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The Contribution of Interleukin-8 Rs4073 Genotypes to Triple Negative Breast Cancer Risk in Taiwan

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Abstract. *Background/Aim: Triple negative breast cancer (TNBC) is one of the most challenging breast cancer types. Interleukin-8 (IL-8) is a pro-tumorigenic cytokine, promoting tumor proliferation and migration. This study aimed to examine the contribution of IL-8 rs4073 genotypes to breast cancer risk and provide a summary of related literature. Materials and Methods: IL-8 genotypic profiles were determined among 1,232 breast cancer cases and 1,232 controls via polymerase chain reaction-restriction fragment length polymorphism methodology. Results: The IL-8 rs4073 AT and AA genotypes had significantly lower prevalence in the case group compared to control group. Allelic frequency analysis showed that individuals carrying the A allele have relatively decreased risk for breast cancer. The stratification analysis showed that IL-8 rs4073 genotypes were protective markers for those with younger (≤55) age. Conclusion: IL-8 rs4073 A allele is a novel predictor for breast cancer, especially TNBC.*

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Breast cancer is the most prevalent and death-causing cancer among women worldwide (1). Although the survival rates of breast cancer patients have been improved with modern medical care, the breast cancer-related global death rate remains high (1). Among the several subtypes, triple negative breast cancer (TNBC) occupies 15%-20% of all invasive breast cancers and is characterized by a high metastasis and recurrence rate and poor survival (2, 3). Globally, TNBC is in lack of effective drugs. In Taiwan, breast cancer has the highest incidence among all cancers (4, 5). It is reported that high-caloric intake, high-fat diets, early menarche age, late menopause age, together with overweight/obesity, and exposure to pollutants are typical breast cancer risk factors in Taiwan (6). Several studies have investigated biomarkers and genetic variants for the early detection of breast cancer. For instance, carriers of *BRCA1*/*BRCA2* mutations are at higher risk of breast cancer (7-10). However, *BRCA1* and *BRCA2* genetic variants can explain the etiology of a small percentage of breast cancer cases. Translational scientists are interested in identifying biomarkers for TNBC (11-13).

Chronic inflammation can induce DNA damage and may lead to carcinogenesis (14-16). Interleukin-8 (IL-8, also named CXCL8), is related to inflammation, and plays a role in many cellular processes including intersection of cancer plasticity, angiogenesis, and immune suppression (17).

Normally, IL-8 is produced by many cell types such as monocytes, neutrophils, fibroblasts, macrophages, and endothelial cells (18, 19). Under certain abnormal conditions, IL-8 can also be produced by tumor cells, promoting the pro-tumorigenic processes of angiogenesis and cancer cell proliferation (20, 21). IL-8 has been shown to influence the processes of tumorigenesis of several types of cancer, such as melanoma, lung, colorectal, pancreatic, prostate and breast cancer (22). In literature, IL-8 has been reported to be over-expressed in some types of tumor cells, such as gastric, prostate, and breast cancer, and involved in invasion and metastasis (23-26). From a genomic viewpoint, several polymorphic sites in *IL-8*, T−251A, C+781T, C+1633T, and A+2767T, have associated with susceptibility to cancer (27). Among them, T−251A (rs4073) is the most commonly studied. The *IL-8* rs4073, located in its promoter region, has been reported to be involved in the regulation of its protein levels. In detail, the A allele is responsible for a higher protein expression compared to the T allele (28, 29). Elevated IL-8 levels have been further reported to be associated with lung, colorectal, gastric, and breast cancer recurrence (30-33). Studies have shown that *IL-8* genotypes are indeed associated with the risk for several types of cancer, including oral cancer (34, 35), nasopharyngeal cancer (27), lung cancer (36, 37), gastric cancer (38, 39), hepatoma (40), and prostate cancer (41). Regarding breast cancer, several studies have investigated the association of *IL-8* rs4073 polymorphism and risk to breast cancer in Tunisia (42, 43), China (44), and Italy (45). However, conclusive evidence is still lacking.

