The association of oestrogen receptor α-haplotype with cardiovascular risk factors in the British Women’s Heart and Health Study

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KEYWORDS
ESR1; Oestrogen receptor; Hormone replacement; High density lipoprotein cholesterol

Aims One previous study among women with established coronary heart disease found a gene–treatment interaction between the oestrogen receptor gene (ESR1) and hormone replacement in their association with high density lipoprotein cholesterol (HDL-c). We aimed to replicate these findings in a general population sample.

Methods and results Cross-sectional associations were assessed in a study of 3404 women from 23 towns across Britain who were aged 60–79 at the time of assessment and were described as white by the examining nurse. Women with the T-A haplotype [constructed from two single nucleotide polymorphisms (SNPs) in the first intron of ESR1: c454-397T>C (rs2234693) and c454-351A>G (rs9340799)], which was predicted to be associated with reduced oestrogen response, were more likely to have been past ([per haplotype odds ratio 1.16 (95% CI 1.01, 1.33), P = 0.02] or to be current users ([per haplotype odds ratio 1.19 (95% CI 0.99, 1.42), P = 0.05]) of hormone replacement. However, there was no association between haplotype or either SNP and HDL-c or any other cardiovascular disease risk factors.

Conclusion Women with the T-A haplotype are more likely to use hormone replacement. However, genotyping of ESR1 rs2234693 or rs9340799 in post-menopausal women to tailor hormone replacement is unlikely to markedly improve cardiovascular risk.

Introduction

In biological and epidemiological studies, oestrogens have been shown to have beneficial effects on the cardiovascular system via favourable effects on lipid profiles and via vasodilatory, anti-inflammatory, fibrinolytic, and anti-proliferative effects.1,2 Despite these findings, large well-conducted randomized controlled trials show that hormone replacement does not protect post-menopausal women from coronary heart disease (CHD).3,4 These disparities may be explained by confounding in the observational studies,5,6 and/or differences in the study populations between the observational studies and randomized controlled trials.7,8 Women using hormone replacement in the observational studies were largely peri-menopausal when they started treatment and the most common reason for being prescribed hormones was menopausal symptoms. In contrast, participants in the randomized controlled trials were largely post-menopausal, with mean ages of 60 years.3,4 Although there was no strong evidence of an interaction with age at randomization in the women’s health initiative trial,4,9 randomized trial evidence to date cannot rule out the possibility that women with peri-menopausal symptoms (possibly indicative of low oestrogen) may benefit from the use of hormone replacement to prevent CHD.8

The effects of oestrogen on the vascular system are mediated by two distinct oestrogen receptors (ESRs), ESR1 and ESR2, encoded by two separate genes, which are both expressed in endothelial cells and vascular smooth muscle cells.10,11 Animal and human studies suggest that ESR1 is the major mediator of the atheroprotective effect of oestrogen.12–14 The ESR1 gene (ESR1) is located at chromosome 6q24.1 and has six domains encoded by eight exons. Associations between a number of polymorphisms in ESR1 and various phenotypes that are known to be affected by endogenous oestrogens, including breast cancer,15 osteoporosis,16 hysterectomy, and age at menopause,17 have been reported. Most of these studies have focused on two single nucleotide polymorphisms (SNPs) c454-397T>C (rs2234693) and c454-351A>G (rs9340799) located in the first intron of ESR1 46 base pairs apart from each other.

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and 397 and 351 base pairs upstream of exon 2, respectively. The results of these studies suggest a decrease in oestrogen response (implied by an increase in risk of phenotypes associated with decreased oestrogen activity) for individuals with the T and A alleles, respectively. However, the mechanisms by which these polymorphisms act on ESR1 are not fully established. There is some evidence that they affect ESR1 expression by altered binding of transcription factors.18,19 But further research is required to determine the molecular level mechanisms of these SNPs and to determine whether they are functional or whether they are in linkage disequilibrium (LD) with a truly functional, but as yet unknown, variant elsewhere in ESR1.

