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Genetically predicted 17beta-estradiol, cognitive function and depressive symptoms in women: A Mendelian randomization in the Guangzhou Biobank Cohort Study

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Conflict of interests: In addition to Kar Keung Cheng's appointment at University of Birmingham, he is affiliated to Department of General Practice at Peking University Health Science Centre. The latter receives support from Pfizer China to support the training of family doctors (approximately US$100,000 a year for 2014-16). The authors have no other conflict of interest, financial or otherwise.

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Figure: 1

Appendix: 1

Keywords: Estrogen; cognitive function; depressive symptoms; Mendelian randomization analysis

Running head: Estrogen and cognition and depression using MR

List of abbreviations

DNA: Deoxyribonucleic acid

HEPA: Health-enhancing physical activity

HRT: Hormone replacement therapy

GBCS: Guangzhou Biobank Cohort Study

GDS: Geriatric Depression Scale

GHHARE: The Guangzhou Health and Happiness Association for the Respectable Elders

GWAS: Genome wide association studies

IPAQ: International Physical Activity Questionnaire

KEEPS: The Kronos Early Estrogen Prevention Study

MET: Metabolic equivalent

MMSE: Mini Mental State Examination

RCT: Randomized controlled trial

SNP: Single nucleotide polymorphism

WHIMS: Women's Health Initiative Memory Study

Abstract

Objective: The role of estrogen in cognitive function and depressive symptoms is controversial due to discrepancies between results from randomized controlled trials (RCT) and observational studies. Mendelian randomization analysis may provide further insights concerning the role of estrogen in these outcomes as it assesses the effect of lifelong endogenous exposure but is less vulnerable to confounding than observational studies.

Method: We used separate sample instrumental variable analysis to estimate the association of log 17β estradiol with cognitive function (Delayed 10 word recall, and Mini Mental State Examination (MMSE)) and depressive symptoms (Geriatric Depression Scale (GDS)) in older Chinese women of the Guangzhou Biobank Cohort Study (GBCS, n=3,086). The estimate was derived based on the Wald estimator, the ratio of the association of genetic determinants (rs1008805 and rs2175898) of log 17β-estradiol with cognitive function and depressive symptoms in GBCS and the association of log 17β-estradiol with genetic determinants in the sample of young women in Hong Kong (n=236).

Results: Genetically predicted 17β-estradiol was not associated with delayed 10-word recall (0.42 words per log increase in 17β-estradiol (pmol/L), 95% confidence interval (CI) -0.49 to 1.34) MMSE (0.39 per log increase in 17β-estradiol (pmol/L), 95% CI -0.87 to 1.65) or GDS (0.24 per log increase in 17β-estradiol (pmol/L), 95% CI -0.57 to 1.05).

Conclusion: These results were largely consistent with evidence from RCTs and did not show any beneficial effect of estrogen on cognitive function and depressive symptoms. However, larger Mendelian randomization analyses are needed to identify any minor effects.

Introduction

There are known sex differences in neurological function such as lower prevalence of mild cognitive impairment among women (1), but higher prevalence of depression (2). It has been speculated that estrogen, the female sex hormone, may explain these differences as estrogen may improve neural plasticity and the health of the neurovascular unit (3), but may also modulate mood via the serotonergic system (3). Observational studies consistently showed that hormone replacement therapy (HRT) users had a lower risk of dementia (3), but less so for depression (3), than non-users. The subsequent Women's Health Initiative Memory Study (WHIMS) showed HRT had adverse effect on cognition (4), whilst findings from trials of estrogen or HRT for depression have been inconsistent (5-7). HRT users differed from non-users in terms of socioeconomic position and lifestyle, so unmeasured confounding may explain the discrepant results between these two study designs (8). However, the response to HRT could depend on the timing of initiation, a phenomenon known as the timing hypothesis (3, 9). Specifically, HRT initiated around the time of menopause may improve cognition but reduce cognition if HRT is initiated years after menopause. Different HRT formulations (as estrone, estradiol or estradiol with progesterone) may also have different effects on cognitive function (3). Replication using an alternative design, comparing cognition and depression in women with genetically determined differences in estrogen, may help elucidate the role of estrogen. A separate sample Mendelian randomization study, which utilizes genetic predictors of the exposure, in this case estrogen, may be an alternative for several reasons. First, genetic make-up is randomly allocated at conception analogous to the randomization process in randomized controlled trials (RCTs), giving estimates of association that are less susceptible to the unmeasured confounding that may bias observational studies (10). Mendelian randomization has increasingly been used to ascertain

