

## RESEARCH ARTICLE

# The G801A Polymorphism in the CXCL12 Gene and Risk of Breast Carcinoma: Evidence from a Meta-Analysis Including 2,931 Subjects

Yong Xia\*, Xu-Guang Guo, Tian-Xing Ji

### Abstract

More and more evidence indicates that the G801A polymorphism in the CXCL12 gene might be associated with susceptibility to breast carcinoma in humans being. However, individually published results have been inconsistent. The purpose of this meta-analysis was to investigate the association between the G801A polymorphism in the CXCL12 gene and breast carcinoma risk. A complete search strategy was done by the electronic databases including PubMed and Chinese Biomedical Literature Database. A meta-analysis including seven individual studies was carried out in order to explore the association between the G801A polymorphism in the CXCL12 gene polymorphisms and breast carcinoma. The pooled odds ratios (ORs) and their corresponding 95% confidence intervals (95% CIs) between the G801A polymorphism in the CXCL12 gene and breast carcinoma risk were assessed by the random-effects model. A significant relationship between the G801A polymorphism in the CXCL12 gene and breast carcinoma was discovered in an allelic genetic model (OR: 1.214, 95% CI: 1.085-1.358,  $p=0.001$ ), a homozygote model (OR: 1.663, 95% CI: 1.240-2.232,  $p=0.001$ ), a heterozygote model (OR: 1.392, 95% CI: 1.190-1.629,  $p=0.000$ ), a recessive genetic model (OR: 1.407, 95% CI: 1.060-1.868,  $p=0.018$ ) and a dominant genetic model (OR: 1.427, 95% CI: 1.228-1.659,  $p=0.000$ ). On sub-group analysis based on ethnicity, significance was observed between the European group and the mixed group. A significant relationship was found between the G801A polymorphism in the CXCL12 gene and breast carcinoma risk. Individuals with the A allele of the G801A polymorphism in the CXCL12 gene are under a higher risk for breast carcinoma.

**Keywords:** Breast carcinoma - breast cancer - meta-analysis - G810A - polymorphism - CXCL12

*Asian Pac J Cancer Prev*, 15 (6), 2857-2861

### Introduction

Breast carcinoma is the most prevalent aggressive carcinoma in female people globally. Breast carcinoma consist of 22.9% of invasive carcinomas in women and 16% of all female carcinomas. Worldwide, breast carcinoma caused 458,503 deaths in 2008 (Siegel et al., 2013; Desantis et al., 2014).

Varies widely around the world, the incidence of breast carcinoma is the lowest in developing countries and the greatest in the developed countries. In the twelve different areas in the world, the annual age-standardized incidence rates per 100,000 women are as follows: in North America, 90; Western Europe, 78; Oceania, 74; Northern Europe, 73; Southern Europe, 56; Eastern Europe, 49; South and Central America, 42; North Africa and Western Asia (Donnelly et al., 2013), 28; South-Eastern Asia, 26; sub-Saharan Africa, 22; South Central Asia, 22; and in Eastern Asia, 18 (Desantis et al., 2014).

The number of incidents worldwide has increased dramatically since the 1970s, a phenomenon partly

attributed to the contemporary lifestyle. Breast carcinoma is highly related to age with only 5% of all breast carcinomas being present in women under 40 years old. There were larger than 41,000 newly diagnosed cases of breast carcinomas registered in England in 2011, around 80% of these cases were in women age 50 or older. Approximately 232,340 recent cases of invasive breast tumours and 39,620 breast tumor deaths are expected to occur among US women in 2013 (Siegel et al., 2013; Desantis et al., 2014).

