterone and estradiol and the risk of EAC or gastric cardia adenocarcinoma in men,[12] but these findings were not supported by the results of the present study. No previous study has specifically assessed the association between endogenous FSH or LH levels and risk of EAC or BE.

FSH and LH are essential gonadotropins, stimulating the secretion of sex steroids in both sexes.[29-31] Elevated levels of these hormones have been associated with some health problems, e.g. increased FSH levels may cause infertility in women,[32] and higher LH levels may contribute to cognitive deficits in Alzheimer's disease.[33] The increased risk of EAC and BE associated with higher FSH levels observed in this study is in line with previous findings of a decreased risk of EAC associated with more childbearing and breastfeeding.[34] Interestingly, the receptors of both FSH and LH are highly expressed in the human lower esophagus, i.e. where EAC and BE arise. According to the Bgee dataBase for Gene Expression Evolution, a

database to retrieve and compare gene expression patterns in multiple species, the expression levels of FSH and LH receptors are in fact highest in the lower esophagus among all anatomical entities with available expression data in human.[35] Yet, the specific downstream mechanisms after binding to their receptors in EAC development are unclear. Notably, the FSH receptor has been found to be selectively expressed on the endothelial surface of the blood vessels of a wide range of tumors,[36] indicating an angiogenesis-related mechanism for the potential involvement of FSH in tumor development. The specificity of associations observed only with FSH and LH in the present study suggests that these two hormones may be involved in the development of EAC through pathways independent of other sex hormones. It should be noted that two genetic variants used for predicting FSH and LH levels (rs11031005 and rs11031002) in this study are in linkage disequilibrium with a functional polymorphism in the promoter of *FSHB* gene, which codes for the beta polypeptide of FSH.[20] In addition, previous studies have generated conflicting findings regarding the direction of effect of these variants on FSH and LH levels,[37-39] although we assumed the minor allele would be negatively associated with FSH levels and positively associated with LH levels based on the results of the only relevant GWAS.[20] Overall, the specific etiologic roles and mechanisms of FSH and LH in EAC development remain to be identified.

EAC has a poor prognosis, with an overall 5-year survival rate below 20% in Western populations.[2] Clarifying the role of sex hormones in the development of this cancer may unravel novel targets for prevention and treatment. If an important role of FSH and LH in EAC development is confirmed in future research, it may be worth evaluating potential therapeutic targets, e.g. blocking FSH receptors signaling in the prevention of EAC among high-risk individuals and as adjuvant therapy to counteract tumor recurrences in patients who have undergone curatively intended treatment.

This study is, to the best of our knowledge, the first Mendelian randomization analysis of associations between endogenous sex hormone levels and the risk of EAC and BE. We used data from many high-quality GWASs, which have been merged and analyzed in collaboration through large consortia. A weakness is the lack of a replication analysis in an independent sample, but the availability of such a sample collection will depend on future large-scale collaborative endeavors because of the relatively low incidence of EAC. However, the observed associations, particularly for FSH levels, are less likely to be due to chance considering the consistent findings in separate analyses of EAC, BE, and EAC/BE, as well as in both sexes. In a Mendelian randomization analysis, the genetic variants are ideally strongly associated with the endogenous exposure of interest to avoid weak-instrument problems, i.e. biased results if the 'exclusion restriction' is violated or lowered statistical power,[40] which might be a limitation in the present study. Because only a limited number of genetic variants predicting sex hormone levels have been identified from existing GWAS, the instrumental variables used in this study were based on no more than five genetic variants or even single variants only. This could have reduced the statistical power, particularly in the analyses with relatively weak instruments. Specifically, only one or two SNPs have been used for predicting endogenous FSH and LH levels, and these SNPs only account for a small proportion of the variations in FSH and LH levels (Supplementary Table 6). Thus, the estimated associations between genetically predicted sex hormone levels and risk of EAC or BE were probably biased towards the null in this Mendelian randomization analysis. Potential pleiotropy of the SNPs used for predicting sex hormone levels could not be ruled out. Notably, a few SNPs used in this study correlated moderately or strongly, including the pair of rs11031005 predicting FSH levels and rs11031002 predicting LH levels (r^2 of linkage disequilibrium 0.79). Thus, the observed specific genetic instrument-outcome associations might be partially attributable to correlations between sex hormones. In addition, although all

selected sex hormone-associated SNPs have been confirmed by GWAS in populations of European descent,[18-23] we were unable to verify the validity of the instrumental variables in the study due to unavailability of directly measured sex hormone levels. Taken together, the findings of the present study need to be interpreted with caution when making causal inferences.