whether risk factors for poor cognitive function, such as lipids and alcohol use, are causal (11, 12). Second, Mendelian randomization studies assess lifelong effects which is potentially more relevant to the timing hypothesis than randomized controlled trials where mainly postmenopausal women were recruited (4). On the other hand, unlike randomized controlled trials which assess the effect of exogenous exposures, Mendelian randomization studies assess the effect of endogenous exposure, and hence may be less susceptible to problems such as pleiotropic effect of interventions used in RCTs (13). Third, a separate sample Mendelian randomization study allows estimates of effects where the genetic association for the exposure comes from a different sample than the genetic associations for the outcome, which is a more efficient and less biased design (14) that enables estimation of lifelong effects of an exposure, such as estrogen that changes with age. Genetic determinants of estrogen exposure in young women may be used to determine the effects of estrogen in older women because it is less susceptible to measurement error (15), and so may give more reliable estimates (14). We have previously used this approach to examine the role of testosterone and estrogen in cardiovascular disease risk factors and inflammation (16-18). In this study, we examined the relation of lifelong exposure to estrogen with cognition and depressive symptoms using a Mendelian randomization study among Southern Chinese women to clarify the role of lifelong estrogen exposure.

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 (19). First, 237 young women (mean age 21.0 years) were recruited from the University of Hong Kong, restricted to those with 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 4th day to 7th day of the menstrual cycle for 17β-estradiol assessment, by immunoassay (Ortho Clinical Diagnostics Vitros Eci), and deoxyribonucleic acid (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+ years) from GBCS, an ongoing collaboration of Guangzhou Number 12 Hospital, the Universities of Hong Kong and Birmingham, UK (20). Recruitment of participants was in 3 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 (50US cents). About 7% of permanent Guangzhou residents aged 50+ 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 (20). Cognitive function was assessed using the test

of new learning ability (10-word list learning task) from a test battery developed for the Consortium to Establish a Registry for Alzheimer's Disease (21). Four words "pole", "shore", "cabin", and "engine" were replaced with "corner", "stone", "book", and "stick" as in the adapted Consortium 10-word list learning task (22), and "butter" and "queen" were replaced by "soy sauce" and "chairman" as these are more culturally appropriate. During the learning phase, the 10-word list was read out to the participant who was then asked to recall immediately the words they remembered; this was repeated 3 times. After a 5-minute period of distraction, during which the interview was continued, the participant was then asked to recall as many of the 10 words as he or she was able, giving the delayed recall score out of 10. In phase 3 and the subsequent follow up for all phases, we additionally used the Mini Mental State Examination (MMSE) (23). Three of the 11 tasks in the original MMSE were modified to be culturally appropriate and consistent with other adaptations for Chinese populations (24). Orientation in place was adapted according to geographical divisions of China and screening setting to: "country", "province", "city", "hospital", and "floor". In the 3-word registration and recall "table" and "penny" were replaced by "newspaper" and "train", while the third word remained as "apple", to ensure all three words were frequently used two-character Chinese words. The modified MMSE has the same scale as the original MMSE (24), hence the psychometric properties of the measures should be similar. Depressive symptoms were assessed by the Chinese version of the 15-item Geriatric Depression Scale (GDS) which has been used before in Chinese (25).

Fasting blood samples were collected at baseline 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^{0}C for all apart from a subset of phase 3 participants whose DNA was extracted from fresh blood and stored at -80^{0}C (12). 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 prior to participation.

DNA extraction and SNP 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 (12). SNPs were selected from genes (*ESR1*, *ESR2* and *CYP19A1* (26-29)) functionally relevant to estradiol or breast cancer, with minor allele frequency >5% in Chinese (30). Five SNPs (rs2175898 from *ESR1*, rs1256030 and rs1256031 from *ESR2*, and rs10046 and rs1008805 from *CYP19A1*) were analyzed at the Centre 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, California). 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 10-20ng/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 delayed 10-word recall test score and modified MMSE score for cognitive function, and GDS score for depressive symptoms. The adapted Consortium 10-word list learning task has been validated as a culturally and educationally sensitive tool for identifying dementia in population-based research in developing countries (22). The modified MMSE has been used before (31-33). The delayed 10-word recall test, MMSE and GDS were also administered to all participants (phases 1-3) at follow up, so we used the test scores from follow up for those without baseline test scores, because MMSE and GDS was not administered at baseline in phases 1 and 2.