Since the genetic background of Taiwanese is unique, geographically demarcated, and representative of East Asia, we aimed to examine the contribution of *IL-8* rs4073 genotypes to breast cancer risk in Taiwan. In addition, we want to provide a summary of the literature to increase understanding of *IL-8* genotype's contribution to breast cancer risk.

Materials and Methods

Patients. Up to 1,232 female cases diagnosed with breast cancer in China Medical University Hospital in central Taiwan were recruited. The same number of healthy subjects was recruited from the Health Examination Cohort. The participants are all Taiwanese and the detailed procedure has been previously published (12, 46). Among all the breast cancer cases, 194 were identified as TNBC cases. Our study was approved and supervised by the Institutional Review Board (DMR-99-IRB-108). Specific demographic characteristics are summarized in Table I.

Genotyping methodology for IL-8 rs4073. Peripheral blood was collected from all the study participants and genomic DNA was extracted within 24 h (47-49). The genotypes at *IL-8* rs4073 were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as previously published (36, 49, 50). Briefly, the forward and reverse primer sequences were 5'-TCATCCATGATCTTGTTCTA-3' and 5'-GGAAAACGCTGTAG GTCAGA-3', respectively. The PCR conditions were as follows: pre-denaturation at 94˚C for 2 min; followed by 35 cycles of 94˚C degeneration for 20 s, 57˚C annealing for 20 s, 72˚C extension for 20 s; and 72˚C extension for 20 min. The adducts were cut with *Mfe* I. The digestible A-allele adducts were cut into two fragments of 449 + 75 bps, while T-allele adducts remained intact.

Statistical analysis. Typical *Student's t*-test was used in evaluating the difference between the cases' and controls' age. Pearson's chi-square test was used for evaluating the differential distribution of the *IL-8* genotypes. The associations between the *IL-8* genotypes and breast cancer risk were examined *via* calculating the odds ratios (ORs) and specific 95% confidence intervals (CIs) in stratification analysis. Results with $p<0.05$ were considered statistically significant.

Results

Demographic characteristics of the study population. The age, menarche age, age at birth of first child, menopause age, personal habits, tumor sites, TNBC status and family history of the recruited 1,232 breast cancer cases and the 1,232 healthy subjects are summarized and compared in Table I. There was no difference between the case and control groups in regards to age, age at menarche, age at first child birth, and age at menopause (all $p>0.05$) (Table I). There were more smokers and alcohol drinkers among the breast cancer patients than the controls (both $p<0.0001$) (Table I). Last, there were 194 TNBC cases and 97.2% of cases had unilateral breast cancer (Table I).

The genotypes of IL-8 rs4073 in the population. The genotypic distributions of *IL-8* rs4073 among the controls and the breast cancer cases are shown in Tables II. The frequencies of *IL-8* rs4073 genotypes among the controls fit the Hardy-Weinberg equilibrium ($p=0.2306$). The *IL-8* rs4073 genotypes were differently distributed between the breast cancer and healthy control groups (p for trend$=3.93*10^{-6}$). In detail, the *IL-8* rs4073 hetero-variant AT and homo-variant AA genotypes were associated with a significantly decreased breast cancer risk, compared with the wild-type TT genotype (OR=0.67 and 0.64, 95%CI=0.56-0.80 and 0.51-0.81, $p=0.0001$ and 0.0002, respectively). Furthermore, AA genotype carriers had a significantly lower risk for breast cancer than those carrying TT+AT genotypes in the recessive model (OR=0.79, 95%CI=0.64-0.98, $p=0.0344$). AT+AA genotype carriers had a significantly lower risk for breast cancer than TT carriers in the dominant model (OR=0.66, 95%CI=0.56-0.78, $p=0.0001$). Variant AT and AA genotype carriers at *IL-8* rs4073 have a decreased risk for breast cancer.

