Plasma estrogen levels, estrogen receptor gene variation, and ischemic arterial disease in postmenopausal women: the three-city prospective cohort study.

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Abstract:	Background: In older postmenopausal women, high levels of endogenous estrogen have been related to adverse health outcomes including ischemic arterial disease (IAD). Whether estrogen receptor alpha (ESR1) and beta (ESR2) polymorphisms modulate the effects of estradiol on IAD has not been investigated. Methods: In the Three-City prospective cohort study among subjects over 65 years, we used a case-cohort design in which plasma levels of 17β-estradiol were measured. After exclusion of postmenopausal women using hormone therapy, a random subcohort of 533 women and 105 incident cases of first IAD events over 4-year of follow-up were analyzed. Five common polymorphisms of ESR1 and ESR2 were genotyped. Hazard ratios (HRs) of IAD for 1-standard deviation increase in estradiol levels by genotypes were estimated from Cox models after adjustment for cardiovascular risk factors and correction for multiple testing. We also investigated the role of haemostatis and inflammation as potential mediators. Results: Neither estradiol nor IAD risk were significantly associated with estrogen receptor polymorphisms. Overall, IAD risk increased with estradiol (HR:1.40, 95%CI:1.11-1.77). Stratified analysis by genotypes showed that estradiol was positively related to IAD risk in women with ESR1 rs9340799-AA genotype but not in women with AG/GG genotype (HR:1.62, 95%CI:1.22-2.17 and HR:1.03, 95%CI:0.81-1.30, respectively; p for interaction<0.05). Additional adjustment for haemostatic variables reduced the HR by about one third in women carrying rs9340799-AA genotype (HR:1.41, 95%CI:1.06-1.90).

	Conclusion: ESR1 rs9340799 genotype may modify IAD risk related to high endogenous estradiol levels in older postmenopausal women. Hypercoagulability may act as a mediator.
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Dear Editor,

Please find enclosed our article entitled " **Plasma estradiol, estrogen receptor gene variation and ischemic arterial disease in postmenopausal women: the Three-City prospective Cohort Study** " submitted for publication as an original article. In this paper, we found that *ESR1 rs9340799* genotype may modify IAD risk related to high endogenous estradiol levels in older postmenopausal women and hypercoagulability may act as a mediator.

Best regards,

Valérie Scarabin-Carré

Plasma estradiol, estrogen receptor gene variation and ischemic arterial disease in postmenopausal women: the Three-City prospective Cohort Study

Short title: **Estradiol, Estrogen Receptor polymorphisms and Ischemic Arterial Disease**

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ABSTRACT:

Background: In older postmenopausal women, high levels of endogenous estrogen have been related to adverse health outcomes including ischemic arterial disease (IAD). Whether estrogen receptor alpha (*ESR1*) and beta (*ESR2*) polymorphisms modulate the effects of estradiol on IAD has not been investigated.

Methods: In the Three-City prospective cohort study among subjects over 65 years, we used a case-cohort design in which plasma levels of 17β-estradiol were measured. After exclusion of postmenopausal women using hormone therapy, a random subcohort of 533 women and 105 incident cases of first IAD events over 4-year of follow-up were analyzed. Five common polymorphisms of *ESR1* and *ESR2* were genotyped. Hazard ratios (HRs) of IAD for 1-standard deviation increase in estradiol levels by genotypes were estimated from Cox models after adjustment for cardiovascular risk factors and correction for multiple testing. We also investigated the role of haemostatis and inflammation as potential mediators.

Results: Neither estradiol nor IAD risk were significantly associated with estrogen receptor polymorphisms. Overall, IAD risk increased with estradiol (HR:1.40, 95%CI:1.11-1.77). Stratified analysis by genotypes showed that estradiol was positively related to IAD risk in women with *ESR1 rs9340799*-AA genotype but not in women with AG/GG genotype (HR:1.62, 95%CI:1.22-2.17 and HR:1.03, 95%CI:0.81-1.30, respectively; p for interaction<0.05). Additional adjustment for haemostatic variables reduced the HR by about one third in women carrying *rs9340799*-AA genotype (HR:1.41, 95%CI:1.06-1.90).

Conclusion: *ESR1 rs9340799* genotype may modify IAD risk related to high endogenous estradiol levels in older postmenopausal women. Hypercoagulability may act as a mediator.

INTRODUCTION

Few epidemiologic studies have been conducted to assess the association between circulating levels of endogenous estradiol and the risk of chronic diseases in postmenopausal women (1). Despite an important estrogen deficiency in postmenopausal women, high plasma estradiol levels have been related to several adverse health outcomes including breast cancer (2), dementia (3), frailty (4) and all-cause mortality (5). In addition, previous data reported a positive relation of endogenous estrogens with coronary heart disease (CHD) (6, 7) and stroke (8) but these associations were not independent of traditional cardiovascular risk factors such as diabetes and body mass index (BMI). In a recent study, we found that high plasma level of endogenous estradiol was an independent predictor of ischemic arterial disease (IAD) in older postmenopausal women (9).

Estrogens exert their actions via two distinct types of estrogen receptors (ER): ER-α and ER-β, encoded by the estrogen receptor α gene (*ESR1*) located on chromosome 6 and the estrogen receptor β gene (*ESR2*) located on chromosome 14, respectively. ERs are expressed in many tissues especially in vascular endothelial and smooth muscle cells of cerebral and coronary arteries and may play a role in the development of cardiovascular disease (10, 11). Previous studies suggested that the *rs2234693* and *rs9340799* single nucleotide polymorphism might influence the expression of *ESR1* (12) and affect the function of estrogen on arterial disease including CHD and stroke (13, 14). However, a recent meta-analysis concluded to a lack of association between *ESR1* polymorphisms and CHD (15). Conflicting results about the association of *ESR1* polymorphisms with the risk of stroke have been also reported (16, 17). Similarly, data on association of *ESR2* polymorphisms (including *rs1256049* and *rs1271572*) with the risk of CHD or stroke have not been consistent (18, 19).