Statistical analysis

In the sample of young Chinese women from Hong Kong, we established genetic predictors of log 17β-estradiol based on 2 SNPs from stepwise linear regression starting with 5 SNPs with replication in 1,000 bootstrapping samples, as described previously (17). In the GBCS sample, we tested for Hardy-Weinberg equilibrium at the SNP locus on a contingency table of observed-versus-predicted frequencies with an exact test. We used ANOVA to assess whether genetically predicted log 17β-estradiol was associated with potential confounders We used separate sample instrumental variable analysis to estimate the association of log 17β-estradiol with cognitive

function and depressive symptoms in the sample of older Chinese women from GBCS by using *suest* (seemingly unrelated regression command in Stata) to generate the Wald estimates from the ratio of the association of genetic determinants of log 17β-estradiol with cognitive function and depressive symptoms in GBCS and the association of genetic determinants of log 17β-estradiol with log 17β-estradiol in the sample of young women (34). Figure 1 shows the flow chart of the study.

All statistical analyses were conducted using Stata 13.1 (StataCorp LP, College Station, Texas, USA).

Results

Among the young Chinese women samples (n=236, one participant was excluded because of invalid 17β-estradiol), the F-statistic for genetically predicted log 17β-estradiol was 13.2, with an adjusted R^2 of 4.9%, suggesting that the analyses is unlikely to be susceptible to weak instrument bias. Among the 22,067 women in all 3 phases of GBCS, SNP analysis was available for 3,316 women, with availability depending on the phase of recruitment and other logistical concerns. Among these 3,316 women, 3,096 (93.4%) had all selected SNPs. However, these women were more likely to have a higher education level and to have had non-manual jobs (Appendix 1).The 2 SNPs used to generate the genetic score did not deviate from the Hardy Weinberg equilibrium (p=0.42 for rs1008805; and p=0.73 for rs2175898) in the GBCS sample. Of the 3,096 women, 1,821 had MMSE from baseline and 1,245 from follow-up, 1,824 had GDS from baseline and 1,261 from follow-up and 2,943 had delayed 10 word recall from baseline and 143 from follow up. Table 1 shows genetically predicted log 17β-estradiol, obtained from a previously derived

genetic prediction rule ((0.1×rs1008805-0.1×rs2175898+4.7) (17), was unrelated to age, smoking, alcohol use, physical activity, education and longest held occupation among older Southern Chinese women from GBCS.

Table 2 shows the instrumental variable estimates for the association of log 17β-estradiol with delayed 10-word recall, MMSE and GDS. Log 17β-estradiol was not associated with delayed 10 word recall score (0.42 per unit increase in log 17β-estradiol, 95% confidence interval (CI) -0.49 to 1.34), MMSE scores (0.39 per unit increase in log 17β-estradiol, 95% CI -0.87 to 1.65) or GDS scores (0.24 per unit increase in log 17β-estradiol, 95% CI -0.57 to 1.05) among older Southern Chinese women.

Discussion

This study, to our knowledge, is the first study to examine the effect of lifelong exposure to estrogen on cognitive function or depressive symptoms using a separate sample Mendelian randomization design. This study adds by showing that there is no evidence for any protective effect of estrogen on cognitive function, consistent with the WHIMS and other RCTs (4, 35), or on depressive symptoms, consistent with RCTs (5, 6). By replicating the findings from previous RCTs, this study also demonstrates the utility of the Mendelian randomization study design as a useful alternative or, ideally, precursor to RCTs.

Although we used a separate sample Mendelian randomization design which is less susceptible to the confounding often found in observational studies, limitations exist. First, as with all study designs, Mendelian randomization has assumptions which are not always testable. Specifically, we are unable to assess if the genetic variants affected cognitive function or depressive symptoms only via their associations with estrogen, i.e. the exclusion restriction criteria, as we did not have genetic variants, estrogen, and the outcomes all measured for the same women. Second, we did not use estrogen related genetic variants from genome wide association studies (GWAS), because no such studies have reliably identified estrogen related genetic polymorphisms in Chinese women. Therefore, we have established our own genetic prediction rule in a young Chinese women sample using stepwise regressions and bootstrapping methods, as described previously.(17) Mendelian randomization is susceptible to confounding by population stratification but the participants had the same genetic origin. Fourth, our study was powered to detect a change of 0.23 standard deviation in the outcomes, for example an MMSE score of 0.8 per increase in one log unit change in estrogen (pmol/L). We could not detect smaller changes due to estrogen which could be important to population health but possibly are less clinically relevant. Fifth, women who did not return for follow up had lower socio-economic position than those who returned for follow up (36). Furthermore, some participants were chosen based on their change in cognitive function from baseline for a case control study examining genetic determinants of cognitive function (37). However, selection bias would only occur if those with a specific relation of estrogen related genetic variants with cognitive function or depressive symptoms did not return or were included in the case control study, which is unlikely. Moreover, a sensitivity analysis excluding the women cases gave very similar estimates (data not shown). Lastly, our study only focused on estrogen and may not be relevant