In summary, this Mendelian randomization analysis based on GWAS data from high-quality studies provides the first line of evidence of a role of endogenous FSH and LH levels in the etiology of EAC and BE. Whether the observed associations are causal remains to be confirmed in independent samples with valid instruments or in randomized controlled trials, if ethical and feasible.

References

1. Xie SH, Lagergren J. The Male Predominance in Esophageal Adenocarcinoma. Clin Gastroenterol Hepatol 2016;14:338-347 e1.
2. Coleman HG, Xie SH, Lagergren J. The Epidemiology of Esophageal Adenocarcinoma. Gastroenterology 2018;154:390-405.
3. Xie SH, Lagergren J. Social group disparities in the incidence and prognosis of oesophageal cancer. United European Gastroenterol J 2018;6:343-348.
4. Singh S, Sharma AN, Murad MH, et al. Central adiposity is associated with increased risk of esophageal inflammation, metaplasia, and adenocarcinoma: a systematic review and meta-analysis. Clin Gastroenterol Hepatol 2013;11:1399-1412 e7.
5. Lagergren K, Mattsson F, Lagergren J. Abdominal fat and male excess of esophageal adenocarcinoma. Epidemiology 2013;24:465-6.
6. Derakhshan MH, Liptrot S, Paul J, et al. Oesophageal and gastric intestinal-type adenocarcinomas show the same male predominance due to a 17 year delayed development in females. Gut 2009;58:16-23.
7. Palmer S, Albergante L, Blackburn CC, et al. Thymic involution and rising disease incidence with age. Proc Natl Acad Sci U S A 2018;115:1883-1888.
8. Taneja V. Sex Hormones Determine Immune Response. Front Immunol 2018;9:1931.
9. Cook MB, Wood S, Hyland PL, et al. Sex steroid hormones in relation to Barrett's esophagus: an analysis of the FINBAR Study. Andrology 2017;5:240-247.
10. Petrick JL, Falk RT, Hyland PL, et al. Association between circulating levels of sex steroid hormones and esophageal adenocarcinoma in the FINBAR Study. PLoS One 2018;13:e0190325.
11. Cook MB, Wood SN, Cash BD, et al. Association between circulating levels of sex steroid hormones and Barrett's esophagus in men: a case-control analysis. Clin Gastroenterol Hepatol 2015;13:673-82.
12. Petrick JL, Hyland PL, Caron P, et al. Associations Between Prediagnostic Concentrations of Circulating Sex Steroid Hormones and Esophageal/Gastric Cardia Adenocarcinoma Among Men. J Natl Cancer Inst 2019;111:34-41.
13. Boef AG, Dekkers OM, le Cessie S. Mendelian randomization studies: a review of the approaches used and the quality of reporting. Int J Epidemiol 2015;44:496-511.
14. Levine DM, Ek WE, Zhang R, et al. A genome-wide association study identifies new susceptibility loci for esophageal adenocarcinoma and Barrett's esophagus. Nat Genet 2013;45:1487-93.
15. Gharahkhani P, Fitzgerald RC, Vaughan TL, et al. Genome-wide association studies in oesophageal adenocarcinoma and Barrett's oesophagus: a large-scale meta-analysis. Lancet Oncol 2016;17:1363-1373.
16. Laurie CC, Doheny KF, Mirel DB, et al. Quality control and quality assurance in genotypic data for genome-wide association studies. Genet Epidemiol 2010;34:591-602.
17. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009;5:e1000529.
18. Coviello AD, Haring R, Wellons M, et al. A genome-wide association meta-analysis of circulating sex hormone-binding globulin reveals multiple Loci implicated in sex steroid hormone regulation. PLoS Genet 2012;8:e1002805.