The allelic frequencies of IL-8 rs4073 in the population. An allelic frequency distribution analysis for the *IL-8* rs4073 was performed to validate the results deduced from Table II and Table III. Variant A allele frequency was lower (35.1%) in the breast cancer group than that (41.6%) in the control group (OR=0.76, 95%CI=0.68-0.85, $p=0.0001$, Table III).

Table I. *Demographics of the 1,232 breast cancer patients and 1,232 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)	0.59b
Personal habits							
Cigarette smokers	86	7.0%		170	13.8%		0.0001*[a]
Alcohol drinkers	91	7.4%		162	13.1%		0.0001*[a]
TNBC cases							
Yes				194	15.7%		
No				1038	84.3%		
Tumor sites							
Unilateral				1198	97.2%		
Bilateral				34	2.8%		
Family history							0.6264
First degree (Mother, sister, and daughter)		46	3.7%	55	4.5%		
Second degree		5	0.4%	6	0.5%		
No history		1181	95.9%	1171	95.0%		

[a]Chi-square or [b]unpaired Student's *t*-test; *statistically significant; TNBC: triple negative breast cancer.

Table II. *Interleukin-8 rs4073 genotypes among the 1,232 patients with breast cancer and 1,232 healthy controls.*

Genotype	Controls		Patients		OR (95%CI)	*p*-Value[a]
	n	%	n	%		
rs4073						
TT	431	35.0%	552	44.8%	1.00 (Reference)	
AT	578	46.9%	496	40.3%	0.67 (0.56-0.80)	0.0001*
AA	223	18.1%	184	14.9%	0.64 (0.51-0.81)	0.0002*
p_{trend}						$3.93*10^{-6}$*
p_{HWE}						0.2306
Carrier comparison						
TT+AT	1009	81.9%	1048	85.1%	1.00 (Reference)	
AA	223	18.1%	184	14.9%	0.79 (0.64-0.98)	0.0344*
TT	431	35.0%	552	44.8%	1.00 (Reference)	
AT+AA	801	65.0%	680	55.2%	0.66 (0.56-0.78)	0.0001*

[a]Based on chi-square test without Yates's correction; OR: odds ratio; CI: confidence interval; p_{trend}: *p*-value for trend analysis; p_{HWE}: *p*-value for Hardy-Weinberg equilibrium analysis; *statistically significant.

IL-8 rs4073 genotype was associated with age. The genotyping results are stratified by age among the cases and controls (Table IV). Interestingly, the variant AT and TT genotypes at *IL-8* rs4073 were associated with a decreased risk for breast cancer in those who are younger than or equal to 55 years old (OR=0.64 and 0.58, 95%CI=0.52-0.78 and 0.44-0.76, *p*=0.0001 and 0.0001, respectively). On the contrary, AT and TT genotypes at *IL-8* rs4073 were not associated with altered risk for breast cancer for those who were older than 55 years old (OR=0.78 and 0.90, 95%CI=0.55-1.09 and 0.56-1.42, *p*=0.1667 and 0.7261, respectively).

IL-8 rs4073 genotypes were associated with TNBC status. We examined whether *IL-8* rs4073 genotype could be used as a biomarker for the prediction of TNBC risk. Thus, the

Table III. *Distribution of allelic frequencies for interleukin-8 rs4073 among 1,232 patients with breast cancer and 1,232 healthy controls.*

Allele	Controls, n	%	Cases, n	%	OR (95%CI)	*p*-Value[a]
rs4073						
T	1440	58.4%	1600	64.9%	1.00 (Reference)	
A	1024	41.6%	864	35.1%	0.76 (0.68-0.85)	0.0001*

[a]Based on chi-square test without Yates's correction; OR: odds ratio; CI: confidence interval; *statistically significant.

