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Lack of association between the *aryl hydrocarbon receptor* rs2066853 polymorphism and breast cancer: A meta-analysis on *Ahr* polymorphism and breast cancer

Y. Li[1]*, H.Z. Qin[2]*, Q. Song[1], X.D. Wu[1] and J.H. Zhu[3]

[1]Department of Internal Medicine-Oncology, Chinese PLA General Hospital, Beijing, China
[2]Department of Surgical Oncology, Chinese People's Liberation Army Hospital, Beijing,China
[3]Department of Internal Medicine-Oncology, The First Affiliated Hospital, Chinese PLA General Hospital, Beijing,China

*These authors contributed equally to this study.
Corresponding author: J.H. Zhu
E-mail: jianhuazhudoc@yeah.net

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ABSTRACT. Published data regarding the association between *aryl hydrocarbon receptor (Ahr)* rs2066853 polymorphism and the risk of breast cancer shows conflicting results. We performed a meta-analysis on 2999 patients and 3050 controls from three related case-control studies to estimate the association between Ahr rs2066853 polymorphism and the risk of breast cancer. The protocol was approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida (America NIH Publication No. 86-231985 Revision). According to the three eligible populations, the odds ratios (ORs), 95% confidence intervals (CIs) on the risk of breast cancer for the genotypes *GA vs GG*, *AA vs GG*, and *A vs G*

were 1.06 (0.81-1.40), 0.96 (0.81-1.13), and 1.02 (0.85-1.22), respectively. The OR (95%CI) for *GA + AA vs GG* was 1.05 (0.80-1.37). Furthermore, after multi-variates adjustment, the ORs (95%CIs) were 1.05 (0.80-1.38) for *GA vs GG*, and 0.92 (0.76-1.10) for *AA vs GG*. This meta-analysis suggests that *Ahr* (rs2066853) polymorphism would not modify the risk of breast cancer. However, further research should be conducted to provide more evidence.

Key words: *Ahr*; Gene; Polymorphism; Breast cancer; Meta-analysis

INTRODUCTION

The *aryl hydrocarbon receptor (Ahr)* is a member of the basic helix-loop-helix/PER-AHR nuclear translocator (ARNT)-SIM superfamily of nuclear receptors (Tan et al., 2010). It regulates a wide range of developmental and toxicological processes including cell proliferation and xenobiotic metabolism (Meyer and Perdew, 1997; Marlowe and Puga, 2005; Kawajiri and Fujii-Kuriyama, 2007). In addition, it is regarded to play a contributory role in cancer (Nebert et al., 2004; Bradshaw et al., 2008).

Ahr is a key regulator of transcriptional expression for cytochrome P450 (Sangrajrang et al., 2009). *Ahr* rs2066853 (Arg554Lys) is located in exon 10, a region that encompasses a major portion of the trans-activation domain of this gene (Long et al., 2006). Some studies have explored the relationship between *Ahr* and the risk of cancers, including lung cancer (Kawajiri et al., 1995; Cauchi et al., 2001) and bladder cancer (Zhang et al., 2002). Furthermore, previous research has also been conducted to determine the relationship between *Ahr* rs2066853 polymorphism and the risk of breast cancer. However, the results of these studies were inconclusive. Therefore, we conducted a meta-analysis on all eligible case-control studies to estimate the association between *Ahr* (rs2066853) polymorphism and the risk of breast cancer.

MATERIAL AND METHODS

Search strategy

Literature searches were conducted using the databases PubMed, EMBASE, and the COCHRANE Library, which were in English. In addition, the Chinese databases VIP, CNKI, and Sinomed (up to Sep. 18, 2014) were also used. The following keywords and subject terms were included: ‘Ahr’ or ‘aryl hydrocarbon receptor gene’ and ‘breast cancer’. References of received articles were also further investigated.

Inclusion criteria

Studies included in this meta-analysis required the following criteria: (a) being a case-control study, (b) individual studies involved only unrelated study participants, and (c) the relationship between the *Ahr* polymorphism and breast cancer was evaluated.

Exclusion criteria

Case reports, review articles, editorials, clinical guidelines, and information articles for pa-

tients were all excluded. Individual studies in which information regarding *Ahr* polymorphism were insufficiently described were also rejected.

Data extraction

Literature research was independently conducted by two investigators (Y.L. and H.Z.Q.), and the studies were then screened for inclusion and appraisal. Discrepancies were adjudicated by third party persons who were familiar with the related studies. Agreements were reached following discussions. Data were collected from each publication including the authors, year of publication, country, ethnicity, journal, study design, sample size, resources of controls, and information regarding *Ahr* polymorphism. The Newcastle-Ottawa-Scale (NOS) was used to quantify study quality (Cota et al., 2013).