19. Prescott J, Thompson DJ, Kraft P, et al. Genome-wide association study of circulating estradiol, testosterone, and sex hormone-binding globulin in postmenopausal women. PLoS One 2012;7:e37815.
20. Ruth KS, Campbell PJ, Chew S, et al. Genome-wide association study with 1000 genomes imputation identifies signals for nine sex hormone-related phenotypes. Eur J Hum Genet 2016;24:284-90.
21. Prins BP, Kuchenbaecker KB, Bao Y, et al. Genome-wide analysis of health-related biomarkers in the UK Household Longitudinal Study reveals novel associations. Sci Rep 2017;7:11008.
22. Ohlsson C, Wallaschofski H, Lunetta KL, et al. Genetic determinants of serum testosterone concentrations in men. PLoS Genet 2011;7:e1002313.
23. Jin G, Sun J, Kim ST, et al. Genome-wide association study identifies a new locus JMJD1C at 10q21 that may influence serum androgen levels in men. Hum Mol Genet 2012;21:5222-8.
24. Sun BB, Maranville JC, Peters JE, et al. Genomic atlas of the human plasma proteome. Nature 2018;558:73-79.
25. Cook MB, Corley DA, Murray LJ, et al. Gastroesophageal reflux in relation to adenocarcinomas of the esophagus: a pooled analysis from the Barrett's and Esophageal Adenocarcinoma Consortium (BEACON). PLoS One 2014;9:e103508.
26. Hoyo C, Cook MB, Kamangar F, et al. Body mass index in relation to oesophageal and oesophagogastric junction adenocarcinomas: a pooled analysis from the International BEACON Consortium. Int J Epidemiol 2012;41:1706-18.
27. Cook MB, Kamangar F, Whiteman DC, et al. Cigarette smoking and adenocarcinomas of the esophagus and esophagogastric junction: a pooled analysis from the international BEACON consortium. J Natl Cancer Inst 2010;102:1344-53.
28. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. Int J Epidemiol 2013;42:1497-501.
29. Filicori M. The role of luteinizing hormone in folliculogenesis and ovulation induction. Fertil Steril 1999;71:405-14.
30. Howles CM. Role of LH and FSH in ovarian function. Mol Cell Endocrinol 2000;161:25-30.
31. Araujo AB, Wittert GA. Endocrinology of the aging male. Best Pract Res Clin Endocrinol Metab 2011;25:303-19.
32. Baird DT, Collins J, Egozcue J, et al. Fertility and ageing. Hum Reprod Update 2005;11:261-76.
33. Burnham VL, Thornton JE. Luteinizing hormone as a key player in the cognitive decline of Alzheimer's disease. Horm Behav 2015;76:48-56.
34. Cronin-Fenton DP, Murray LJ, Whiteman DC, et al. Reproductive and sex hormonal factors and oesophageal and gastric junction adenocarcinoma: a pooled analysis. Eur J Cancer 2010;46:2067-76.
35. Bastian F, Parmentier G, Roux J, et al. Bgee: Integrating and Comparing Heterogeneous Transcriptome Data Among Species. DILS: Data Integration in Life Sciences. Lecture Notes in Computer Science. Volume 5109, 2008:124-131.
36. Radu A, Pichon C, Camparo P, et al. Expression of follicle-stimulating hormone receptor in tumor blood vessels. N Engl J Med 2010;363:1621-30.
37. Schuring AN, Busch AS, Bogdanova N, et al. Effects of the FSH-beta-subunit promoter polymorphism -211G->T on the hypothalamic-pituitary-ovarian axis in normally cycling women indicate a gender-specific regulation of gonadotropin secretion. J Clin Endocrinol Metab 2013;98:E82-6.

38. Grigorova M, Punab M, Ausmees K, et al. FSHB promoter polymorphism within evolutionary conserved element is associated with serum FSH level in men. Hum Reprod 2008;23:2160-6.
39. Grigorova M, Punab M, Zilaitiene B, et al. Genetically determined dosage of follicle-stimulating hormone (FSH) affects male reproductive parameters. J Clin Endocrinol Metab 2011;96:E1534-41.
40. Davies NM, von Hinke Kessler Scholder S, Farbmacher H, et al. The many weak instruments problem and Mendelian randomization. Stat Med 2015;34:454-68.

