ANTICANCER RESEARCH *41*: 4387-4393 (2021)
doi:10.21873/anticanres.15243

Contribution of Interleukin-12A Genotypes to Breast Cancer Risk

YUN-CHI WANG[1,2*], ZHI-HONG WANG[3*], TE-CHUN SHEN[2*], WEN-SHIN CHANG[1,2], SI-ZEIN HUANG[4], CHIEN-CHIH YU[5], JAW-CHYUN CHEN[6], YU-CHEN HSIAU[2], JAI-SING YANG[7], CHIA-WEN TSAI[1,2] and DA-TIAN BAU[1,2,8]

[1]*Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;*
[2]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[3]*Department of Food Nutrition and Health Biotechnology, Asia University, Taichung, Taiwan, R.O.C.;*
[4]*Institute of New Drug Development, China Medical University, Taichung, Taiwan, R.O.C.;*
[5]*School of Pharmacy, China Medical University, Taichung, Taiwan, R.O.C.;*
[6]*Department of Medicinal Botanicals and Health Applications, Da-Yeh University, Changhua, Taiwan, R.O.C.;*
[7]*Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[8]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: Breast cancer incidence is highest among women worldwide, and practical markers for personalized therapeutic strategies are few. Interleukin-12 (IL-12) is a cytokine that is reported to be significantly lower in healthy controls than breast cancer cases, however, its genotypic contribution to carcinogenesis has never been revealed in breast cancer. We examined whether IL-12A rs568408 and rs2243115 genotypes contribute to elevated breast cancer risk and summarized related literature among other cancers. Materials and Methods: IL-12A genotypic profiles were determined among 1,232 breast cancer cases and 1,232 healthy controls via polymerase chain reaction–restriction fragment length polymorphism methodology. Results: The variant genotypes of IL-12A rs568408 and rs2243115 were not found to be significantly associated with elevated breast cancer risk (both p>0.05). Conclusion: IL-12A rs568408 and rs2243115 genotypes may not serve as good predictors of breast cancer risk.*

*These Authors contributed equally to this study.

Correspondence to: Da-Tian Bau and Chia-Wen Tsai, Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 (Ext. 5805), e-mail: artbau2@gmail.com

Key Words: Breast cancer, genotype, interleukin-12A, polymorphism, Taiwan.

Breast cancer is the most invasive and death-causing cancer among women (1). Although the modern care and medication systems have developed very rapidly to improve the survival rates of breast cancer patients, the breast cancer-related global death rate has still kept its raising step (1). In Taiwan, breast cancer has the highest incidence among cancers, and during the past decade breast cancer has kept threatening women's health and life very severely (2, 3). The risk factors for breast cancer among Taiwanese females have been epidemiologically proven to be high-caloric intake, high-fat diets, early onset of menarche, relatively late menopause, overweight or obesity, high levels of mental stress, exposure to pollutants, and other unrevealed factors (4).

Interleukin 12 (IL-12) has been found to be produced by activated antigen-presenting cells, such as dendritic cells, macrophages, and monocytes. As a heterodimeric proinflammatory cytokine, IL-12 is formed by a 35,000 dalton light chain (known as p35 encoded by *IL12A*) and a 40,000 dalton heavy chain (known as p35 encoded by *IL-12B*). Originally, studies have identified IL-12 as a stimulatory factor for natural killer cells and maturation factor for lymphocytes (5, 6). IL-12 has been found to stimulate the cytolytic capacity of natural killer cells, enhancing their interferon-γ (IFNγ) production capacity (7, 8). However, IL-12 is involved in several other processes in addition to affecting natural killer cells. IL-12 played a central role in type 1 (Th1) innate resistance and adaptive immunity (7, 8), and is essential for the differentiation of naive CD4+ T cells to Th1 cells (9, 10). In a mice model, mice deficient in IL-12 subunit p35 developed earlier, with more papilloma,

Table I. *Demographics and life style habits of the 1232 breast cancer patients and the 1232 healthy controls.*