Statistical analysis

Unadjusted odds ratio (OR) with corresponding 95% confidence interval (CI) of each selected study was first calculated. The pooled OR was examined using the Z-test. Heterogeneity among studies was measured by the Q-statistic test and I-square statistic test. Both fixed-models using the Mantel-Haenszel method and random-effect models were included in this meta-analysis.

Hardy-Weinberg (H-W) equilibrium was assessed using Pearson Chi-square test for the controls in each study.

Potential publication bias was accessed by Funnel plot and Egger's linear regression. All analyses were performed by the software Stata, version 8.0 (Stata Corp, College Station, TX, USA). The tests were two-sided. Statistical significance was defined as $P < 0.05$.

RESULTS

Study characteristics and meta-analysis database

A total of nine potential papers were found according to our search terms from the databases PubMed, EMBASE, and the COCHRANE Library (restricted to human research). No related paper in Chinese was found. Among the nine papers, three of those focused on the function of *Ahr* in breast cancer cells (Abdelrahim et al., 2003; Zhao et al., 2012; Tarnow et al., 2013). One study was based on a cohort study among in patients (Long et al., 2007). Two studies did not show sufficient information on *Ahr (rs2066853)* polymorphism (Georgitsi et al., 2007; Tan et al., 2010). Therefore, a total of three individual studies (Le Marchand et al., 2005; Long et al., 2006; Sangrajrang et al., 2009) were included in this meta-analysis. Data from 2999 patients and 3050 controls were obtained from the included. Breast cancer in patients was confirmed by clinical as well as other assistant examinations.

A dataset based on the extracted information from each included report was established (Table 1). Quality assessment for the eligible studies according to the NOS is shown in Table 2.

Quantitative synthesis

The average relative frequencies of the *A* allele, *AA* genotype, and *GA* genotype from the three populations were 31.85, 10.76, and 40.65% in breast cancer patients and 31.29, 10.98, and 38.31% in the controls, respectively. The genotype distributions of *Ahr* (rs2066853) in controls from only the first and second eligible study populations satisfied the H-W equilibrium ($P > 0.05$).

Table 1. Characteristics of literatures included in this analysis.

ID	First author	Year	Country	Ethnicity	Source of controls	Genotyping method	Sample size (case/control)	Polymorphisms distribution of *Ahr* rs2066853 (case/control)			Allele distribution of *Ahr* rs2066853 (case/control)		OR (95%CI)	
								G/G	G/A	A/A	G	A	GA *vs* GG	AA *vs* GG
1	Sangrajrang S	2009	Thailand	Asian	HB	TaqMan	570/497	238/245	260/189	59/48	736/679	378/285	1.34 (1.02,1.76)	1.23 (0.79,1.92)
2	Long JR	2006	China	Asian	PB	High-throughput sequencing	1090/1183	472/444	455/516	113/139	1399/1404	681/794	0.82 (0.69,0.99)	0.76 (0.58,1.01)
3	Marchand LL	2005	UAS	Mixed	PB	PCR/RFLP	1339/1370	721/756	463/456	155/158	1905/1968	773/772	1.1 (0.9,1.3)	1.0 (0.7,1.3)

ID: study id; HB: hospital based; PB: population based.

Table 2. Quality assessment for eligible studies according to NOS.

ID	First author	Selectiona	Comparabilitya	Exposurea
1	Sangrajrang S	3	2	2
2	Long JR	4	2	2
3	Marchand LL	4	2	2

The NOS for case-control study: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. Therefore, a maximum of four stars can be given for Selection, three stars for Outcome. A maximum of two stars can be given for Comparability. More stars mean higher quality of the eligible studies. a: means the number of stars.

Compared with the *GG* genotype, no statistically significant relationship was found between *GA*, *AA* and the risk of breast cancer. The odds ratios (ORs), 95% confidence intervals (CIs), and the Pheterogeneity values for *GA* and *AA* on the risk of breast cancer were 1.06 (0.81, 1.40), 0.003, 0.96 (0.81, 1.13), and 0.106, respectively. At the same time, the *A* allele also did not significantly increase the risk of breast cancer, as compared with the *G* allele. The corresponding OR (95%CI) was 1.02 (0.85, 1.22), $P_{heterogeneity}$ = 0.006. Compared with the *GG* genotype, *GA+ AA* genotypes also did not modify the risk of breast cancer with OR (95%CI) and $P_{heterogeneity}$ value of 1.05 (0.80, 1.37) and 0.002, respectively.

Following multi-variates adjustment, *GA*, *AA* genotypes still did not modify the risk of breast cancer as compared with the *GG* genotype. The corresponding ORs (95%CIs) and $P_{heterogeneity}$ values were 1.05 (0.80, 1.38) and 0.006 for *GA vs GG*. ORs (95%CIs) and $P_{heterogeneity}$ for *AA vs GG* were 0.92 (0.76, 1.10) and 0.154, respectively

Publication bias

Funnel plots and Egger's tests were conducted to examine publication bias (Figure 1). No publication bias was found for *GA vs* GG, P = 0.602.