Table 1. Characteristics of selected genetic variant associated with sex hormone levels from previous genome-wide association studies

Sex(s)	Hormone	SNP	Chr.	Position	Gene	Minor/ major allele	Effect/ other allele	Beta *	*P* †	Call rate
Male	Sex hormone-binding globulin	rs12150660	17	7521915	*SHBG*	T/G	T/G	0.110	4×10^{-80}	0.99
Male	Sex hormone-binding globulin	rs2411984	17	47445751	*ZNF652*	A/G	A/G	0.034	2×10^{-10}	0.98
Male	Sex hormone-binding globulin	rs7910927	10	65138910	*JMJD1C*	G/T	T/G	-0.050	1×10^{-25}	1.00
Male	Sex hormone-binding globulin	rs293428	4	69591782	*UGT2B15*	G/A	A/G	-0.029	3×10^{-8}	1.00
Female	Sex hormone-binding globulin	rs12150660	17	7521915	*SHBG*	T/G	T/G	0.087	6×10^{-30}	0.99
Female	Sex hormone-binding globulin	rs7910927	10	65138910	*JMJD1C*	G/T	T/G	-0.046	2×10^{-13}	1.00
Female	Sex hormone-binding globulin	rs780093	2	27742603	*GCKR*	T/C	T/C	-0.041	9×10^{-11}	1.00
Female	Sex hormone-binding globulin	rs727428	17	7537792	*FXR2/SHBG/ SAT2/ATP1B2*	T/C	T/C	-0.126	2×10^{-16}	1.00
Both	Sex hormone-binding globulin	rs1042522 ‡	17	7520197	*TP53*	G/C	G/C	-0.127	1×10^{-15}	1.00
Both	Dehydroepiandrosterone sulphate	rs78900934	1	101738121	*PPIAP7*	A/G	A/C	0.050	6×10^{-12}	1.00
Both	Dehydroepiandrosterone sulphate	rs2911280	16	81591313	*CMIP*	A/G	A/G	0.090	6×10^{-10}	0.99
Both	Dehydroepiandrosterone sulphate	rs148982377	7	99075038	*ZNF789*	C/T	C/T	-0.255	2×10^{-14}	1.00
Male	Testosterone	rs12150660	17	7521915	*SHBG*	T/G	T/G	1.103	1×10^{-41}	0.99
Male	Testosterone	rs6258	17	7534678	*SHBG*	T/C	T/C	-2.856	2×10^{-22}	1.00
Male	Testosterone	rs10822184	10	65337153	*JMJD1C*	C/T	T/C	-0.058	1×10^{-8}	0.99
Male	Testosterone	rs727428	17	7537792	*SHBG*	T/C	T/C	-0.073	1×10^{-12}	1.00
Male	Dihydrotestosterone	rs72829446	17	7552123	*SHBG*	T/C	T/C	0.164	9×10^{-10}	0.98
Male	Dihydrotestosterone	rs727428	17	7537792	*SHBG*	T/C	T/C	-0.103	1×10^{-11}	1.00
Both	Progesterone	rs112295236	11	62915346	*SLC22A9*	G/C	G/C	0.255	8×10^{-12}	0.99

Both	Progesterone	rs34670419	7	99130834	*ZKSCAN5*	T/G	T/G	-0.346	6×10^{-14}	0.99
Both	Estradiol	rs117585797	12	6011490	*ANO2*	A/C	A/C	Single variant	2×10^{-8}	0.98
Both	Follicle-stimulating hormone	rs11031005	11	30226356	*FSHB*	C/T	C/T	Single variant	2×10^{-8}	0.99
Both	Luteinizing hormone	rs11031002	11	30215261	*FSHB*	A/T	A/T	0.221	4×10^{-9}	1.00
Both	Luteinizing hormone	rs139643250	19	49517146	*RUVBL2*	T/C	T/C	-0.68	3×10^{-50}	
Both	Free androgen index	rs117145500	16	52947630	*LOC643714*	C/A	C/A	Single variant	2×10^{-8}	0.99

[*] Changes per effect allele in µmol/L for dehydroepiandrosterone sulphate, in unit/L for luteinizing hormone, and in nmol/L for other hormones.