The foremost risk factors for breast carcinoma are female sex and older age. Extra potential risk factors include: genetics, deficiency of childbearing or deficiency of breastfeeding, higher levels of human hormones, certain dietary patterns, and obesity. Some genetic susceptibility may play a minute role in most cases. Overall, however, it believed that genetics is supposed to be the foremost cause of 5-10% of all cases. In those with zero, one or two affected relatives, the risk of breast carcinoma before the age of 80 is 7.8%, 13.3%, and 21.1% with a subsequent mortality from the disease of 2.3%, 4.2%, and 7.6%

Department of Clinical Laboratory Medicine, the Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China  
\*For correspondence: gysyxy@gmail.com

respectively. For those with a major degree relative with the disease, the risk of breast carcinoma between the age of 40 and 50 is double that of the general population.

Contemporary studies have reported that C-X-C motif chemokine 12(CXCL12) G801A polymorphism contributes to carcinoma susceptibility. CXCL12 also known as the stromal cell-derived factor 1 (SDF-1) is a chemokine protein that in humans is encoded by the CXCL12 gene. Razmkhah et al. showed that SDF-1 may be regarded as factors increasing the susceptibility of Iranian women to breast carcinoma(Razmkhah et al., 2005a; 2005b). However, Kruszyna et al. indicates that CXCL12 G801A polymorphism does not constitute a risk factor for breast carcinoma (Kruszyna et al., 2010).

To reveal the relationship between CXCL12 G801A polymorphism and breast tumor risk, we performed this meta-analysis by pooling all eligible studies.

## Materials and Methods

### Publication search and inclusion criteria

The following keywords were searched in electronic databases such as PubMed, Web of Science, China Biological Medicine Database: “carcinoma of breast”, “breast carcinoma”, “breast carcinoma”, “carcinoma of breast”, “breast neoplasms”, “breast neoplasms”, “C-X-C motif chemokine 12”, “CXCL12”, “stromal cell-derived factor 1”, “SDF-1”, “genetic variation” and “polymorphism.” Other relevant studies were also found in the indexed references of the retrieved literatures. The latest research was updated on December 20, 2013, with publication years ranging from 2004 to 2013.

The studies were selected based on the following inclusion criteria: studies that evaluate the G801A polymorphism in the CXCL12 gene association with female breast carcinoma susceptibility; studies that diagnosis of carcinoma was confirmed by a histopathological analysis; case-control or cohort studies published in official journals; studies did not contain overlapping data and studies that conform to the Hardy-Weinberg equilibrium (HWE). The main reasons for exclusion of studies were: animal studies; duplicated publications; no control population; pure cell studies; the study only involved a case population; not concerned with carcinoma risk; and no usable data described. All records were chosen by all the authors independently according to the inclusion criteria and reached consensus on each record.

### Data extraction

The data were carefully extracted from all eligible publications independently by two of the authors according to the inclusion criteria referred above. Disagreement was resolved by discussion between the two authors. If these two authors could not achieve a consensus, then a third author was consulted to resolve the dispute. Studies that did not follow the inclusion criteria, those sheer double publications, or those that provided inadequate data were excluded. If the identical data appeared in different studies, the data were used only once. The following data were collected: the first author’s name, the year of publication, region, the number of genotypes, genotypes, study design,

matching criteria, the total number of cases and controls and HWE.

### Statistical analyses

Five genetic models were used, including allelic (distribution of A allelic frequency of the G801A polymorphism in the CXCL12 gene), recessive (AA vs GA+GG), dominant (AA+GA vs GG), homozygous (AA vs GG), and heterozygous (GA vs GG)(Thakkinstian et al., 2005). The odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were utilized to compare the association between the G801A polymorphism in the CXCL12 gene and breast carcinoma. Chi-square-based Q-tests were utilized to calculate the heterogeneity between the individual studies with significance set at  $p < 0.05$  level(Cochran 1968). The random-effect model was utilized to assess the pooled OR (DerSimonian and Laird method) if there was heterogeneity among the individual studies(Mantel et al., 1959). Otherwise, the fixed-effect model was utilized (the Mantel-Haenszel method). The pooled OR was determined through Z test with significance is fixed at  $p < 0.05$  level.