A number of studies have shown that these two ESR1 SNPs, c454-397T>c and c454-351A>G, are associated with cardiovascular disease risk factors, including dyslipidaemia,20,21 coronary reactivity,22 body mass index,23 and atherosclerosis.24 Recently, a prospective cohort study found a positive association between a haplotype formed from these two SNPs (representing the T and A alleles) and CHD risk in women,18 although a second study (the largest to date and the only one with adequate power to test this association) found no associations between these two SNPs or their haplotypes and incident CHD in either women or men.25 In one small study (n = 300), (a subgroup of the participants, all with CHD, in the Estrogen Replacement and Atherosclerosis randomized controlled trial) a gene–treatment interaction was found in which there was a greater increase in high density lipoprotein cholesterol (HDL-c) due to oestrogen replacement among women who were homozygous for the rare alleles of the two ESR1 SNPs c454-397T>C and c454-351A>G.20 On this basis of these findings, Herrington et al. concluded that genetic screening to tailor decisions about hormone replacement use in post-menopausal women might result in important health benefits for ‘at risk’ women defined by their genotype. In the same study, evidence for a similar gene–oestrogen treatment interaction with E-selectin was found, but there was no such interaction found with C-reactive protein.19 However, in that study gene–hormone replacement interactions for 10 sequence variants in ESR1 were assessed with a number of cardiovascular disease risk factor outcomes and, taking these multiple tests into account, the statistical evidence for an interaction was weak given the prior probabilities (0.004 and 0.07 for the interactions of statistical evidence for an interaction was weak given the comes and, taking these multiple tests into account, the

Methods

Study population

Full details of the selection of participants in the British Women’s Heart and Health Study have been previously reported.27,28 In brief, between 1999 and 2001 4286 women aged 60–79, who were randomly selected from 23 British towns were interviewed, examined, completed medical questionnaires, and had detailed reviews of their medical records. These women have been followed-up over a median (interquartile range) of 4.7 (3.1–5.4) years by flagging with the National Health Service Central Register (NHSCR) for mortality data and review of their medical records every 2 years.

Cardiovascular risk factors

Blood samples were taken after a minimum of 6 h fast. Plasma glucose was measured using a glucose oxidase method using a Flacor 600 automated analyser. Serum insulin was measured using an ELISA assay which does not cross-react with proinsulin.26 Insulin resistance was estimated according to the homeostasis model assessment as the product of fasting glucose (mmol/L) and insulin (μU/mL) divided by the constant 22.5.31 HDL-c and triglyceride levels were measured using a Hitachi 717 automated analyser and reagents supplied by Roche Diagnostics. Fibrinogen was assayed in stored citrated plasma by the Clauss assay in a MDA-180 automated coagulometer (Organon Teknika), and C-reactive protein by a high-sensitivity immunonephelometric assay on a ProSpec protein analyser (Dade-Behring).

Standard procedures were used to assess blood pressure, height (standing and seated, from which trunk length was derived), weight, and waist and hip circumference as previously described.27,28 Information on occupational social class, parity, birth weight of first born offspring, hysterectomy, smoking, ever being diagnosed with osteoporosis, ever experiencing a germ or hip fracture, and physical activity were obtained from either the baseline research nurse interview or the self-complete questionnaire, previously reported.27,28 The three indicators of psychological distress were assessed: current use of anti-depressant medication, self-report of ever being diagnosed by a doctor with depression, and the EQ5D (EuroQol: http://www.evr.nl/bmg/imta/eq-net/EQ5d.htm) mood question. Participants brought all of their medications to a nurse interview. Medications were coded using the British National Formulary (http://www.bnf.org/), with anti-depressants being any medication in Section 4.3 (anti-depressants). Participants whose response to the EQ5D mood question was that they were ‘today feeling either moderately or extremely anxious and/or depressed’ were coded as scoring positively in terms of psychological distress.

Because we had limited power to detect an association of genetic variants with CHD events, we did not intend to examine this as a primary outcome. However, we do present the percentage of women with prevalent or incident CHD by haplotype. Prevalent CHD was defined as any woman with a self-report of a doctor diagnosis of angina or myocardial infarction and/or evidence in medical record review at baseline of either of these diagnoses, and incident CHD was defined as any woman who had an underlying cause of death from CHD (ICD10 codes I20–I25, I51.6) or a new diagnosis (in someone who was previously disease free) of angina or myocardial infarction in the medical record reviews during the follow-up period up to 31 December 2004.

