

## Polymorphisms of interleukin (IL)-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and IL-18 and the risk of ovarian cancer

Abigail W. Bushley, Robert Ferrell, Katharine McDuffie, Keith Y. Terada, Michael E. Carney, Pamela J. Thompson, Lynne R. Wilkens, Ko-Hui Tung, Roberta B. Ness, Marc T. Goodman\*

*Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, HI 96813, USA*

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### Abstract

**Objective.** Recent studies of ovarian cancer have suggested a role for inflammation in carcinogenesis. Data from a population-based case-control study in Hawaii were examined to assess the relation between polymorphisms in cytokines involved with the inflammatory response, specifically members of the interleukin (IL) family and the incidence of ovarian cancer.

**Patients and methods.** The analysis of 182 epithelial ovarian cancer cases and 219 controls focused on the polymorphisms in the following genes: *IL-1 $\alpha$* , *IL-1 $\beta$* , *IL-6*, *IL-10*, and *IL-18*. Genotype data were obtained from blood samples collected in participants' homes, and reproductive, demographic, and lifestyle histories were collected during interview.

**Results.** There were no significant odds ratios (ORs) for ovarian cancer by allelic variants in any of the IL genes after adjusting for age, ethnicity, education, oral contraceptive pill use, pregnancy, and history of tubal ligation. Although there was a significantly reduced risk of ovarian cancer risk among women with an *IL-1 $\alpha$*  (–4845) T allele compared to women with two G alleles (OR: 0.59; 95% confidence interval: 0.37–0.97) after adjustment for age and ethnicity, the trend was not significant ( $p = 0.10$ ). Further examination of the data suggested that women with at least one *IL-18* variant allele (a G to C transition at position –137) were at significantly decreased risk of advanced ovarian cancer (OR: 0.51; 95% confidence interval: 0.28–0.90) compared to women with the *IL-18* GG genotype. There was a significant difference in the risk of ovarian cancer associated with the *IL-18* C allele by stage at diagnosis ( $p = 0.04$  for homogeneity in the ORs): cases with *IL-18* GC or CC genotypes were less likely to be diagnosed at regional/distant stages. Analysis of the data within ethnic subgroups revealed a significant positive association of the heterozygous *IL-18* GC genotype with ovarian cancer risk among Native Hawaiian women (OR: 9.96; 95% CI: 1.88–52.90). The OR for ovarian cancer was not significant for Native Hawaiian women homozygous for the *IL-18* C allele, but only one case and control had the *IL-18* CC genotype.

**Conclusions.** Overall, this study does not support an association of selected *IL-1 $\alpha$* , *IL-1 $\beta$* , *IL-6*, *IL-10*, or *IL-18* polymorphisms with the risk for ovarian cancer. However, the *IL-18* G137C variant may be a marker for ovarian cancer progression or metastasis.

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**Keywords:** Polymorphism; Ovarian Cancer; Carcinogenesis

### Introduction

Ovarian cancer is the fourth leading cause of cancer death among women in the United States, and it is estimated

that 16,000 women in the United States will die from this disease in 2004 [1]. Established risk factors for ovarian cancer include family history and gene mutations, such as *BRCA1* and *BRCA2*; while a decreased risk is associated with high parity, use of oral contraceptives, and breastfeeding, each of which suppress ovulation. Research has suggested that damage to the ovarian epithelium resulting from repeated ovulation may play a role in the development of carcinoma at this site [2–4]. Repeated cellular repair at

*Abbreviations:* IL, Interleukin; OR, Odds ratio; CI, Confidence interval.

\* Corresponding author. Etiology Program, Cancer Research Center of Hawaii, University of Hawaii, 1236 Lauhala Street, Honolulu, HI 96813. Fax: +1 808 586 2982.

the site of ovulation, with associated inflammation and repair, increases the chances of mutation. Inflammation may also be caused by environmental or infectious agents, such as talc or *Chlamydia trachomatis*, traveling through the lower to the upper genital tract [4,5]. Decreased risk of ovarian cancer is associated with tubal ligation and hysterectomy, both of which sever this pathway [5,6].

