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Lack of association between the *ESR1* rs9340799 polymorphism and age at menarche: a meta-analysis

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ABSTRACT. It has been reported that the estrogen receptor alpha (*ESR1*) rs9340799 polymorphism is associated with age at menarche (AAM). However, recent investigations have generated inconsistent results. This study aimed to establish a more precise estimation of the association between this polymorphism and AAM. A meta-analysis was conducted based on an *in silico* literature search using PubMed. Six studies presenting continuous data, including *ESR1* rs9340799 genotype frequencies, were selected. Effect size was estimated using Hedges' adjusted *g* with 95% confidence intervals (CIs), which were calculated based on the standardized mean difference between groups of subjects and different genotypes. No evidence of an association

between the *ESR1* rs9340799 polymorphism and AAM was found in the pooled continuous data under any genotype comparison (AA *vs* GG+AG: Hedges' g = -0.085, 95%CI = -0.202-0.032, P = 0.156; GG *vs* AA+AG: Hedges' g = 0.143, 95%CI = -0.041-0.327, P = 0.129; A *vs* G: Hedges' g = 0.187, 95%CI = -0.032-0.406, P = 0.095). Moreover, a funnel plot generated using this data was found to be symmetrical using the Egger (P = 0.797) and Begg tests (P = 0.851), indicating the absence of publication bias. In summary, our meta-analysis shows that the *ESR1* rs9340799 polymorphism is not a significant, independent contributing factor to AAM. To validate this finding, further studies involving larger numbers of participants are needed.

Key words: Meta-analysis; Age at menarche; *ESR1*

INTRODUCTION

Age at menarche (AAM) is an important trait concerning women's health. Menarche, the first menstruation, marks the beginning of female reproductive life and is considered one of the most important events in puberty. It is well-known that early onset of menarche is associated with elevated risk of breast and endometrial cancers (Peeters et al., 1995; Kaaks et al., 2002). Late menarche decreases the likelihood of coronary heart disease; however, it may increase susceptibility to Alzheimer's disease and osteoporosis (Ito et al., 1995; Rees, 1995). Therefore, from a clinical point of view, understanding the potential factors responsible for AAM may shed light on the pathophysiology of these diseases.

The effects of several factors, including environmental and lifestyle influences, have been considered in relation to AAM, in particular, height, weight, and body mass index. Increased fat uptake and early maternal menarche have been reported as positive predictive factors of precocious menarche, while sports activity seems to delay the onset of menstruation (Merzenich et al., 1993; Chie et al., 1997). Twin and family studies have suggested that approximately 53-74% of the variation in AAM can be attributed to genetic factors (Kaprio et al., 1995; Sharma, 2002). Several genes have been reported to be associated with this event, such as estrogen receptor alpha (*ESR1*; Stavrou et al., 2002; Long et al., 2005), sex hormone-binding globulin, androgen receptor, and cytochrome P450c17α (Jorm et al., 2004; Chang et al., 2005; Xita et al., 2005).

AAM is dependent on the maturity of the female reproductive system and that of other endocrine organs. Estrogen plays an important role in the maturation and function of the reproductive system, and can stimulate hyperplasia of mammary gland epithelial cells, mainly through interaction with estrogen receptor (ESR) and the modification of downstream gene expression. ESR has two major forms, alpha and beta. The former, encoded by the *ESR1* gene, is of relevance to AAM. Human *ESR1* comprises eight exons spanning more than 300 kb of chromosome 6q25.1. A number of studies have investigated potential associations between *ESR1* polymorphisms and AAM in humans (Stavrou et al., 2002; Gorai et al., 2003; Xu et al., 2005; Mitchell et al., 2008; Kulik-Rechberger et al., 2010; Silva et al., 2010). However, their results have been inconsistent. Therefore, the aim of this study was to investigate the putative relationship between the *ESR1* rs9340799 polymorphism and AAM using a meta-analysis.