Fisher’s exact test was utilized to evaluate the HWE, and significance was set at  $p < 0.05$  level. Funnel plot asymmetry was assessed using Egger’s linear regression test, a linear regression approach for measuring funnel plot asymmetry on the natural logarithm scale of OR. The funnel plot was used to estimate the potential publication bias(Stuck et al., 1998). Significance of interception was determined using the t-test suggested by Egger;  $p < 0.05$  was considered to indicate a significant publication bias. All statistical tests were performed utilizing STATA version 10.0 software (Stata Corporation, College Station, TX, USA).

## Results

### Characteristics of eligible studies

Of the 16 articles (Zafiroopoulos et al., 2004; Razmkhah et al., 2005a; 2005b; Dimberg et al., 2007; Hassan et al., 2008; de Oliveira et al., 2009; Hinton et al., 2009; Kruszyna et al., 2010; Ma et al., 2010; de Oliveira et al., 2011; Chuang et al., 2012; Gong et al., 2012; Lin et al., 2012; Shen et al., 2012; de Oliveira et al., 2013;

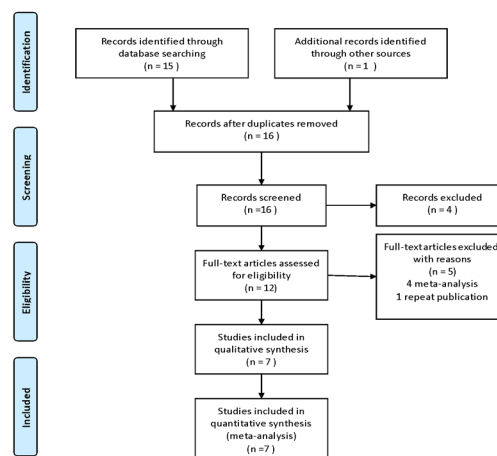


Figure 1. Flow Chart of the Study Selection Process

Kontogianni et al., 2013;) that were initially identified in the search strategy, 16 studies were removed, including 4 studies during the title/abstract review, and 5 studies including one repeat publication and 4 meta-analysis during the full-text review (Figure 1). Seven studies satisfied all of the criteria and were listed in this report. Five studies were carried out in European countries, two conducted in Asian countries. No study was discarded for deviating from the HWE. The data were collected from

1374 breast carcinoma cases and 1557 controls (Table 1, Figure 1).

*Pooled analysis*

A significant association between the G801A polymorphism in the CXCL12 gene and breast carcinoma was found in an allelic genetic model (OR: 1.214, 95%CI: 1.085-1.358,  $p=0.001$ , Pheterogeneity=0.868), a homozygote model (OR: 1.663, 95%CI: 1.240-

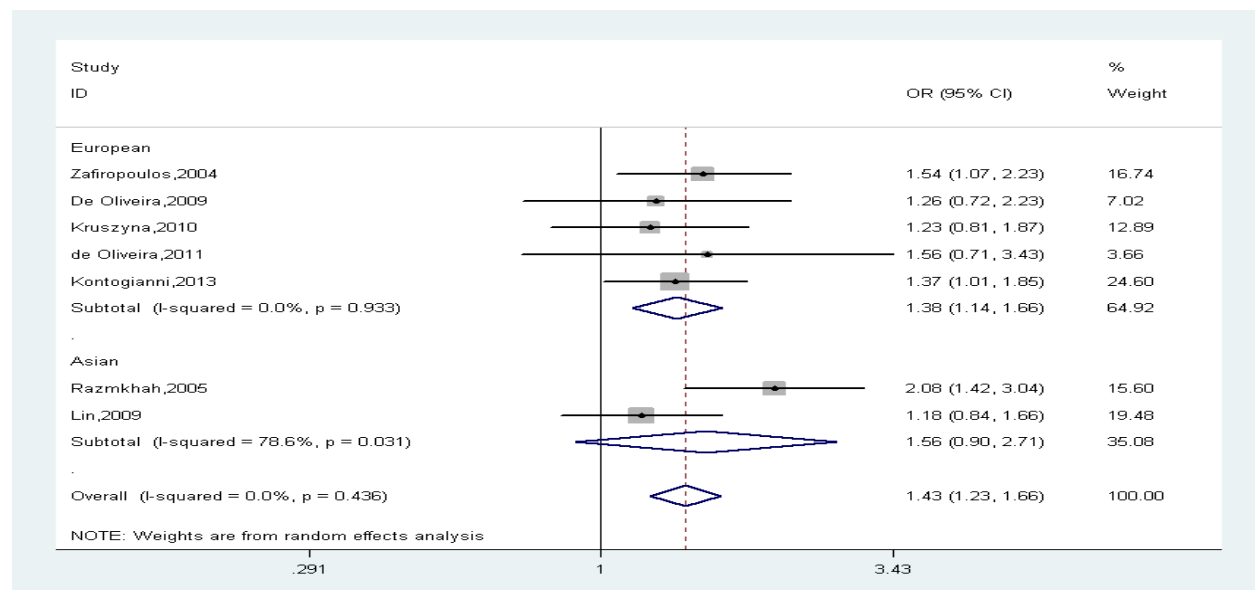
**Table 1. Characteristics of Studies of CXCL12 G810A Polymorphism Included in this Pooled Analysis**