Despite the potential role of estrogens and their receptors in the development of IAD, no study has evaluated whether ER polymorphisms could modulate the effects of estradiol on IAD. Therefore, we investigated the risk of CHD and ischemic stroke related to plasma estradiol levels by *ESR1* and *ESR2* polymorphisms in a cohort study among postmenopausal women over 65 years, who did not use hormone therapy. In addition, since estradiol is known to be associated with haemostatic and

inflammation parameters (20, 21), their role was evaluated as potential mediators in the association of both estradiol and ER polymorphisms with IAD risk.

METHODS

Population study

The Three-City (3C) study is a large ongoing French prospective cohort designed to evaluate the risk of dementia, CHD and stroke, in subjects over 65 years. The study was approved by the Ethics Committee of the University Hospital of Kremlin-Bicêtre and a written informed consent was obtained for all participants. A detailed methodology of the study has been previously described (22). Briefly, 3,649 men and 5,645 women, not institutionalized, registered on electoral rolls in 3 French cities (Bordeaux, Dijon and Montpellier), were recruited between 1999 and 2001. Baseline data were collected for each participant by trained psychologists or nurses using standardized questionnaires during a face-to-face interview. Moreover, each subject underwent a clinical examination to measure blood pressure, weight and height.

Baseline covariates

Information on sociodemographic characteristics, education, medical history, medication use, smoking status and consumption of alcohol were systematically collected at baseline. Women were classified as current hormone therapy users if they used hormone therapy at any time during the 3 months before the inclusion. BMI was calculated as weight in kilograms divided by height in meters squared. Hypertension status was defined as a high blood pressure measurement (systolic blood pressure ≥140mmHg and/or diastolic blood pressure ≥90mm Hg) or antihypertensive treatment at inclusion. Participants were categorized as diabetic if their fasting blood glucose level exceeded 1.26g/L (or 7mmol/L) or if they used current treatment for diabetes at baseline. Hypercholesterolemia was considered present if the fasting blood total cholesterol level was above 2.40g/L or if the subjects

were treated for hypercholesterolemia. Smoking status was studied in three categories (never, past and current).

Follow-up and event ascertainment

Every 2 years after inclusion, the participants have been reexamined for the detection of dementia and IAD events. For this study, we focused on data collected over the 4-year follow-up. IAD consisted of either CHD or ischemic stroke during the follow-up. These events were adjudicated within an independent group of experts using medical documentation. CHD was defined as a hospitalization for either stable or unstable angina pectoris, coronary dilatation, artery by-pass, myocardial infarction, or CHD death. Non-fatal CHD were ascertained using hospital charts and practitioners' reports. CHD deaths were validated by reviewing hospital records, medical data obtained from family physicians or specialists and proxy interviews (coded I210 to 219, I251 to 259, I461 and R960 according to 10th version of the International Classification Disease) as described (23). Stroke events were defined as a rapid onset of a neurological deficit lasting more than 24 hours and confirmed by a lesion compatible with an acute stroke on computed tomography or magnetic resonance imaging of the brain. Ischemic or hemorrhagic stroke events were classified by a review of brain imaging. Our study focused on IAD and hemorrhagic stroke events were therefore excluded. For subjects who presented with both CHD and ischemic stroke during follow-up, we used the first cardiovascular event that occurred.

Case-cohort study

A case-cohort study was designed from the 3C study to investigate the association of blood biomarkers with cardiovascular risk and dementia (24). The case-cohort study design was described firstly by Prentice (25) and consists of a random sample of the original cohort together with all incidents cases of this cohort. In practice, after exclusion of men (n=1,264), current users of hormone therapy (n=120), women with a personal history of CHD or stroke at baseline (n=86) and lost for

follow-up (n=16), the subcohort included 537 women (522 non-cases and 15 cases) to which 91 incident cases of IAD outside the subcohort were added. The detailed flowchart has been described (9). Data on all ER polymorphisms were missing for 5 subjects. The final sample therefore consisted of 623 women, 518 non-cases and 105 incident cases of a first IAD event including 67 CHD and 38 ischemic strokes.

Blood collection and laboratory methods

At baseline, blood samples were collected for more than 90% of the full cohort. Plasma samples were available for all the subjects included in the case-cohort study. EDTA plasmas were obtained after one centrifugation at 3,000g and immediately stored at -80°C in 1-mL plastic tubes.

Plasma total estradiol was measured with a sensitive direct radioimmunoassay (RIA) using an Orion Diagnostica device (Spectria, Espoo, Finland). The minimum detectable concentration was 2pg/mL (7.3pmol/L). The intraassay and interassay coefficient of variation (CV) were 17.6% and 18.1% respectively, for total estradiol concentrations of 3.2pg/mL (12pmol/L) and were 2.8% and 5.8% respectively, for total estradiol concentrations of 24pg/mL (88pmol/L).

DNA was extracted from white blood cells (Puregene Kit, Qiagen, France) and stored at-80°C. Genotyping of ER polymorphisms was performed by Kbiosciences (Hoddesdon Herts, UK) using their competitive allele specific polymerase chain reaction (PCR) single-nucleotide polymorphism (SNP) genotyping system (KASPar). Fluorescence scanning in a BMG Labtech Pherastar scanner analyzed the amplified PCR products and the results were interpreted with KlusterCaller 1.1 software. The error rate for the KASPar assay system is less than 0.3%. The two most commonly studied *ESR1* polymorphisms were investigated: *rs2234693* and *rs9340799* (otherwise known as *Pvu*II and *Xba*I), which are located at position 397 and 351 of intron 1, respectively. Three *ESR2* polymorphisms were genotyped: *rs1256049* (otherwise known as *Rsa*I), *rs4986938* (otherwise known as *Alu*I) and rs1271572, which are located in position 1082 of exon 5, in position 1730 of the 3'-untranslated region of exon 8 and in the promoter region, respectively. The selection of these polymorphisms was based

on previously published associations with risk of CHD and stroke (15, 17, 18) and possible functional significance (12, 26, 27).

