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Polymorphism of interleukin (IL)-4 receptor is associated with the risk and the prognosis of epithelial ovarian cancer in Korean women

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Objective : The aim of current investigation was to analyze the association between a single nucleotide polymorphism (SNP) in interleukin (IL)-4R Ile50Val and epithelial ovarian cancer (EOC) susceptibility and its prognosis in Korean women.

Methods : The blood samples of 98 EOC patients and 321 cancer-free control subjects were collected. Polymorphisms in IL-4R Ile50Val were determined using TaqMan method. Allele frequency and genotype distribution in the EOC group were compared with those of the control group. Thereafter association between this polymorphism and clinicopathologic factors such as stromal invasion, histologic type, surgical stage, and survival within the case group was evaluated.

Results : In the EOC group, the frequency of *Ile* allele was 52.0% which is significantly higher than that of the control group, 46.3% (p=0.014).There was significantly elevated risk of EOC in patients with *Ile* allele, with Odds Ratio of 2.49 (*Ile/Ile+Ile/Val* versus *Val/Val*, 95% Confidence Internal: 1.34-4.59). In subgroup analysis within the cancer group, this polymorphism showed no significant difference in clinicopathologic parameters such as stromal invasion, histologic type, and surgical stage. However the survival analysis showed the poor survival in the patients with *Val/Val* genotype.

Conclusion : IL-4R Ile50Val polymorphism is associated with the susceptibility and the survival of EOC in Korean women.

Key Words **:** Interleukin-4 Receptor, Single nucleotide polymorphism, Epithelial ovarian cancer

INTRODUCTION

Ovarian cancer is the leading cause of gynecological cancer death in the USA.[1] Despite significant improvements in surgical treatment and chemotherapy, the overall 5-year survival rate remains poor.[1] Such an unsatisfactory survival rate is mainly due to low chance of early detection resulting in more than 60% of all patients being classified as stage III and IV at the time of diagnosis.[1] Therefore, new predictive and prognostic factors for ovarian cancer are in need to improve early detection rate and the efficacy of clinical management.

High-affinity interleukin-4 receptor (IL-4R) is expressed on a variety of nonhematopoietic tumor cells such as melanoma, ovarian, breast, and renal carcinoma cells.[2] IL-4R is a heterodimeric comprising the IL-4Rα and γc chains.[3] In fact, signaling through the receptors of IL-4 has also some direct biological effects on these nonhematopoietic tumor cells, such as the production of IL-6.[4,5] Recently, it was reported that there exist polymorphic site on the IL-4R gene which was associated with an increased risk of atopy[6,7] and renal cell carcinoma (RCC).[8] This polymorphic site results in the substitution of Ile for Val (Ile50Val) in the extracellular and cytoplasmic domain. Functional assays have shown that substitution of Ile for Val enhances signal transduction via the IL-4R by augmenting the activation of STAT6. Therefore, it is believed that the Ile allele, which strengthens signals through the IL-4R, was highly dis-

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tributed in patients with atopy or RCC by their genetic effects of predisposing the host immune system to be more susceptible for the diseases.[6-8]

To clarify the significance of the IL-4R gene polymorphism in epithelial ovarian cancer (EOC) patients, we have examined how Ile50Val polymorphism influenced the incidence and prognosis in a series of 98 sporadic EOCs in Korean women. Hereby we report the association between the polymorphism of IL-4R Ile50Val and the risk and the survival for EOC for the first time.

MATERIALS AND METHODS

1. Sample procurement and DNA preparation

The blood samples of 98 primary epithelial ovarian cancer patients who underwent surgery and/or chemotherapy at Seoul National University Hospital from 1999 to 2002 were collected. Final diagnoses were made on the basis of pathology reports of the specimens obtained by surgery. As a control, blood samples of 321 subjects who admitted at the same hospital with benign diseases were also obtained. All of the subjects in the control group took exploratory laparotomy or pelviscopic surgery including the procedure of ovarian evaluation with inspection and/or palpation in order to confirm the lack of ovarian neoplasia. The study protocol was approved by an Institutional Review Board, and signed consents were obtained from all subjects after they had been advised of the study nature.

After defrosting, samples were incubated overnight with 100 ng/ml proteinase K (Sigma Chemical Co., St. Louis, MO, USA) at 37^{o}C with agitation. DNA was then purified by sequential phenol/chloroform extraction and salt/ethanol precipitation. DNA was dissolved in 70μl TE buffer [10 mM Tris (pH 7.8), 1 mM EDTA], and its concentration was determined by measurements of OD 260.