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At baseline assessment, women were asked about current and past use of hormone replacement including type of preparation, duration of use, and age at commencing use. Of the 4286 participants, 543 (12.7%) indicated that they were past users and 368 (8.6%) that they were current users of hormone replacement. Of the past users, 66% did not know the name or type of preparation, 27% had used a combined oestrogen–progestogen preparation, 4% had used unopposed oestrogen, and 4% had not used hormone replacement (they had used tibolone or raloxifene). Of the current users, only 9% did not know the name or type, 40% were taking a combined preparation, 39% unopposed oestrogen, and 12% were using tibolone or raloxifene. Among the subgroup of women aged 60–64 in our study, 41% reported that they had ever used hormone replacement (this proportion includes self-report of past and current use irrespective of whether they knew the name of the preparation or not or whether they reported use of tibolone or raloxifene rather than hormone replacement). This proportion is similar to the proportion (45%) of women aged 50–64 in the UK Million women study who reported ever use of hormones (including non-oestrogen preparations such as tibolone).22 In our main analyses, those women who had not or were not currently using hormone replacement (i.e. tibolone or raloxifene users) were categorized as non-users and those who did not know the type of therapy that they had used were all assumed to have used or be using hormone replacement (on the basis that the majority of those who did know what they were using were in fact using either opposed or unopposed oestrogen). Thus in the main analysis, 524 women were defined as past users and 324 as current users of hormone replacement. In one sensitivity analysis, all women who did not know the type of therapy they were on were categorized as non-users, and in a second, these women were excluded from the analysis. The results of these two sensitivity analyses did not differ substantively from those presented here, though the effects were less precisely estimated. All of those who were currently using unopposed oestrogen had had a hysterectomy.

Genotyping and haplotype construction

DNA was extracted from K-EDTA whole blood samples by salting out procedure.33 All genotyping were performed by KBioscience (http://www.kbioscience.co.uk). SNPs were genotyped using the KASPar chemistry, which is a competitive allele-specific PCR SNP genotyping system using FRET quencher cassette oligos (http://www.kbioscience.co.uk/genotyping/genotyping-chemistry.htm).

We used the genotype data for each of the two SNPs to infer the haplotype alleles present in the population by using the programme PHASE (version 2.1.1).34 PHASE uses a Bayesian statistical method for reconstructing haplotypes from population genotype data.34 We decided a priori to examine the effect of possession of a haplotype representing the T allele of c454-351A and the A allele of c454-351A>G on hormone replacement and cardiovascular disease risk factors, as this haplotype has been found in a prospective study to be associated with CHD risk.18 In addition, we repeated all analyses with each of the SNPs separately in order to specifically examine whether we could replicate the earlier results of Herrington et al.35 with respect to an interaction between haplotype and hormone replacement and HDLc.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was tested at each SNP locus on a contingency table of observed vs. predicted genotypic frequencies using an exact test.35 LD parameters $D^\prime$ and $r^2$ values were calculated using the Stata package ‘pwld’ (www.gene.cimr.cam.ac.uk/clayton). Uncertainty in haplotype assignment was not taken into account in the analysis because the estimated posterior probabilities of correct haplotype inferences were higher than 0.99 for all women included in the analysis.

Our a priori hypothesis was of a linear trend (one degree of freedom) association with increasing risk of hormone replacement and cardiovascular risk factors with each additional T-A haplotype from 0 to 2. We used logistic regression to examine the association between haplotype and past or current use of hormone replacement. Linear regression was used to examine the association of haplotype with continuous outcome variables in the whole sample and separately in women who had ever (past or current) and never used hormone replacement. F-tests were computed to assess statistical evidence for a haplotype–hormone replacement interaction and a single SNP-hormone replacement interaction. Consistent with the findings of Herrington et al.,20 our a priori hypothesis was that among those who were homozygous for the rare alleles of either SNP the effect of hormone replacement on HDL-c would be greater than among those without these alleles. F-tests are one-sided; all other statistical tests were two-sided. It is not useful to select arbitrary alpha levels to determine whether a result is significant; we therefore present exact P-values for all associations.36 We have undertaken a number of tests of association but these are hypothesis testing on the basis of previous literature. However, in the discussion, we consider our findings with respect to different methods of dealing with multiple testing. All analyses were conducted using Stata version 9.