Laboratory methods for haemostatic variables and CRP have been described (20, 24, 28, 29). Briefly, fibrinogen was measured in citrated plasmas by the kinetic method of Clauss and von Willebrand factor and fibrin D-dimer by ELISAs. Thrombin generation was measured by the calibrated automated thrombography. We used peak height (highest value of thrombin generated), and endogenous thrombin potential (ETP; total quantity of thrombin generated) in our analyses. High-sensitivity serum C-reactive protein (hs-CRP) was measured using a particle-enhanced turbidimetric immunoassay.

Statistical analyses

Chi-square tests were used to compare the distribution of ER genotypes with those predicted under the Hardy-Weinberg equilibrium in the subcohort. Pairwise linkage disequilibrium between the different polymorphisms was analyzed by using Thesias software in the subcohort (30).

Baseline characteristics of all subjects in the subcohort are presented as frequencies for categorical variables and arithmetic means with standard deviations for continuous variables that presented a normal distribution. Variables with a positive skewed distribution were log-transformed and values were expressed as geometric means and interquartile ranges. Baseline characteristics of subjects in the subcohort were compared by *rs9340799* genotypes using $\chi 2$ tests and 2-tailed Student's *t*-tests. Mean levels of total estradiol were compared by ER polymorphisms in women of the subcohort using ANOVA tests adjusted for study center and traditional cardiovascular risk factors including BMI, diabetes, hypertension, hypercholesterolemia and smoking status. In addition, Pearson's correlations were estimated between haemostatic variables and estradiol by ER genotype in the subcohort.

The association of both estradiol levels and ER polymorphisms with IAD risk and their interactions were investigated using weighted Cox proportional-hazards models adapted for the case-cohort design by the method of Barlow (31, 32). Because age is sharply associated with IAD risk, age as time-scale was used as recommended (33). The proportional hazards assumption was checked for each model. Additive, dominant and recessive models were initially examined to determine which genetic model best described the data. Therefore, results are presented assuming a genotype model in dominant model with a 2-level categorical variable. The hazard ratios (HRs) and 95% confidence intervals (95%CI) by ER genotypes were estimated for 1-standard deviation increase in the log-transformed estradiol distribution. Estradiol x ER genotypes interaction terms were introduced into statistical models. The HRs for IAD were adjusted for study center and traditional cardiovascular risk factors including BMI, diabetes, hypertension, hypercholesterolemia and smoking status. In addition, given that five SNPs were investigated, Bonferroni correction for multiple comparisons was used. The HRs for IAD were further adjusted for haemostatic and inflammation markers including fibrinogen, thrombin generation, von Willebrand factor, fibrin D-dimer and hs-CRP, to evaluate the part of the excess risk explained by these variables. Of note, data on haemostatic and inflammation variables were missing for 25 subjects.

For each ER polymorphism, the risk of IAD was also investigated by tertiles of estradiol distribution and the low tertile was chosen as the reference group. Tests for linear trend across the 3 categories of estradiol levels were used to investigate the significance of the variables, after having evaluated the linearity of the associations. To assess the linearity of the relation of estradiol levels with IAD risk, we used tests based on the difference in the log-likelihood between two models of prediction (one with 2 dummy variables corresponding to the tertiles of the estradiol levels and the other including the qualitative ordinal variable in 3 categories). All tests were non-significant and we did not reject the hypothesis of linearity.

Finally, a separate analysis was performed for CHD and ischemic stroke, including the first arterial event. Similarly to analyses on IAD risk, the HRs of CHD and ischemic stroke in relation to estradiol levels were estimated by ER polymorphisms with a multivariate Cox model.

231 Statistical analyses were performed with the Statistical Analysis System software version 9.2
232 (SAS Institute Inc, Cary, NC).
233

RESULTS

Baseline characteristics are described for 533 women in the whole subcohort and according to *rs9340799* genotype (table 1). The distribution of *ESR1 rs9340799* polymorphisms in the subcohort was similar to those expected in Caucasian subjects, as follows: 45.6%, 42.9%, and 11.5% for AA, AG and GG respectively. No significant difference was detected among traditional cardiovascular risk factors between women with *rs9340799*-AA genotype and those with *rs9340799*-AG/GG genotype. Mean levels of total estradiol were similar across *rs9340799* genotypes. There was no significant association between estradiol levels and other ER polymorphisms (data not shown).

The genotypic frequencies of ER polymorphisms were in agreement with the Hardy–Weinberg equilibrium. The two *ESR1* polymorphisms were in strong linkage disequilibrium and so were the three *ESR2* polymorphisms (all D' ranged from 0.90 to 1.00).

No significant association was found between IAD risk and the different ER polymorphisms (data not shown). After adjustment for traditional cardiovascular risk factors, plasma estradiol levels were positively associated with IAD risk in the whole population sample (HR:1.40 for 1-SD log estradiol change, 95%CI:1.11-1.77). Adjusted HR for IAD events and 95% CI in relation to estradiol levels by ER polymorphisms are given in table 2 before and after Bonferroni correction. With regard to *ESR1 rs9340799* polymorphism, the risk of IAD was positively associated with total estradiol in women with AA genotype but not in women with AG/GG genotype (HR:1.62, 95% CI:1.22-2.17 and HR:1.03, 95%CI:0.81-1.30, respectively; p for interaction<0.05). In women with AA genotype, high levels of estradiol increased significantly the risk of IAD compared to the lowest estradiol levels (HR: 3.78, 95%CI:1.59-8.99 for the higher tertile vs the lower tertile, p for linear trend<0.01). After Bonferroni correction for multiple testing, the estradiol x *rs9340799* genotype interaction remained significant. Similar associations were observed for *rs2234693* polymorphism, although the interaction between estradiol levels and *rs2234693* polymorphism was not significant. No significant interaction was detected between estradiol levels and ER-β polymorphisms on IAD risk.

