A meta-analysis on association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk

Eric Tzyy Jiann Chong^a, Lucky Poh Wah Goh^a, Ping-Chin Lee^{a,*}

^aBiotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia

Accepted 2nd October 2018

Abstract. Breast cancer remains a challenging disease globally due to its heterogeneity and complexity, with an annual increase rate of 3.1%. Cytochrome P450 2E1 (*CYP2E1*) rs2031920 and rs3813867 polymorphisms that reside in the 5'-flanking promoter region of the gene are in a complete linkage disequilibrium and have been associated with breast cancer risk, but the findings are inconsistent and inconclusive. Therefore, we investigated the association of the *CYP2E1* rs2031920 and rs3813867 polymorphisms with the risk of breast cancer through a meta-analysis. After literature search, eight eligible studies were included in this meta-analysis with a total of 3650 breast cancer cases and 3607 controls. Our meta-analysis revealed no significant association of the *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk in all comparison models including the allelic (OR = 0.968, 95% CI = 0.855-1.097; $p = 0.612$), heterozygous (OR = 1.003, 95% CI = 0.869-1.159; $p = 0.963$), homozygous (OR = 0.792, 95% CI = 0.519-1.207; $p = 0.278$), dominant (OR = 0.987, 95% CI = 0.858-1.134; $p = 0.851$), and recessive (OR = 1.265, 95% CI = 0.831-1.924; $p = 0.273$). In conclusion, this meta-analysis suggests that the *CYP2E1* rs2031920 and rs3813867 polymorphisms are not associated with the risk of breast cancer.

Keywords: breast cancer, *CYP2E1*, meta-analysis, single nucleotide polymorphisms

INTRODUCTION

Breast cancer is the most common global incidence in women and it has been reported to increase from approximately of 0.64 million cases in 1980 to 1.64 million cases in 2010, with an increase rate of 3.1% per year (Forouzanfar *et al.*, 2011). In general there are two types of breast cancer, namely non-invasive breast cancer and invasive breast cancer (Sharma *et al.*, 2010). The susceptibility of certain genes combining with environmental factors plays an important role in developing breast cancer (Lichtenstein *et al.*, 2000). Studies have shown that consumption of alcohol increases the level of estrogen that may subsequently stimulate the proliferation of breast

cancer cells (Purohit, 2000) or the production of reactive oxygen species during alcoholic metabolism that results in breast cancer through the damage of DNA (Wright *et al.*, 1999).

Cytochrome P450 2E1 (*CYP2E1*) of the cytochrome P450 gene family is involved in metabolization of more than 70 different chemicals with diverse structures (Boniza *et al.*, 2009) and it can reduce molecular oxygen to highly reactive forms that may result in DNA damage and carcinogenesis (Danko & Chaschin, 2005). The rs2031920 and rs3813867 polymorphisms that reside in the 5'-flanking promoter region of the *CYP2E1* gene were

* Author for correspondence: Assoc. Prof. Dr. Ping-Chin Lee, Biotechnology Programme, Faculty of Science and Natural Resources, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia. Email – leepe@ums.edu.my

previously reported to be in a complete linkage disequilibrium (Watanabe *et al.*, 1990), and these polymorphisms have been empirically demonstrated to alter the transcription activity of the *CYP2E1* gene (Hayashi *et al.*, 1991; Watanabe *et al.*, 1994) that may modulate the progression and metastasis of breast cancer (Leung *et al.*, 2013). To date, there are many case-control studies that assessed the association of the rs2031920 and rs3813867 polymorphisms with the risk of breast cancer, but the findings are inconsistent and inconclusive (Anderson *et al.*, 2012; Choi *et al.*, 2003; Chong *et al.*, 2016; Khedhaier *et al.*, 2008; McCarty *et al.*, 2012; Sangrajrang *et al.*, 2010; Wu *et al.*, 2006; Zgheib *et al.*, 2013). Hence, we investigated the association of the *CYP2E1* rs2031920 and rs3813867 polymorphisms with the risk of breast cancer through a meta-analysis.

