RESEARCH ARTICLE

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Meta-Analysis of Association between *PALB2* Polymorphisms and Breast Cancer

Ali Dianatpour, Sepideh Faramarzi, Soudeh Ghafouri-Fard*

Abstract

Background: Previous studies have assessed associations between single nucleotide polymorphisms (SNPs) of the Partner and localizer of *BRCA2 (PALB2)* gene and risk of breast cancer. However, the results of these studies are not consistent. **Materials and Methods:** We designed a meta-analysis to obtain a more reliable appraisal of the association between SNPs in the *PALB2* gene and the susceptibility to breast cancer. We searched PubMed, Google scholar and Embase databases and selected six studies with sufficient data to estimate the pooled odds ratios (ORs) and 95% confidence intervals (CIs). **Results:** Statistical analyses showed that the rs120963 was associated with breast cancer risk in allelic (OR (95% CI) = 1.33 (1.18-1.49)), homozygous (OR (95% CI) = 1.74 (1.31-2.32)), dominant (OR (95% CI) = 1.42 (1.22, 1.65)) and recessive (OR (95% CI) = 1.54 (1.17, 2.03)) models. The rs249954 and rs16940342 were associated with breast cancer risk in allelic (OR (95% CI) = 1.13 (1.04, 1.23) and 1.12 (1.01, 1.24) respectively) and dominant (OR (95% CI) = 1.23 (1.09, 1.39) and 1.18 (1.04, 1.33) respectively) models. The rs249935 and rs447529 SNPs were associated with breast cancer in homozygous (OR (95% CI) = 0.67 (0.46, 0.97) and 0.51 (0.30, 0.89) respectively) and recessive (OR (95% CI) = 0.65 (0.45, 0.95) and 0.51 (0.30, 0.88) respectively) models. **Conclusions:** The current meta-analysis shows the associations between five SNPs of *PALB2* and breast cancer risk and confirms the results of previous studies regarding the role of this gene in the pathogenesis of breast cancer.

Keywords: *PALB2*- breast cancer- polymorphism- meta-analysis

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Introduction

Breast cancer is a molecularly heterogeneous disorder associated with high mortality and morbidity (Seifi-Alan et al., 2013; Iranpour et al., 2016). Among the most important factors contributing in breast cancer are genes involved in maintenance of genomic integrity (Walsh and King, 2007). Partner and localizer of *BRCA2 (PALB2)* encodes a protein which co-localizes with *BRCA2* in nuclear foci, enhances its stability in chromatin and nuclear matrix and facilitates its tumor suppression effect (Xia et al., 2006). In addition, it provides the functional link between BRCA1 and *BRCA2*. It coheres directly to BRCA1, and makes the molecular scaffold for establishment of the BRCA1-*PALB2*-*BRCA2* complex. Its interaction with BRCA1 is mainly facilitated via apolar bonding between their corresponding coiled-coil domains. Noticeably, BRCA1 mutations detected in cancer patients abolish such interactions resulting in insufficiency of homologous recombination (HR) repair. In brief, *PALB2* makes the molecular link between the BRCA proteins and its mutations lead to defective HR repair, genomic instability and carcinogenesis (Sy et al., 2009). As revealed by in vitro studies, many of *BRCA2* functions including G2/M checkpoint, replication fork shields and repair of double strand breaks by HR depend on its interaction with *PALB2* (Hartford et al., 2016). Germ line mutations in *PALB2* have been shown to increase breast cancer risk in different populations (Rahman et al., 2007; Cao et al., 2009; Antoniou et al., 2014). However, breast cancer susceptibility is best explained by a "polygenic" model, in which several loci confer breast cancer risk, with each locus having only a minor influence (Pharoah et al., 2002). Consequently, in addition to high risk mutations, many low-penetrance variants such as single nucleotide polymorphisms (SNPs) in *PALB2* might interactively affect the risk of breast cancer development (Cao et al., 2010). The associations between *PALB2* SNPs and breast cancer risk have been evaluated in different populations (Chen et al., 2008; Cao et al., 2010; Guenard et al., 2010; Jiang et al., 2016). However, the results of these studies are inconclusive and inconsistent in some cases. Such inconsistency might be explained by the moderately small sample size of former studies or the inherent genetic heterogeneity of breast cancer in diverse populations. Therefore, we designed a meta-analysis to resolve these incompatible results and to achieve a conclusive decision regarding the role of *PALB2* variants in genetic

*Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. *For Correspondence: s.ghafourifard@sbmu.ac.ir*

susceptibility to breast cancer. The selection of *PALB2* for the meta-analysis was based on the previous reports indicating its co-localization with *BRCA2* and its putative role in the pathogenesis of breast cancer.