Estradiol was positively correlated to fibrinogen (r=0.21, p<0.01), peak height of thrombin generation (r=0.10, p=0.13) and hs-CRP (r=0.38, p<0.01) in women with *rs9340799*-AA genotype. After additional adjustment for haemostatic variables, the HR for IAD risk in women with AA genotype (HR:1.58 for 1-SD log estradiol change, 95%CI:1.21-2.05) was reduced to 1.41 (95%CI:1.06-1.90), suggesting that hypercoagulability explained 29 % of the excess risk of IAD. Adjustment for hs-CRP alone or combined with haemostatic variables made no substantial change to the results (data not shown).

When the analyses were stratified by type of arterial events, total estradiol was positively associated with CHD risk in women with *rs9340799*-AA genotype, but not in women with *rs9340799*-AG/GG genotype (adjusted and corrected HR: 1.93, 95% CI: 1.28-2.91 and HR: 1.01, 95% CI: 0.76-1.35, respectively; p for interaction<0.05). No significant interaction was found between *rs9340799* genotype and estradiol for the risk of ischemic stroke.

DISCUSSION

In this prospective study, neither estradiol levels nor IAD risk were significantly associated with ER polymorphisms in older postmenopausal women. However, we found that genetic variations in *ESR1* modified the risk of IAD related to high endogenous estradiol levels. Plasma estradiol levels were positively associated with IAD risk in women carrying *rs9340799*-AA genotype but not in women carrying *rs9340799*-AG/GG genotype. The excess risk of IAD in women with AA genotype was partially explained by hypercoagulability. No interaction between estradiol levels and other ER polymorphisms was observed in relation with the risk of IAD.

Many studies have investigated the association of ER polymorphisms with the risk of IAD but previous data led to conflicting results. The reasons for such discrepancies include differences in study population, design and analysis, together with a strong heterogeneity in quality of investigations (15). Importantly, while the effect of ER polymorphisms on IAD may be different for women and men, few data were stratified by sex. Furthermore, use of hormone therapy and/or menopause status were not taken into account in most studies among women and ultimately, only few studies focused on postmenopausal women. A population-based prospective cohort study including 3,488 older postmenopausal women showed a positive association of *ESR1 rs2234693*-T/*rs9340799*-A haplotype with the risk of myocardial infarction (34). However, although the results were adjusted for use of hormone therapy, no data were given among women not using estrogens. Consistently, another study excluding women using hormone therapy, reported an increased risk of stroke in women carrying *ESR1 rs2234693*-TT (17). By contrast, a Japanese case-control study including highly selected postmenopausal women reported that the *ESR1 rs2234693*-CC and *rs9340799*-GG genotypes were more common in women with coronary artery disease than in controls (35).With regard to *ESR2* polymorphisms, the Women's Health Study showed that *rs1256049*-G and *rs1271572*-T alleles increased CHD risk but not stroke risk, whereas *rs4986938* polymorphism was associated with neither CHD nor stroke (18). Most other investigations among postmenopausal women failed to detect an association of either CHD or stroke risk with *ESR1* and *ESR2* polymorphisms (16, 19, 36-38).

Therefore, our data are consistent with these null findings which are also supported by a meta-analysis (15).

Few studies investigated the interaction between estradiol and ER polymorphisms in relation to health outcomes in postmenopausal women. Interestingly, a dutch case-cohort study showed that the positive association between estradiol and breast cancer risk was more pronounced among women with *rs2234693*-CT/ TT genotype than among those with *rs2234693*-CC genotype (39). This result may be relevant to our finding because both *rs2234693* and *rs9340799* are in linkage disequilibrium. To our knowledge, the 3C study is the first to show that *ESR1* polymorphisms may modulate the association between plasma estradiol levels and IAD risk. As previously described, plasma estradiol levels were positively associated with IAD risk in the whole population irrespective of ER genotypes (9). Adjustment for traditional cardiovascular risk factors yielded similar results suggesting no major mediator role of obesity, diabetes, hypercholesterolemia, hypertension and smoking status. Therefore, we can hypothesize that the relation between estradiol an IAD risk among women with *rs9340799*-AA genotype could be driven by other mechanisms involved in atherothrombosis such as inflammation and hypercoagulability. Both estradiol levels and IAD risk are known to be positively associated with CRP in postmenopausal women (21, 29, 40). Moreover, one study reported that the *rs2234693*-T allele carriers exhibited an enhanced inflammatory response compared to the CC homozygotous (41). Of note, while *rs2234693* and *rs9340799* polymorphisms are in linkage disequilibrium, inflammation could be similarly increased in women with *rs9340799*-AA genotype as those with *rs2234693*-TT genotype. Nevertheless, in our study, the additional adjustment for hs-CRP did not change the results, suggesting that the inflammation did not explain the excess of IAD risk. Alternatively, given that high estradiol levels and IAD risk are known to be associated with hypercoagulability(20, 24, 28, 42), the association between estradiol and IAD risk could also be mediated through haemostatic variables. Interestingly, a nested case-control study in the Women's Health Initiative trials reported an increased response of plasmin-antiplasmin to hormone therapy in women with *rs9340799*-AA genotype compared to women with *rs9340799*-AG or *rs9340799*-GG genotypes(38). Although exogenous and endogenous estrogens might have different metabolic and vascular effects, it can be suggested that

high endogenous estradiol levels play a role in the thrombotic component of IAD through blood coagulation and fibrinolysis activation. In our study, hypercoagulability explained a part of the relation between estradiol and IAD risk in women with *rs9340799*-AA genotype. Further adjustment for hs-CRP did not change the results, suggesting that changes in coagulation pathway play a more important role in the development of IAD than inflammation. Other unmeasured haemostatic variables could also be involved in the association of IAD risk with estradiol in women with *rs9340799*-AA genotype and further data on clotting factors are required.