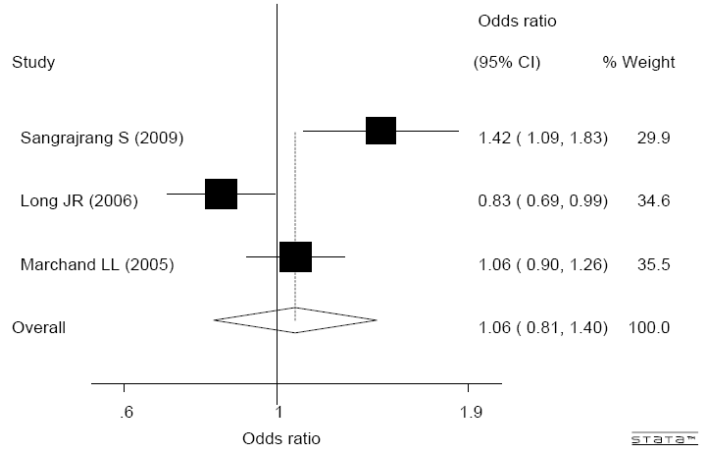

Figure 1. Association of Breast cancer with the Ahr rs2066853 genotype by random model (*GA vs GG*) plotted as an OR Forest plots with 95%CI Heterogeneity = 11.46 p = 0.003, I^2 = 82.6%, z = 0.44 P = 0.659. Black square indicates the value of OR; size of the square is inversely proportional to its variance. Horizontal line denotes 95% confidence interval (CI) of OR; black diamond indicates pooled results; studies were ordered by published year.

DISCUSSION

The present meta-analysis consisting of data compiled from three related case-control studies explored the relationship between *Ahr* (rs2066853) polymorphism and the risk of breast cancer. We did not observe any significant increase in breast cancer development in *A* (rs2066853) allele carriers. Furthermore, no statistically significant relationship was found following multi-variates adjustments. Heterogeneity among eligible studies was found, and therefore, random-effect models were used in this analysis. No evidence of publication bias was found in this meta-analysis, and all three studies received high quality score according to the NOS.

Ahr is found in multiple human organs, and is highly expressed in different kinds of cancers (Vezina et al., 2009; Liu et al., 2013; Wang et al., 2013). Sangrajrang et al (2009) found that among the women in Thailand, *GA* heterozygotes of *Ahr* (rs2066853) increased the risk of breast cancer, while in Chinese women, this polymorph showed the contrary influence on the risk of breast cancer (Sangrajrang et al., 2006). However, neither of such findings was replicated in other studies including those included in this meta-analysis. Pooled analysis based on the multivariate adjustment ORs (95%CIs) did not find any statistically significant relationship between *GA/AA* (rs2066853) genotypes and the risk of breast cancer. Therefore, our meta-analysis suggests that the *A* allele on the *Ahr* gene does not modify the risk of breast cancer.

Some limitations in our meta-analysis should be considered when interpreting the results. Since only three studies were included in this meta-analysis with low between-study, sensitivity analysis was not performed. In addition, language limitation may have hindered information interpretation. Furthermore, lacking the original data of the reviewed studies, evaluation of results was limited. It is possible that other factors such as gene-gene, gene-environment, and even different polymorphic loci of the same gene may modulate breast cancer risk. In spite of these limitations, our meta-analysis also included several advantages. First, a large number of cases and controls were pooled, which significantly increased the statistical power of the analysis. Secondly, the quality of all case-control studies included in the current meta-analysis was considered satisfactory, and met our inclusion criterion.

In conclusion, this meta-analysis suggested that *A* allele of *Ahr (*rs2066853) did not significantly modify the risk of breast cancer. However, because of the comparatively insufficient published studies included, we were not able to systematically analyze the relationship between *Ahr (*rs2066853) and the risk of breast cancer. More evidence from epidemiologic researches is needed to provide a more clear characterization of the role of *Ahr (*rs2066853), and whether it exerts any influence on genetic susceptibility to breast cancer development.

Conflicts of interest

The authors declare no conflict of interest.

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Y. Li and H.Z. Qin searched the materials independently. Y. Li analyzed the data involved in this meta-analysis, and explained the results. H. Qin, Q. Song, X.D. Wu, and J.H. Zhu checked the materials, and explained the results. J.H. Zhu supported the research.

REFERENCES

Abdelrahim M, Smith R 3rd and Safe S (2003). Aryl hydrocarbon receptor gene silencing with small inhibitory RNA differentially modulates Ah-responsiveness in MCF-7 and HepG2 cancer cells. *Mol. Pharmacol.* 63: 1373-1381.