Materials and Methods

Literature search

The studies included in the meta-analysis were chosen by searching the PubMed, Google scholar and Embase databases from their establishment to January 2018 using the following keywords: "*PALB2* gene or Partner and localizer of *BRCA2*" AND "breast cancer or breast carcinoma". We also assessed all references in of the retrieved studies to detect extra research not included in the databases. We just assessed researches written in English. Figure 1 shows the flowchart of course of selection of articles for inclusion in the current meta-analysis.

Inclusion criteria

The following criteria was adopted to include studies in the current meta-analysis: (1) a case-control study scheme; (2) a concentration on the association of *PALB2* SNPs with breast cancer risk; (3) satisfactory data to determine the odds ratio (OR), confidence interval (CI) and P value; (4) compliance of genotype distribution of all control groups with Hardy-Weinberg equilibrium (HWE).

Data extraction and quality assessment

Two researchers (AD and SF) evaluated eligible studies and extracted first author's name, publication year, sample size, country, ethnicity, source of DNA used for genotyping, and frequencies of each genotype in certain study groups. The quality of the studies was evaluated using Newcastle-Ottawa scale (NOS) (Stang, 2010). In the cases of missing data in the obtained studies, the mentioned researchers gathered the missing data through communication (via email) with the corresponding authors.

Statistical analyses

All analyses were performed in the RevMan (v.5.1) software (http://www.cochrane.org/revman). We assessed the association between *PALB2* SNPs and breast cancer using Z test to appraise the significance of the pooled odds ratios (OR). ORs were computed in allelic (wild type (W) versus minor (M)), homozygote (WW versus MM), dominant (WW+WM versus MM) and recessive (WW versus WM+MM) models. The grade of heterogeneity between the studies was evaluated using Q-test and I2 parameter. The values up to 40% were considered acceptable. Based on the calculated P values, OR and 95% CI (for the pooled odds ratio) were computed using random-effect model or fixed-effect model in heterogeneous or homogenous states respectively. Publication bias was evaluated using Begg's and Egger's tests. The level of significance was set at $P<0.05$.

Results

Results of literature search

After initial assessment of titles and abstracts of found manuscripts, 201 manuscripts were reviewed for inclusion (Figure 1). A total of 187 studies were filtered due to unsuitable study design, unsuitable type of study (studies other than original research) or language. Fourteen studies were subjected to detailed evaluation. Finally, we chose six articles for inclusion in the current meta-analysis (Chen et al., 2008; Cao et al., 2010; Guenard et al., 2010; Loizidou et al., 2010; Leyton et al., 2015; Jiang et al., 2016). Table 1 shows the characteristics of studies included in the meta-analysis and the obtained NOS scores.

Meta-analysis

Statistical analyses showed that the rs120963 was associated with breast cancer risk in allelic (OR (95% CI) = 1.33 (1.18-1.49)), homozygous (OR (95% CI) = 1.74 (1.31-2.32)), dominant (OR (95% CI) = 1.42 (1.22, 1.65)) and recessive (OR (95% CI) = 1.54 (1.17, 2.03)) models. The rs249954 and rs16940342 were associated with breast cancer risk in allelic (OR (95% CI) = 1.13 (1.04, 1.23) and 1.12 (1.01, 1.24) respectively) and dominant (OR (95% CI) = 1.23 (1.09, 1.39) and 1.18 (1.04, 1.33) respectively) models. The rs249935 and rs447529 SNPs were associated with breast cancer in homozygous (OR (95% CI) = 0.67 (0.46, 0.97) and 0.51 (0.30, 0.89) respectively) and recessive (OR (95% CI) = 0.65 (0.45, 0.95) and 0.51 (0.30, 0.88) respectively) models. Other SNPs were not associated with breast cancer risk in any of the assessed genetic models. Figures 2-5 show forest plots for the assessed SNPs in the allelic, homozygous, dominant and recessive models respectively. Next, we analyzed associations between mentioned SNPs and breast cancer risk in distinct ethnic