Author	Year	Region	Ethnicity	Cases			Control			Genotyping	Study design	Matching criteria	Sample size (Cases/Control)	HWE
				GG	GA	AA	GG	GA	AA					
Zafropoulos	2004	Greece	European	98	136	30	101	92	19	PCR-RFLP	Case-control	Age, sex, ethnicity	264/212	Yes
Razmkhah	2005	Iran	Asian	105	139	34	101	67	13	PCR-RFLP	Case-control	Age, sex, ethnicity	278/181	Yes
De Oliveira	2009	Brazil	European	59	41	3	61	32	4	PCR-RFLP	Case-control	Age, sex, ethnicity	103/97	Yes
Lin	2009	China	Asian	106	98	16	175	136	23	PCR-RFLP	Case-control	Age, sex, ethnicity	220/234	Yes
Kruszyna	2010	Poland	European	123	61	9	136	58	5	PCR-RFLP	Case-control	Age, sex, ethnicity	193/199	Yes
de Oliveira	2011	Brazil	European	32	21	2	37	15	2	PCR-RFLP	Case-control	Age, sex, ethnicity	55/54	Yes
Kontogianni	2013	Greece	European	114	118	29	247	198	35	PCR-RFLP	Case-control	Age, sex, ethnicity	261/480	Yes

