RESEARCH ARTICLE

Current Evidence on the Association between rs3757318 of *C6orf97* and Breast Cancer Risk: a Meta-Analysis

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Abstract

Background: A common genetic variant rs3757318, located in intron of *C6orf97*, was firstly identified to be associated with breast cancer (BC) risk by a genome-wide association (GWA) study. However, subsequent validation studies with different ethnicities have yielded conflicting results. Materials and Methods: We performed a meta-analysis to synthesize all available data for evaluating the precise effect of this variant on BC susceptibility. Results: A total of 8 articles containing 11 studies with 62,891 cases and 65,635 controls were included in this meta-analysis. When compared to the G allele, the rs3757318-A allele was significantly associated with BC risk with the pooled OR of 1.21 (95% CI=1.15 - 1.29, *P*<0.001) but with obvious between-study heterogeneity (*P*=0.040). Stratified analysis suggested that diversity of ethnicity along with control source may explain part of the heterogeneity. Similarly, significant associations were also identified in heterozygote, homozygote, dominant and recessive genetic models. Sensitivity and publication bias analyses indicated robust stability of our results. Conclusions: Our present meta-analysis demonstrated that the variant rs3757318 is associated with increased BC risk. Nevertheless, further studies are needed to clarify the underlying biological mechanisms.

Keywords: Breast cancer - risk - rs3757318 - *C6orf97* - meta-analysis

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Introduction

Breast Cancer (BC) is the most common malignancy and leading cause of cancer death among women in the world with an estimate of 1.38 million new cases diagnosed and 458,000 deaths occurred in 2008 (Jemal et al., 2011). As a developing country, the morbidity and mortality of BC have grown rapidly in both urban and rural areas of China (Yang et al., 2006). BC has become the leading cause of malignancy of females in China according to the surveillance data from the third cancer death investigation (Chen, 2009). Similarly, like most of other cancers, BC is a heterogeneous disease and the precise underlying mechanism has not been elucidated. Nonetheless, accumulated evidence suggested that genetic susceptibility plays an important role in the risk of BC, since the family history is one of the main risk factors for BC (Antoniou et al., 2006).

The most important finding of genetic susceptibility analysis for BC was that the *BRCA1* and *BRCA2* genes were identified by linkage analysis and positional cloning in the 1990s (Miki et al., 1994; Wooster et al., 1995). However, the inherited mutations in *BRCA1* and *BRCA2* only accounted for a small fraction of BC susceptibility though they conferred a high risk for BC (Nathanson et al., 2001). Most of the unexplained fraction of relative risk of BC was likely to be a consequence of a combination of low-penetrance genetic variants (Antoniou et al., 2002). Genome-wide association (GWA) study provides a comprehensive approach to identify genetic variants to be associated with cancer risk unconstrained by existing knowledge. And it can detect common variants with small effect but great public health impact to the cancer risk. Since the first GWA study about BC was reported in 2007, many GWA studies were published and many variants were identified to be associated with BC risk (Easton et al., 2007; Stacey et al., 2007; Zheng et al., 2009; Turnbull et al., 2010), among which, rs3757318 located in 6q25.1 lies ~200 kb upstream of ESR1 in an intron of *C6orf97* was firstly identified by Turnbull et al. (2010).

Although there were many statistical evidence of variant rs3757318 for BC risk, the results from replication studies were still conflicting. This may be caused by the restriction of sample size or ethnic diversity. In addition, individual studies may have deficient ability to achieve a reliable conclusion. Meta-analysis as a comprehensive process to clarify the incompatible results in genetic association studies by increasing the sample size have

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confirmed many variants to be associated with BC risk (Chen et al., 2012; Guo et al., 2012). Therefore, we performed this meta-analysis to determine the effect of this variant on BC susceptibility.

Materials and Methods

Search strategy

We performed a systematic search for literatures using PubMed, ISI Web of Science and China National Knowledge Infrastructure databases up to February 28th 2014. The search query combined the following terms of "rs3757318 or *C6orf97*" and "breast cancer, breast neoplasm, or breast carcinoma." We also scrutinized the reference lists from reviews and other relevant publications to further identify useful studies.