The purpose of this pilot study was to examine the association of ovarian cancer risk with genetic polymorphisms in several cytokines involved in inflammation, specifically IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, IL-10, and IL-18. This analysis also focused on potential differences in genetic risk by ethnic group, histology, and stage, which may influence the etiology of this malignancy [7].

## Materials and methods

### Population and data collection

Eligible cases for this population-based, case-control study in Hawaii comprised all patients with histologically confirmed, primary, epithelial ovarian cancer diagnosed between July 1, 1993 and June 30, 2002, and identified through Hawaii Tumor Registry personnel in any of the major hospital centers in the state [8]. Eligible women were 18 to 84 years of age and were residents of Hawaii. Interview information was obtained from 277 (70%) of the 394 ovarian cancer cases eligible for participation in the study. The control pool consisted of population-based lists of female Oahu residents who were included in the random sample interviewed annually by the Hawaii Health Survey Program of the Hawaii Department of Health. This source was supplemented with women age 65 and older who were Health Care Financing Administration participants on Oahu. Potential controls were randomly selected from the pool so that the ethnic (e.g., Chinese) and 5-year age group distribution would match that of the case group with a 1:1 ratio. Controls were required to report whether or not they had undergone oophorectomy and, if so, whether one or both ovaries had been removed. Eligible controls had to have at least one intact ovary. Four hundred ninety nine women meeting these eligibility criteria were contacted to participate in the study. Interviews were obtained for 342 (69%) of these women, with 157 (31%) eligible women with unknown whereabouts or refusing to participate.

The study was described to all participants prior to obtaining written consent. Trained interviewers administered the majority (>95%) of surveys in subjects' homes. The questionnaire included information on diet, reproductive and gynecologic histories, exogenous hormones, contraception, tobacco smoking, and other lifestyle practices [9].

The Hawaii Tumor Registry does not collect each of the specific components of the American Joint Committee on Cancer TNM or FIGO (International Federation of Gynecology and Obstetrics) system. However, the registry does

use a summary stage variable developed by the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program to group invasive ovarian cancer cases into one of four categories: localized, regional, distant, and unknown [10]. Although the FIGO and SEER Summary Stage systems are not directly interconvertible, the following provides a general translation. Localized ovarian cancer has infiltrated the epithelium, but invasion has not spread beyond the organ (FIGO stages IA and IB). Regional cancer has spread beyond the ovary by direct extension to adjacent tissue and/or regional lymph node involvement (FIGO stages IC, IIA–C). Distant or metastatic ovarian cancer includes tumor cells that have broken away from the primary tumor and have begun to grow in a new, remote location beyond the pelvis (FIGO stages IIIA–C, IV). Histological type was classified as mucinous and non-mucinous (including serous, endometrioid, and clear cell types), based on the classification scheme of the World Health Organization [11].

### Specimen collection and analysis

Blood was drawn from 198 (71%) of the interviewed cases and 238 (70%) of the interviewed controls. A subset of 182 cases and 219 controls were selected for genotyping based on an invasive stage at diagnosis, completeness of data, and adequacy of sample. Laboratory personnel were blinded to the case-control status of the subjects. DNA was purified from peripheral blood leukocytes by SDS/proteinase K treatment and phenol/chloroform extraction.

The genetic polymorphisms which were examined in this analysis included the following transitions: *IL-1 $\alpha$*  C to T at position –889; *IL-1 $\alpha$* , G to T at position at –4845; *IL-1 $\beta$* , G to A at position –3957; *IL-6*, C to G at position –174; *IL-10*, T to C at position –1082; *IL-10*, T to C at position –819; and *IL-18*, G to C at position –137. Genotypes were determined with the 5' -nuclease assay using an ABI 7900 sequence detection system (Applied Biosystems) [12].