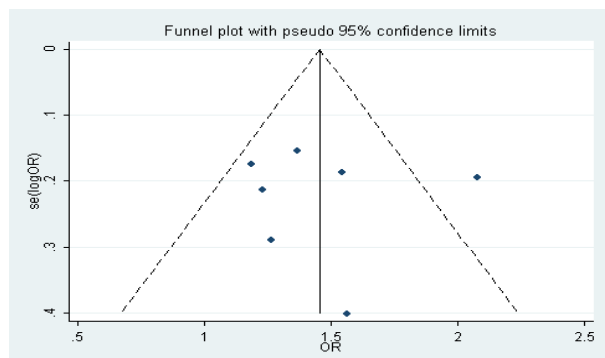
\*HWE, Hardy-Weinberg equilibrium

**Table 2. Meta-Analysis of the Association between the G801A Polymorphism in the CXCL12 Gene and Breast Cancer**

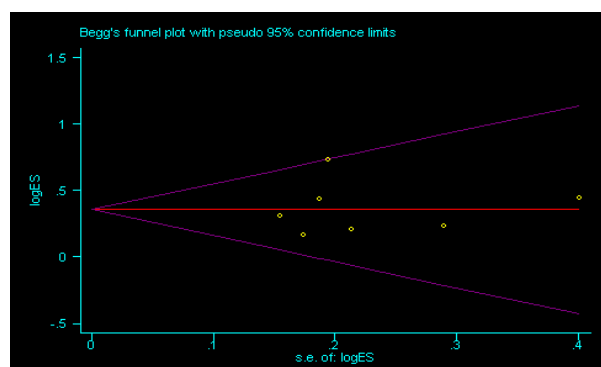
Polymorphism	Population	Number of studies	Test of association			Test of heterogeneity		
			OR	95%CI	p value	Model	p value	I <sup>2</sup>
A allele	Overall	7	1.214	1.085-1.358	0.001	REM	0.868	0.00%
	European	5	1.201	1.045-1.381	0.01	REM	0.996	0.00%
	Asian	2	1.247	0.939-1.656	0.128	REM	0.132	55.90%
AA versus GG	Overall	7	1.663	1.240-2.232	0.001	REM	0.711	0.00%
	European	5	1.65	1.141-2.386	0.008	REM	0.87	0.00%
	Asian	2	1.695	0.785-3.657	0.179	REM	0.114	59.90%
GA versus GG	Overall	7	1.392	1.190-1.629	0	REM	0.528	0.00%
	European	5	1.34	1.103-1.628	0.003	REM	0.894	0.00%
	Asian	2	1.528	0.920-2.536	0.101	REM	0.058	72.30%
AA versus GA+GG	Overall	7	1.407	1.060-1.868	0.018	REM	0.843	0.00%
	European	5	1.424	0.999-2.029	0.051	REM	0.828	0.00%
	Asian	2	1.379	0.820-2.318	0.225	REM	0.27	17.90%
AA+GA versus GG	Overall	7	1.427	1.228-1.659	0	REM	0.436	0.00%
	European	5	1.379	1.144-1.662	0.001	REM	0.933	0.00%
	Asian	2	1.559	0.897-2.708	0.115	REM	0.031	78.60%



**Figure 2. Forest Plot of Breast Carcinoma Associated with The G801A Polymorphism in the CXCL12 Gene under Dominate Genetic Model**



**Figure 3. Funnel Plot for Studies of the Association of Breast Tumor and The G801A Polymorphism in the CXCL12 Gene**



**Figure 4. Egger Plot of Studies of the Association of breast Carcinoma and The G801A Polymorphism in the CXCL12 Gene**

2.232,  $p=0.001$ , Pheterogeneity=0.711), a heterozygote model (OR: 1.392, 95%CI: 1.190-1.629,  $p=0.000$ , Pheterogeneity=0.528), a dominant genetic model (OR: 1.427, 95%CI: 1.228-1.659,  $p=0.000$ , Pheterogeneity=0.436), a recessive genetic model (OR: 1.407, 95%CI: 1.060-1.868,  $p=0.018$ , Pheterogeneity=0.843) as shown in Table 2 and Figure 2.

The results from the five genetic models (an allelic genetic model, a dominant genetic model, a recessive genetic model, a homozygote model and heterozygote model) were positive and the heterogeneity between the individual studies did not exist which indicated that there was an intensively positive association between the G801A polymorphism in the CXCL12 gene and breast carcinoma risk.

#### Bias diagnosis

The publication bias of the studies was assessed using the funnel plot and Egger's test. Publication bias was not seen in the funnel plot (Figure 3). No statistically significant difference was found in the Egger's test ( $p=0.130$ ), indicating low publication bias in the current meta-analysis (Figure 4).

## Discussion

The current study suggested a significant association between the G801A polymorphism in the CXCL12 gene and breast carcinoma in an allelic genetic model, a homozygote model, a heterozygote model, a dominant

genetic model and a recessive genetic model. The A allele of the G801A polymorphism in the CXCL12 gene may be the susceptibility gene for breast carcinoma. This result was the strength of this meta-analysis.