MATERIAL AND METHODS

Identification and eligibility of relevant studies

The PubMed/MEDLINE database was searched using various combinations of the query terms "*ESR1*", "polymorphism", "age at menarche", or "menarche" and "rs9340799" (the final search was performed on December 28, 2015). Any human population-based association study with continuous results that reported means and standard deviations of AAM according to *ESR1* rs9340799 genotype, and provided sample sizes for each genotype group was selected, regardless of the study size. Furthermore, citations included in the identified articles were used as a source of additional publications.

Data extraction

Two investigators independently extracted data for analysis. Results were compared and disagreements resolved by consensus. In instances of overlapping data being published by the same investigator in multiple studies, only the most recent report was included in the meta-analysis.

Statistical analysis

For each genotype comparison, pooled standardized mean differences (SMDs) and 95% confidence intervals (CIs) were calculated to measure the strength of any association between the *ESR1* rs9340799 polymorphism and AAM. For continuous outcomes, Hedges' adjusted *g* was used to measure effect size (Hedges and Olkin, 1985). This commonly used estimator of effect is calculated based on the SMD between two groups. Heterogeneity across studies was examined using the *Q*-statistic, for which P values < 0.05 were considered significant (Lau et al., 1997). Random- and fixed-effect models were used to conduct the meta-analysis when heterogeneity was found to be present and absent, respectively.

Potential publication bias was assessed using the Begg test (the funnel-plot method) and the Egger linear regression test, for which P values < 0.05 were considered significant (Egger et al., 1997). All statistical analyses were performed with the Stata software (version 8.2; StataCorp, College Station, TX, USA).

RESULTS

Eligible studies

The literature search and subsequent selection process based on the inclusion criteria identified six studies including continuous outcomes regarding the association between the *ESR1* rs9340799 polymorphism and AAM. In total, the eligible investigations included 1400 women (Table 1).

Meta-analysis

The results of our meta-analysis testing the association between the *ESR1* rs9340799 polymorphism and AAM, and those of the heterogeneity test, are shown in Table 2 and Figure 1.

Overall, rs9340799 variant genotypes were not found to be associated with AAM under any genetic model (AA *vs* GG+AG: Hedges' *g* = -0.085, 95%CI = -0.202-0.032, P = 0.156; GG *vs* AA+AG: Hedges' *g* = 0.143, 95%CI = -0.041-0.327, P = 0.129; AA *vs* GG: Hedges' *g* = 0.187, 95%CI = -0.032-0.406, P = 0.095).

Table 1. Summary of the eligible studies used in our meta-analysis of the association between the *ESR1* rs9340799 polymorphism and age at menarche (AAM).

Author	Year	Country	N	AAM, GG genotype (years, means ± SD)	N	AAM, AG genotype (years, means ± SD)	N	AAM, AA genotype (years, means ± SD)	P
Silva	2010	Brazil	40	13.3 ± 1.8	219	13.3 ± 1.9	14	13.3 ± 1.9	>0.05
Kulik-Rechberger	2010	Poland	19	12.94 ± 0.76	57	13.15 ± 1.00	51	13.12 ± 0.96	>0.05
Mitchell	2008	USA	17	13.0 ± 1.2	65	12.7 ± 1.5	68	12.6 ± 1.2	1.54
Hong Xu	2005	China	14	13.4 ± 1.3	147	13.7 ± 1.4	229	13.5 ± 1.4	0.57
Gorai	2003	Japan	9	14.5 ± 1.6	97	13.8 ± 1.3	209	13.8 ± 1.3	0.456
Stavrou	2002	USA	35	13.36 ± 1.24	56	12.80 ± 1.14	54	12.75 ± 1.35	0.017

SD = standard deviation.

Table 2. Hedges' *g* values and 95% confidence intervals concerning the relationship between age at menarche and the *ESR1* rs9340799 polymorphism under different genetic models.