2. Polymerase chain reaction (PCR) and SNP detection

We conducted SNP analysis using the TaqMan assay method, which is constitutes polymerase chain reaction and probing. The sequences of the PCR primers used were 5'-GCGAGTGGAAGATGAATGGT and 5'-CGCTGGGCTTGAAGGAG. TaqMan assays were carried out in a total volume of 5.0μl, containing 1.0μl (20 ng/μl) of genomic DNA, 2x TaqMan Universal Master Mix 2.5μl, Probe-FAM (5μM) 0.1μl , VIC (5μM) 0.1μl, primer-forward (20 pM) 0.15μl, primer-reverse (20 pM) 0.15μl, and D.W. 1.0μl. This solution was then incubated for 2 min at 50^{o}C, 10 min at 95^{o}C, and subjected to 40 amplification cycles (15 sec at 95^{o}C, 60 sec at 60^{o}C), with a final extension for 7 min at 40^{o}C.

3. Statistical analysis

Differences in the genotype distribution from those expected by the Hardy-Weinberg equilibrium, and the significance of differences in the observed frequencies of the SNP in ovarian cancer group and control group were assessed by the Chi-square test. $p<0.05$ was considered statistically significant. The risks associated with individuals' alleles and genotypes were calculated as the Odds ratio (OR) with 95% confidence intervals. Associations between genotypes and clinicopathologic parameters were evaluated by Fisher's exact test. Survival curves were generated by the Kaplan-Meier method, and differences between groups defined by genotypes of the IL-4R gene were compared by the log-rank test.

RESULTS

1. IL-4R Ile50Val and the risk of EOC

Allele frequencies and genotype distributions in the cancer and control groups are summarized in Table 1. In the control group, the polymorphism demonstrated Hardy-Weinberg equilibrium, while the genotype distribution of case group was not in Hardy-Weinberg equilibrium. In the EOC group, the frequency of *Ile* allele was 52.0% which is significantly higher than that of the control group, 46.3% (p=0.014). The genotype distribution also shows significant difference between two groups, with higher frequency of *Ile/Val* in the EOC group (67.3%), compared with 48.9% of *Ile/Val* frequency in the control group. These results

Table 1. Allele frequency and genotype distribution of IL-4R Ile50Val in epithelial ovarian cancer patients and controls

	Control (n=321)	EOC (n=98)	p-value	OR	95% CI
Allele frequency					
Ile	268 (46.3%)	102 (52.0%)	0.014	1.51	1.10-2.09
Val	374 (53.7%)	94 (48.0%)		1	ref
Genotype distribution					
Ile/Ile	70 (21.8%)	18 (18.4%)	0.003	1.73	0.80-3.71
Ile/Val	157 (48.9%)	66 (67.3%)		2.82	1.50-5.30
Val/Val	94 (29.3%)	14 (14.3%)		1	ref
Ile/Ile+Ile/Val	227 (70.7%)	84 (85.7%)		2.49*	1.34-4.59

OR; odds ratio, CI; confidence interval
**Ile/Ile+Ile/Val* versus Val/Val

Table 2. Association with clinicopathologic parameters according to IL-4R Ile50Val genotype in epithelial ovarian cancer patients

Clinicopathologic parameter	*Val/Val*	*Ile/Val+Ile/Ile*	p-value
Stromal Invasion			
Borderline malignancy	1	17	0.455
Invasive cancer	13	67	
Histologic type			
Serous	8	43	0.777
Others	6	41	
Surgical stage			
I/II	3	36	0.152
III/IV	11	48	

mean that similar to the atopic or RCC population, the frequency of *Ile* allele among EOC patients is higher than controls with Odds Ratio (OR) of 2.49 (*Ile/Ile+Ile/Val* versus *Val/Val*, 95% Confidence Internal (CI): 1.34-4.59).

These findings suggest that allele associated with enhanced signaling of the IL-4 is correlated with increased EOC susceptibility in Korean women.

2. IL-4R Ile50Val and the prognosis of EOC

Then we analyzed the correlation between the genotypes and the clinicopathologic parameters of EOC within the case group. Genotypes were classified into two categories, *Ile/Ile +Ile/Val* genotype and *Val/Val* genotype. No significant association of genotypes was found with respect to each phenotype including stromal invasion, histologic type, and surgical stage (Table 2). Interestingly, however, the survival analysis showed significant difference among genotypes. Fig. 1A demonstrates that *Val/Val* genotype had a significantly lower survival compared with those with other genotypes (p=0.0011) in all the EOC patients. Fig. 1B shows the survival curve for the patients with stage III or more adjusting the compounding effect of surgical stage on the survival, which also shows lower survival of *Val/Val* genotype (p=0.0047). These results indicate that *Val/Val* genotype has an effect on the poor prognosis which is contrary to the results from the analysis with susceptibility for EOC.