Results

Of the 4286 women who participated in the study, eight refused consent for genetic testing and 15 were described by the research nurse as non-white; these women are not considered further here. Of the remaining 4263 women, 3404 (80%) had complete data for both SNPs, and for these women, estimated probabilities of correct haplotype inferences varied from 0.996 to 1.000. Genotype failure occurred in 3% of samples for both SNPs and there was exact agreement of genotype between the first and a repeat blood sample in a random sub-sample of 42 women. Use of hormone replacement therapy (HRT), age, HDL-c, triglycerides, insulin, glucose, and other characteristics did not differ between those women with genotype data and those without these data (all $P > 0.4$). We conducted a sensitivity analysis to determine the influence of missing data on any of the findings presented here. For this we used the method of multiple imputation by chained equation to generate 10 data sets with imputed values for the missing data (using the ice procedure in Stata).37 There was no difference between results using these imputed data sets and those presented here. There was no evidence of departure from HWE for either SNP (Table 1). The two SNPs were in moderate LD: $D^\prime/r^2$ values were 0.94/0.53. The distribution of the T-A haplotype in this population (Table 1) was similar to that found among European origin women and men by Schuit et al.18 Of the 3404 women included in this analysis, 406 (11.9%) were past hormone replacement users and 269 (7.9%) were current users.

Haplotype was associated with past and current use of hormone replacement and with those women with one or two copies of the T-A haplotype being more likely to have used hormone replacement than those who did not carry the haplotype (Table 2). Haplotype was, unexpectedly, associated with social class, though not with other socioeconomic or behavioural risk factors (Table 2). The unadjusted odds ratio of past use of hormone replacement per additional copy of the T-A haplotype was 1.16 (95% CI 1.01, 1.33), and this was not substantively altered by adjustment for childhood and adult social class, educational attainment, smoking, and physical activity: 1.17 (95% CI 1.01, 1.34).
The unadjusted odds ratio of current hormone replacement use was 1.19 (95% CI 0.99, 1.42), and this was not changed by adjustment for covariates. There was no strong statistical evidence of any interactions between childhood or adult social class and haplotype in their associations with hormone use (all P-values for interaction ≥0.3). Adjustment for other characteristics listed in Table 2 had no effect on the associations of the T-A haplotype with hormone replacement use. Genotype was not associated with the use of statins, aspirin, or blood pressure medication (data not shown), and adjustment for these medications did not affect the association of genotype with hormone replacement use.

In the whole sample, there was weak statistical evidence that each additional T-A haplotype was associated with reduced insulin resistance (P = 0.05), lower fibrinogen levels (P = 0.04) and smaller waist:hip (P = 0.06). These associations are in the opposite direction to what would be expected from the association of the haplotype with reduced oestrogen response. However, these weak associations were not fully explained by the association of haplotype with social class, with adjustment for adult and childhood social class, smoking and physical activity; all three of these associations attenuated towards the null (all P-values >0.2). Haplotype was not related to HDL-c or other cardiovascular disease risk factors (Table 2). There was no strong evidence that haplotype was related to any other oestrogen-related outcomes such as nulliparity, offspring birth weight, hysterectomy, osteoporosis, or trunk length. The magnitudes and directions of haplotype associations with HDL-c and all outcomes were similar in women who had never used hormone replacement and those who had ever (either past or current) used it, and there was no statistical evidence of a gene–hormone replacement interaction with any outcome (all P-values >0.6). We did also examine interactions between genotype and current hormone use and between genotype and past hormone use with all outcomes and found no strong statistical evidence of any interactions (all P-values >0.5).

Consistent with our findings for the haplotype analysis, women with the T or A allele of each individual SNP were more likely to be past or current hormone replacement users (all P-values >0.01), but there were no associations between each SNP and cardiovascular disease risk factors and no gene–hormone replacement interactions (all P-values >0.2). Figures 1 and 2 show the mean levels of HDL-c for each SNP with women stratified by hormone use and genotype. These demonstrate our failure to replicate the earlier results of Herrington et al. with a P-value of 0.97 for the interaction between c454-397T>C and hormone replacement use and that for the interaction between c454-351A>G and hormone replacement being 0.64.