MATERIALS AND METHODS

Literature search strategy and inclusion criteria. A systematic literature search was performed in the PubMed database for studies reporting the association on *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk until 31 August 2016, using the following keywords: “*CYP2E1*” or “*CYP450 2E1*” or “Cytochrome P450 2E1”, “polymorphism” or “variant”, “rs2031920” or “rs3813867” or “C-1019T” or “G-1259C” and “breast” or “breast cancer” or “breast carcinoma” or “breast tumor”. The following inclusion criteria were specified: (1) case-control study assessing the association on *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk; (2) sufficient data on genotype distributions of the *CYP2E1* rs2031920 and rs3813867 polymorphisms were provided. To avoid duplication, only studies with the most recent data were included when the study populations were overlapped.

Data extraction. Data including first author's name, year of publication, studied population, total sample size for both cases and controls, and genotype distribution in both cases and controls were extracted from all the included studies. Data

extraction from included studies were individually conducted by two investigators and disagreements were solved through discussion.

Statistical analyses. The Hardy-Weinberg equilibrium (HWE) for each of the included study was calculated using the Online Encyclopedia for Genetic Epidemiology software (Rodriguez *et al.*, 2009). Odd ratio (OR) with 95% confidence interval (95% CI) was calculated for five genetic comparison models including the allelic (c2 vs. c1), heterozygous (c1/c2 vs. c1/c1), homozygous (c2/c2 vs. c1/c1), dominant (c1/c2+c2/c2 vs. c1/c1) and recessive (c1/c1+c1/c2 vs. c2/c2) using the Comprehensive Meta Analysis Ver. 2.2.064 (Biostat, Inc., USA). The heterogeneity of each genetic comparison study was determined by the I^2 (a value in %) and Q (a p -value) statistical tests. Fixed effect model (Mantel-Haenszel method) was used to calculate the pooled OR (Mantel & Haenszel, 1959) for I^2 value less than 50% and $p \geq 0.10$ while the random effect model (DerSimonian-Laird method) was used to calculate the pooled OR for I^2 value more than 50% and $p < 0.10$ (DerSimonian & Laird, 1986). Publication bias was determined using the funnel plot supported with Egger's test (Egger *et al.*, 1997) and Begg's test (Begg & Mazumdar, 1994), and a p -value > 0.05 was considered not statistically significant for both tests. All p -values in this meta-analysis were two-sided.

RESULTS AND DISCUSSION

Eight studies with a total of 7257 subjects (3650 breast cancer cases and 3607 controls) were included in this meta-analysis after literature search based on the inclusion criteria and detailed assessment (Anderson *et al.*, 2012; Choi *et al.*, 2003; Chong *et al.*, 2016; Khedhaier *et al.*, 2008; McCarty *et al.*, 2012; Sangrajrang *et al.*, 2010; Wu *et al.*, 2006; Zgheib *et al.*, 2013) (Figure 1). Among all studies, four were conducted in Asian populations, two were in non-Asian populations and two were in mixed populations. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was the most frequent approach used for genotyping among all studies followed by hydrolysis-probe real-time

PCR and MassARRAY. All study populations were within the HWE except for study by McCarty *et al.* (2012). The characteristic of the included studies was summarized in Table 1.

Figure 1. Schematic diagram of literature searching process for this meta-analysis.

In this meta-analysis, all pooled ORs were calculated using the fixed model (Mantel-Haenszel method) based on the absent of heterogeneity among all included studies (Table

2). The variant c2 allele was not associated with the risk of breast cancer in the allelic comparison model (OR=0.968, 95% CI=0.855-1.097; $p=0.612$) (Figure 2). Besides that, forest plot for all genotypic comparison models including the heterozygous (OR=1.003, 95% CI=0.869-1.159; $p=0.963$) (Figure 3), homozygous (OR=0.792, 95% CI=0.519-1.207; $p=0.278$) (Figure 4), dominant (OR=0.987, 95% CI=0.858-1.134; $p=0.851$) (Figure 5) and recessive (OR=1.265, 95% CI=0.831-1.924; $p=0.273$) (Figure 6) revealed no significant evidence to associate the *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk. Although previous studies showed that the presence of at least one c2 allele increased the risk of breast cancer in the Malaysian population (Chong *et al.*, 2016) and subjects with at least one c1 allele elevated the risk of breast cancer in the Taiwanese population (Wu *et al.*, 2006), the findings of this meta-analysis were statistically reliable and robust as there was no individual study significantly influenced the pooled OR when individual studies were subsequently removed in the sensitivity test.