### Data analysis

The odds ratios (ORs) associated with different levels of the exposure variables were evaluated by unconditional multiple logistic regression modeling case-control status [13]. Odds ratios and 95% confidence intervals (CI) were computed by exponentiating the regression coefficients (coefficient  $\pm 1.96 \times$  SE) for the binary indicator variables representing the interleukin genotypes. Because sample sizes were limited in some instances and we were concerned with the instability in the ORs, multivariate adjustment included age, as a continuous variable, and ethnicity by indicator variables (Caucasian, Asian, other). In addition to age and ethnicity, we also included education (continuous), oral contraceptive pill use (ever vs. never), pregnancy history (ever vs. never), and history of tubal ligation (yes vs. no) in main effects models shown

in Table 2. Gene-dosage effects were modeled by assigning the value 1, 2, and 3 to an *IL* trend variable according to the subject's number of variant alleles (zero, one and two variant alleles, respectively). The effect of genotype on ovarian cancer risk was investigated within subgroups defined by exposure variables, such as ethnicity and pregnancy. Subgroups of ethnicity were more specifically defined for these analyses, divided into the following subgroups: Caucasian, Japanese, and Native Hawaiian. The genotype effects were statistically tested across subgroups by the likelihood ratio test comparing a model including main effects terms for genotype and subgroup membership, with one including the main effects terms and interaction terms between genotype and subgroup. In subgroup analyses involving stage at diagnosis and histologic type, polytomous logistic regression models were performed [13]. These models compared each subgroup of cases against controls. For instance, one model compared localized cases against all controls, and regional/distant cases against all controls, simultaneously. The effects of genotype on ovarian cancer risk were compared between stage or histology groups by the Wald test.

## Results

The age distribution of cases and controls was similar, with mean age of both cases and controls being 54.7 years (Table 1). The majority of cases were 'other', followed by Asian and Caucasian. The 'other' ethnic category consisted mainly of Native Hawaiian cases (39/72 = 54.2%) and controls (37/70 = 52.9%). We found a reduction in the risk of ovarian cancer associated with greater years of education, a higher number of full-term pregnancies, longer duration of oral contraceptive pill use, and a history of tubal ligation. Tobacco smoking, age at menarche, age at menopause, estrogen/hormone replacement therapy (premarin), and alcohol use were not clearly associated with risk in this analysis subset (data not shown).

The overall and ethnic-specific genotype distributions of the interleukin polymorphisms were found to be in Hardy–Weinberg equilibrium. There were no significant ORs for ovarian cancer associated with specific polymorphisms in the interleukin cytokine alleles among all subjects combined (Table 2). Although there was a significantly reduced risk of ovarian cancer risk among women with an *IL-1 $\alpha$*  (–4845) *T* allele compared to women with two *G* alleles (OR: 0.59; 95% CI: 0.37–0.97) after adjustment for age and ethnicity, the trend was not significant.

Polytomous logistic regression models were used to analyze the risk of ovarian cancer associated with the interleukin genotypes by stage at diagnosis and histologic type, after adjustment for age and ethnicity. We found a significant negative relation between the *IL-18 GC* or *CC* genotype and ovarian cancer risk among those cases diagnosed at the regional/distant stage (Table 3). There

Table 1  
Distribution of subject demographics and risk factor information

Variable	Cases ( <i>n</i> = 182)		Controls ( <i>n</i> = 219)		Odds ratio <sup>a</sup>	95% Confidence interval	<i>p</i> for trend <sup>b</sup>
	<i>N</i>	%	<i>N</i>	%			
<i>Age (years)</i>							
<45	40	22	47	21			
45–54	55	30	72	33			
55–64	38	21	45	21			
>64	49	27	55	25			
<i>Ethnicity</i>							
Caucasian	40	22	70	32			
Asian	70	38	70	32			
Other	72	40	79	36			
<i>Education (years)</i>							
<13	71	39	54	25	1 <sup>c</sup>		
13–14	61	34	84	38	0.6	0.3–0.9	
15	30	16	47	21	0.5	0.3–0.9	
>15	20	11	34	16	0.5	0.3–1.0	0.02
<i>Number of full term pregnancies</i>							
Never	43	24	33	15	1 <sup>c</sup>		
1	33	18	36	16	0.6	0.3–1.2	
2	43	24	63	29	0.5	0.3–0.9	
>3	63	34	87	40	0.5	0.3–0.9	0.01
<i>Oral contraceptive pill use (years)</i>							
Never	110	61	71	32	1 <sup>c</sup>		
<3.0	33	18	65	30	0.2	0.1–0.4	
3.0–5.3	22	12	27	12	0.4	0.2–0.8	
>5.3	17	9	56	26	0.1	0.06–0.3	<0.0001
<i>History of tubal ligation</i>							
No	155	85	155	71	1 <sup>c</sup>		
Yes	27	15	64	29	0.4	0.3–0.7	

<sup>a</sup> Data were adjusted by unconditional logistic regression for age and ethnicity.