Genotype comparison	Hedges' *g* pooled SMD (95%CI), P	Heterogeneity P value	Begg's test P value	Egger's test P value
AA *vs* GG+AG	-0.085 (-0.202-0.032), 0.156	0.946	0.851	0.797
AA+AG *vs* GG	0.143 (-0.041-0.327), 0.129	0.138	0.851	0.865
A *vs* G	0.187 (-0.032-0.406), 0.095	0.295	0.851	0.710

SMD = standardized mean difference; CI = confidence interval.

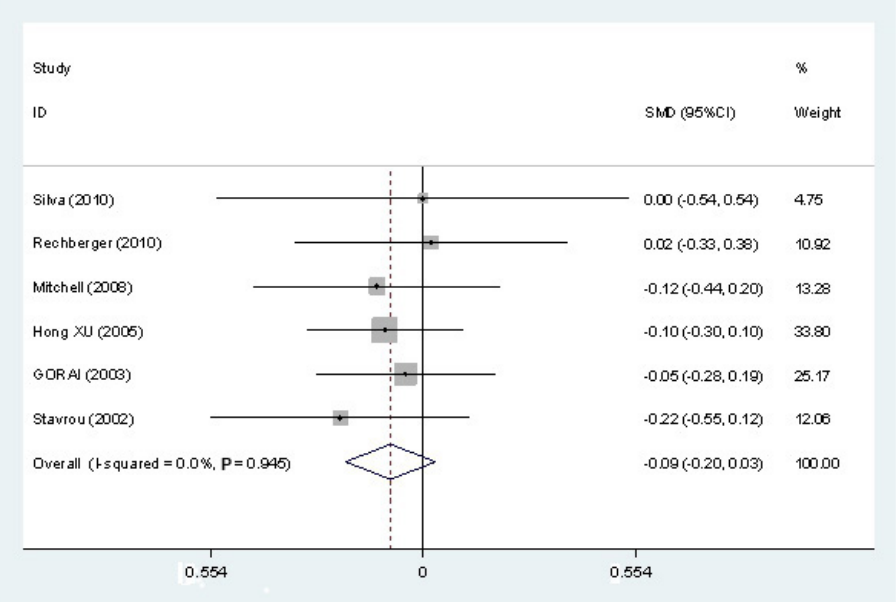

Figure 1. Forest plot of standardized mean differences (SMDs) concerning the association between age at menarche and the *ESR1* rs9340799 polymorphism for the AG+GG *vs* AA genotype comparison. Squares and horizontal lines represent study-specific SMDs and 95% confidence intervals (CIs), respectively. The area of each square signifies study-specific weight, while the diamond depicts the pooled SMD and 95%CI.

Publication bias

The Egger test was performed and a funnel plot was generated to estimate any publication bias present among the selected literature. As shown in Figure 2, the Egger test provided statistical evidence of funnel plot symmetry, based on the dominant genetic model (AA *vs* AG+GG: P = 0.797), suggesting the absence of publication bias.