Increased IAD risk in women with elevated estradiol levels and carrying *rs9340799*-AA genotype does not necessarily imply that relationship is causal. First, our findings could be due to the chance. Second, studied genetic variants may be highly correlated with other variants within *ESR1* or nearby genes that are themselves "causal" in the development of IAD, suggesting that the ER polymorphisms are not the only factor that determines the effect of estrogen on IAD. Few studies support the functional importance of *ESR1* polymorphisms altering the quantity or the quality of ER-α transcripts or resulting proteins and the underlying mechanisms related to a direct effect of ER polymorphism in vascular biology remain to be clarified (12, 27, 43).

The strengths of our study include the prospective population-based and multicenter cohort design with a high participation rate during the 4 years of follow-up. Moreover, baseline data were collected by a direct interview and high-quality methods of ascertainment of cardiovascular risk factors and disease outcome were used. In addition, estradiol measurements and genotyping were conducted without knowledge of the case or non-case status, using validated methodology. Furthermore, our genetic-association study cannot be influenced by stratification population or heterogeneity because most participants were Caucasian.

Our study has several limitations. Firstly, the small number of incident cases may result in a lack of statistical power, especially for subgroup analyses by CHD or stroke event. In addition, multiple comparisons by different polymorphisms could result in falsely positive findings. However, our results remained significant after Bonferroni correction for multiple testing. Secondly, our

population study included older women who were aged 73 years on average and mostly Caucasian. Our data cannot therefore be generalized to younger postmenopausal women or other ethnics. With regard to the sex steroid hormones assays, especially at low levels of estradiol in postmenopausal women, conventional RIAs with preceding purification steps would provide more reliable and accurate measurements of plasma estradiol as compared with direct RIA (44). However, measurement error related to direct RIA would bias our analysis toward the null hypothesis, resulting in a potential underestimation of the true associations (1). On the other hand, the blood concentration of estrogens does not necessarily reflect the biologically active forms at the tissue level, as they are dependent on the local enzyme activity and the binding on the protein transporters such as sex hormone binding globulin.

In conclusion, *ESR1 rs9340799*-AA genotype may increase IAD risk related to high endogenous estradiol levels in older postmenopausal women and hypercoagulability may explain a part of this excess of risk. These innovative results have the potential to improve the stratification of IAD risk in postmenopausal women whose IAD risk may depend upon both plasma estradiol levels and *ESR1* genotype and their interaction. However, further investigations with larger sample size are needed to replicate these findings and to assess the interaction between estradiol levels and *ESR1* genetics polymorphism on CHD and stroke risk separately.

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REFERENCES:

1. **Rosner W, Hankinson SE, Sluss PM, Vesper HW, Wierman ME** 2013 Challenges to the Measurement of Estradiol: An Endocrine Society Position Statement. Journal of Clinical Endocrinology & Metabolism 98:1376-1387
2. **Key T, Appleby P, Barnes I, Reeves G** 2002 Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. Journal of the National Cancer Institute 94:606-616
3. **Ravaglia G, Forti P, Maioli F, Bastagli L, Montesi F, Pisacane N, Chiappelli M, Licastro F, Patterson C** 2007 Endogenous sex hormones as risk factors for dementia in elderly men and women. Journals of Gerontology Series a-Biological Sciences and Medical Sciences 62:1035-1041
4. **Carcaillon L, Garcia-Garcia FJ, Tresguerres JAF, Avila GG, Kireev R, Rodriguez-Manas L** 2012 Higher Levels of Endogenous Estradiol are Associated with Frailty in Postmenopausal Women from the Toledo Study for Healthy Aging. Journal of Clinical Endocrinology & Metabolism 97:2898-2906
5. **Maggio M, Ceda GP, Lauretani F, Bandinelli S, Ruggiero C, Guralnik JM, Metter EJ, Ling SM, Paolisso G, Valenti G, Cappola AR, Ferrucci L** 2009 Relationship between higher estradiol levels and 9-year mortality in older women: the Invecchiare in Chianti study. Journal of the American Geriatrics Society 57:1810-1815
6. **Rexrode KM, Manson JE, Lee IM, Ridker PM, Sluss PM, Cook NR, Buring JE** 2003 Sex hormone levels and risk of cardiovascular events in postmenopausal women. Circulation 108:1688-1693
7. **Chen Y, Zeleniuch-Jacquotte A, Arslan AA, Wojcik O, Toniolo P, Shore RE, Levitz M, Koenig KL** 2011 Endogenous hormones and coronary heart disease in postmenopausal women. Atherosclerosis 216:414-419
8. **Lee JS, Yaffe K, Lui LY, Cauley J, Taylor B, Browner W, Cummings S** 2010 Prospective study of endogenous circulating estradiol and risk of stroke in older women. Archives of neurology 67:195-201
9. **Scarabin-Carre V, Canonico M, Brailly-Tabard S, Trabado S, Ducimetiere P, Giroud M, Ryan J, Helmer C, Plu-Bureau G, Guiochon-Mantel A, Scarabin PY** 2012 High level of plasma estradiol as a new predictor of ischemic arterial disease in older postmenopausal women: the three-city cohort study. Journal of the American Heart Association 1:e001388
10. **Mendelsohn ME, Karas RH** 1999 The protective effects of estrogen on the cardiovascular system. New England Journal of Medicine 340:1801-1811
11. **Gruber CJ, Tschugguel W, Schneeberger C, Huber JC** 2002 Mechanisms of disease - Production and actions of estrogens. New England Journal of Medicine 346:340-352
12. **Herrington DM, Howard TD, Hawkins GA, Reboussin DM, Xu JF, Zheng SL, Brosnihan KB, Meyers DA, Bleecker ER** 2002 Estrogen-receptor polymorphisms and effects of estrogen replacement on high-density lipoprotein cholesterol in women with coronary disease. New England Journal of Medicine 346:967-974
13. **Deroo BJ, Korach KS** 2006 Estrogen receptors and human disease. Journal of Clinical Investigation 116:561-570
14. **Saltiki K, Alevizaki M** 2007 Coronary heart disease in postmenopausal women; the role of endogenous estrogens and their receptors. Hormones (Athens, Greece) 6:9-24
15. **Lluis-Ganella C, Lucas G, Subirana I, Escurriol V, Tomas M, Senti M, Sala J, Marrugat J, Elosua R** 2009 Qualitative assessment of previous evidence and an updated meta-analysis confirms lack of association between the ESR1 rs2234693 (PvuII) variant and coronary heart disease in men and women. Atherosclerosis 207:480-486
16. **Molvarec A, Szeplaki G, Kovacs M, Szeplaki Z, Fazakas A, Prohaszka Z, Fust G, Karadi I** 2007 Estrogen receptor alpha (ESR1) PvuII and XbaI gene polymorphisms in ischemic stroke in a Hungarian population. Clinica Chimica Acta 382:100-105