[†] *P* value for the association between the single nucleotide variant and the specific sex hormone measure as reported in the original genome-wide association study.

[‡] Replacing rs1641549 of high linkage disequilibrium (r^2=0.95) due to low call rate (0.44).

Chr.: chromosome; SNP: single nucleotide polymorphism.

Table 2. Characteristics in study participants, number (%)

Characteristic	Control participants (N=2127)	Esophageal adenocarcinoma patients (N=2488)	Barrett's esophagus patients (N=3247)
Age, years			
<50	301 (14.2)	185 (7.4)	438 (13.5)
50-59	533 (25.1)	540 (21.7)	766 (23.6)
60-69	736 (34.6)	878 (35.3)	1002 (30.9)
70-79	521 (24.5)	688 (27.7)	827 (25.5)
≥80	36 (1.7)	177 (7.1)	207 (6.4)
Missing	0 (0)	20 (0.8)	7 (0.2)
Mean ± standard deviation	61.7 ± 11.2	65.1 ± 10.4	63.1 ± 12.0
Sex			
Male	1670 (78.5)	2173 (87.3)	2454 (75.6)
Female	457 (21.5)	315 (12.7)	793 (24.4)
Recurrent reflux symptoms			
No	1411 (66.3)	956 (38.4)	1042 (32.1)
Yes	344 (16.2)	845 (34.0)	1164 (35.9)
Missing	384 (18.1）	687 (27.6)	1041 (32.1)
Body mass index			
<25	772 (36.3)	241 (9.7)	596 (18.4)
25-29.9	918 (4.2)	442 (17.8)	1178 (36.3)
≥30	420 (19.7)	295 (11.9)	919 (28.3)
Missing	17 (0.8)	1510 (60.7)	554 (17.1)
Mean ± standard deviation	27.0 ± 4.7	28.4 ± 5.2	28.7 ± 5.1
Tobacco smoking			
Never	866 (40.7)	563 (22.6)	1065 (32.8)
Ever	1249 (58.7)	1664 (66.9)	1964 (60.5)
Missing	12 (0.6)	261 (10.5)	219 (6.7)

Table 3. Associations between genetic risk scores or single nucleotide variants of sex hormones and the risk of esophageal adenocarcinoma (EAC) and Barrett's esophagus (BE)

Sex	Hormone	Missing *	EAC OR (95% CI) †	BE OR (95% CI) †	EAC/BE OR (95% CI) †
			Single nucleotide variants		
Male	Follicle-stimulating hormone	3	1.17 (1.03, 1.34)	1.12 (0.99, 1.27)	1.14 (1.01, 1.27)
Female	Follicle-stimulating hormone	0	1.29 (0.96, 1.73)	1.26 (1.00, 1.59)	1.28 (1.03, 1.59)
Male	Estradiol	101	1.23 (0.69, 2.18)	0.74 (0.40, 1.35)	0.95 (0.57, 1.60)
Female	Estradiol	29	0.28 (0.03, 2.43)	0.49 (0.14, 1.68)	0.42 (0.13, 1.35)
Male	Free androgen index	73	1.09 (0.91, 1.31)	1.08 (0.90, 1.29)	1.09 (0.93, 1.28)
Female	Free androgen index	20	0.82 (0.52, 1.30)	0.74 (0.52, 1.05)	0.78 (0.56, 1.08)
			Genetic risk scores		
Male	Luteinizing hormone	91	0.92 (0.87, 0.99)	0.99 (0.93, 1.06)	0.96 (0.91, 1.02)
Female	Luteinizing hormone	22	0.93 (0.79, 1.09)	0.88 (0.77, 0.99)	0.89 (0.79, 1.00)
Male	Sex hormone-binding globulin	171	0.96 (0.91, 1.04)	0.99 (0.93, 1.05)	0.98 (0.93, 1.04)
Male	Sex hormone-binding globulin ‡	102	0.97 (0.91, 1.04)	1.00 (0.94, 1.07)	0.99 (0.94, 1.05)
Female	Sex hormone-binding globulin	14	1.04 (0.89, 1.21)	0.97 (0.86, 1.09)	0.99 (0.89, 1.11)
Female	Sex hormone-binding globulin ‡	0	0.97 (0.84, 1.13)	1.00 (0.90, 1.13)	1.00 (0.90, 1.12)
Male	Dehydroepiandrosterone sulphate	81	0.98 (0.92, 1.05)	0.98 (0.92, 1.05)	0.98 (0.92, 1.04)
Female	Dehydroepiandrosterone sulphate	24	1.15 (0.97, 1.36)	0.98 (0.88, 1.11)	1.02 (0.91, 1.14)
Male	Progesterone	72	0.99 (0.93, 1.06)	0.97 (0.91, 1.04)	0.98 (0.92, 1.04)