Characteristic	Controls (n=1,232)			Patients (n=1,232)			*p*-Value
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)							
<40	359	29.1%		362	29.4%		0.89[a]
40-55	558	45.3%		547	44.4%		
>55	315	25.6%		323	26.2%		
Age at menarche (years)			12.4 (0.7)			12.1 (0.6)	0.79[b]
Age at birth of first child (years)			29.4 (1.2)			29.8 (1.4)	0.63[b]
Age at menopause (years)			48.8 (1.8)			49.3 (2.0)	0.59[b]
Personal habits							
Cigarette smokers	86	7.0%		170	13.8%		<0.0001[a]*
Alcohol drinkers	91	7.4%		162	13.1%		<0.0001[a]*
Tumor site							
Unilateral				1198	97.2%		
Bilateral				34	2.8%		
Family history							
First degree (Mother, sister, and daughter)				55	4.5%		
Second degree				6	0.5%		
No history				1171	95%		

[a]Chi-square or b unpaired Student's *t*-test; *Statistically significant.

indicating that IL-12 may be a tumor suppressor (10). In support, mice lacking IL-12 receptor chain (IL-12Rβ2) also developed a faster growing B16 melanoma (11). In addition, it has been shown that exogenous administration of IL-12 effectively inhibited the growth of transplanted tumors including sarcoma, melanoma, lung carcinoma, and breast carcinoma (12-14). On the contrary, a recent study on human samples showed that IL-12 levels were elevated in breast cancer patients than normal controls (15).

Human *IL-12A* and *IL-12B* genes are located at chromosomes 3 and 5, respectively. Several molecular epidemiologic studies have explored the influences of *IL-12* polymorphisms on susceptibility to various cancers, such as oral cancer (16), lung cancer (17), hepatocellular carcinoma (18-22), colorectal cancer (23-25), gastric cancer (26, 27) and cervical cancer (28, 29). However, the functional roles of *IL-12* genotypes on cancer risk are still uncertain. The most commonly studied polymorphic site was rs568408 in *IL-12A* gene, perhaps owing to its effect on *IL-12* gene expression, reduction of protein synthesis, and subsequently carcinogenesis. Another polymorphic site, *IL-12A* rs2243115, is located in 5'UTR, and its functional significance has never been examined. In this study, we aimed to investigate the influence of *IL-12A* genotypes on the risk of breast cancer in Taiwan.

Materials and Methods

Breast cancer sample collecting methodology. A total of 1,232 female cases diagnosed with breast cancer were recruited from the China Medical University Hospital. The same number of healthy controls was recruited from the Health Examination Cohort. The detail procedure has been published previously (30, 31). All participants contributed their peripheral blood samples for the genotyping after providing informed consent. This study was approved and supervised by the Institutional Review Board (DMR-99-IRB-108). Selected characteristics and personal habits of participants are recorded in Table I.

IL-12A polymerase chain reaction (PCR)-restriction fragment length polymorphism genotyping methodology. Genomic DNA from the peripheral blood of each participant was extracted using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) (32, 33) and further processed as we described previously (34, 35). For *IL-12A* rs2243115 and rs568408, the forward primers introduce a mismatched A to replace C and a mismatched T to replace C, respectively, at -3 bp from the polymorphic sites to create *Bst NI*- and *Nde I*- (New England BioLabs, Ipswich, MA, USA) restriction sites, respectively. For *IL-12A* rs568408, the primers were 5'-AGAAAAGACCTGTGAACAAAACGACT-3' (forward) and 5'-AGATGGCTCACTAGATGCCAGG-3' (reverse). For *IL-12A* rs2243115, the primers were 5'-GAAGGATGGGACYA TTACATCCATAT-3' (forward) and 5'-CAGGATGGATATTTTCCC TTCT-3' (reverse). The wild-type T allele of *IL-12A* rs2243115 presented a fragment of 122 bp, while the variant G allele of *IL-12A* rs2243115 produces DNA fragments of 93 and 29 bp. The wild-type G allele of *IL-12A* rs568408 produces DNA fragments of 98 and 23 bp and the variant A allele of *IL-12A* rs568408 produces a fragment of 121 bp.