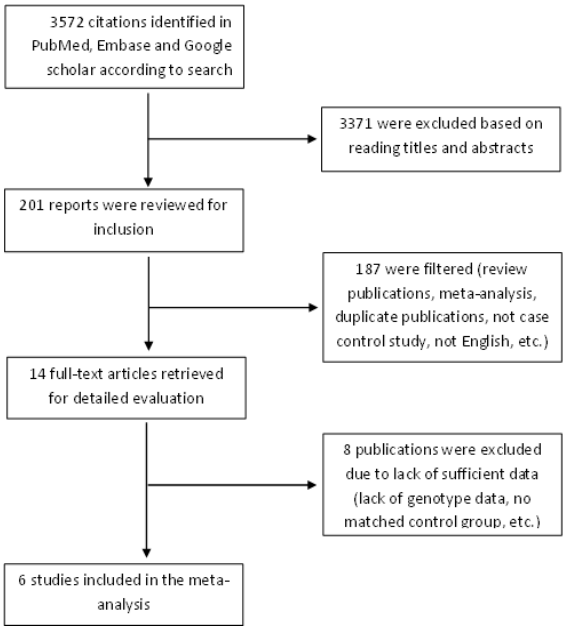

Figure 1. A Systematic Flow Chart Depicting the Sequence of Selection of Articles for This Meta-analysis.

Table 1. Characteristics of Studies Included in the Meta-analysis

Study Name	Year	Country	Source of DNA	Ethnicity	No. of Cases	No. of Controls	Genotype frequency in controls			Genotype frequency in cases			NOS Score	HWE/ Chi-Square
							WW	WR	RR	WW	WR	RR		
rs45532440														
Loizidou et al.	2010	Cyprus	Blood	Caucasian	1,102	1,159	1,035	120	4	972	126	4	7	0.7931/0.07
Gue´nard et al.	2010	Canada	Blood	French Canadian	96	95	92	2	1	88	8	0	8	0.0000/22.74
rs249954														
Chen et al.	2008	China	Blood	Asian	1,010	1,033	412	477	144	331	524	155	7	0.7521/0.10
Cao et al.	2009	China	Blood	Asian	660	756	281	361	113	256	321	82	7	0.8667/0.03
Gue´nard et al.	2010	Canada	Blood	French Canadian	96	86	55	29	2	56	36	4	8	0.4175/0.66
Jiang et al.	2016	China	Blood	Asian	351	360	176	156	28	131	174	46	7	0.4153/0.66
rs45551636														
Loizidou et al.	2009	Cyprus	Blood	Caucasian	1,104	1,170	1,076	92	2	1,010	92	2	7	0.9817/0.000
Gue´nard et al.	2010	Canada	Blood	French Canadian	96	89	87	1	1	95	1	0	8	0.0000/38.8
Leyton et al.	2015	Chile	Blood	-	436	809	786	23	0	413	22	1	7	0.6817/0.17
rs45478192														
Loizidou et al.	2009	Cyprus	Blood	Caucasian	1,109	1,107	1,107	0	0	1,109	0	0	7	Monomorphic
Gue´nard et al.	2010	Canada	Blood	French Canadian	96	84	84	0	0	95	1	0	8	-
rs16940342														
Chen et al.	2008	China	Blood	Asian	1,031	1,052	620	377	55	561	423	47	7	0.8135/0.05
Cao et al.	2009	China	Blood	Asian	660	756	473	252	31	400	233	27	7	0.7228/0.12
Jiang et al.	2016	China	Blood	Asian	351	360	233	109	18	206	122	23	7	0.2653/1.24
rs249935														
Chen et al.	2008	China	Blood	Asian	1,035	1058	762	264	32	720	290	25	7	0.1226/2.38
Cao et al.	2009	China	Blood	Asian	660	756	512	214	30	470	176	14	7	0.2048/1.61
Jiang et al.	2016	China	Blood	Asian	351	360	219	129	12	202	142	7	7	0.1801/1.80
rs120963														
Chen et al.	2008	China	Blood	Asian	997	1,008	488	436	84	428	459	110	7	0.3307/0.95
Jiang et al.	2016	China	Blood	Asian	351	360	235	116	9	168	157	26	7	0.2274/1.46
rs8053188														
Cao et al.	2009	China	Blood	Asian	660	756	745	11	0	647	13	0	7	0.8403/0.04
Gue´nard et al.	2010	Canada	Blood	French Canadian	96	92	87	5	0	92	4	0	8	0.7887/0.07
rs447529														
Cao et al.	2009	China	Blood	Asian	660	756	519	209	28	471	177	12	7	0.2293/1.44
Jiang et al.	2016	China	Blood	Asian	351	360	213	133	14	204	139	8	7	0.2245/1.47
rs152451														
Gue´nard et al.	2010	Canada	Blood	French Canadian	96	94	86	7	1	80	14	2	8	0.0758/3.15
Leyton et al.	2015	Chile	Blood	-	436	809	674	127	8	351	79	6	7	0.4633/0.54