Selection criteria

Studies were included in this meta-analysis if they met the following criteria: (1) the studies investigated the association of rs3757318 and BC risk using either case-control or cohort design; (2) sufficient genotypic or allelic frequency information or the odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were reported; (3) the genotypes in controls conformed to hardy-Weinberg Equilibrium (HWE); (4) the diagnosis of BC patients was confirmed histologically or pathologically. If the same or overlapping samples were reported in multiple publications, only the well-studied or largest one was finally included. In addition, the studies focused on other species and male were also excluded.

Data extraction

The following pieces of information were extracted from each included study: name of the first author, publication year, ethnicity of the participants, country where the study was carried, the study design and method, genotyping method, number of cases and controls, numbers of three genotypes and alleles' frequencies in cases and controls or the corresponding ORs and 95% CIs. When an article reported results on different subgroups according to ethnicity or BC type, we considered each subgroup as a separate study in this meta-analysis.

Statistical analysis

The strength of association between rs3757318 and BC risk was assessed by the ORs and their corresponding 95% CIs. The pooled ORs were performed for allelic model (A *vs* G), heterozygote model (GA *vs* GG), homozygote model (AA *vs* GG), dominant model (GA + AA *vs* GG), and recessive model (AA *vs* GA + GG). The Cochran's χ^2 -based Q statistic test and I^2 statistics were used to assess the heterogeneity among studies and the heterogeneity was considered significant when $P<0.05$ for Q test. The fixed-effects model was applied to calculate the pooled ORs when the heterogeneity was negligible; otherwise, the random-effects model was employed (DerSimonian et al., 1986).

Sensitivity analysis was conducted to assess the stability of the results by removing each study in turn from the total and re-analyzed the remainder, while the cumulative analysis was performed to investigate the chronology trend of the association between rs3757318 and BC risk through assortment of studies with publication time. The publication bias was evaluated by funnel plot and Egger's test (Egger et al., 1997). All P values were two-tailed with a significant level at 0.05. All statistical analyses were performed using the Stata version 10.0.

In addition, subgroup analyses were carried out according to ethnicity (Asian or European) and control source (hospital-based or population-based) under the allelic model. The stratified analyses were not conducted in other models due to the insufficient information.

Results

Characteristics of included studies

The systematic literature search and study selection procedures for association between rs3757318 and BC risk was displayed in Figure 1. A total of 14 potentially relevant reports were initially identified after an extensive search. However, only 8 publications (Turnbull et al., 2010; Yoshimoto et al., 2011; Sueta et al., 2012; Barzan et al., 2013; Chen et al., 2013; Michailidou et al., 2013; Mizoo et al., 2013; Xia et al., 2013) met the inclusion criteria. The publication reported by Barzan et al. (2013) provided two separated results on Asian and European populations was considered as two different case-control studies. Besides, the study reported by Yoshimoto N et al. applied two different case subgroups (Estrogen Receptor (ER) positive and ER negative) with the same set of control was also considered as two individual studies. Additionally, the study reported by Michailidou et al. (2013) separately offered their results according to the study design was considered as one GWA study and one replication study. Finally, 8 articles containing 11 studies with 62,891 cases and 65,635 controls were included in this meta-analysis, of which, 7 studies were conducted in Asian (4 studies in Japanese, 3 studies in Chinese), and 4 were conductedT in European (1 study in British, 1 study in German and 2 studies in mixed European populations). The study characteristics were summarized in Table 1.

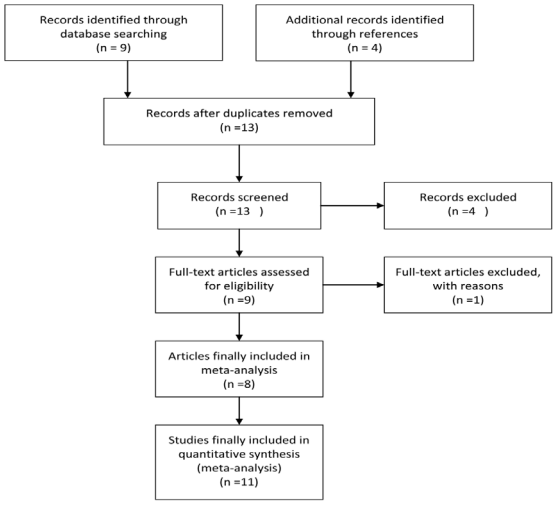

Figure 1. Flow Diagram of the Study Selection Procedure

Table 1. Characteristics of the Studies about rs3757318 and BC Risk Included in the Meta-analysis