to the effect of combined treatment (i.e. estrogen and progestin). However, the WHIMS showed combined treatment did not prevent cognitive impairment (38). The Kronos Early Estrogen Prevention Study (KEEPS) showed positive effect of HRT on mood although only for those receiving oral treatment (39), whether comparing effects by formulation for mood was part of the original protocol is unclear (39).

The discrepancies between observational studies and RCTs could be an indicator of confounding by healthier attributes among users compared to non-users of HRT in observational studies (8, 35). Notwithstanding this likely explanation, alternative explanations such as the timing hypothesis may explain this discrepancy (9), in particular WHIMS mainly recruited participants several years after their menopause (4). The null findings from our study, which examined the lifelong effect of estrogen, do not provide evidence in support of the timing hypothesis and is consistent with the WHIMS analysis restricting to participants aged 50-55 (40). Similarly, our null findings on estrogen and depressive symptoms are consistent with the more recent RCTs (5, 6). Taking these studies together suggests no strong protective effect of estrogen on long term cognitive function or depressive symptoms in the general population.

Our study adds evidence concerning the effect of estrogen on cognitive function and depressive symptoms using separate sample Mendelian randomization, and there is no strong evidence for an effect or evidence for the timing hypothesis although the issue of statistical power may account for the lack of evidence. However, replication in a larger Mendelian randomization study may examine the possibility of smaller effects of estrogen on these outcomes, with

corresponding implications for public health and clinical practice, assuming the benefits outweigh any risks.

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Figure legend:

Figure 1: Flow chart of the study. A separate-sample instrumental variable analysis with genetic instruments, i.e. Mendelian randomization analysis, was used. See detailed in the Methods