<sup>b</sup> Based on a likelihood ratio test comparing models with and without a trend variable that was assigned median values for the categories.

<sup>c</sup> Reference category.

was also a significant difference in the OR for ovarian cancer associated with the *IL-18 C* allele for regional/distant cases when compared to the OR for localized cases (*p* = 0.04). There were no differences in risk associated with *IL-18* genotype in cases with mucinous or non-mucinous histologic types. Additional adjustment for education, oral contraceptive pill use, pregnancy, and tubal ligation had little influence on the odds ratios.

Distant ovarian cancer was more common among Native Hawaiian women (50% of cancers) than among Japanese women (40% of cancers) or Caucasian women (40% of cancers). However, when the analyses were stratified by ethnic group, no significant differences in ovarian cancer risk were found by *IL-18* genotype after adjustment for age (Table 4). After additional adjustment of the ORs for education, oral contraceptive pill use, pregnancy, and tubal ligation, a significant positive association of ovarian cancer among women with the *GC* genotype compared to women with the *GG* genotype of

Table 2  
Odds ratios for the association of interleukin genotypes with ovarian cancer

Genotype	No. cases	No. controls	OR <sup>a,b</sup>	95% CI <sup>b</sup>	<i>p</i> for trend <sup>c</sup>	OR <sup>d,b</sup>	95% CI <sup>b</sup>	<i>p</i> for trend <sup>c</sup>
<i>IL-1α</i> –889								
CC	140	150	1 <sup>e</sup>			1 <sup>e</sup>		
CT	32	59	0.64	0.38–1.06		0.62	0.36–1.08	
TT	6	7	1.12	0.35–3.52	0.25	1.31	0.38–4.54	0.32
CT or TT	38	66	0.68	0.42–1.11		0.68	0.41–1.15	
<i>IL-1α</i> –4845								
GG	146	151	1 <sup>e</sup>			1 <sup>e</sup>		
GT	30	61	0.55	0.33–0.92		0.56	0.32–0.96	
TT	6	7	1.05	0.33–3.29	0.10	1.28	0.37–4.40	0.19
GT or TT	36	68	0.59	0.37–0.97		0.62	0.37–1.04	
<i>IL-1β</i> –3957								
GG	150	167	1 <sup>e</sup>			1 <sup>e</sup>		
GA	29	49	0.74	0.43–1.26		0.74	0.42–1.33	
AA	2	3	1.06	0.17–6.73	0.35	1.96	0.29–13.2	0.56
GA or AA	31	52	0.75	0.44–1.28		0.78	0.44–1.38	
<i>IL-6</i> +174								
CC	143	163	1 <sup>e</sup>			1 <sup>e</sup>		
CG	34	46	1.13	0.62–2.06		1.32	0.68–2.56	
GG	5	9	1.04	0.30–3.51	0.78	1.32	0.36–4.84	0.44
CG or GG	39	55	1.12	0.62–2.02		1.32	0.69–2.52	
<i>IL-10</i> –1082								
TT	56	75	1 <sup>e</sup>			1 <sup>e</sup>		
TC	85	78	1.58	0.98–2.54		1.54	0.92–2.58	
CC	39	65	1.06	0.57–1.96	0.61	1.18	0.61–2.28	0.46
TC or CC	124	143	1.43	0.91–2.25		1.44	0.88–2.35	
<i>IL-10</i> –819								
TT	128	146	1 <sup>e</sup>			1 <sup>e</sup>		
TC	42	50	1.13	0.66–1.93		1.00	0.56–1.78	
CC	11	23	0.78	0.32–1.85	0.83	0.65	0.26–1.66	0.50
TC or CC	53	73	1.06	0.65–1.41		0.92	0.53–1.60	
<i>IL-18</i> –137								
GG	127	139	1 <sup>e</sup>			1 <sup>e</sup>		
GC	48	71	0.82	0.52–1.29		0.91	0.56–1.48	
CC	7	9	0.97	0.34–2.75	0.51	0.93	0.30–2.83	0.73
GC or CC	55	80	0.83	0.54–1.29		0.91	0.57–1.46	