**Discussion**

**ESR1 and hormone replacement use**

In this large cohort of post-menopausal women, we have found an association between ESR1 and use of hormone replacement, with increased risk of past and current use among those women with one or more copy of the T-A haplotype, which has been found to be associated with phenotypes indicative of a reduced oestrogen response in other studies.\(^{15-17}\) This finding suggests that there may be a genetic influence on hormone use. One possibility is that women who carry the T-A haplotype of ESR1 have lower oestrogen response and consequently more marked menopausal symptoms. However, we found no association between haplotype and oestrogen-related outcomes such as trunk length, osteoporosis, parity, offspring birth weight, and hysterectomy. A second possibility is that personality type is influenced by ESR1 and that this also affects the likelihood of a woman using HRT. One study has found an association between a TA dinucleotide repeat in ESR1 and anxiety traits \(^{38}\) and a second between this repeat and personality traits of non-conformity, irritability, and indirect aggression.\(^{39}\) We were unable to specifically assess these associations because we have neither data on this dinucleotide repeat nor detailed assessment of personality traits in our study. However, the genetic variants in ESR1 that we examined were not associated with three indicators of psychological distress, and the association between the T-A haplotype and hormone use that we found was not affected by adjustment for these indicators of psychological distress. Finally, the association may be a chance finding. Although we undertook multiple statistical tests in this manuscript, most were hypothesis-driven. However, a Bonferroni correction for all haplotype–phenotype associations would give an alpha value of 0.0017, which our associations for current and past use of HRT do not meet. Future larger studies and replication studies of the association between ESR1 and hormone replacement use should additionally focus on menopausal symptoms and personality traits to further explore whether the association is robust and what possible mechanisms might explain it.

**ESR1 and cardiovascular risk factors**

In this data set, we found a weak and unexpected association between haplotype and social class that seemed to underlie weak associations, in the opposite direction to that anticipated on the basis of the hypothesized effect.
Table 2  Hormone replacement and other characteristics by ESR1 T-A haplotype among British women aged 60–79 (n = 3404)

<table>
<thead>
<tr>
<th></th>
<th>Copies of haplotype 1 (T-A)</th>
<th>P-value for linear trend</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (n = 683)</td>
<td>1 (n = 1738)</td>
</tr>
<tr>
<td>Past use of HRT, n (%)</td>
<td>70 (10.3)</td>
<td>207 (11.9)</td>
</tr>
<tr>
<td>Current use HRT, n (%)</td>
<td>44 (6.4)</td>
<td>137 (7.9)</td>
</tr>
<tr>
<td>Continuous outcomes, mean (SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) 69.1 (5.4)</td>
<td>68.8 (5.5)</td>
<td>68.9 (5.4)</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L) 6.67 (1.29)</td>
<td>6.62 (1.21)</td>
<td>6.63 (1.19)</td>
</tr>
<tr>
<td>HDL-c (mmol/L) 1.64 (0.44)</td>
<td>1.66 (0.46)</td>
<td>1.66 (0.47)</td>
</tr>
<tr>
<td>Triglyceride (mmol/L)</td>
<td>1.68 (1.63, 1.74)</td>
<td>1.66 (1.62, 1.69)</td>
</tr>
<tr>
<td>Insulin resistance HOMA-Ra 1.70 (1.62, 1.79)</td>
<td>1.69 (1.64, 1.74)</td>
<td>1.60 (1.53, 1.68)</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)a 1.84 (1.69, 2.00)</td>
<td>1.77 (1.68, 1.87)</td>
<td>1.72 (1.61, 1.86)</td>
</tr>
<tr>
<td>Fibrinogen (g/L) 3.47 (0.73)</td>
<td>3.47 (0.71)</td>
<td>3.40 (0.68)</td>
</tr>
<tr>
<td>Systolic BP (mmHg) 147.3 (25.2)</td>
<td>147.0 (25.0)</td>
<td>147.3 (25.7)</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>79.6 (12.0)</td>
<td>79.4 (11.6)</td>
</tr>
<tr>
<td>BMI (kg/m²) 27.8 (5.0)</td>
<td>27.6 (5.1)</td>
<td>27.4 (4.9)</td>
</tr>
<tr>
<td>Waist:hip 0.822 (0.071)</td>
<td>0.819 (0.069)</td>
<td>0.816 (0.066)</td>
</tr>
<tr>
<td>Total height (mm) 1588.4 (61.1)</td>
<td>1586.9 (62.4)</td>
<td>1588.7 (60.7)</td>
</tr>
<tr>
<td>Trunk length (mm) 829.0 (34.7)</td>
<td>830.3 (36.7)</td>
<td>831.0 (35.5)</td>
</tr>
<tr>
<td>Age at menopause (years) 47.5 (4.6)</td>
<td>47.5 (4.9)</td>
<td>47.8 (4.7)</td>
</tr>
<tr>
<td>Age at menarche (years) 13.3 (1.7)</td>
<td>13.4 (1.7)</td>
<td>13.3 (1.6)</td>
</tr>
<tr>
<td>Sex-adjusted offspring birth weight (kg)b 3.24 (0.53)</td>
<td>3.24 (0.52)</td>
<td>3.26 (0.52)</td>
</tr>
<tr>
<td>Binary outcomes, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incident or prevalent CHD 194 (28.6)</td>
<td>524 (30.5)</td>
<td>286 (30.0)</td>
</tr>
<tr>
<td>Hysterectomy 199 (29.1)</td>
<td>488 (28.1)</td>
<td>265 (27.0)</td>
</tr>
<tr>
<td>Osteoporosis 51 (8.5)</td>
<td>141 (9.1)</td>
<td>86 (9.7)</td>
</tr>
<tr>
<td>Hip or wrist fracture 91 (15.9)</td>
<td>190 (13.0)</td>
<td>136 (15.9)</td>
</tr>
<tr>
<td>Nulliparous 63 (10.1)</td>
<td>153 (9.9)</td>
<td>88 (9.9)</td>
</tr>
<tr>
<td>Adult manual social class 388 (56.8)</td>
<td>1024 (58.9)</td>
<td>507 (51.6)</td>
</tr>
<tr>
<td>Childhood manual social class 558 (81.7)</td>
<td>1406 (80.9)</td>
<td>767 (78.0)</td>
</tr>
<tr>
<td>Left full-time education at or before legal minimum age 211 (35.0)</td>
<td>594 (37.1)</td>
<td>325 (35.0)</td>
</tr>
<tr>
<td>Current smoker 75 (11.0)</td>
<td>218 (12.5)</td>
<td>89 (9.1)</td>
</tr>
<tr>
<td>Physically inactive 138 (20.2)</td>
<td>328 (18.9)</td>
<td>192 (19.6)</td>
</tr>
<tr>
<td>Ever been diagnosed with depression 110 (16.1)</td>
<td>290 (16.7)</td>
<td>134 (13.6)</td>
</tr>
<tr>
<td>Currently taking, anti-depressants 84 (12.3)</td>
<td>207 (11.9)</td>
<td>108 (11.0)</td>
</tr>
<tr>
<td>Positive on euroQol mood question 154 (22.6)</td>
<td>399 (23.0)</td>
<td>221 (22.5)</td>
</tr>
</tbody>
</table>