First author	Published year	Ethnicity	Country	Study type	Study method	Control source	Genotype method	Case/control	HWE
Turnbull C[12]	2010	European	UK	GWAS	CC	population-based	Illumina	3659/4897	Yes
Yoshimoto N[17]	2011	Asian	Japan	Replication	CC	hospital-based	Taqman	635/264	Yes
		Asian	Japan	Replication	CC	hospital-based	Taqman	136/264	Yes
Sueta A[18]	2011	Asian	Japan	Replication	CC	hospital-based	Taqman	697/1,394	Yes
Barzan D[19]	2013	Asian	China	Replication	CC	hospital-based	Sequenom MassArray system	984/2206	Yes
		European	Germany	Replication	CC	population-based	Sequenom MassArray system	311/960	Yes
Michailidou k[20]	2013	European	Mixed	GWAS	CC	population-based	Illumina, Affymetrix	10052/12575	Yes
		European	Mixed	Replication	CC	Mixed	Illumina Infinium array	45290/41880	Yes
Chen W [21]	2013	Asian	China	Replication	CC	hospital-based	Taqman	477/534	Yes
Xia P [22]	2013	Asian	China	Replication	CC	hospital-based	Sequenom MassArray system	185/199	Yes
Mizoo T [23]	2013	Asian	Japan	Replication	CC	hospital-based	Taqman	465/462	Yes

Abbreviation: CC, case-control study; UK, United Kingdom; Yes, conform to HWE

Table 2. Meta-Analysis of rs3757318 in Association with BC Risk

	Genetic model	OR(95% CI)[a]	*P*	I^2	*P* for heterogeneity	*P* for Egger's test
overall						
Overall (n=11)	A *vs* G	1.21 (1.15-1.29)	< 0.001	47.5	0.04	0.613
Overall (n=4)	GA *vs* GG	1.21 (1.04-1.41)	0.016	50.1	0.111	0.046
	AA *vs* GG	1.48 (1.08-2.02)	0.015	61.5	0.05	0.355
Overall (n=5)	Dominant model	1.20 (1.07-1.35)	0.002	57.3	0.053	0.508
	Recessive model	1.33 (1.04-1.69)	0.024	33.4	0.199	0.494
Ethnicity						
Asian (n=7)	A *vs* G	1.20 (1.06-1.35)	0.003	56.2	0.033	0.243
European (n=4)	A *vs* G	1.18 (1.14-1.22)	< 0.001	28.9	0.239	0.256
Control source						
Hospital-based (n=7)	A *vs* G	1.20 (1.06-1.35)	0.003	56.2	0.033	0.243
Population-based (n=3)	A *vs* G	1.24 (1.17-1.31)	< 0.001	0	0.572	0.756
Mixed (n=1)	-	-	-	-	-	-

a: The ORs were calculated in fixed- or random-effects models

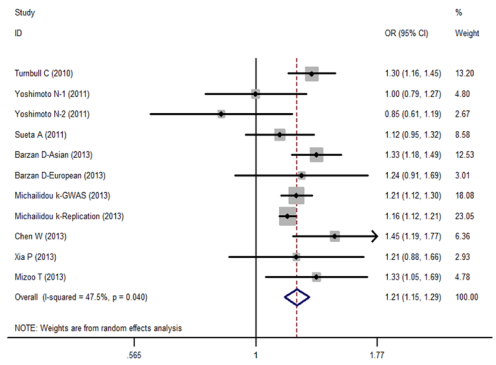

Figure 2. The Forest Plot of Association of rs3757318 with BC Risk in Allelic Model

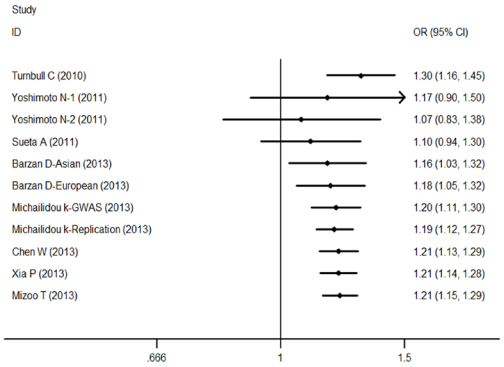

Figure 3. The Cumulative Meta-analysis of rs3757318 with BC Risk Under Allelic Model