17. **Munshi A, Sharma V, Kaul S, Al-Hazzani A, Alshatwi AA, Manohar VR, Rajeshwar K, Babu MS, Jyothy A** 2010 Estrogen receptor alpha genetic variants and the risk of stroke in a South Indian population from Andhra Pradesh. Clinica Chimica Acta 411:1817-1821
18. **Rexrode KM, Ridker PM, Hegener HH, Buring JE, Manson JE, Zee RYL** 2007 Polymorphisms and haplotypes of the estrogen receptor-beta gene (ESR2) and cardiovascular disease in men and women. Clin Chem 53:1749-1756
19. **Markoula S, Milionis H, Lazaros L, Spengos K, Vassilopoulou S, Chatzistefanidis D, Kargiotis O, Georgiou I, Kyritsis AP** 2012 Associations of ESR2 AluI (G/A) polymorphism with ischemic stroke in Caucasians. Journal of the Neurological Sciences 316:126-130
20. **Canonico M, Brailly-Tabard S, Gaussem P, Setiao J, Rouaud O, Ryan J, Carcaillon L, Guiochon-Mantel A, Scarabin PY** 2012 Endogenous oestradiol as a positive correlate of plasma fibrinogen among older postmenopausal women: a population-based study (the Three-City cohort study). Clinical endocrinology 77:905-910
21. **Maggio M, Ceda GP, Lauretani F, Bandinelli S, Corsi AM, Giallauria F, Guralnik JM, Zuliani G, Cattabiani C, Parrino S, Ablondi F, Dall'Aglio E, Ceresini G, Basaria S, Ferrucci L** 2011 SHBG, Sex Hormones, and Inflammatory Markers in Older Women. Journal of Clinical Endocrinology & Metabolism 96:1053-1059
22. **Alperovitch A** 2003 Vascular factors and risk of dementia: Design of the three-city study and baseline characteristics of the study population. Neuroepidemiology 22:316-325
23. **Alperovitch A, Bertrand M, Jougla E, Vidal JS, Ducimetiere P, Helmer C, Ritchie K, Pavillon G, Tzourio C** 2009 Do we really know the cause of death of the very old? Comparison between official mortality statistics and cohort study classification. European Journal of Epidemiology 24:669-675
24. **Carcaillon L, Alhenc-Gelas M, Bejot Y, Spaft C, Ducimetiere P, Ritchie K, Dartigues JF, Scarabin PY** 2011 Increased Thrombin Generation Is Associated With Acute Ischemic Stroke but Not With Coronary Heart Disease in the Elderly The Three-City Cohort Study. Arteriosclerosis Thrombosis and Vascular Biology 31:1445-1451
25. **Prentice RL** 1986 On the design of synthetic case-control studies. Biometrics 42:301-310
26. **Rosenkranz K, Hinney A, Ziegler A, Hermann H, Fichter M, Mayer H, Siegfried W, Young JK, Remschmidt H, Hebebrand J** 1998 Systematic mutation screening of the estrogen receptor beta gene in probands of different weight extremes: Identification of several genetic variants. Journal of Clinical Endocrinology & Metabolism 83:4524-4527
27. **Jeng MH, Shupnik MA, Bender TP, Westin EH, Bandyopadhyay D, Kumar R, Masamura S, Santen RJ** 1998 Estrogen receptor expression and function in long-term estrogen-deprived human breast cancer cells. Endocrinology 139:4164-4174
28. **Carcaillon L, Gaussem P, Ducimetiere P, Giroud M, Ritchie K, Dartigues JF, Scarabin PY** 2009 Elevated plasma fibrin D-dimer as a risk factor for vascular dementia: the Three-City cohort study. Journal of Thrombosis and Haemostasis 7:1972-1978
29. **Straczek C, Ducimetiere P, Barberger-Gateau P, Helmer C, Ritchie K, Jouven X, Carcaillon L, Amouyel P, Tzourio C, Empana JP** 2010 Higher level of systemic C-reactive protein is independently predictive of coronary heart disease in older community-dwelling adults: the three-city study. Journal of the American Geriatrics Society 58:129-135
30. **Tregouet DA, Garelle V** 2007 A new JAVA interface implementation of THESIAS: testing haplotype effects in association studies. Bioinformatics (Oxford, England) 23:1038-1039
31. **Barlow WE** 1994 Robust variance estimation for the case-cohort design. Biometrics 50:1064-1072
32. **Barlow WE, Ichikawa L, Rosner D, Izumi S** 1999 Analysis of case-cohort designs. Journal of clinical epidemiology 52:1165-1172
33. **Korn EL, Graubard BI, Midthune D** 1997 Time-to-event analysis of longitudinal follow-up of a survey: choice of the time-scale. American journal of epidemiology 145:72-80
34. **Schuit SCE, Oei HHS, Witteman JCM, van Kessel CHG, van Meurs JBJ, Nijhuis RL, van Leeuwen J, de Jong FH, Zillikens MC, Hofman A, Pols HAP, Uitterlinden AG** 2004 Estrogen