groups (Table 2). In Asians, significant associations were found between the rs249935 breast cancer in homozygous and recessive models, rs249954 and breast cancer in dominant and allelic models, and the rs120963 and breast cancer in all inheritance models. Finally, the rs447529 was associated with breast cancer in this ethnic group only in recessive model.

Assessment of publication bias

The funnel plots were illustrated to evaluate the existence of publication bias in the meta-analysis of the mentioned SNPs in allelic, homozygous, dominant and recessive models (Supplementary Figure 1A-D respectively). The shape of the funnel plot was symmetrical implying lack of publication bias.

Discussion

PALB2 encodes a protein with fundamental role in HR and maintenance of genome stability (Leyton et al., 2015). Biallelic *PALB2* mutations are associated with increased susceptibility to cancers along with hypersensitivity to DNA-damaging materials (Reid et al., 2007). Based on the functional interaction between *PALB2* and the breast cancer susceptibility gene *BRCA2*, *PALB2* SNPs have been considered as putative risk factors for breast cancer development. In the present meta-analysis, we assessed the associations between 10 SNPs within this gene and risk of breast cancer. We found that the rs120963 was associated with breast cancer risk in all assessed genetic models. This SNP resides approximately 16.5 kb from the 3'-end of *PALB2* and was associated with

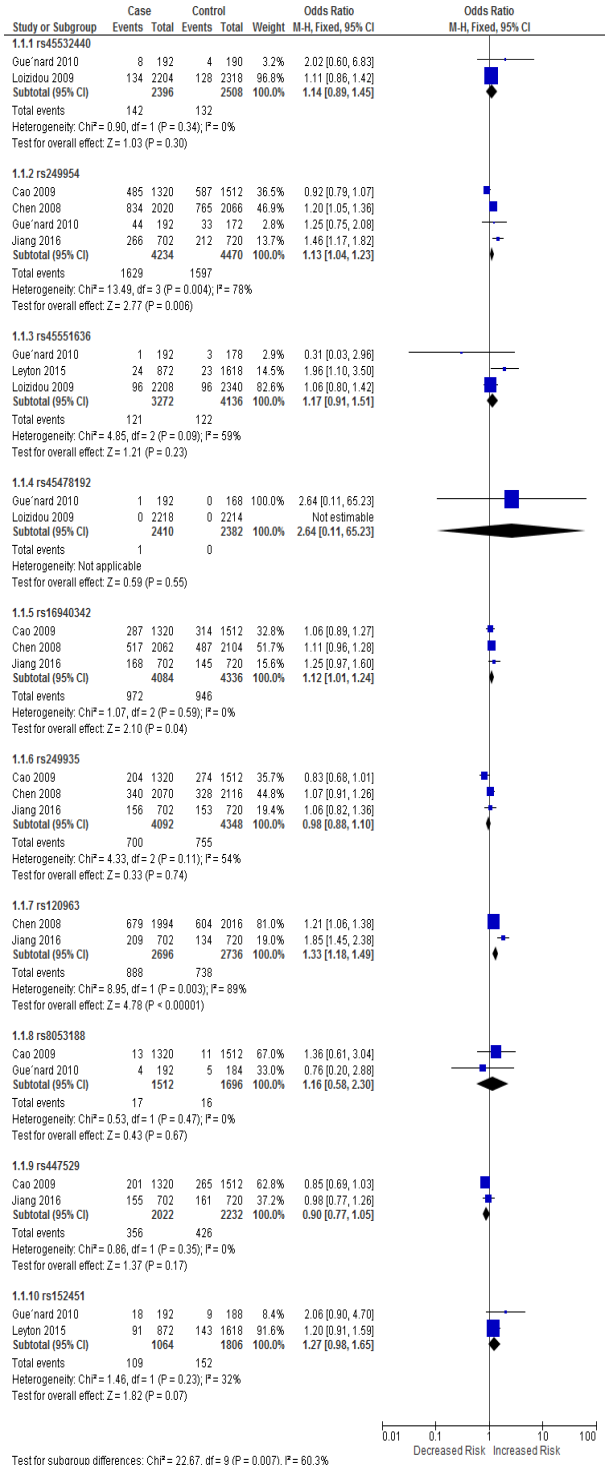

Figure 2. Forest Plot of the Risk for *PALB2* Polymorphisms in Allelic Model. The error bars indicate 95% CIs. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

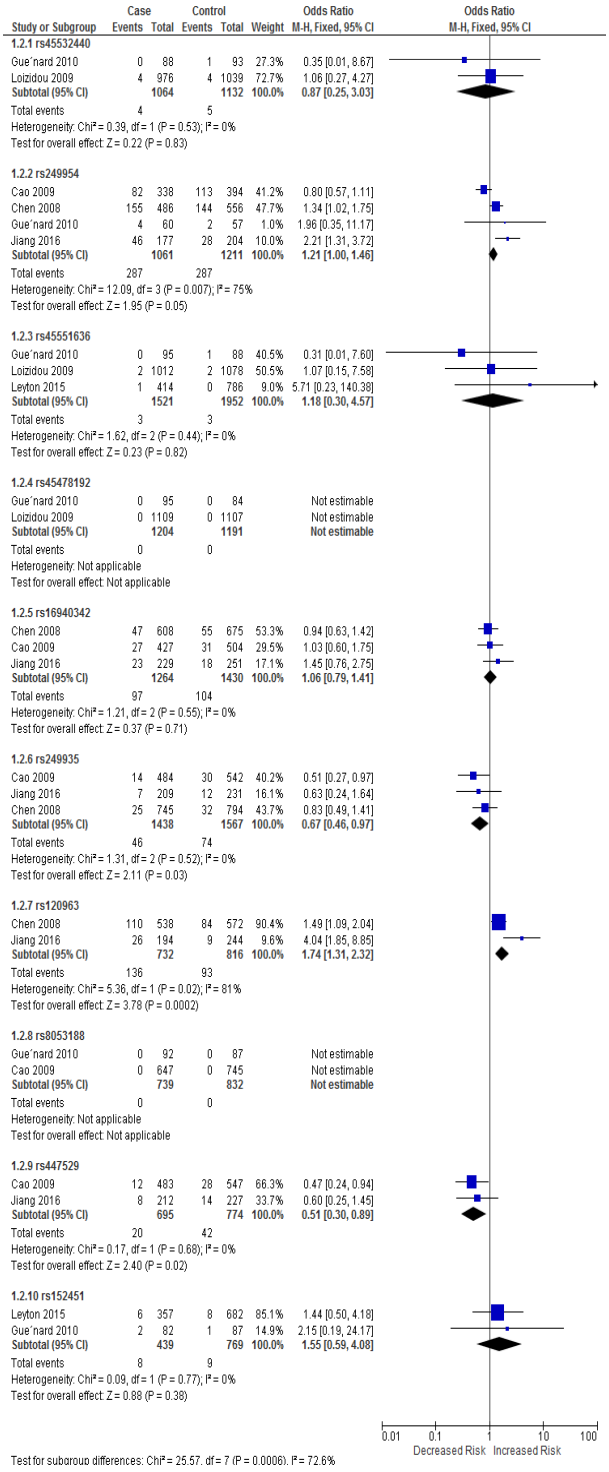

Figure 3. Forest Plot of the Risk for *PALB2* Polymorphisms in Homozygous Model. The error bars indicate 95% CIs. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