The association between rs3757318 and BC risk in the overall meta-analysis

The random-effects model was employed to pool the comprehensive OR under the allelic model due to the significant between-study heterogeneity was observed (*P*=0.040). When compared to the G allele, the rs3757318-A allele conferred to a risk factor for BC with the pooled OR of 1.21 (95% CI=1.15-1.29, *P*<0.001) (Figure 2). In addition, significant associations of rs3757318 and BC risk were identified in the other four models (genotypic GA *vs* GG model: OR=1.21, 95% CI=1.04 - 1.41, $P_{heterogeneity}$=0.111; genotypic AA *vs* GG model: OR=1.48, 95% CI=1.08 - 2.02, $P_{eterogeneity}$=0.050; dominant model: OR=1.20, 95% CI=1.07 - 1.35, $P_{heterogeneity}$=0.053; recessive model: OR=1.33, 95% CI=1.04 - 1.69, $P_{heterogeneity}$=0.199) (shown in Table 2).

Stratified analysis

To evaluate the potential source of heterogeneity among studies, stratified analysis was conducted under the allelic model. As shown in Table 2, When stratified by ethnicity, statistically significant associations were detected among Asians and Europeans with the ORs of 1.20 (95% CI=1.06-1.35, $P_{heterogeneity}$=0.033) and 1.18 (95% CI=1.14-1.22, $P_{heterogeneity}$=0.239), respectively. The between-study heterogeneity was significantly reduced in Europeans but still remained in Asians. After stratifying by control source, heterogeneity was disappeared in population-based subgroup, whereas in hospital-based subgroup, the heterogeneity was still existed. Meanwhile, significant relationship of rs3757318 and BC risk were found in the both two subgroups.

Sensitivity analyses and cumulative meta-analysis

Sensitivity analysis was performed in an attempt to assess the effects of each individual study on the pooled OR owing to the significant heterogeneity across studies was found. As shown in Table 3, the pooled OR for the allelic model was similar before and after omission of each study. It suggested that the meta-analysis for rs3757318

Table 3. Sensitivity Analysis Under the Allelic Model

Study	I^2	P for heterogeneity	OR(95% CI)
Turnbull C	45.2	0.058	1.18 (1.15-1.22)
Yoshimoto N-1	46.9	0.05	1.19 (1.16-1.23)
Yoshimoto N-2	40.4	0.088	1.19 (1.16-1.23)
Sueta A	51.3	0.03	1.22 (1.15-1.30)
Barzan D-Asian	41.3	0.082	1.18 (1.15-1.22)
Barzan D-European	52.6	0.025	1.21 (1.14-1.29)
Michailidou k-GWAS	52.2	0.027	1.21 (1.13-1.31)
Michailidou k-Replication	39.3	0.096	1.24 (1.18-1.29)
Chen W	40.7	0.086	1.19 (1.15-1.22)
Xia P	52.7	0.025	1.21 (1.14-1.29)
Mizoo T	50.5	0.033	1.21 (1.14-1.28)
Combined	47.5	0.04	1.21 (1.15-1.29)

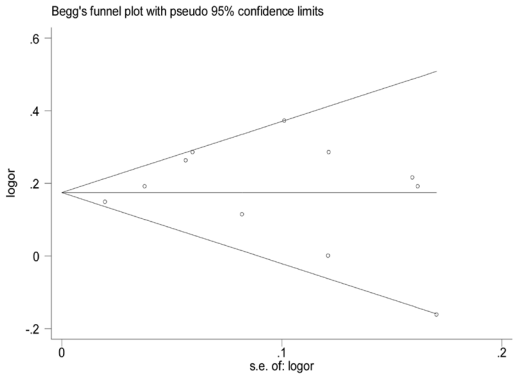

Figure 4. Begg's Funnel Plot for Publication Bias Under the Allelic Model

and BC risk was reliable and stable. In addition, narrowed 95% CIs for the pooled ORs with each accumulation of more studies under allelic model indicated the progressively increasing precision of the estimation by continual adding more studies. Simultaneously, significant associations of rs3757318 and BC risk were found all over the time (Figure 3).

Publication bias

Begg's funnel plot (Figure 4) and Egger's test (Table 2) were performed to estimate the publication bias of the literatures. No significant publication bias was observed except for the genotypic GA *vs* GG model (P=0.046) which may be due to the limited studies.

