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Original article

# Genetically predicted 17beta-estradiol and cardiovascular risk factors in women: a Mendelian randomization analysis using young women in Hong Kong and older women in the Guangzhou Biobank Cohort Study

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ABSTRACT

*Purpose:* The role of estrogen in cardiovascular health remains contested with discrepancies between findings from randomized controlled trials and observational studies. Mendelian randomization, which assesses the effect of lifelong endogenous exposure, may help elucidate these discrepancies.
*Methods:* We used separate sample instrumental variable analysis to estimate the association of log 17β-estradiol with factors related to cardiovascular disease risk (systolic and diastolic blood pressure, lipids, fasting glucose, body mass index, waist hip ratio, and waist circumference) and Framingham score, a predictor of 10-year risk of ischemic heart disease events, in older Chinese women from the Guangzhou Biobank Cohort Study (GBCS, $n = 3092$). The estimate was derived using the Wald estimator, that is, the ratio of the association of genetic determinants (rs1008805 and rs2175898) of log 17β-estradiol with cardiovascular disease risk factors and Framingham score in GBCS and the association of these genetic determinants with log 17β-estradiol in a sample of young women from Hong Kong ($n = 236$).
*Results:* Genetically, higher 17β-estradiol was not associated with any cardiovascular disease-related risk factor or with Framingham score (−0.01, 95% confidence interval = −1.34 to 1.31).
*Conclusions:* Lifetime exposure to estrogen does not appear to be cardioprotective via the cardiovascular disease-related risk factors examined.



## Introduction

The role of estrogen in cardiovascular disease and its risk factors has been intensively investigated for many years. Initially, it was thought that estrogen protects against cardiovascular disease because women have lower cardiovascular disease rates than men, and the menopause precedes an increase in cardiovascular deaths. However, randomized controlled trials (RCTs) suggest that hormone replacement therapy (HRT) has a little overall effect on cardiovascular disease or its risk factors but decreases LDL-cholesterol slightly [1], decreases diabetes, and increases the risk of thrombosis and stroke [2]. Nevertheless, findings from these RCTs have been contested [3], and the possibility that estrogens have some cardiovascular benefits remains a topic of active experimental investigation [4]. First, some hormone replacement trials mainly used estrogen and progestin, whose effects may differ from estrogen alone [2]. For example, in the Women's Health Initiative trial, estrogen and progestin together may increase the risk of ischemic heart disease, whereas estrogen only had no effect [2]. Second, the effect of estrogen on cardiovascular risk may depend on other factors, such as timing of HRT initiation [4,5] or the presence of atherosclerosis, that is, when atherosclerosis is absent, estrogen protects but when atherosclerosis is present, estrogen is

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detrimental [6]. Given the risk of breast cancer and venous thrombosis from estrogen administration, further major RCTs of estrogen are unlikely, but the role of estrogen in cardiovascular disease remains an important topic relevant to the widespread use of oral contraceptives including estrogen. In this situation replication, or otherwise, using a different approach can make a key contribution. In this study, for the first time, we used a separate sample Mendelian randomization analysis to assess the lifelong effect of estrogen on cardiovascular disease risk factors, overall and in women without cardiovascular disease.

Mendelian randomization analysis, that is, instrumental variable analysis with genetic instruments, is increasingly used to evaluate the causal role of risk factors in disease, particularly when RCTs are unavailable. Mendelian randomization may also enable assessment of the effects of endogenous rather than exogenous exposure, which may differ, because Mendelian randomization tests a causal pathway whereas an RCT tests an intervention which can have unknown pleiotropic effects [7]. Genetic polymorphisms associated with the exposure are randomly allocated during conception, so this resembles the randomization process in RCTs and hence is less susceptible to confounding [8]. Mendelian randomization analyses have clarified the role of many factors in cardiovascular disease etiology, such as C-reactive protein [9]. However, a conventional Mendelian randomization analysis is sensitive to measurement error of the exposure, which may lead to inflated estimates [10]. Separate sample Mendelian randomization analysis, where a genetic prediction rule is generated in a sample less susceptible to measurement error, may alleviate this problem [11]. We have successfully implemented this approach to examine the effect of testosterone on cardiovascular disease risk factors and of testosterone and estrogen on inflammation in older people [12–14]. In this study, we examined the relation of lifelong exposure to estrogen with cardiovascular disease risk factors using a Mendelian randomization analysis among Southern Chinese women to clarify the role of lifelong estrogen exposure in health.