Table IV. *Interleukin-8 rs4073 genotype in breast cancer risk after stratification by age.*

Genotype	Younger (≤55), n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value	Elder (>55), n		OR (95%CI)[a]	aOR (95%CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
TT	309	410	1.00 (ref)	1.00 (ref)		122	142	1.00 (ref)	1.00 (ref)	
AT	433	365	0.64 (0.52-0.78)	0.58 (0.45-0.72)	0.0001*	145	131	0.78 (0.55-1.09)	0.73 (0.51-1.12)	0.1667
AA	175	134	0.58 (0.44-0.76)	0.51 (0.38-0.67)	0.0001*	48	50	0.90 (0.56-1.42)	0.86 (0.48-1.35)	0.7261
Total	917	909				315	323			
ptrend					<0.0001*					0.3386

[a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusted of sex, smoking and alcohol drinking status; p_{trend}, *p*-value for rend analysis; *statistically significant; CI: confidence interval; aOR: adjusted odds ratio; *statistically significant.

Table V. *Association of interleukin-8 rs4073 genotypes with breast cancer risk stratified with TNBC, non-TNBC, or healthy controls.*

Genotype	Control	Non-TNBC	OR, 95%CI	*p*-Value[a]	TNBC	OR, 95%CI	*p*-Value[a]
TT	431	454	1.00 (Ref)		98	1.00 (Ref)	
AT	578	419	0.69 (0.57-0.83)	0.0001*	77	0.58 (0.42-0.86)	0.0015*
AA	223	165	0.70 (0.55-0.89)	0.0048*	19	0.37 (0.22-0.63)	0.0002*
Total	1232	1038			194		
p_{trend}				0.0001*			<0.0001*

[a]Based on chi-square test without Yates's correction; OR: odds ratio; CI: confidence interval; TNBC: triple negative breast cancer; p_{trend}: *p*-value for trend analysis; *statistically significant.

breast cancer patients were further stratified into TNBC or non-TNBC patients. The results showed that among both the TNBC and non-TNBC cases, the protective effects of *IL-8* rs4073 AT and AA genotypes were statistically significant (*p*=0.0015 and 0.0002 for TNBC and 0.0001 and 0.0048 for non-TNBC groups, respectively).

Discussion

Most cytokines are involved in carcinogenesis. Among them, IL-8 is a pro-tumorigenic mediator, promoting angiogenesis and cancer cell proliferation (20, 21, 51). Elevated levels of IL-8 have been reported to correspond to an increased breast cancer severity (52), as well as other types of cancer, including melanoma (53), pancreatic (54), gastric (55, 56), colorectal (57, 58), renal (59), prostate (60), and ovarian cancer (61). As for breast cancer, the induction of epithelial-to-mesenchymal transition (EMT) subsequently increased the activity of the IL-8/IL-8R cytokine signaling in cancer cells (1). In addition, IL-8 secreted from mesenchymal cells was able to induce EMT in surrounding epithelial cells, which was essential for the acquisition and maintenance of the metastatic phenotype of breast cancer cells (62). Furthermore, inhibition of IL-8 receptors resulted in a significant decrease in the invasive ability of breast cancer cells (62).

However, the contribution of *IL-8* genotypes to breast cancer remains elusive, especially in Taiwan. In the present study, we examined the genotype profile of a representative population of 1,232 patients with breast cancer and 1,232 controls in Taiwan (Table I). Among breast cancer cases, the prevalence of *IL-8* rs4073 AT and AA genotypes was significantly lower than that in controls (Table II). The results

Table VI. *Summary of interleukin-8 rs4073 genotype in breast cancer risk.*

First author (reference)	Published year	Studies ethnicity	Genotypes for controls TT: AT:AA	Genotypes for cases TT:AT:AA	Highlight findings
Smith (63)	2004	England	76 : 105 : 54	37 : 63 : 19	No specific association
Snoussi (42)	2006	Tunisian	72 : 110 : 54	65 : 157 : 86	AT and AA genotypes contributed to higher risk
Vogel (64)	2006	Denmark	78 : 167 : 116	88 : 160 : 113	No specific association
Snoussi (43)	2010	Tunisian	92 : 138 : 71	84 : 201 : 124	AT and AA genotypes contributed to higher risk
Wang (44)	2014	China	102 : 213 : 186	51 : 231 : 192	TT genotype contributed to lower risk
Zhang (65)	2017	China	43 : 191 : 213	78 : 174 : 190	TT genotype contributed to higher risk
Wang	Current	Taiwan	431 : 578 : 223	552: 496 : 184	AT and AA genotypes contributed to lower risk