Note. Data were missing for some genotypes, so totals may vary.

<sup>a</sup> Data were adjusted by unconditional logistic regression for age and ethnicity.

<sup>b</sup> OR, odds ratio; CI, confidence interval.

<sup>c</sup> Trend based on a variable assigned the value 1, 2, or 3 according to the subject's number of C alleles (0, 1, or 2, respectively).

<sup>d</sup> Data were adjusted by unconditional logistic regression for age, ethnicity, education, oral contraceptive pill use, pregnancy, and tubal ligation.

<sup>e</sup> Reference category.

the *IL-18* allele was observed among Native Hawaiian women (OR: 9.96; 95% CI: 1.88–52.90) (data not shown). The OR for ovarian cancer did not follow a monotonic trend with the number of C alleles (*p* for trend 0.02), but the homozygous CC genotype group in Native Hawaiians subjects was small (1 case, 1 control). The OR for ovarian cancer was 7.97 (95% CI: 1.75–36.32) among Native Hawaiian women with either GC or CC genotypes compared to women with the GG genotype after adjustment by age, education, oral contraceptive pill use, pregnancy, and tubal ligation (data not shown). Stratification of cases and controls by ethnic group did not reveal

any significant associations between the other interleukin genetic variants and the risk of ovarian cancer.

## Discussion

This study did not identify a significant association of genetic polymorphisms of *IL-1α*, *IL-1β*, *IL-6*, *IL-10*, or *IL-18* with the risk of ovarian cancer. Further analyses stratified by ethnicity did not reveal any significant difference between the distribution of cases and controls for any of the cytokine alleles, except perhaps for *IL-18*. Previous

Table 3

Odds ratios<sup>a</sup> for the association of IL-18 genotype with ovarian cancer by stage and histologic type

Group	GG		GC			CC			<i>p</i> for <sup>c</sup> trend	GC or CC			<i>p</i> for homogeneity <sup>d</sup>
	No.	OR <sup>b</sup>	No.	OR	(95% CI)	No.	OR	(95% CI)		No.	OR	(95% CI)	
Controls	139		71			9				80			
Cases <sup>1</sup>													
Localized stage	48	1	27	1.23	(0.70–2.18)	5	1.72	(0.53–5.58)	0.30	32	1.28	(0.74–2.22)	
Regional/distant stage	79	1	19	0.51	(0.28–0.93)	2	0.47	(0.10–2.28)	0.03	21	0.51	(0.28–0.90)	0.04
Cases													
Mucinous histology	22	1	12	1.37	(0.63–2.99)	1	1.01	(0.12–8.77)	0.54	13	1.33	(0.62–2.87)	
Nonmucinous histology	105	1	36	0.72	(0.44–1.17)	6	0.95	(0.32–2.82)	0.31	42	0.74	(0.46–1.18)	0.35

Note. Two cases were missing stage information.

OR, odds ratio; CI, confidence interval.

<sup>a</sup> Adjusted by polytomous logistic regression (compares all controls with cases in each stage or histology group) for age and ethnicity.

<sup>b</sup> Reference category.

<sup>c</sup> Trend based on a variable assigned the value 1, 2, or 3 according to the subject = s number of C alleles (0, 1, or 2, respectively).