## Materials and methods

### *Sources of data*

Two groups of women of different ages from the same genetic background, that is, from Hong Kong and Guangzhou, the capital of Guangdong, in Southern China, were recruited. Most Hong Kong residents are first, second, or third generation migrants from Guangdong [15]. First, 237 young women (mean age = 21.0 years) were recruited with restriction to those with both parents and at least three grandparents born in Hong Kong or Guangdong and not taking hormone-related medication. Morning blood samples were taken on the fourth day to seventh day of the menstrual cycle for 17β-estradiol assessment, by immunoassay (Ortho Clinical Diagnostics, VITROS ECi) and DNA extraction. Self-administered questionnaires were used to collect information, such as socioeconomic position and health status. Second, we used a sample of older women (50 or more years) from Guangzhou Biobank Cohort Study (GBCS), an ongoing collaboration of Guangzhou Number 12 Hospital, the Universities of Hong Kong and Birmingham, UK [16]. Recruitment of participants was in three phases. All participants were permanent residents of Guangzhou and members of "The Guangzhou Health and Happiness Association for the Respectable Elders" (GHHARE), a community social and welfare association unofficially aligned with the municipal government. Membership is open to older people for a monthly fee of 4 Yuan (50 US cents). About 7% of permanent Guangzhou residents aged 50 or more years are members of GHHARE, of whom 11% (about 10,000 participants) enrolled for each of phases one, two, and three. The inclusion criteria were that they were capable of consenting, ambulatory, and not receiving treatment modalities which, if omitted, may result in immediate life-threatening risk, such as chemotherapy or radiotherapy for cancer, or dialysis for renal failure. The methods of measurement have previously been reported [16]. Standing height was measured without shoes to the nearest 0.1 centimeter. Weight was measured in light clothing to the nearest 0.1 kg. Hip circumference was measured at the greatest circumference round the buttocks below the iliac crest. Waist circumference was measured horizontally around the smallest circumference between the ribs and iliac crest or at the level of the naval for obese participants. Body mass index was calculated as the weight in kilograms divided by the square of height in meters. We recorded seated blood pressure as the average of the last two or three measurements, using the Omron 705CP sphygmomanometer (Omron Corp., Kyoto, Japan). Fasting low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and glucose levels were determined with a Shimadzu CL-8000 clinical chemical analyzer (Shimadzu Corp., Kyoto, Japan) in the hospital laboratory. Fasting blood samples were collected at recruitment in phase 3 or at follow-up for participants recruited in other phases. Samples were stored, as whole blood or as buffy coat and sera, at −80°C for all apart from a subset of phase 3 participants whose DNA was extracted from fresh blood and stored at −80°C [17]. The University of Hong Kong-Hospital Authority Hong Kong West Cluster Joint Institutional Review Board approved the study. The Guangzhou Medical Ethics Committee of the Chinese Medical Association approved GBCS, including the use of genetic data. All participants gave written, informed consent before participation.

### *DNA extraction and single nucleotide polymorphism analysis*

DNA was extracted using QIAamp DNA Blood Midi Kit (Catalog No. 51185) for fresh blood in Hong Kong, phenol-chloroform extraction for fresh blood in GBCS and magnetic bead extraction for previously stored specimens in GBCS [17]. single nucleotide polymorphism (SNP)s were selected from genes (*ESR1*, *ESR2*, and *CYP19A1*) [18–21] functionally relevant to estradiol or breast cancer, with minor allele frequency >5% in Chinese [22]. Five SNPs (rs2175898 from *ESR1*, rs1256030 and rs1256031 from *ESR2*, and rs10046 and rs1008805 from *CYP19A1*) were analyzed at the Center for Genomic Sciences of the University of Hong Kong, for the Hong Kong sample, and a commercial company (Beijing Capital Bio Corporation) in Beijing, for the GBCS sample, using a Mass ARRAY system (Sequenom, San Diego, CA). For DNA quality analysis, we used spectrophotometry for most of the samples and gel electrophoresis for four duplicate check controls and six randomly selected samples in each DNA sample plate. The determined sample concentration and A260/280 ratios were 10–20 ng/uL and 1.7–2.0, respectively. A call rate <80% was considered failure. All SNPs passed with a call rate >95%.

### *Exposure*

The exposure was genetically predicted log 17β-estradiol (pmol/L).

### *Outcome*

The outcomes were systolic and diastolic blood pressure, LDL-cholesterol, HDL-cholesterol, triglycerides, glucose, body mass index, waist hip ratio, waist circumference, and Framingham score. The Framingham score overestimates absolute risk of cardiovascular disease in Chinese populations [23] but provides a risk ranking. The Framingham score was calculated from age, LDL-