496 receptor alpha gene polymorphisms and risk of myocardial infarction. Jama-Journal of the
497 American Medical Association 291:2969-2977
498 35. **Lu H, Higashikata T, Inazu A, Nohara A, Yu WX, Shimizu M, Mabuchi H** 2002 Association of
499 estrogen receptor-alpha gene polymorphisms with coronary artery disease in patients with
500 familial hypercholesterolemia. Arteriosclerosis Thrombosis and Vascular Biology 22:817-823
501 36. **Matsubara Y, Murata M, Kawano K, Zama T, Aoki N, Yoshino H, Watanabe G, Ishikawa K,**
502 **Ikeda Y** 1997 Genotype distribution of estrogen receptor polymorphisms in men and
503 postmenopausal women from healthy and coronary populations and its relation to serum
504 lipid levels. Arteriosclerosis Thrombosis and Vascular Biology 17:3006-3012
505 37. **Kjaergaard AD, Ellervik C, Tybjaerg-Hansen A, Axelsson CK, Gronholdt MLM, Grande P,**
506 **Jensen GB, Nordestgaard BG** 2007 Estrogen receptor alpha polymorphism and risk of
507 cardiovascular disease, cancer, and hip fracture - Cross-sectional, cohort, and case-control
508 studies and a meta-analysis. Circulation 115:861-871
509 38. **Rossouw J, Bray P, Liu JM, Kooperberg C, Hsia J, Lewis C, Cushman M, Bonds D, Hendrix S,**
510 **Papanicolaou G, Howard T, Herrington D** 2011 Estrogen Receptor Polymorphisms and the
511 Vascular Effects of Hormone Therapy. Arteriosclerosis Thrombosis and Vascular Biology
512 31:464-U433
513 39. **Onland-Moret NC, van Gils CH, Roest M, Grobbee DE, Peeters PH** 2005 The estrogen
514 receptor alpha gene and breast cancer risk (The Netherlands). Cancer Causes Control
515 16:1195-1202
516 40. **Stork S, Bots ML, Grobbee DE, van der Schouw YT** 2008 Endogenous sex hormones and C-
517 reactive protein in healthy postmenopausal women. J Intern Med 264:245-253
518 41. **Mysliwska J, Rutkowska A, Hak L, Siebert J, Szyndler K, Rachon D** 2009 Inflammatory
519 response of coronary artery disease postmenopausal women is associated with the IVS1-
520 397T > C estrogen receptor alpha polymorphism. Clinical immunology (Orlando, Fla 130:355-
521 364
522 42. **Danesh J, Lewington S, Thompson SG, Lowe GD, Collins R, Kostis JB, Wilson AC, Folsom AR,**
523 **Wu K, Benderly M, Goldbourt U, Willeit J, Kiechl S, Yarnell JW, Sweetnam PM, Elwood PC,**
524 **Cushman M, Psaty BM, Tracy RP, Tybjaerg-Hansen A, Haverkate F, de Maat MP, Fowkes FG,**
525 **Lee AJ, Smith FB, Salomaa V, Harald K, Rasi R, Vahtera E, Jousilahti P, Pekkanen J,**
526 **D'Agostino R, Kannel WB, Wilson PW, Tofler G, Arocha-Pinango CL, Rodriguez-Larralde A,**
527 **Nagy E, Mijares M, Espinosa R, Rodriquez-Roa E, Ryder E, Diez-Ewald MP, Campos G,**
528 **Fernandez V, Torres E, Marchioli R, Valagussa F, Rosengren A, Wilhelmsen L, Lappas G,**
529 **Eriksson H, Cremer P, Nagel D, Curb JD, Rodriguez B, Yano K, Salonen JT, Nyyssonen K,**
530 **Tuomainen TP, Hedblad B, Lind P, Loewel H, Koenig W, Meade TW, Cooper JA, De Stavola**
531 **B, Knottenbelt C, Miller GJ, Cooper JA, Bauer KA, Rosenberg RD, Sato S, Kitamura A, Naito**
532 **Y, Palosuo T, Ducimetiere P, Amouyel P, Arveiler D, Evans AE, Ferrieres J, Juhan-Vague I,**
533 **Bingham A, Schulte H, Assmann G, Cantin B, Lamarche B, Despres JP, Dagenais GR,**
534 **Tunstall-Pedoe H, Woodward M, Ben-Shlomo Y, Davey Smith G, Palmieri V, Yeh JL,**
535 **Rudnicka A, Ridker P, Rodeghiero F, Tosetto A, Shepherd J, Ford I, Robertson M, Brunner E,**
536 **Shipley M, Feskens EJ, Kromhout D, Dickinson A, Ireland B, Juzwishin K, Kaptoge S,**
537 **Lewington S, Memon A, Sarwar N, Walker M, Wheeler J, White I, Wood A** 2005 Plasma
538 fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality: an
539 individual participant meta-analysis. Jama 294:1799-1809
540 43. **Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, Li X, Hawkins GA, Reboussin DM,**
541 **Xu J, Zheng SL, Meyers DA, Bleecker ER** 2002 Common estrogen receptor polymorphism
542 augments effects of hormone replacement therapy on E-selectin but not C-reactive protein.
543 Circulation 105:1879-1882
544 44. **Stanczyk FZ, Jurow J, Hsing AW** 2010 Limitations of direct immunoassays for measuring
545 circulating estradiol levels in postmenopausal women and men in epidemiologic studies.
546 Cancer Epidemiol Biomarkers Prev 19:903-906
547