Female	Progesterone	19	0.95 (0.81, 1.11)	0.97 (0.86, 1.09)	0.96 (0.86, 1.07)
Male	Testosterone	89	0.95 (0.89, 1.02)	0.97 (0.91, 1.03)	0.96 (0.91, 1.02)
Male	Testosterone ‡	20	0.97 (0.90, 1.03)	0.99 (0.93, 1.06)	0.98 (0.92, 1.04)
Male	Dihydrotestosterone	129	1.03 (0.97, 1.10)	0.97 (0.91, 1.04)	1.00 (0.95, 1.06)

* Number of missing values of genetic risk score or single nucleotide variant.

† Odd ratios (95% confidence intervals) of per allele increase in estradiol, follicle-stimulating hormone and free androgen index and odds ratios of per standard deviation increase in genetic risk score for the remaining, adjusted for age (continuous) and the first four genetic principal components.

‡ Excluding SNPs rs12150660 and rs727428.

Supplementary Table 1. Distribution of study participants by study

Location		EAC cases	BE case	Control participants	Total
Barrett's and Esophageal Adenocarcinoma Consortium (BEACON)					
Australia	Australia-wide	236	0	248	481
	Queensland, Australia	0	326	323	649
Europe	Sheffield, England	102	167	0	269
	Sweden-wide	64	0	116	180
	Ireland	194	199	218	611
North America	Northern California, United States	0	242	215	457
	Washington & New Jersey, United States	56	0	114	170
	Rochester, Minnesota, United States	503	814	0	1317
	Toronto, Ontario, Canada	248	0	259	507
	Raleigh, North Carolina, United States	0	100	0	100
	Washington, United States	0	157	167	324
	Washington, United States	0	296	0	296
	Nova Scotia, Canada	54	115	92	261
	Los Angeles, California, United States	59	0	438	497
	Subtotal in BEACON	1516	2416	2187	6119
Barrett's Oesophagus Gene Study					
Europe	United Kingdom-wide excluding Northern Ireland	0	882	0	882
Stomach and Oesophageal Cancer Study					
Europe	United Kingdom-wide excluding Northern Ireland	1003	0	0	1003
Total genotyped		2519	3298	2187	8004
Total analyzed in this study		2488	3247	2127	7862

Supplementary Table 2. Assessment of directional pleiotropy using the MR-Egger method

Sex	Hormone	EAC		BE		EAC/BE	
		Intercept (SE)	*P* *	Intercept (SE)	*P* *	Intercept (SE)	*P* *
Male	Sex hormone-binding globulin	-0.021 (0.05)	0.680	0.021 (0.040)	0.599	0.013 (0.037)	0.724
Female	Sex hormone-binding globulin	-0.121 (0.127)	0.343	-0.092 (0.101)	0.361	-0.112 (0.091)	0.219
Male	Dehydroepiandrosterone sulphate	0.110 (0.066)	0.098	0.106 (0.066)	0.112	0.111 (0.055)	0.045
Female	Dehydroepiandrosterone sulphate	0.255 (0.030)	0.263	0.125 (0.082)	0.128	0.231 (0.121)	0.055
Male	Testosterone	-0.048 (0.037)	0.192	-0.009 (0.037)	0.810	-0.028 (0.030)	0.352

* The *P* value of the intercept is a test of directional pleiotropy.