DISCUSSION

The low survival rate of EOC is due to the intra-abdominal location of tumors, the relative paucity of early symptoms, and the lack of a screening method for early detection. To improve therapeutic efficacy and survival rate, it is crucial to set up early detection tool. Therefore, we investigated the association of genetic polymorphism with EOC to understand molecular event involved in the development of EOC.

In this hospital-based case-control study, we found a significantly increased risk for EOC in the patients with

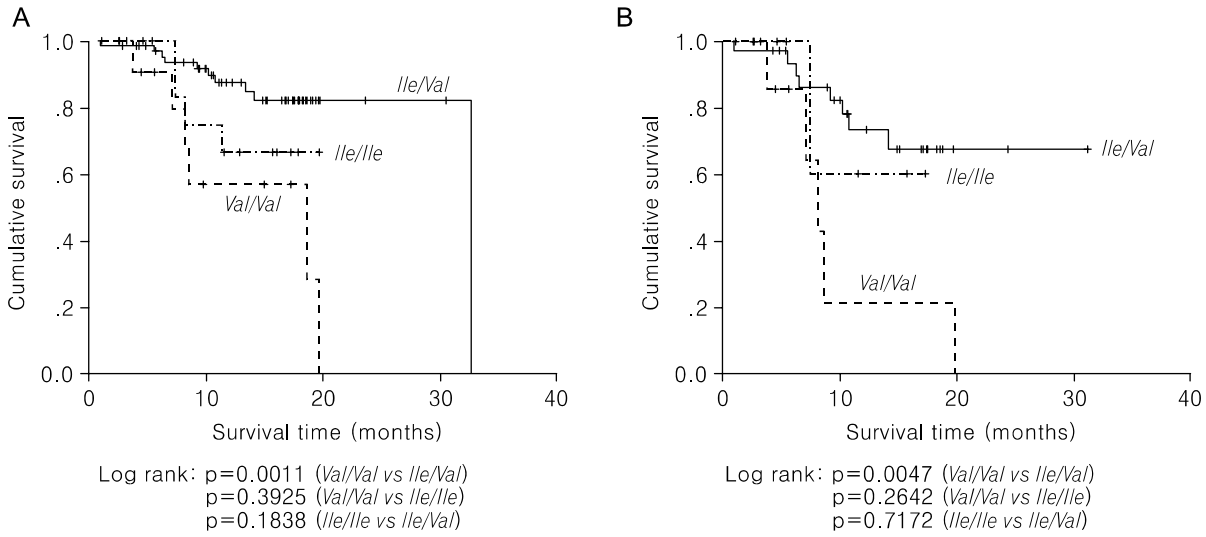

Fig. 1. Survivals according to each genotype of IL-4R Ile50Val in EOC patients. (A) Survival curve for all the patients. (B) Survival curve for the patients with stage III or more EOC according to each genotype of IL-4R Ile50Val.

IL4R *Ile* allele and a poor survival in the patients with *Val/Val* genotype, suggesting that the IL4R Ile50Val polymorphism may play a role in the oncogenic process and the progression of EOC.

The role of IL-4R in carcinogenesis is currently being investigated. High-affinity interleukin-4 receptor (IL-4R) is expressed on a variety of nonhematopoietic tumor cells such as melanoma, ovarian, breast, and renal carcinoma cells.[2] It has also been known that IL-4R mRNA is detected in the majority of ovarian cancer biopsies, ovarian cancer cell lines and xenografts in spite of the absence of IL-4 mRNA.[9] Therefore we could hypothesize that if the secretion of proinflammatory cytokine such as IL-4 is activated, the signal enhancement of IL-4 ligand is depend on the affinity or bioactivity of IL-4R. Although, the exact roles of IL-4 and its signaling pathway on EOC are still unknown and require further examination, it is possible that the dominant genetic effects of the polymorphisms on increasing risks of EOC are attributable to the direct effects of signals through the IL-4R.