BMI, body mass index; BP, blood pressure; HOMA-R, homeostasis model assessment of insulin resistance.

aGeometric means and 95% CI of geometric mean.

bFor first born offspring. n = 2602 with at least one birth and who could recall their first offspring’s birth weight.
of the T-A haplotype, with insulin resistance and some other cardiovascular disease risk factors. Because genes are randomly allocated at conception, we cannot think of any biological reason for the association with social class and it is likely that this finding is a chance finding. The association of the T-A haplotype with hormone use was anticipated a priori. Those with social class were not and the failure for these associations to reach a Bonferroni corrected level of statistical significance is likely to be important.

We found no strong statistical evidence of associations between haplotype and cardiovascular disease risk factors that are thought to be associated with oestrogen action. A recent prospective study of 2617 (272 incident events) men and 3791 (168 incident events) post-menopausal women found an increased risk of acute myocardial infarction and total incident CHD with carriage of one or two T-A haplotypes among women but no association in men (no evidence of a statistical interaction with sex was presented and CIs suggested that the effect was actually consistent in both sexes). However, in that study (as in ours), there was no association of haplotype with body mass index, lipid levels, blood pressure, or diabetes in either sex, and the authors concluded that the effect of ESR1 on CHD risk in women was mediated by other factors, possibly a direct action of oestrogen on endothelial function. Similarly, in the Framingham offspring study (1739 men and women), C454-397T>C was associated with prevalent myocardial infarction (59 events) and major cardiovascular events (83 events), but there were no associations with lipids, blood pressure, or diabetes. Of note, the authors of the Framingham study concluded that the association was largely driven by an effect in men, as there were too few events among women for separate analyses among them. Other studies have found no association between this genotype and CHD in either sex. In particular, the largest study to date including 3657 patients with myocardial infarction and 1211 controls with angiographically normal coronary arteries found no association between the genotypes and haplotypes examined in our study and CHD in either sex. We also found no evidence of an association between haplotype and CHD. However, we had limited power within this study to be able to detect an association and therefore this was not our primary aim. Two recent studies have found associations of ESR1 with LDL-c particle size, suggesting that this gene may be related to lipid metabolism. In the British Women’s Heart and Health Study, we do not have information on LDL-c particle size to enable us to examine this association.