Breast tumor is a type of carcinoma originating from breast tissue, most commonly from the innermost lining of milk ducts or the lobules that supply the ducts with milk. The most common type of breast tumor is ductal carcinoma, which starts in the lining of the milk ducts (thin tubes that carry milk from the lobules of the breast to the nipple). Another type of breast tumor is lobular carcinoma, which starts in the lobules (milk glands) of the breast. Invasive breast carcinoma is a breast tumor that has spread from where it began in the breast ducts or lobules to surrounding normal tissue. Breast carcinoma takes place in both men and women, although male breast carcinoma is infrequent. Breast carcinoma kills more women in the United States than any carcinoma except lung tumor. No one knows why some women get breast carcinoma, but there are a number of risk factors. Risks that you cannot change include age, genes, personal factors, being overweight, using menopausal hormone therapy (also called hormone replacement therapy), taking birth control pills, drinking alcohol, not having children or having your first child after age 35 or having dense breasts.

Some people have hereditary mutations that make them more likely to develop breast carcinoma. The most frequent gene defects are found in the BRCA1 and BRCA2 genes. These genes normally produce proteins that provide protection from carcinoma. If a parent passes you a malfunctioning gene, you are at an increased risk of breast carcinoma. Women with one of these weaknesses have up to an 80% chance of getting breast carcinoma sometime during their life.

Some genetic susceptibility may play a negligible role in most cases. Overall, however, genetics believes that it the foremost cause of 5-10% of all cases. In those with zero, one or two affected relatives, the risk of breast carcinoma before the age of 80 is 7.8%, 13.3%, and 21.1% with a subsequent mortality from the disease of 2.3%, 4.2%, and 7.6% respectively. For those with a foremost degree relative with the disease, the risk of breast carcinoma between the age of 40 and 50 is double that of the general population.

In less than 5% of cases, genetics plays a more noticeable role by causing a hereditary breast-ovarian carcinoma syndrome. This includes those who carry the BRCA1 and BRCA2 gene mutation. These mutations account for up to 90% of the total heritable influence with a risk of breast carcinoma of 60-80% of those affected. Other significant mutations are : p53 (Li-Fraumeni syndrome), PTEN (Cowden syndrome), and STK11 (Peutz-Jeghers syndrome), CHEK2, ATM, BRIP1, and PALB2. In 2012, researchers said that there are four genetically distinct types of breast carcinoma and that in each type, hallmark genetic changes lead to numerous carcinomas.

Overall, a meaningful association exists between the G801A polymorphism in the CXCL12 gene and carcinoma risk. This finding seems to indicate that the genetic variant in the CXCL12 gene may crucially modify the susceptibility of carcinomas. Hence, the



G801A polymorphism in the CXCL12 gene may modify the susceptibility of carcinomas though changing the expression of CXCL12 gene. The mechanism needs additional investigation.

Meta-analysis is a retrospective study that is subject to the methodological deficiencies of the included studies and numerous specific details merit consideration in the ongoing meta-analysis. A key consideration is that our consequences are based on unadjusted estimates and a more precise analysis stratified by different lifestyle related habits and distinct grades of breast carcinoma could be performed if individual data were available. An additional consideration is that large-scale studies on the relationship between the G801A polymorphism in the CXCL12 gene and breast carcinoma are still inadequate. CXCL12 is impacted not only by the G801A polymorphism in the CXCL12 gene, but also by ecological factors, such as the concentration of blood sugar, insulin, triglycerides, and so on. Nevertheless, the total number of subjects provided in the present part of the analysis comprises the largest sample size so far. Finally, as with any meta-analysis of published consequences, the quality of our meta-analysis depends on that of the characteristic studies. Ideally we would want to pool individual level data. However, this is not achievable in the present study. These considerations may distort our results.

In conclusion, our study proves that the G801A polymorphism in the CXCL12 gene is associated with breast tumor risk. Nevertheless, large-scale and well-designed investigations are needed to study gene-gene and gene-environment interactions on the G801A polymorphism in the CXCL12 gene and breast carcinoma risk, which may eventually lead to better inclusive understanding of the probable roles in tumorigenesis. It remains to be seen whether the association with additional carcinoma types, including colorectal carcinoma, can be validated in a large case-control series.

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