breast cancer risk in Chinese population as revealed by independent studies (Chen et al., 2008; Jiang et al., 2016). Moreover, the rs249954 and rs16940342 were associated with breast cancer risk in allelic and dominant models. The rs249935 and rs447529 SNPs were associated with breast cancer in homozygous and recessive models. Other SNPs were not associated with breast cancer risk in any of the assessed genetic models. The rs152451 and rs45551636 are located in exons 4 and 9 of this gene respectively and have been previously identified through sequence analysis of the complete coding region of *PALB2* in 100 probands from South-American breast cancer families negative for BRCA1 and *BRCA2* point mutations (Leyton et al., 2015). However, our meta-analysis revealed no association between these variants and breast cancer risk. For the rs45532440, rs45478192

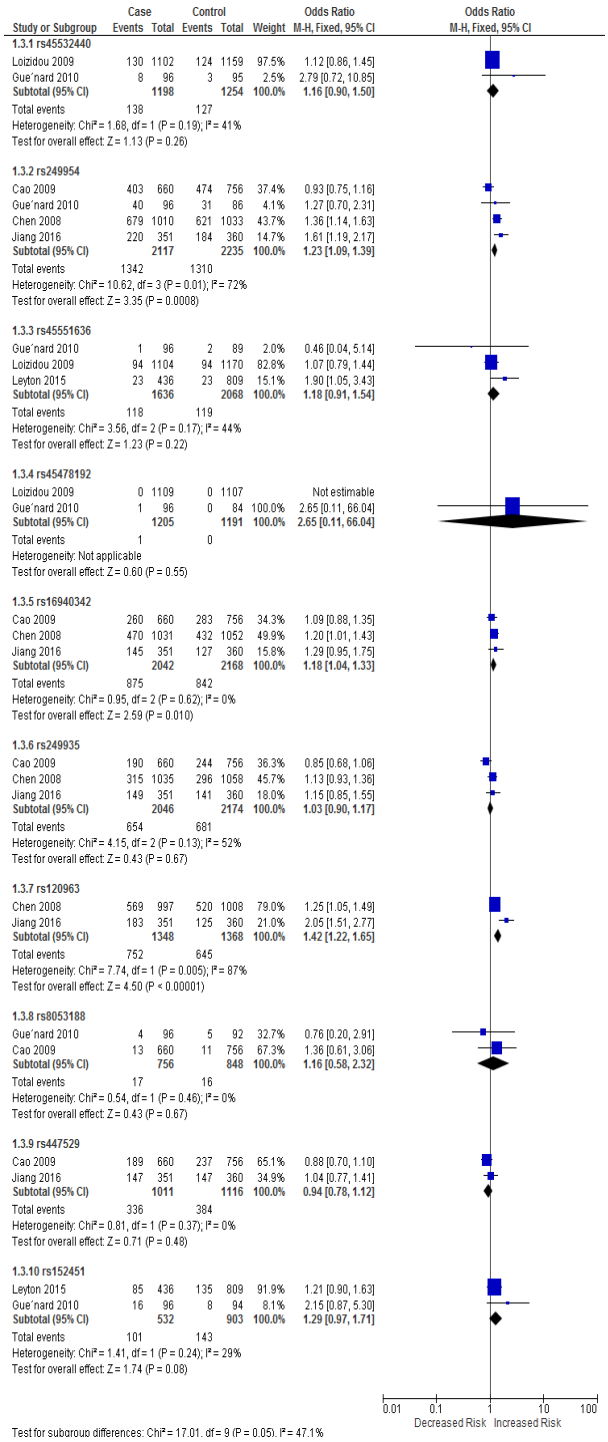

Figure 4. Forest Plot of the Risk for *PALB2* Polymorphisms in Dominant Model. The error bars indicate 95% CIs. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