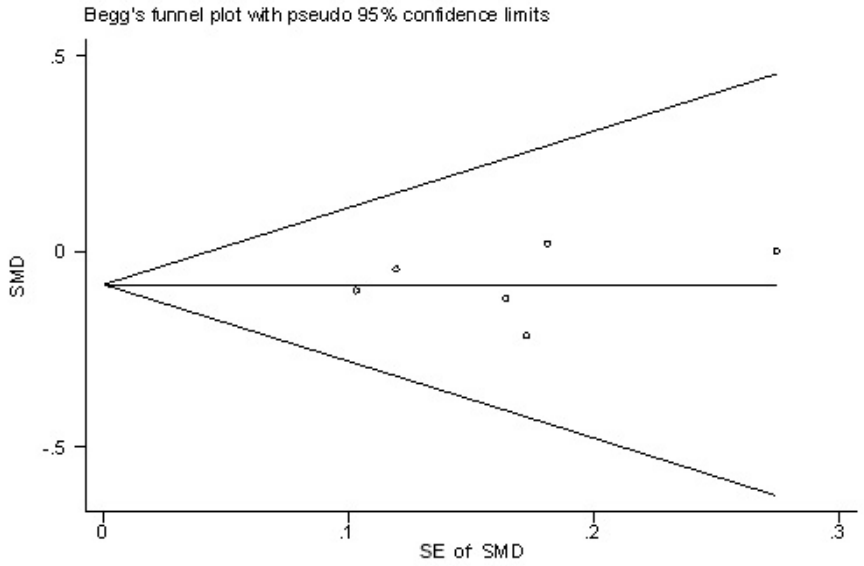

Figure 2. Funnel plot assessing publication bias in our meta-analysis of the relationship between the *ESR1* rs9340799 polymorphism and age at menarche, based on the AA *vs* AG+GG model. SMD = standardized mean difference; SE = standard error.

DISCUSSION

Menarche is the first menstruation, marking the beginning of a woman's reproductive life, and therefore representing one of the most important events in female puberty. AAM is a genuinely complex attribute determined by interactions between myriad factors, including environmental, genetic, and socioeconomic elements. Recognized influences on AAM include nutrition, exercise, socioeconomic status, psychosocial stimuli, childhood experience, rural or urban birthplace, and general health (Long et al., 2005). Moreover, over the past two centuries, AAM in humans has been decreasing, although it is unclear whether this trend has leveled off or even reversed in recent years. This gradual reduction is typically attributed to socioeconomic improvements in various human populations over the last 200 years (Onland-Moret et al., 2005). Nevertheless, the contribution of genetics to AAM may be more important than environment or socioeconomics. Given the complicated nature of AAM, the potential number of genes involved may be very large. For example, the Skeletal Gene Database lists several hundred genes related to bone biology (Ho et al., 2000).

Moreover, different research groups have obtained evidence of associations with several candidate genes, including those in biochemical pathways involved in ovarian estrogen production and signaling. The estrogen hormone exerts its genomic and nongenomic effects via proteins encoded by the *ESR1* and *ESR2* genes. The importance of estrogens and their biochemical signaling pathways in human physiology and pathology has been corroborated by a vast amount of data collected by multiple, independent biomedical researchers, spanning diverse disciplines.

We performed a systematic literature review by means of a meta-analysis of the association between the *ESR1* rs9340799 polymorphism and AAM, with no evidence of publication bias among the included reports. Of the six studies reporting continuous outcomes, only one (Stavrou et al., 2002) described a positive result, suggesting increased AAM among US women carrying the A allele and decreased AAM among those with the G allele. By contrast, the other studies examined demonstrated no significant difference between *ESR1* rs9340799 genotypes in terms of AAM (Gorai et al., 2003; Xu et al., 2005; Mitchell et al., 2008; Kulik-Rechberger et al., 2010; Silva et al., 2010). Besides the small sample sizes, differences in ethnicity may be a major reason for such disagreement. The pooled SMD from our meta-analysis suggested no significant association between the *ESR1* rs9340799 polymorphism and AAM.

The two ESR subtypes (alpha and beta) derive from distinct genes on separate chromosomes. The gene encoding *ESR1*, the primary receptor for estrogen, is located on chromosome 6q25.1 and spans >140 kb, consisting of eight exons separated by seven introns. As this subtype is the main mediator of the protective effect of estrogen (Figtree et al., 2009), variation in *ESR1* was the focus of this meta-analysis.

Polymorphisms of *ESR1* were first recognized over two decades ago, shortly after the genomic structure of chromosome 6 had been established. Recent studies of variations within *ESR1* have identified single nucleotide polymorphisms (SNPs) associated with several diseases, including breast, prostate, and endometrial cancer, osteoporosis, and neurodegenerative diseases (Gennari et al., 2005; Liu et al., 2005).