Statistical methodology. Student's *t*-test was adapted for comparing the difference of age between the cases and controls. Pearson's chi-square test was adapted for comparing the distribution of the *IL-12A* genotypes between the two groups. The associations between the *IL-12A* genotypes and cancer risk were evaluated *via* the calculated

Table II. *Distribution of IL-12 rs568408 and rs2243115 genotypes among 1232 breast cancer cases and 1232 healthy controls.*

IL-12A genotype		Cases		Controls		OR (95%CI)	*p*-Value[a]
		n	%	n	%		
rs568408	GG	861	69.9%	880	71.4%	1.00 (Reference)	
	AG	320	26.0%	314	25.5%	1.04 (0.87-1.25)	0.6604
	AA	51	4.1%	38	3.1%	1.37 (0.89-2.11)	0.1486
	AG+AA	371	30.1%	352	28.6%	1.08 (0.91-1.28)	0.4006
	P_{trend}						0.3391
rs2243115	TT	1018	82.6%	1032	83.8%	1.00 (Reference)	
	GT	196	15.9%	186	15.1%	1.07 (0.86-1.33)	0.5537
	GG	18	1.5%	14	1.1%	1.30 (0.64-2.63)	0.4593
	GT+GG	212	17.4%	200	16.2%	1.07 (0.87-1.33)	0.5054
	P_{trend}						0.6514

OR: Odds ratio; CI: confidence interval. [a]Based on chi-squared test without Yate's correction.

odds ratios (ORs) and 95% confidence intervals (CIs). Any result with $p<0.05$ was considered as statistically significant.

Results

The age, menarche age, age at birth of first child, menopause age, tumor sites, family history, and personal habits for the 1232 breast cancer patients and the 1232 controls are presented in Table I. First, there was no difference between the case and control groups regarding age, age at menarche, age at first child birth, and age at menopause (all $p>0.05$) (Table I). Second, the proportions of smokers and alcohol drinkers were found to be higher in the breast cancer group than those in the control group (both $p<0.0001$), which indicates that cigarette smoking and alcohol drinking habits are associated with breast cancer risk for Taiwanese (Table I). Last, the prevalent tumor sites of breast cancer were unilateral (97.2%) among the investigated Taiwanese breast cancer patient population (Table I).

The genotypic frequency distribution of *IL-12A* rs568408 and rs2243115 among the controls and cases of breast cancer in Taiwan is summarized in Table II. First, the genotypic frequencies for the two SNPs investigated fit well with the Hardy–Weinberg equilibrium for the control group ($p>0.05$). Second, there was no significant difference in the distribution of *IL-12A* rs568408 genotypes between the breast cancer and control groups (p for trend=0.3391). The frequencies of the heterozygous AG and homozygous GG of *IL-12A* rs568408 were 26.0% and 4.1% in the breast cancer group and not significantly different from those in the control group (25.5% and 3.1%, respectively). Neither the AG (OR=1.04, 95%CI=0.87-1.25, p=0.6604) nor AA (OR=1.37, 95%CI=0.89-2.11, p=0.1486) genotype at *IL-12A* rs568408 can serve as a predictive biomarker for breast cancer. In addition, combining AG with AA genotypes at *IL-12A* rs568408 also did not alter the risk for breast cancer compared with the wild-type GG genotype (OR=1.08, 95%CI=0.91-1.28, p=0.4006; Table II). Similarly, there was no significant difference in the distribution of *IL-12A* rs2243115 genotypes between breast cancer and control groups (p for trend=0.6514). The frequencies of the GT and GG of *IL-12A* rs2243115 were 15.9% and 1.5% for the breast cancer group, not significantly different from those of the control group (15.1% and 1.1%, respectively). Also, neither the GT (OR=1.07, 95%CI=0.86-1.33, p=0.5537) nor GG (OR=1.30, 95%CI=0.64-2.63, p=0.4593) genotype at *IL-12A* rs2243115 can serve as a predictive biomarker for breast cancer. In addition, combining GT with GG at *IL-12A* rs2243115 also did not alter the risk for breast cancer among Taiwanese compared with the wild-type TT genotype (OR=1.07, 95%CI=0.87-1.33, p=0.5054) (Table II).

The allelic frequency distribution of *IL-12A* rs568408 and rs2243115 is shown in Table III. Supporting the temporary conclusion from Table II, the presence of variant allele A at *IL-12A* rs568408 was not associated with an increased risk of breast cancer compared with the wild-type allele G (OR=1.10, 95%CI=0.95-1.28, p=0.2192). Similarly, the variant G allele of *IL-12A* rs2243115 was not associated with an increased risk of breast cancer (OR=1.09, 95%CI=0.90-1.33, p=0.3715; Table III).