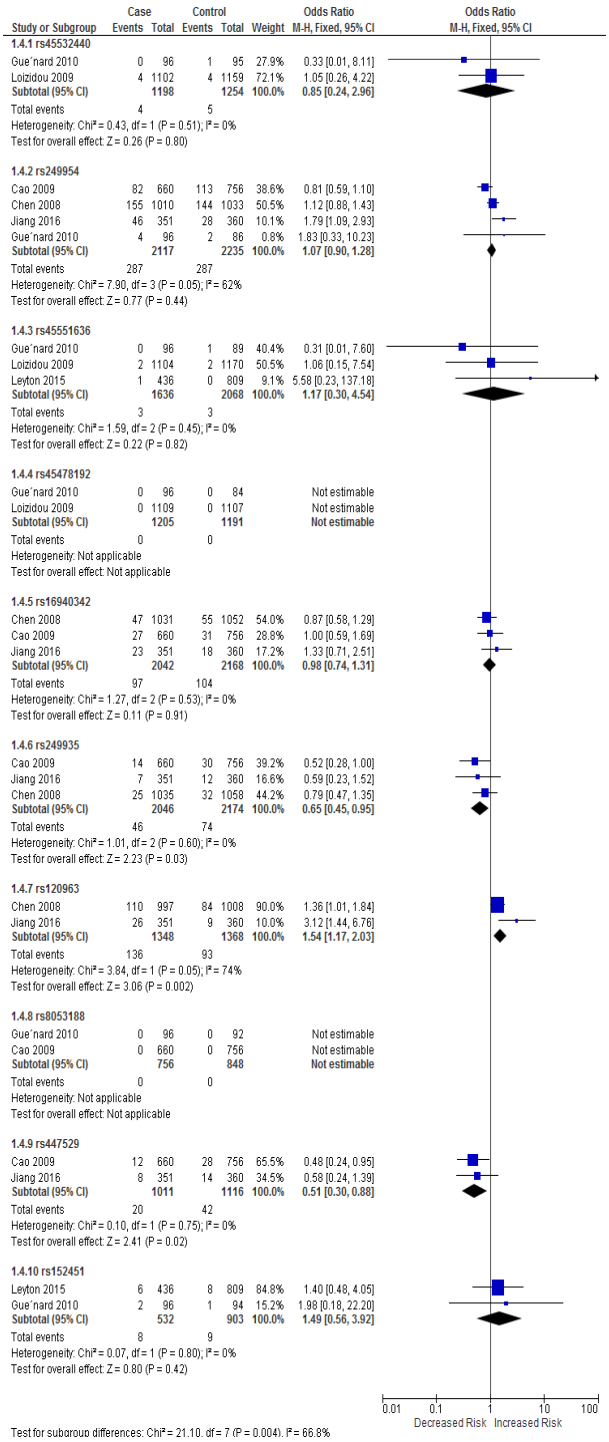

Figure 5. Forest Plot of the Risk for *PALB2* Polymorphisms in Recessive Model. The error bars indicate 95% CIs. Solid squares represent each study in the meta-analysis. Solid diamonds represent pooled OR.

and rs8053188 the results of the meta-analysis were in line with the results of individual studies regarding lack of association with breast cancer risk.

In brief, our study provides further evidences for participation of *PALB2* in breast cancer risk and warrants future studies to explore the biological functions of these variants. As certain genetic variations in *PALB2* alter its functional interactions with *BRCA2* in DNA repair mechanisms and cell cycle control (Teo et al., 2013), it is possible that functional polymorphisms within this gene also affect such interactions.

Based on the detected association between five SNPs and breast cancer, we suggest the possibility of combined effects of certain SNPs in conferring breast cancer risk which should be assessed through haplotype analysis in future studies.

Our study has some limitations. First, due to the relative small number of eligible studies which assessed association of each *PALB2* SNP with breast cancer risk, we could not perform subgroup analysis. Subgroup

Table 2. Meta-analyses of *PALB2* Polymorphisms and Risk of Breast Cancer in Ethnicity-based Subgroups (NA: not assessed)