Sequence variation in this gene is one of the most potent mechanisms responsible for modulation of its expression. Susceptibility to restriction digestion by endonucleases has greatly expanded the capability of researchers to investigate *ESR1* polymorphisms, particularly through the use of *Pvu*II and *Xba*I. The two corresponding SNPs are located in the first intron, 397 and 351 bp upstream of exon 2, with the *Xba*I restriction site polymorphism consisting of an A-to-G transition (rs9340799). These sequence variations may have functional consequences by modifying *ESR1* expression through altered transcription factor binding, and influencing alternative splicing (Herrington et al., 2002).

The present meta-analysis summarizes data from six observational studies with continuous outcomes testing the effects of the *ESR1* rs9340799 polymorphism on AAM. Overall, our results suggest that there is no association between this variant and AAM based on the pooled dataset, comprising a multiethnic study population. Although menarcheal age is affected by nutrition and other environmental and lifestyle factors, such information was not available in the present study, and therefore was not used to adjust the raw data. There is a need for further well-designed, large studies to validate the association between the *ESR1* rs9340799 polymorphism and AAM in different populations, while also taking into consideration nutritional and environmental influences on AAM.

Conflicts of interest

The authors declare no conflict of interest.

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REFERENCES

Chang JH, Gertig DM, Chen X, Dite GS, et al. (2005). *CYP17* genetic polymorphism, breast cancer, and breast cancer risk factors: Australian Breast Cancer Family Study. *Breast Cancer Res.* 7: R513-R521. http://dx.doi.org/10.1186/bcr1040

Chie WC, Liu YH, Chi J, Wu V, et al. (1997). Predictive factors for early menarche in Taiwan. *J. Formos. Med. Assoc.* 96: 446-450.

Egger M, Davey Smith G, Schneider M and Minder C (1997). Bias in meta-analysis detected by a simple, graphical test. *BMJ* 315: 629-634. http://dx.doi.org/10.1136/bmj.315.7109.629

Figtree GA, Noonan JE, Bhindi R and Collins P (2009). Estrogen receptor polymorphisms: significance to human physiology, disease and therapy. *Recent Pat. DNA Gene Seq.* 3: 164-171. http://dx.doi.org/10.2174/187221509789318397

Gennari L, Merlotti D, De Paola V, Calabrò A, et al. (2005). Estrogen receptor gene polymorphisms and the genetics of osteoporosis: a HuGE review. *Am. J. Epidemiol.* 161: 307-320. http://dx.doi.org/10.1093/aje/kwi055

Gorai I, Tanaka K, Inada M, Morinaga H, et al. (2003). Estrogen-metabolizing gene polymorphisms, but not estrogen receptor-alpha gene polymorphisms, are associated with the onset of menarche in healthy postmenopausal Japanese women. *J. Clin. Endocrinol. Metab.* 88: 799-803. http://dx.doi.org/10.1210/jc.2002-020353

Hedges LV and Olkin I (1985). Statistical methods for meta-analysis. Academic Press, Orlando.

Herrington DM, Howard TD, Brosnihan KB, McDonnell DP, et al. (2002). Common estrogen receptor polymorphism augments effects of hormone replacement therapy on E-selectin but not C-reactive protein. *Circulation* 105: 1879-1882. http://dx.doi.org/10.1161/01.CIR.0000016173.98826.88

Ho NC, Jia L, Driscoll CC, Gutter EM, et al. (2000). A skeletal gene database. *J. Bone Miner. Res.* 15: 2095-2122. http://dx.doi.org/10.1359/jbmr.2000.15.11.2095

Ito M, Yamada M, Hayashi K, Ohki M, et al. (1995). Relation of early menarche to high bone mineral density. *Calcif. Tissue Int.* 57: 11-14. http://dx.doi.org/10.1007/BF00298989

Jorm AF, Christensen H, Rodgers B, Jacomb PA, et al. (2004). Association of adverse childhood experiences, age of menarche, and adult reproductive behavior: does the androgen receptor gene play a role? *Am. J. Med. Genet. B. Neuropsychiatr. Genet.* 125B: 105-111. http://dx.doi.org/10.1002/ajmg.b.20114

Kaaks R, Lukanova A and Kurzer MS (2002). Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol. Biomarkers Prev.* 11: 1531-1543.