Discussion

Cytokine-mediated immunity is thought as one of the important gate-keeping mechanisms in carcinogenesis (36). IL-12 not only play an important role in immune responses, but also in impaired neo-angiogenesis in tumors (8, 37). IL-12 is evaluated as one of the most feasible cytokines that can contribute to the immunotherapy of cancer (38). However, the association of *IL-12* genotypes with carcinogenesis is seldomly revealed. In the current study, the contributions of

Table III. *Distributions of IL-12 rs568408 and rs2243115 allelic frequencies among 1232 breast cancer cases and 1232 healthy controls.*

Allele		Cases		Controls		OR (95%CI)	*p*-Value[a]
		n	%	n	%		
rs568408	Allele G	2042	82.9%	2074	84.2%	1.00 (reference)	
	Allele A	422	17.1%	390	15.8%	1.10 (0.95-1.28)	0.2192
rs2243115	Allele T	2232	90.6%	2250	91.3%	1.00 (reference)	
	Allele G	232	9.4%	214	8.7%	1.09 (0.90-1.33)	0.3715

OR: Odds ratio; CI: confidence interval. [a]Based on Chi-square test without Yate's correction.

Table IV. *A concise summary of literature regarding the association of IL-12 rs568408 genotype with the risk for various types of cancer.*

Authors (Ref)	Published year	Population	Cancer	Case, n	Control, n	Highlight findings
Wang	Current	Taiwanese	Breast	1232	1232	No significance
Chen *et al*. (29)	2009	Chinese	Cervical	404	404	AG+AA genotypes are associated with increased cancer risk
Roszak *et al*. (28)	2012	Polish	Cervical	405	450	No significance
Sun *et al*. (24)	2015	Chinese	Colorectal	257	236	AG+AA genotypes are associated with increased cancer risk
Tao *et al*. (39)	2012	Chinese	Esophageal	426	432	AG+AA genotypes are associated with increased cancer risk
Hou *et al*. (27)	2007	Polish	Gastric	302	428	No significance
Liu *et al*. (20)	2011	Chinese	HCC	831	844	AG+AA genotypes are associated with increased cancer risk
Lee *et al*. (41)	2007	Chinese	Lung	119	113	No significance
Wu *et al*. (17)	2018	Taiwanese	Lung	358	716	No significance
Li *et al*. (16)	2020	Taiwanese	Oral	958	958	No significance
Wang *et al*. (40)	2013	Chinese	Osteosarcoma	106	210	AG+AA genotypes are associated with increased cancer risk

Literature was surveyed by using the Endnote software, updated on 2021/07/14. HCC: Hepatocellular carcinoma.

Table V. *A concise summary of literature regarding the association of IL-12 rs2243115 genotype with the risk for various types of cancer.*

Authors (Ref)	Published year	Population	Cancer	Case, n	Control, n	Highlight findings
Wang	Current	Taiwanese	Breast	1232	1232	No significance
Sima *et al*. (45)	2012	Chinese	Brain (Glioma)	170	222	GT+GG genotypes are associated with increased cancer risk
Chen *et al*. (29)	2009	Chinese	Cervical	404	404	No significance
Sun *et al*. (24)	2015	Chinese	Colorectal	257	236	No significance
Sun *et al*. (46)	2013	Chinese	Esophageal	368	370	No significance
Yin *et al*. (26)	2015	Chinese	Gastric	234	466	No significance
Liu *et al*. (20)	2011	Chinese	HCC	831	844	No significance
Wu *et al*. (17)	2018	Taiwanese	Lung	358	716	No significance
Wang *et al*. (40)	2013	Chinese	Osteosarcoma	106	210	No significance
Li *et al*. (16)	2020	Taiwanese	Oral	958	958	No significance

Literature was surveyed by using the Endnote software, updated on 2021/07/14. HCC: Hepatocellular carcinoma.

IL-12A rs568408 and rs2243115 genotypes to breast cancer susceptibility were investigated for the first time in a Taiwanese population. From the genotyping results, neither *IL-12A* rs568408 nor rs2243115 were found to be suitable genomic biomarkers for predicting breast cancer risk among Taiwanese (Table II). The findings were further validated by the allelic frequency analysis that showed that neither of the variant alleles at *IL-12A* rs568408 and rs2243115 were associated with increased breast cancer risk (Table III). Although the data seemed to be negative, it is very valuable and worth of notice. We summarized the literature for a comprehensive understanding of the association of both *IL-12A* rs568408 and rs2243115 with various types of human cancer risk in Table IV and Table V.