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Table legends:

Table 1. Baseline characteristics of participants in subcohort of 3C study by *ESR1 rs9340799* genotype

Table 2. Hazard Ratios of IAD events in relation to 1 SD log increase of estradiol levels by ER polymorphisms among 623 postmenopausal women of 3C case-cohort study

Table 1. Baseline characteristics of participants in subcohort of 3C study by *ESR1 rs9340799* genotype

	All subjects	*ESR1 rs9340799* Genotype**		
		AA	AG/GG	
	(n=533)	(n=241)	(n=288)	p value*
Characteristic				
Age, mean (SD), y	74.4 (5.3)	74.8 (5.2)	74.1 (5.4)	0.11
BMI, mean (SD), kg/m²	25.7 (4.8)	25.8 (4.7)	25.7 (4.9)	0.89
Hypertension, n (%)	407 (76.4)	186 (45.9)	219 (54.1)	0.76
Diabetes, n (%)	38 (7.2)	17 (44.7)	21 (55.3)	0.89
Hypercholesterolomia, n (%)	338 (63.4)	160 (47.6)	176 (52.4)	0.21
Smoking, n (%)				
Never	435 (81.6)	202 (46.8)	230 (53.2)	0.33
Past	73 (13.7)	27 (37.5)	45 (62.5)	
Current	25 (4.7)	12 (48.0)	13 (52.0)	
Total Estradiol, GM (IQR), pg/mL	5.24 (3.51-8.00)	5.23 (3.57-7.90)	5.28 (3.15-8.33)	0.87

* p value obtained from t-tests or χ^2 tests for all characteristics except for total estradiol (ANOVA adjusted for study center, BMI, diabetes, hypertension, hypercholesterolemia and smoking status)

** 4 missing values

GM : Geometric Mean ; IQR : Interquartile Range

Table 2. Hazard Ratios of IAD events in relation to 1 SD log increase of estradiol levels by ER polymorphisms among 623 postmenopausal women of 3C case-cohort study

		Ischemic Arterial Disease** (n=623)									
		No. of Events	HR (95% CI)	Marginal p†	Corrected pλ	No. of Events	HR (95% CI)	Marginal p†	Corrected pλ	Marginal p interaction	Corrected p interactionλ
	rs2234693*	TT (n=190)				TC/CC (n=421)					
	Total Estradiol, pg/mL										
	For 1 SD log	29	1.52 (1.06-2.18)	**0.02**	0.11	74	1.15 (0.94-1.42)	0.18	0.91	0.13	0.65
	T1 < 4.21	4	1 [reference]	**0.04**	0.22	20	1 [reference]	0.44	1.00		
	T2 [4.21-7.27[	13	3.39 (1.02-11.29)			28	1.61 (0.90-2.89)				
	T3 ≥ 7.27	12	3.09 (0.98-9.82)			26	1.25 (0.65-2.38)				
ESR1	***rs9340799****	AA (n=280)				AG/GG (n=338)					
	Total Estradiol, pg/mL										
	For 1 SD log	47	1.62 (1.22-2.17)	**<0.01**	**<0.05**	56	1.03 (0.81-1.30)	0.84	1.00	**0.01**	**<0.05**
	T1 < 4.21	7	1 [reference]	**<0.01**	**<0.05**	18	1	0.54	1.00		
	T2 [4.21-7.27[	19	3.14 (1.33-7.45)			21	1.22 (0.65-2.27)				
	T3 ≥ 7.27	21	3.78 (1.59-8.99)			17	0.76 (0.36-1.61)				
	rs1256049*	GG (n=559)				AG/AA (n=56)					
	Total Estradiol, pg/mL										
	For 1 SD log	100	1.22 (1.01-1.48)	**0.04**	0.20	5	3.06 (0.53-17.76)	0.21	1.00	0.30	1.00
	T1 < 4.21	23	1 [reference]	0.10	0.50	3	1				
	T2 [4.21-7.27[	40	1.79 (1.06-3.02)			1	NA				
	T3 ≥ 7.27	37	1.60 (0.91-2.82)			1	NA				
	rs4986938*	AA (n=100)				AG/GG (n=516)					
	Total Estradiol, pg/mL										
	For 1 SD log	21	1.85 (1.17-2.94)	**<0.01**	**<0.05**	84	1.09 (0.88-1.34)	0.42	1.00	0.10	0.50
ESR2	T1 < 4.21	1	1	**<0.01**	**<0.05**	25	1	0.75	1.00		
	T2 [4.21-7.27[	12	22.47 (2.20-229.87)			29	1.03 (0.60-1.76)				
	T3 ≥ 7.27	8	9.65 (0.91-102.43)			30	1.10 (0.62-1.95)				
	rs1271572*	GG (n=206)				GT/TT (n=404)					
	Total Estradiol, pg/mL										
	For 1 SD log	36	1.36 (0.95-1.94)	0.09	0.47	67	1.20 (0.96-1.51)	0.11	0.54	0.68	1.00
	T1 < 4.21	8	1	0.22	1.00	16	1	0.37	1.00		
	T2 [4.21-7.27[	14	1.96 (0.85-4.51)			27	1.57 (0.82-2.99)				
	T3 ≥ 7.27	14	1.74 (0.68-4.44)			24	1.37 (0.69-2.73)				

* 12 missed values for *rs2234693,* 5 for *rs9340799,* 8 for *rs1256049,* 7 for *rs4986938* and 13 for *rs1271572*

** Including 67 CHD and 39 stroke events

† P value for continuous variable and p for trend for variable in tertiles

λ P value after Bonferroni correction

Model adjusted for study center, BMI, diabetes, hypertension, hypercholesterolemia and smoking status

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