Kaprio J, Rimpelä A, Winter T, Viken RJ, et al. (1995). Common genetic influences on BMI and age at menarche. *Hum. Biol.* 67: 739-753.

Kulik-Rechberger B, Skorupski P, Bogusiewicz M, Miotła P, et al. (2010). Height at menarche is influenced by estrogen receptor alpha gene polymorphisms. *J. Endocrinol. Invest.* 33: 332-338. http://dx.doi.org/10.1007/BF03346595

Lau J, Ioannidis JP and Schmid CH (1997). Quantitative synthesis in systematic reviews. *Ann. Intern. Med.* 127: 820-826. http://dx.doi.org/10.7326/0003-4819-127-9-199711010-00008

Liu X, Fan XL, Zhao Y, Luo GR, et al. (2005). Estrogen provides neuroprotection against activated microglia-induced dopaminergic neuronal injury through both estrogen receptor-alpha and estrogen receptor-beta in microglia. *J. Neurosci. Res.* 81: 653-665. http://dx.doi.org/10.1002/jnr.20583

Long JR, Xu H, Zhao LJ, Liu PY, et al. (2005). The oestrogen receptor alpha gene is linked and/or associated with age of menarche in different ethnic groups. *J. Med. Genet.* 42: 796-800. http://dx.doi.org/10.1136/jmg.2004.028381

Merzenich H, Boeing H and Wahrendorf J (1993). Dietary fat and sports activity as determinants for age at menarche. *Am. J. Epidemiol.* 138: 217-224.

Mitchell ES, Farin FM, Stapleton PL, Tsai JM, et al. (2008). Association of estrogen-related polymorphisms with age at menarche, age at final menstrual period, and stages of the menopausal transition. *Menopause* 15: 105-111.

Onland-Moret NC, Peeters PH, van Gils CH, Clavel-Chapelon F, et al. (2005). Age at menarche in relation to adult height: the EPIC study. *Am. J. Epidemiol.* 162: 623-632. http://dx.doi.org/10.1093/aje/kwi260

Peeters PH, Verbeek AL, Krol A, Matthyssen MM, et al. (1995). Age at menarche and breast cancer risk in nulliparous women. *Breast Cancer Res. Treat.* 33: 55-61. http://dx.doi.org/10.1007/BF00666071

Rees M (1995). The age of menarche. *ORGYN* 1995: 2-4.

Sharma K (2002). Genetic basis of human female pelvic morphology: a twin study. *Am. J. Phys. Anthropol.* 117: 327-333. http://dx.doi.org/10.1002/ajpa.10055

Silva IV, Rezende LC, Lanes SP, Souza LS, et al. (2010). Evaluation of *Pvu*II and *Xba*I polymorphisms in the estrogen receptor alpha gene (*ESR1*) in relation to menstrual cycle timing and reproductive parameters in post-menopausal women. *Maturitas* 67: 363-367. http://dx.doi.org/10.1016/j.maturitas.2010.08.006

Stavrou I, Zois C, Ioannidis JP and Tsatsoulis A (2002). Association of polymorphisms of the oestrogen receptor alpha gene with the age of menarche. *Hum. Reprod.* 17: 1101-1105. http://dx.doi.org/10.1093/humrep/17.4.1101

Xita N, Tsatsoulis A, Stavrou I and Georgiou I (2005). Association of *SHBG* gene polymorphism with menarche. *Mol. Hum. Reprod.* 11: 459-462. http://dx.doi.org/10.1093/molehr/gah178

Xu H, Long JR, Li MX and Deng HW (2005). Interaction effects between estrogen receptor alpha and vitamin D receptor genes on age at menarche in Chinese women. *Acta Pharmacol. Sin.* 26: 860-864. http://dx.doi.org/10.1111/j.1745-7254.2005.00122.x