of allelic frequency analysis supported the idea that individuals carrying the A allele have decreased risk for breast cancer (Table III). The stratification analysis showed that *IL-8* rs4073 genotypes were associated with younger (£55) age (Table IV). Furthermore, *IL-8* rs4073 genotypes can serve as a biomarker for both TNBC and non-TNBC subtypes (Table V). Cigarette smoking and alcohol drinking contribute to breast cancer risk in Taiwan (Table I). However, the associations of *IL-8* rs4073 genotypes with smoking and drinking were not specific (all $p<0.05$, data not shown).

There are only a few studies examining the contribution of *IL-8* rs4073 genotypes to breast cancer risk in the literature. We have summarized the contribution of *IL-8* rs4073 genotypes to breast cancer risk (Table VI). As early as in 2004, Smith *et al*. firstly investigated the contribution of *IL-8* rs4073 genotypes to breast cancer risk; however, no specific association of *IL-8* rs4073 was found among a small English population containing 235 controls and 119 breast cancer cases (63). In 2006, Vogel *et al*. carried out a similar investigation in Denmark, however, no positive association was found (64). In the same year, Snoussi *et al*. found a positive association between *IL-8* rs4073 AT and AA genotypes and higher breast cancer risk in a Tunisian population containing 308 breast cancer cases and 236 healthy controls (42). Four years later, the same group enlarged the investigated population to 409 breast cancer cases and 301 controls, and again found no association between *IL-8* rs4073 AT and AA genotypes and higher breast cancer risk (43). In 2014, Wang *et al*. examined the genetic contribution of *IL-8* rs4073 in 474 breast cancer cases and 501 controls in China. They found that TT genotypes of *IL-8* rs4073 contributed to lower risk of breast cancer (44). In contrast, in 2017, Zhang *et al*. found that TT genotypes of *IL-8* rs4073 contributed to a higher risk of breast cancer (65). The inconsistency cannot be explained since they investigated a similar population with the same methodology, and their control/case numbers were also the similar (442 cases and 447 controls). The samples of the current study are genetically and geographically conserved. Our findings are more consistent with those of Zhang *et al*.'s. To the best of our knowledge, we are the first to examine the association of *IL-8* rs4073 with the risk of TNBC, finding that genotypes of *IL-8* rs4073 can indeed serve as a predictor for the occurrence of TNBC. It is undeniable that there are differences among different ethnicities, and various types of breast cancer cases collected in different studies may also contribute to difference in the findings. All findings are valuable, but they need to be validated in larger sample size and various populations. Most of all, the researchers should keep good record about the subtypes of breast cancer samples.

In conclusion, the results of this study showed that *IL-8* rs4073 genotypes, especially the A allele, may serve as an indicator for decreased risk of breast cancer. More importantly, it can server as a protective marker for TNBC. There are many ongoing clinical trials evaluating the addition of IL-8 targeting strategies to immune-based therapies, and the *IL-8* rs4073 marker for breast cancer, especially TNBC, can add an extra value and importance to IL-8.

Conflicts of Interest

The Authors declare no conflict of interest in relation to this study.

Authors' Contributions

Research design: Wang YC, Wang ZH and Yen JH; patient and questionnaire summaries: Lee HT, Shen YC and Shen TC; experimental work: Wang YC, Chang WS, CH SU and Yang JS; statistical analysis: Wang ZH, Chen KY and Yen CM; article writing: Wang YC and Tsai CW; review and revision: Tsai CW and Bau DT.

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