Table 1. Characteristic of the included studies in this meta-analysis.

Reference	Popula- tion	Sample size (case/ control)	Case			Control			HW E (χ^2) ^a
			c1/c1	c1/c2	c2/c2	c1/c1	c1/c2	c2/c2	
Anderson <i>et al.</i> (2012)	Mixed	883/927	817	63	3	867	59	1	1.28
Choi <i>et al.</i> (2003)	Korean	329/332	232	83	14	224	95	13	3.19
Chong <i>et al.</i> (2016)	Malaysian	71/260	40	29	2	181	74	5	1.59
Khedhaier <i>et al.</i> (2008)	Tunisian	304/244	296	8	0	232	12	0	0.19
McCarty <i>et al.</i> (2012)	Mixed	1028/1048	957	64	7	974	70	4	23.99
Sangrajrang <i>et al.</i> (2010)	Thai	546/473	400	135	11	340	115	18	1.96
Wu <i>et al.</i> (2006)	Taiwanese	262/225	162	94	6	134	77	14	0.58
Zgheib <i>et al.</i> (2013)	Lebanese	227/98	219	8	0	95	3	0	0.10

^aIn Hardy-Weinberg equilibrium (HWE) if $\chi^2 < 3.84$.

Table 2. Association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with the risk of breast cancer in different comparison models.

Genetic comparison	Model	OR (95% CI); <i>p</i>	Heterogeneity (<i>I</i> ²); <i>p</i>	Egger's test (<i>t</i>); <i>p</i>	Begg's test (<i>z</i>); <i>p</i>
Allelic (c2 vs. c1)	Fixed	0.968 (0.855-1.097); 0.612	24.693; 0.232	0.297; 0.776	0.742; 0.458
Heterozygous (c1/c2 vs. c1/c1)	Fixed	1.003 (0.869-1.159); 0.963	9.140; 0.359	0.064; 0.951	0.742; 0.458
Homozygous (c2/c2 vs. c1/c1)	Fixed	0.792 (0.519-1.207); 0.278	39.445; 0.143	1.573; 0.191	1.315; 0.188
Dominant (c1/c2+c2/c2 vs. c1/c1)	Fixed	0.987 (0.858-1.134); 0.851	15.360; 0.309	0.199; 0.849	0.495; 0.621
Recessive (c1/c1+c1/c2 vs. c2/c2)	Fixed	1.265 (0.831-1.924); 0.273	38.570; 0.149	1.388; 0.238	0.939; 0.348

Figure 2. Forest plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under allelic comparison model (c2 vs. c1).

Figure 3. Forest plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under heterozygous comparison model (c1/c2 vs. c1/c1).

Figure 4. Forest plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under homozygous comparison model (c2/c2 vs. c1/c1). Two studies were excluded as no data on c2/c2 genotype was reported (Khedhaier *et al.*, 2008; Zgheib *et al.*, 2013).

Figure 5. Forest plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under dominant comparison model (c1/c2+c2/c2 vs. c1/c1).

Figure 6. Forest plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under recessive comparison model (c1/c1+c1/c2 vs. c2/c2). Two studies were excluded as no data on c2/c2 genotype was reported (Khedhaier *et al.*, 2008; Zgheib *et al.*, 2013).

Funnel plot was performed to test for existing of publication bias in this meta-analysis. The shape of funnel plot under allelic comparison model showed a low possibility of publication bias (Figure 7), which further supported by statistical evidences from Egger's test ($t=0.297$; $p=0.776$) and Begg's test ($\chi^2=0.742$; $p=0.458$). The results of Egger's test and Begg's test for comparison of heterozygous, homozygous, dominant and recessive models also revealed no evidence of publication bias (Table 2). Therefore, we concluded that no publication bias was detected in this meta-analysis.

There are several limitations that should be addressed in this meta-analysis. First, only eight studies with a total of 3650 breast cancer cases and 3607 controls were included in this meta-analysis, which is relatively small. Second, studies that included in this meta-analysis were only obtained from the PubMed database and limited to articles that published in English. Therefore, there is possible for studies that published in other databases or other languages that meet the inclusion criteria but did not include in this meta-analysis. Third, this meta-analysis did not perform subgroup analyses for ethnicity, menopausal

status, lifestyle and environmental factors that have been reported to associate with breast cancer risk (Farvid *et al.*, 2016; Kim *et al.*, 2016; White *et al.*, 2016).