Discussion

Meta-analysis improved research power via increasing the sample size of the study. Therefore, it has been widely used to precisely evaluate the association of genetic factors and BC risk. Zhang et al. (2013) conducted a meta-analysis including 4499 BC cases and 6369 controls and then revealed that common variants in PALB2 gene may increase the risk of BC. In addition, Zhang et al. (2013). found that 657 del 5 mutation in NBS1 gene significantly associated with BC with a meta-analysis including 11 eligible articles (Zhang et al., 2012). As a comprehensive method, meta-analysis overcome the limitation of individual studies' insufficient power which may due to the small sample size. It is an efficient, financial and practical method. Therefore, we performed this research to investigate the association of rs3757318 and BC risk.

The present meta-analysis included 11 studies with 62,891 cases and 65,635 controls to explore the association between rs3757318 and BC risk. The results demonstrated that the A allele conferred to increased risk for BC compared to G allele with the pooled OR being 1.21 (95% CI=1.15-1.29, P<0.001). Cumulative analysis exhibited the narrow trend of the 95% CI, which represented that the evaluation precision of the effect of this variant on BC risk was increased with more samples included in the meta-analysis. In addition, the sensitivity analysis and publication bias analysis indicated the robust stability of our results. Stratified analysis under allelic model was conducted to explore the potential explanation for the significant between-study heterogeneity. When stratified by ethnicity, heterogeneity was significantly reduced in European subgroup but still remained in Asian subgroup. It suggested that ethnicity may interpret part of the heterogeneity, exemplified by the evidence that the minor allele frequency was much higher in Asian than in European. After stratifying by control source, heterogeneity was disappeared in population-based subgroup but not in hospital-based subgroup, possibly due to the more inevitable confounding factors introduced into hospital-based studies than population-based studies. Taken together, we revealed that ethnicity and control source may explain part of the source of heterogeneity.

The variant rs3757318 was located in 6q25.1 lies ~200 kb upstream of the transcript start site of the gene encoding estrogen receptor 1 (ESR1) in an intron of *C6orf97*. ESR1 is a hormone receptor which acts as a transcription factor based on ligand binding. Epidemiological studies have demonstrated that estrogen receptors play an important role in the pathology of BC, and many studies had been conducted to investigate the association of genetic variants in ESR1 and increased BC risk in different ethnicities (Andersen et al., 1994; Cai et al., 2003; Gold et al., 2004). Barzan et al. (2013) hypothesized that rs3757318 may modify the BC susceptibility by influencing the expression pattern of ESR1. However, no other evidences were found to support their hypothesis, and certainly, larger comprehensive and more functional studies are anticipated to further elucidate the underling mechanisms of association between rs3757318 and increased BC risk.

Since this variant was first identified to be related to BC risk in European by Turnbull et al. (2010) in a GWA study, several replication studies have been followed to confirm the association in other races but with conflicting results. Barzan et al. (2013) and Chen et al. (2013) identified positive association between rs3757318 and BC risk in Chinese population but Xia et al. (2013) did not. Barzan et al. (2013) Michailidou et al. (2013) combined 9 GWAS data and 41 replication studies in European population identified that rs3757318 was significantly associated with increased BC risk in European ancestry. However, both Yoshimoto et al. (2011) and Sueta et al. (2012) identified non-significant association between rs3757318 and BC risk in Japanese. Even though Mizoo T et al. (2013) concluded that this variant contributed to the susceptibility of BC in Japanese, no significant associations of rs3757318 and BC risk were identified when classified the participants according to the menopausal status.

Despite the advantage of comprehensive strategy

to integrate all eligible data in the current study, some limitations also should be acknowledged. First, the controls were not uniformly defined in all studies, proved by the fact that some studies were population-based but some were hospital-based. Furthermore, the eligible studies of Asian population included in the meta-analysis were limited in just Chinese and Japanese, which may restrict the extension of the results. Significant association was observed in Chinese but not in Japanese may prompt that this variant was not a risk factor for BC in Japanese. Therefore, more studies conducted in many other countries especially in other Asian countries are needed to further confirm and extend the positive results.

In conclusion, this meta-analysis provided a comprehensive evaluation of the association between rs3757318 and BC risk. The results indicated that the rs3757318 contributed to the susceptibility of BC. Certainly, further more studies are needed to elucidate the biological mechanisms.

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