It is intriguing that the current study reveals the poor survival for patients with *Val/Val* genotype who were supposed to be at the lower risk for EOC development according to association analysis. This result can be interpreted with several possible explanations. First, IL-4 and IL-4R might have different pathways for the initial carcinogenesis and the progression. IL-4 is reported to have an inhibitory effect for the growth of the tumor cells in a dose-dependent manner[4] as well as a promoting effect for transformation and proliferation.[5,10] Although the exact roles of IL-4 and its signaling pathway on EOC are still unknown, it is possible that IL-4R polymorphism has different mechanisms depending on the change of microenvironment as the tumor progresses. Second, such an inconsistency between the susceptibility and the survival may originate from the shortage of knowledge for the functional polymorphisms. As other polymorphisms could exist interacting with the concerned polymorphism, only one single nucleotide polymorphism (SNP) cannot be a satisfying answer for complicated oncogenic process. With the aim of overcoming this pitfall of SNP study, linkage disequilibrium (LD) statistics or haplotype analysis have been developed, but the achievement is still insufficient.

As for the relation of this polymorphism to patients' clinicopathologic parameters, no significant association was found either between the each genotype with phenotypes including stromal invasion, histologic type, surgical stage. Our results may disclose the true pathophysiologic nature

of IL4R Ile50Val polymorphism, but the probability of false-negativity is still remained because of some weak point of polymorphism study in the point of dynamic process of clinical condition. A genotype represents a static value unable to change in response to a new situation, such as exposure to chemotherapy. Also, it may not reflect changes in tumor DNA, such as loss of heterozygosity.[11]

Several limitations in the present study need to be addressed. First, this study has a relatively small sample size especially for EOC group. As pointed earlier, the genotype distribution in EOC group was not in Hardy-Weinberg equilibrium, which makes it difficult to exclude a sampling bias completely. The lack of association between IL4R Ile50Val polymorphism and the clinicopathologic parameters except survival may also be due to small sample size. The estimated sample size required to confirm the association is much larger if the linkage between SNP and phenotype is weaker than expected.

Second the retrospective nature of this study places limitations in regards to the differences in the clinical course of patients in EOC group. Especially the treatment differences among each subject, although the standard treatment for EOC patients is established, could influence the clinical outcome and consequently the prognosis.

In conclusion, this study shows that the IL4R Ile allele may contribute to the etiology of EOC and *Val/Val* genotype is associated with the poor survival in Korean women. To our knowledge, the increased risk of IL4R Ile50Val polymorphism for EOC and the poor survival in *Val/Val* genotype group have not been reported so far. And this polymorphism might be applied to the early detection marker or prognostic factor of EOC. In order to achieve such a usefulness of this polymorphism, the larger scale investigation with other races rather than Korean women is required.

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한국 여성에서 인터류킨-4 수용체의 유전자 다형성(Ile50Val)과 상피성 난소암의 감수성 및 예후와의 연관성

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목적 : 한국 여성에서 인터류킨-4 수용체(IL-4R)의 유전자 다형성(Ile50Val)과 상피성 난소암의 감수성 및 예후와의 연관성을 알아보고자 하였다.

연구 방법 : 1999년부터 2002년까지 서울대학교병원에서 치료 받은 상피성 난소암 환자 98명의 말초혈액검체와 같은 기간동안 양성질환으로 수술 받은 환자 321명의 말초혈액검체를 대조군으로 이용하였다. Taqman방법으로 IL-4R수용체의 유전자 단일염기다형성을 검출하였다. 상피성 난소암 환자군과 대조군의 대립유전자 빈도와 유전자형 분포를 비교하여 상피성 난소암의 감수성과 IL-4R유전자 단일염기다형성 사이의 연관성을 조사하였다. 또한 예후와의 연관성을 알아보기 위해 상피성 난소암 환자군 내에서 수술적 병기, 간질 침윤, 조직학적 분류 및 생존율과 본 다형성과의 연관성을 분석하였다.

결과 : 난소암 환자군에서 Ile대립유전자의 빈도는 52.0%로 대조군에서의 46.3%에 비해 유의하게 높았다(p=0.014). 또한 Ile 대립유전자를 포함하는 군에서 상피성 난소암의 위험도가 유의하게 높았다(OR=2.49, *Ile/Ile+Ile/Val* versus *Val/Val*, 95% CI: 1.34-4.59). 수술적 병기, 간질 침윤, 조직학적 분류 등의 임상병리학적 척도와 IL-4R유전자 단일염기다형성 사이에도 유의한 연관성은 없었으나 Val/Val 유전자형 군에서 생존율이 유의하게 낮았다.

결론 : 이상의 결과를 통해 한국 여성에서 IL-4R 유전자 단일염기다형성은 상피성 난소암에 대한 감수성 및 예후와 연관이 있음을 알 수 있었다.

중심단어 : 인터류킨-4 수용체, 단일염기다형성, 상피성 난소암