<sup>d</sup> For pairwise difference in OR for the TT or TC genotype for regional/distant cases compared with localized cases, or for nonmucinous cases compared with mucinous cases.

studies have revealed some positive relations between *IL-1* genetic polymorphisms and *Helicobacter pylori* infection and gastric cancer (*IL-1β* −31 T allele and *IL-1β* −511 C allele) [14,15], Alzheimer's disease (*IL-1α*, −889 T allele, *IL-1β*+3953 T allele) [16], and possibly rheumatoid arthritis (*IL-1β* exon 5) [17]. The *IL-1α* and *IL-1β* proteins have also been shown to play a role in cervical cancer development and increased ovarian cancer cell growth [18,19]. Our findings are generally consistent with a study of *IL-1* genetic polymorphisms that did not find a relation between allelic variants and ovarian cancer risk [20], although we found a reduced risk of ovarian cancer risk among women with an *IL-1α* (−4845) T allele compared to women with the GG genotype.

Increased *IL-6* protein levels have been associated with malignant ovarian tumors, endometriosis, and cervical cancer [21,22]. An *IL-6* polymorphism (−174 C) was found to influence ovarian cancer phenotype, showing early tumor stage, increasing length of disease-free time, and improving overall survival [23]. We did not find a relation between allelic variation in the *IL-6* gene and ovarian carcinogenesis.

Three single nucleotide polymorphisms in *IL-10* (−1082 T, −819 A, −592A) have been associated with increased susceptibility to skin carcinomas after renal transplantation [24]. A study of vascular endothelial growth factor producing ovarian cancer in mice indicated that the *IL-10* protein suppresses tumor growth, angiogenesis, and dissemination of ovarian cancer cells [25]. Our study did not suggest any association of two *IL-10* polymorphisms with the risk of ovarian cancer.

The present analysis demonstrated a significantly decreased risk of late stage ovarian cancer among women with G to C polymorphism at position −137 in the *IL-18* gene, supporting a role for the *IL-18* G allele in tumor progression and metastasis. *IL-18*, a proinflammatory cytokine that belongs to the *IL-1* family of ligands, induces

interferon (IFN)- $\gamma$  production in T cells and natural killer cells, playing an important part in the T helper-cell type 1 response [26,27]. An anti-tumor effect of *IL-18* has been shown, and *IL-18* has been considered for use as cancer immunotherapy or gene therapy [26–28]. Osaki et al. [28] found delayed emergence and growth retardation of tumors in mice receiving *IL-18* treatment. Wang et al. [29] demonstrated the expression of *IL-18* in normal ovarian epithelial cells, but the ability to process and secrete mature *IL-18* was lost during neoplastic transformation, perhaps a reflection of reduced IFN- $\gamma$  activity in ovarian tumors [30]. Decreased IFN- $\gamma$  production resulting from reduced *IL-18* stimulation may have a direct effect on ovarian carcinoma progression through down-regulation of antiangiogenic factors and up-regulation of pro-vascular endothelial growth factors (VEGF) [31,32]. Although evidence for an anti-tumor role of *IL-18* is emerging, *IL-18* serum levels have been reported to be elevated in ovarian and gastric cancer patients compared with controls [33,34], and circulating concentrations of *IL-18* increase during metastatic disease compared with early disease in cancer patients [35]. Furthermore, *IL-18* has also been shown to increase the expression of vascular cell adhesion molecule-1 and to increase the adhesion of melanoma cells [36]. Thus, the association of *IL-18* with cancer progression and metastasis remains controversial [37].

Although it is unclear why the positive association of the *IL-18* G137C polymorphism with the risk of ovarian cancer was limited to Native Hawaiian women in the fully adjusted model, there are several possible explanations. Native Hawaiian women may have waited longer to be screened for ovarian cancer and also had a higher frequency of the *IL-18* G allele. This would lead to a spurious association between distant stage and the G allele. However, because there is no effective early detection method for ovarian cancer, it is possible that Native Hawaiian women were

Table 4  
Odds ratios<sup>a</sup> for the association of *IL-18* genotype with ovarian cancer by ethnic group

	GG			GC			CC			GC or CC			<i>p</i> for trend <sup>c</sup>			
	No. cases	No. controls	OR <sup>b</sup>	No. cases	No. controls	OR	(95% CI)	No. cases	No. controls	OR	(95% CI)	No. cases		No. controls	OR	(95% CI)
	Japanese	45	50	1	13	17	0.84	(0.37–1.94)	0	2				13	19	0.76
Caucasian	22	30	1	15	35	0.58	(0.25–1.31)	3	5	0.77	(0.16–3.61)	18	40	0.60	(0.27–1.32)	
Native Hawaiian	28	32	1	10	4	2.85	(0.80–10.1)	1	1	1.28	(0.07–22.6)	11	5	2.55	(0.79–8.29)	

<sup>a</sup> Data were adjusted by unconditional logistic regression for age.