BE: Barrett's esophagus; CI: confidence interval; EAC: esophageal adenocarcinoma; OR: odds ratio; SE: standard error.

Supplementary Table 3. Distribution of covariates by genotype rs11031005 predicting follicle stimulating hormone levels in control participants

Covariates	Genotype			*P* value*
	TT	TC	CC	
Recurrent reflux symptoms, n (%)				
No	990 (79.5)	384 (83.5)	37 (74.0)	0.096
Yes	255 (20.5)	76 (16.5)	13 (26.0)	
Body mass index				
Mean ± standard deviation	27.0 ± 4.6	27.0 ± 5.0	26.7 ± 3.8	0.890
Tobacco smoking, n (%)				
Never	614 (40.8)	225 (40.5)	27 (49.1)	0.460
Ever	890 (59.2)	330 (59.5)	28 (50.9)	

* From analysis of variance for body mass index and chi square tests for the other variables

Supplementary Table 4. Distribution of covariates by genotype rs11031002 predicting luteinizing hormone levels in control participants

Covariates	Genotype			*P* value*
	TT	TA	AA	
Recurrent reflux symptoms, n (%)				
No	1012 (79.6)	366 (83.6)	32 (74.4)	0.115
Yes	260 (20.4)	72 (16.4)	11 (25.6)	
Body mass index				
Mean ± standard deviation	27.0 ± 4.6	27.0 ± 5.0	27.0 ± 3.9	0.999
Tobacco smoking, n (%)				
Never	625 (40.7)	218 (41.2)	23 (48.9)	0.526
Ever	910 (59.3)	311 (58.8)	24 (51.1)	

* From analysis of variance for body mass index and chi square tests for the other variables

Supplementary Table 5. Distribution of covariates by genotype rs139643250 predicting luteinizing hormone levels in control participants

Covariates	Genotype			*P* value*
	CC	TC	TT	
Recurrent reflux symptoms, n (%)				
No	1200 (80.2)	185 (81.1)	9 (100.0)	0.312
Yes	297 (19.8)	43 (18.9)	0 (0.0)	
Body mass index				
Mean ± standard deviation	27.0 ± 4.6	27.0 ± 5.0	27.7 ± 5.0	0.858
Tobacco smoking, n (%)				
Never	737 (40.8)	117 (42.4)	7 (58.3)	0.417
Ever	1067 (59.2)	159 (57.6)	5 (41.7)	

* From analysis of variance for body mass index and chi square tests for the other variables

Supplementary Table 5. Assessment of instrument strength for genetic variants predicting follicle-stimulating hormone and luteinizing hormone levels

Sex	Hormone	SNP	Minor allele effect (% standard deviation)	R^2	Number of participants	F *
Male	Follicle-stimulating hormone	rs11031005	-0.232 [1]	0.013	6294	86
Female	Follicle-stimulating hormone	rs11031005	-0.232 [1]	0.013	1565	22
Male	Luteinizing hormone	rs11031002	0.252 [1]	0.016	6285	103
Female	Luteinizing hormone	rs11031002	0.252 [1]	0.016	1560	26
Male	Luteinizing hormone	rs139643250	-0.893 [2]	0.166	6218	1239
Female	Luteinizing hormone	rs139643250	-0.893 [2]	0.166	1548	309

* First-stage F-statistics calculated as $F = \frac{R^2(\mathrm{N}-1-\mathrm{k})}{(1-R^2)\mathrm{k}}$, where R^2 is the proportion of variability in the sex hormone levels explained by the genetic variant, N is the sample size, and k is the number of instrument. [3]

References:

[1] Ruth KS, Campbell PJ, Chew S, et al. Genome-wide association study with 1000 genomes imputation identifies signals for nine sex hormone-related phenotypes. Eur J Hum Genet 2016; 24: 284-90.

[2] Sun BB, Maranville JC, Peters JE, et al. Genomic atlas of the human plasma proteome. Nature 2018; 558: 73-9.

[3] Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol 2011;40: 740-52.