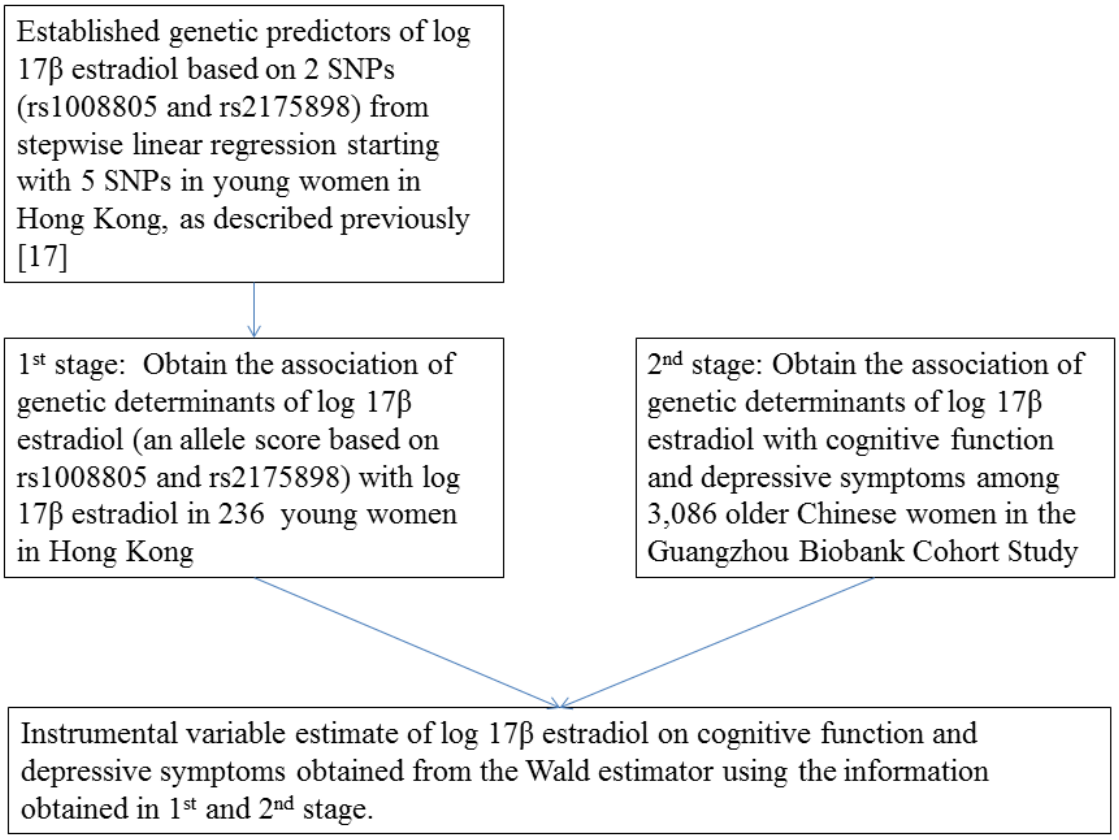

Figure 1

Table 1: Genetically Predicted Log 17β-Estradiol by Lifestyle And Socio-demographics Among Southern Chinese Women In The Guangzhou Biobank Cohort Study, recruited from 2003 to 2008 and followed up till 31 December, 2012

Characteristic		Sample size	Predicted log 17β-estradiol (pmol/L)	[a]p value
Age group	50-54	379	4.73 (0.10)	0.24
	55-59	1061	4.74 (0.10)	
	60-64	696	4.73 (0.10)	
	65-69	538	4.73 (0.10)	
	70-74	289	4.73 (0.09)	
	75-79	94	4.73 (0.10)	
	80+	38	4.74 (0.09)	
Smoking status	Never smokers	2,992	4.7 (0.10)	0.44
	Former smokers	52	4.8 (0.08)	
	Current smokers	45	4.7 (0.10)	
Alcohol status	Never drinkers	2,157	4.7 (0.10)	0.70
	Former drinkers	101	4.7 (0.10)	
	Current drinkers	808	4.7 (0.10)	
[b]Physical activity (IPAQ)	Inactive	194	4.7 (0.10)	0.75
	Minimally active	998	4.7 (0.10)	
	HEPA active	1,903	4.7 (0.10)	
Education	Less than primary	426	4.7 (0.10)	0.26
	Primary	1,004	4.7 (0.10)	
	Junior middle	746	4.7 (0.10)	
	Senior middle	688	4.7 (0.10)	
	Junior college	154	4.7 (0.09)	
	College	77	4.7 (0.10)	
[c]Longest-held occupation	Manual	2,099	4.7 (0.10)	0.72
	Non-manual	638	4.7 (0.10)	
	Others	321	4.7 (0.10)	

[a]P value obtained from ANOVA.

[b]HEPA: Health-enhancing physical activity (i.e. vigorous activity at least 3 days a week achieving at least 1500 metabolic equivalent (MET) minutes per week or activity on 7 days of the week, achieving at least 3000 MET minutes per week (IPAQ: International Physical Activity Questionnaire).

[c]Manual occupations are agricultural worker, factory work or sales and service; non-manual are administrator/ manager, professional/technical, military/disciplined.

Table 2: Effect of Genetically Predicted Log 17β-estradiol On Delayed 10-word Recall, Mini Mental State Examination and Geriatric Depression Scale Scores Among Southern Chinese Women In The Guangzhou Biobank Cohort Study (Recruited from 2003 to 2008 and followed up till 31 December, 2012) Using a Mendelian Randomization Design

Measure	Sample size	Estimate	95% Confidence Interval
Delayed 10 word recall test (per word)	3,086	0.42	-0.49 to 1.34
Mini Mental State Examination (per item)	3,066	0.39	-0.87 to 1.65
Geriatric Depression Scale (per item)	3,085	0.24	-0.57 to 1.05

Appendix 1: Characteristics of participants with or without data among older Chinese women in the Guangzhou Biobank Cohort Study

Characteristics		Participants with data	Participants with missing data	[a]p value
Sample size		3,095	18,946	
Education (%)	Less than primary	13.8	12.8	<0.001
	Primary	32.4	35.4	
	Junior middle	24.1	25.5	
	Senior middle	22.2	20.8	
	Junior college	5.0	4.0	
	College	2.5	1.6	
Sample size		3,058	18,873	
[b]Longest-held	Manual	68.6	67.5	<0.001
Occupation (%)	Non-manual	20.9	18.1	
	Others	10.5	14.4	

[a]P value obtained from chi-square test.
[b]Manual occupations are agricultural worker, factory work or sales and service; non-manual are administrator/ manager, professional/technical, military/disciplined.

Highlights

- Mendelian randomization assesses lifelong exposure and is less prone to confounding
- Mendelian randomization showed no effect of lifelong estrogen on cognition
- Mendelian randomization showed no effect as well on depressive symptoms
- Our study does not support the timing hypothesis as explanation for trial results
- Discrepancies between observational studies and trials is likely due to confounding