Figure 7. Funnel plot showing the association of *CYP2E1* rs2031920 and rs3813867 polymorphisms with breast cancer risk under allelic comparison model (c2 vs. c1) with $t=0.297$ and $p=0.776$ in Egger's test, and $\chi^2=0.742$ and $p=0.458$ in Begg's test.

CONCLUSION

In conclusion, this meta-analysis revealed that the *CYP2E1* rs2031920 and rs3813867 polymorphisms were not associated with breast cancer risk. A comprehensive meta-analysis by including the subgroup analyses such as ethnicity, menopausal status, lifestyle and environmental factors is needed to understand the interplay between these factors and *CYP2E1* polymorphisms with the risk of breast cancer.

REFERENCES

Anderson, L. N., Cotterchio, M., Mirea, L., Ozcelik, H. and Kreiger, N. 2012. Passive cigarette smoke exposure during periods of life, genetic variants, and breast cancer risk among never smokers. *American Journal of Epidemiology* 175: 289-301.

Begg, C. B. and Mazumdar, M. 1994. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 50: 1088-1101.

Boniza, N., Bradamante, V. and Lovric, M. 2009. Genetic polymorphism of metabolic enzymes P450 (CYP) as a susceptibility factor for drug response, toxicity, and cancer

risk. *Archives of Industrial Hygiene and Toxicology* 60: 217-242.

Choi, J. Y., Abel, J., Neuhaus, T., Ko, Y., Harth, V., Hamajima, N., Tajima, K., Yoo, K. Y., Park, S. K., Noh, D. Y., Han, W. S., Choe, K. J., Ahn, S. H., Kim, S. U., Hirvonen, A. and Kang, D. H. 2003. Role of alcohol and genetic polymorphisms of *CYP2E1* and *ALDH2* in breast cancer development. *Pharmacogenetics* 13: 67-72.

Chong, E. T. J., Goh, L. P. W., See, E. U. H., Chuah, J. A., Chua, K. H. and Lee, P. C. 2016. Association of *CYP2E1*, *STK15* and *XRCC1* polymorphisms with risk of breast cancer in Malaysian women. *Asian Pacific Journal of Cancer Prevention* 17: 647-653.

Danko, I. M. and Chaschin, N. A. 2005. Association of *CYP2E1* gene polymorphism with predisposition to cancer development. *Experimental Oncology* 27: 248-256.

DerSimonian, R. and Laird, N. 1986. Meta-analysis in clinical trials. *Controlled Clinical Trials* 7: 177-188.

Egger, M., Smith, G. D., Schneider, M. and Minder, C. 1997. Bias in meta-analysis detected by a simple, graphical test. *British Medical Journal* 315: 629-634.

Farvid, M. S., Cho, E. Y., Eliassen, A. H., Chen, W. Y. and Willett, W. C. 2016. Lifetime grain consumption and breast cancer risk. *Breast Cancer Research and Treatment* 159: 335-345.

Forouzanfar, M. H., Foreman, K. J., Delossantos, A. M., Lozano, R., Lopez, A. D., Murray, C. J. L. and Naghavi, M. 2011. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. *Lancet* 378: 1461-1484.

Hayashi, S., Watanabe, J. and Kawajiri, K. 1991. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *Journal of Biochemistry* 110: 559-565.

Khedhaier, A., Hassen, E., Bouaouina, N., Gabbouj, S., Ahmed, S.B. and Chouchane, L. 2008. Implication of xenobiotic metabolizing enzyme gene (*CYP2E1*, *CYP2C19*, *CYP2D6*, *mEH* and *NAT2*) polymorphisms in breast carcinoma. *BMC Cancer* 8: 109.

Kim, A. E., Lundgreen, A., Wolff, R. K., Fejerman, L., John, E. M., Torres-Mejia, G., Ingles, S. A., Boone, S. D., Connor, A. E., Hines, L. M., Baumgartner, K. B., Giuliano, A., Joshi, A. D., Slattery, M. L. and Stern, M. C. 2016. Red meat, poultry, and fish intake and breast cancer risk among Hispanic and non-Hispanic white women: The Breast Cancer Health Disparities Study. *Cancer Causes & Control* 27: 527-543.