SNP ID	Category	Allelic Model			Homozygote Model			Dominant Model			Recessive Model		
	Ethnicity	OR (95%CI)	I^2 (%)	P	OR (95%CI)	I^2 (%)	P	OR (95%CI)	I^2 (%)	P	OR (95%CI)	I^2 (%)	P
rs45532440	Caucasian	1.11 [0.86, 1.42]	N.A	0.42	1.06 [0.27, 4.27]	N.A	0.93	1.12 [0.86, 1.45]	N.A	0.41	1.05 [0.26, 4.22]	N.A	0.94
	French Canadian	2.02 [0.60, 6.83]	N.A	0.26	0.35 [0.01, 8.67]	N.A	0.52	2.79 [0.72, 10.85]	N.A	0.14	0.33 [0.01, 8.11]	N.A	0.49
rs249954	Asian	1.13 [1.03, 1.23]	85	0.008	1.20 [0.99, 1.46]	83	0.06	1.23 [1.09, 1.40]	81	0.001	1.07 [0.89, 1.27]	73	0.48
	French Canadian	1.25 [0.75, 2.08]	N.A	0.38	1.96 [0.35, 11.17]	N.A	0.45	1.27 [0.70, 2.31]	N.A	0.44	1.83 [0.33, 10.23]	N.A	0.49
rs45551636	Caucasian	1.20 [0.92, 1.55]	71	0.17	1.77 [0.36, 8.73]	0	0.49	1.19 [0.92, 1.56]	66	0.19	1.75 [0.35, 8.65]	0	0.49
	French Canadian	0.31 [0.03, 2.96]	N.A	0.31	0.31 [0.01, 7.60]	N.A	0.47	0.46 [0.04, 5.14]	N.A	0.53	0.31 [0.01, 7.60]	N.A	0.47
rs45478192	French Canadian	2.64 [0.11, 65.23]	N.A	0.55	-	-	-	2.65 [0.11, 66.04]	N.A	0.55	-	-	-
rs16940342	Asian	1.12 [1.01, 1.24]	0	0.04	1.06 [0.79, 1.41]	0	0.71	1.18 [1.04, 1.33]	0	0.01	0.98 [0.74, 1.31]	0	0.91
rs249935	Asian	0.98 [0.88, 1.10]	54	0.74	0.67 [0.46, 0.97]	0	0.03	1.03 [0.90, 1.17]	52	0.67	0.65 [0.45, 0.95]	0	0.03
rs120963	Asian	1.33 [1.18, 1.49]	89	0.000	1.74 [1.31, 2.32]	81	0.000	1.42 [1.22, 1.65]	87	0.000	1.54 [1.17, 2.03]	74	0.002
rs8053188	Asian	1.36 [0.61, 3.04]	N.A	0.46	-	-	-	1.36 [0.61, 3.06]	N.A	0.46	-	-	-
	French Canadian	0.76 [0.20, 2.88]	N.A	0.69	-	-	-	0.76 [0.20, 2.91]	N.A	0.68	-	-	-
rs447529	Asian	0.90 [0.77, 1.05]	0	0.17	0.78 [0.48, 1.26]	78	0.31	0.94 [0.78, 1.12]	0	0.48	0.51 [0.30, 0.88]	0	0.02
rs152451	Caucasian	1.20 [0.91, 1.59]	N.A	0.19	1.44 [0.50, 4.18]	N.A	0.50	1.21 [0.90, 1.63]	N.A	0.22	1.40 [0.48, 4.05]	N.A	0.54
	French Canadian	2.17 [0.95, 4.96]	N.A	0.07	2.15 [0.19, 24.17]	N.A	0.54	2.15 [0.87, 5.30]	N.A	0.10	1.98 [0.18, 22.20]	N.A	0.58

analysis in patients with/ without family history of breast cancer, early/ late onset of breast cancer, distinct tumor subgroups or menopausal status in addition to the ethnic based analysis would explore definite role of *PALB2* in distinct types of breast cancer. As revealed recently, the association of certain SNPs with breast cancer risk may be altered by the tumor pathological features or menopausal status (Jiang et al., 2016). Second, we did not assess linkage disequilibrium (LD) between these common variants. The detected associations between these SNPs and breast cancer risk might be due to high level of LD with another causal variant which should be evaluated in future studies. Consequently, evaluation of associations between *PALB2* SNPs and breast cancer risk in large sample sizes with focus on haplotypes would clarify the complicated mechanism of maintenance of genome integrity by BRCA1 and *BRCA2* proteins in the context of breast cancer.

In conclusion, the current meta-analysis shows the associations between five SNPs of *PALB2* and breast

cancer risk and confirms the results of previous studies regarding the role of this gene in the pathogenesis of breast cancer.

Conflict of interest

None.

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