<sup>b</sup> Reference category.

<sup>c</sup> Trend based on a variable assigned the value 1, 2, or 3 according to the subject's number of C alleles (0, 1, or 2, respectively) OR, odds ratio; CI, confidence interval.

more likely to be diagnosed at a distant stage because their tumors are inherently more aggressive. If the *GG* genotype for *IL-18* is indeed a progression marker, then the higher percentage of *GG* among Native Hawaiian women (86% compared with 72% in Japanese and 43% in Caucasians) is an explanation for the higher proportion of distant cancers among Native Hawaiian women than among Caucasian or Japanese women. One functional study suggesting that persons homozygous for the *IL-18 G* allele at position –137 may have higher levels of *IL-18* mRNA expression than persons with other genotypes is consistent with our findings regarding Native Hawaiians, but this difference was not significant ( $p = 0.17$ ) [38].

Another possible explanation for the inconsistent data trends for the *IL-18* polymorphism could be related to underlying differences in ovarian cancer itself. However, our study did not find a significant difference in risk of ovarian cancer for cases with non-mucinous histologies compared to cases with mucinous histological types among women with either a *GC* or *CC* genotype. If the Native Hawaiian and stage findings are not linked, a more likely explanation for the inconsistency in the results for the *IL-18* gene is the small sample size available for analysis, especially after ethnic stratification, which could have led to spurious associations. There was only one Native Hawaiian case and one control with the *IL-18 CC* genotype. Even when combined with heterozygous Native Hawaiian women, the number of cases increased to only 11. Furthermore, the results may have been confounded by differences in racial admixture between Native Hawaiian cases and controls. The power of this study to detect an association was adequate in comparisons when all cases and controls were used. Given the range of frequencies of the homogeneous wild-type genotype in the present study, 182 cases and 219 controls, 80% power, and a critical value of 0.05 (two-sided), the minimum detectable relative risks range from 1.71 for *IL-18* to 1.82 for *IL-10 -M1082*. The power was minimal for ethnic-specific comparisons; for example, given the frequencies for the homogeneous wild-type genotype for *IL18*, the minimum detectable relative risk was 2.6 for Japanese with 58 cases and 69 controls, 2.8 for Caucasians with 40 cases and 70 controls, and 4.4 for Native Hawaiians with 39 cases and 37 controls.

This study had several strengths, including its population-based design, its multiethnic composition, comprehensive information on a variety of established ovarian cancer risk factors, and the availability of clinical information (histology, stage) for all of the ovarian cancer cases. The modest participation of eligible cases and controls in this study was a concern and may have limited the interpretation of our findings. We have no reason to think that non-response was systematic, especially for the association of genetic factors with risk. Recall bias was probably not a problem for most of the exposure variables because the public's knowledge regarding risk factors for ovarian cancer is not broad. The relatively small sample of study subjects

limited our ability to examine the association of rare alleles with the risk of ovarian cancer, and it is possible that the frequency of the various polymorphisms did not accurately reflect the true frequency of the alleles in the population. Although we think that the case and control groups were broadly representative, there remains a large sampling error.

In conclusion, the results of this study do not support a relation between several specific interleukin cytokine polymorphisms and the risk of ovarian cancer. However, it is possible that the *IL-18 G137C* polymorphism is a marker for advanced disease. The results suggest a possible association between the *IL-18 G137C* polymorphism and ovarian cancer risk among Native Hawaiian women, although this needs to be investigated further. The importance of the role of inflammatory cytokines in ovarian carcinogenesis warrants further research with larger study populations.

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