Leung, T., Rajendran, R., Singh, S., Garva, R., Krstic-Demonacos, M. and Demonacos, C. 2013. Cytochrome P450 2E1 (*CYP2E1*) regulates the response to oxidative stress and migration of breast cancer cells. *Breast Cancer Research* 15: R107.

Lichtenstein, P., Holm, N. V., Verkasalo, P. K., Iliadou, A., Kaprio, J., Koskenvuo, M., Pukkala, E., Skytthe, A. and Hemminki, K. 2000. Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. *New England Journal of Medicine* 343: 78-85.

Mantel, N. and Haenszel, W. 1959. Statistical aspects of the analysis of data from retrospective studies of disease. *Journal of the National Cancer Institute* 22: 719-748.

McCarty, C. A., Reding, D. J., Commings, J., Williams, C., Yeager, M., Burmester, J. K., Schairer, C. and Ziegler, R.G. 2012. Alcohol, genetics and risk of breast cancer in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Breast Cancer Research and Treatment* 133: 785-792.

Purohit, V. 2000. Can alcohol promote aromatization of androgens to estrogens? A review. *Alcohol* 20: 123-127.

Rodriguez, S., Gaunt, T. R. and Day, I. N. M. 2009. Hardy-Weinberg equilibrium testing of biological ascertainment

- for mendelian randomization studies. *American Journal of Epidemiology* 169: 505-514.
- Sangrajrang, S., Sato, Y., Sakamoto, H., Ohnami, S., Khuhaprema, T. and Yoshida, T. 2010. Genetic polymorphism in folate and alcohol metabolism and breast cancer risk: a case-control study in Thai women. *Breast Cancer Research and Treatment* 123: 885-893.
- Sharma, G. N., Dave, R., Sanadya, J., Sharma, P. and Sharma, K. K. 2010. Various types and management of breast cancer: an overview. *Journal of Advanced Pharmaceutical Technology & Research* 1: 109-126.
- Watanabe, J., Hayashi, S. and Kawajiri, K. 1994. Different regulation and expression of the human *CYP2E1* gene due to the *RsaI* polymorphism in the 5'-flanking region. *Journal of Biochemistry* 116: 321-326.
- Watanabe, J., Hayashi, S., Nakachi, K., Imai, K., Suda, Y., Sekine, T. and Kawajiri, K. 1990. *PstI* and *RsaI* in complete linkage disequilibrium at the *CYP2E* gene. *Nucleic Acids Research* 18: 7194.
- White, A. J., Bradshaw, P. T., Herring, A. H., Teitelbaum, S. L., Beyea, J., Stellman, S.D., Steck, S.E., Mordukhovich, I., Eng, S. M., Engel, L. S., Conway, K., Hatch, M., Neugut, A. I., Santella, R. M. and Gammon, M. D. 2016. Exposure to multiple sources of polycyclic aromatic hydrocarbons and breast cancer incidence. *Environment International* 89-90: 185-192.
- Wright, R. M., McManaman, J. L. and Repine, J. E. 1999. Alcohol-induced breast cancer: a proposed mechanism. *Free Radical Biology & Medicine* 26: 348-354.
- Wu, S. H., Tsai, S. M., Hou, M. F., Lin, H. S., Hou, L. A., Ma, H., Lin, J. T., Yeh, F. L. and Tsai, L. Y. 2006. Interaction of genetic polymorphisms in cytochrome P450 2E1 and glutathione S-transferase M1 to breast cancer in Taiwanese women without smoking and drinking habits. *Breast Cancer Research and Treatment* 100: 93-98.
- Zgheib, N. K., Shamseddine, A. A., Geryess, E., Tfayli, A., Bazarbachi, A., Salem, Z., Shamseddine, A., Taher, A. and El-Saghir, N. S. 2013. Genetic polymorphisms of *CYP2E1*, *GST*, and *NAT2* enzymes are not associated with risk of breast cancer in a sample of Lebanese women. *Mutation Research* 747-748: 40-47.