The genotypes of *IL-12A* rs568408 have been reported to be associated with many types of human cancer. For instance, the AG and AA genotypes of *IL-12A* rs568408 have been associated with higher risk of esophageal cancer (39), colorectal cancer (24) and hepatocellular carcinoma (20) in certain Chinese populations. In addition, the variant AG and AA genotypes at *IL-12A* rs568408 have also been associated with higher risk of osteosarcoma in China (40). Furthermore, the same genotypes have been reported to associate with higher risk of cervical cancer in China (29). However, different results have been reported in a study from Poland (28). This may be explained by the different ethnicities investigated or other reasons. However, in both, the sample size is relatively moderate and potential sampling bias cannot be ignored. Consistent with the current study, conducted for the first time on breast cancer, those examining the contribution of *IL-12A* rs568408 to gastric cancer (27), lung cancer (41, 42), and oral cancer (43) were also all negative. These inconsistent results among the various cancers are interesting, and need to be validated in other ethnicities. Noticeably, although in 2017 one meta-analysis reported that *IL-12A* rs568408 genotypes were associated with overall cancer risk (44), we did not find any association between *IL-12A* rs568408 genotypes and breast cancer risk among Taiwanese.

On the other hand, the genotypes of *IL-12A* rs2243115 have also been examined with regard to their association with cancer. Interestingly, in 2012, Sima *et al*. reported that people carrying the GT and GG genotypes at *IL-12A* rs2243115 were at higher risk of brain tumors (glioma), compared with those carrying the wild-type TT genotype (45). This is the only positive association reported up to today. To our knowledge, there is no other study investigating the contribution of *IL-12A* genotypes to breast cancer. Other studies, mainly conducted in China and Taiwan, are all negative. They included cervical cancer (29), colorectal cancer (24), esophageal cancer (46), gastric cancer (26), hepatocellular carcinoma (20), lung cancer (17), osteosarcoma (40), and oral cancer (16).

The current study found no significant association between *IL-12A* genotypes and breast cancer risk. However, the expression levels of IL-12 have been reported to be higher in breast cancer patients than in normal controls (15). Table IV and Table V show that this is the only study conducted in breast cancer patients, using a large and representative population. Of course, it is valuable to validate our findings in other populations. In addition, we have to take into consideration that the phenotype of IL-12 may not be determined only by the genotype of *IL-12A*, but also by the translational and post-translational modifications. Frequently, the genotype of *IL-12B* seems to play a more critical role in determining the serum levels of IL-12. For example, the serum levels of IL-12 have been found to be elevated among type 1 diabetes patients with the *IL-12B* rs3212227 AA genotype compared to those with the AC or CC genotypes (43, 47). *IL-12B* rs3212227 genotypes have been shown to influence IL-12 production or protein expression levels, and finally associate with susceptibility to Th1-mediated diseases, including inflammatory diseases and several types of cancer (42, 48-51). More interesting, IL-12A and IL-12B may control the secretion of each other. The variant genotype *IL-12B* rs3212227 has been correlated with increased secretion of IL-12A but not of IL-12B itself (43).

In conclusion, this study has provided solid data based on a relatively large population, showing that genotypes of *IL-12A* rs568408 and rs2243115 are not predictive biomarkers for breast cancer risk among Taiwanese. These novel findings should be validated in different ethnic groups.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this work.

Authors' Contributions

Research design: Wang YC and Wang ZH; patient and questionnaire summaries: Shen TC, Huang SZ and Yu CC; experimental work: Wang YC, Chang WS and Hsiau YC; statistical analysis: Chen JC, Yang JS and Tsai CW; article writing: Tsai CW and Bau DT; review and revision: Bau DT.

Acknowledgements

The Authors are grateful to the Tissue Bank of China Medical University Hospital and doctors/nurses for their excellent sample collection and technical assistance. The assistance from Yi-Ru Huang, Yu-Hsin Lin, Tzu-Yu Wang, Tzu-Hsuan Wang was very important for the article preparation. This study was supported mainly by China Medical University Hospital (CMU109-ASIA-04).

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Received July 19, 2021
Revised August 3, 2021
Accepted August 5, 2021