Interactions between hormone use and ESR1

It is possible that our null findings of the association between ESR1 and lipid levels and other cardiovascular risk factors masks an effect in women using hormone replacement because it has been suggested that there may be an interaction between hormone replacement and this genotype in its effect on HDL-c. However, unlike one previous small study among women with established CHD, we found no interaction between either ESR1 SNPs and hormone replacement in their association with HDL-c, nor did we find main effects or interactions with any other cardiovascular disease risk factors. The previous study was undertaken in a subgroup of women who had participated in a randomized controlled trial of the effect of hormone replacement on secondary prevention of CHD and therefore had the advantage over our study that the hormone preparation given to women in the intervention arm was known and was the same for all women. However, the results of that study may not be generalizable to post-menopausal women without CHD, an important consideration because the authors of the earlier study considered the value of their findings in terms of the potential for genetic screening to inform decisions around HRT use among women in general. Our findings of the lack of an interaction between ESR1 and hormone replacement use in their association with HDL-c are consistent with two other recent population-based studies, adding further strength to our conclusion that there probably is not an interaction in the general population of post-menopausal women.

Study limitations

Our assessment of hormone replacement use was based on self-report and the majority of past users was unable to describe the type of hormone that they had used. However, most current users could describe the type of preparation and we found a similar association between haplotype and current use to that between haplotype and past use. Further, our results from sensitivity analysis, in which those who could not describe the type of preparation used were either excluded or assumed to be non-users, were similar to the main analyses presented here. Eighty per cent of the eligible participants had complete genotype data. However, there was no evidence of differences between women with genotype and those without genotype data in terms of age, hormone use, cardiovascular risk factors, or other characteristics.

Conclusions

In conclusion, our findings suggest that variants in ESR1 that appear to be associated with oestrogen response may be related to HRT use. However, we found no association between these variants and cardiovascular disease risk factors that are believed to be affected by oestrogen in the whole cohort. Importantly, we failed to replicate the earlier findings of an interaction between ESR1 and hormone replacement in their association with HDL-c. Our findings, together with those of two other recent studies that failed to replicate this previously reported interaction, suggest that it would be premature to consider genetic screening for this variant as a means of targeting HRT. Further large studies are needed to examine the role of ESR1 on propensity to take HRT, relevant novel cardiovascular risk factors, and possible interactions between hormone replacement and ESR1 in their association with CHD events.

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References

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A late complication of a patent foramen ovale amplatzer occluder device

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A 33-year-old woman had an embolic event to her fifth left finger 1-week post-partum. An echocardiogram demonstrated a patent foramen ovale (PFO) and right-to-left shunt with coughing and Valsalva manoeuvre. She underwent percutaneous closure of a PFO with a 25 mm Amplatzer PFO occluder device. No complications were evident post-operatively and echocardiographic findings were normal. Four years later she presented with an episode of left-sided hemi-sensory disturbance with left facial and left arm numbness associated with dysphasia, suggesting a transient cerebral ischaemia. A head CT-scan and carotid ultrasound were normal. She was started on aspirin and referred for transoesophageal echocardiogram. This demonstrated a mobile, flame-shaped thrombus attached to the centre button of the left atrial disc of the PFO device (Panels A and B) with normal appearance of the device and the heart. She was started on warfarin and continued with aspirin and a repeat study 3 months later did not show the left atrial thrombus anymore (Panels C and D). Warfarin was stopped and she was continued on aspirin and remains well.

Percutaneous closure of PFO is becoming increasingly common. Of its reported complications, thrombus formation, particularly on the left side of the device, is especially feared. This is usually prevented by anticoagulation during the procedure and the administration of long-term antiplatelet treatment. Late thrombus formation has been reported up to the first 6 months after implantation. To our knowledge, this is the first documented case of a late left atrial thrombus following the closure of a PFO and may have important clinical implications with the increasing use of percutaneous closure devices.