Bradshaw TD, Stone EL, Trapani V, Leong CO, et al. (2008). Mechanisms of acquired resistance to 2-(4-Amino-3-methylphenyl) benzothiazole in breast cancer cell lines. *Breast Cancer Res. Treat*. 110: 57-68.

Cauchi S, Stücker I, Solas C, Laurent-Puig P, et al. (2001). Polymorphisms of human aryl hydrocarbon receptor (AhR) gene in a French population: relationship with CYP1A1 inducibility and lung cancer. *Carcinogenesis* 22: 1819-1824.

Cota GR, de Sousa MR, Fereguetti TO and Rabello A (2013). Efficacy of anti-leishmania therapy in visceral leishmaniasis among HIV infected patients: a systematic review with indirect comparison. *PLoS Neglected Trop. Dis.* 7: e2195.

Georgitsi M, Karhu A, Winqvist R, Visakorpi T, et al. (2007). Mutation analysis of aryl hydrocarbon receptor interacting protein (AIP) gene in colorectal, breast, and prostate cancers. *Br. J. Cancer* 96: 352-356.

Kawajiri K and Fujii-Kuriyama Y (2007). Cytochrome P450 gene regulation and physiological functions mediated by the aryl hydrocarbon receptor. *Arch. Biochem. Biophys.* 464: 207-212.

Kawajiri K, Watanabe J, Eguchi H, Nakachi K, et al. (1995). Polymorphisms of human Ah receptor gene are not involved in lung cancer. *Pharmacogenetics* 5: 151-158.

Le Marchand L, Donlon T, Kolonel LN, Henderson BE, et al. (2005). Estrogen metabolism- related genes and breast cancer risk: the multiethnic cohort study. *Cancer Epidemiol. Biomarkers Prev.* 14: 1998-2003.

Liu Z, Wu X, Zhang F, Han L, et al. (2013). AhR expression is increased in hepatocellular carcinoma. *J. Mol. Histol.* 44: 455-461.

Long JR, Egan KM, Dunning L, Shu XO, et al. (2006). Population-based case-control study of AhR (aryl hydrocarbon receptor) and CYP1A2 polymorphisms and breast cancer risk. *Pharmacogenet. Genomics* 16: 237-243.

Long JR, Cai Q, Shu XO, Cai H, et al. (2007). Genetic polymorphisms in estrogen- metabolizing genes and breast cancer survival. *Pharmacogenet. Genomics* 17: 331-338.

Marlowe JL and Puga A (2005). Aryl hydrocarbon receptor, cell cycle regulation, toxicity, and tumorigenesis. *J. Cell Biochem.* 96: 1174-1184.

Meyer BK and Perdew GH (1999). Characterization of the AhR-hsp90-XAP2 core complex and the role of the Immunophilin-related protein XAP2 in AhR stabilization. *Biochemistry* 38: 8907-8917.

Nebert DW, Dalton TP, Okey AB and Gonzalez FJ (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J. Biol. Chem.* 279: 23847-23850.

Sangrajrang S, Sato Y, Sakamoto H, Ohnami S, et al. (2009). Genetic polymorphisms of estrogen metabolizing enzyme and breast cancer risk in Thai women. *Int. J. Cancer* 125: 837-843.

Tan KP, Wang B, Yang M, Boutros PC, et al. (2010). Aryl hydrocarbon receptor is a transcriptional activator of the human breast cancer resistance protein (BCRP/ABCG2). *Mol. Pharmacol.* 78: 175-185.

Tarnow P, Tralau T, Hunecke D and Luch A (2013). Effects of triclocarban on the transcription of estrogen, androgen and aryl hydrocarbon receptor responsive genes in human breast cancer cells. *Toxicol. In vitro*: 27: 1467-1475.

Vezina CM, Lin TM and Peterson RE (2009). AHR signaling in prostate growth, morphogenesis, and disease. *Biochem. Pharmacol.* 77: 566-576.

Wang K, Li Y, Jiang YZ, Dai CF, et al. (2013). An endogenous aryl hydrocarbon receptor ligand inhibits proliferation and migration of human ovarian cancer cells. *Cancer Lett.* 9: 153-155.

Zhang DS, Lin GF, Ma QW and Shen JH. (2002). Nonassociation of aryl hydrocarbon receptor genotypes with susceptibility to bladder cancer in Shanghai population. *Acta Pharmacol. Sin.* 23: 188-192.

Zhao S, Kanno Y, Nakayama M, Makimura M, et al. (2012). Activation of the aryl hydrocarbon receptor represses mammosphere formation in MCF-7 cells. *Cancer Lett.* 317: 192-198.