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ORIGINAL ARTICLE

Association between IL-6 Gene (−174 & -572 G/C) Polymorphisms and Endometrial Adenocarcinoma Risk

Hai-ying Wang[1] · **Jin-jun Zhang**[1] · **Xiang-yu Zheng**[1] · **Jian-hua Liu**[2,2] · **Yong-wei Li**[1]

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Abstract We aimed to evaluate the association of IL-6 gene polymorphisms at positions of −174 and −572 and predisposition of endometrial adenocarcinoma (EAC) in a Chinese population. EAC patients have remarkably higher frequency of IL-6 -174 CC genotype [odds ratio (OR) =1.56, 95 % confidence interval (CI) =1.07–2.23; P = 0.03], IL-6 -572 CC genotype (OR =1.93, 95%CI =1.17–3.15; P = 0.01) and IL-6 -174 C allele (OR =1.22, 95 % CI =1.03–1.46; P = 0.04) compared with healthy controls. When stratified with FIGO stage, patients with III-IV EAC have a significantly higher frequency of IL-6 -174 CC genotype (OR =1.66, 95% CI =1.06–2.58; P = 0.02) than healthy controls. The CC genotype of IL-6 gene polymorphisms at positions of −174 and −572 may denote potential high risk of EAC.

Keywords Interleukin-6 · Endometrial adenocarcinoma · Gene polymorphism

✉ Jian-hua Liu
LIUJianh68@126.com; LIUJianhua593@gmail.com

✉ Yong-wei Li
liyw71@126.com

[1] Department of laboratory medicine, Henan Province Chinese Medicine Hospital, Zhenzhou 450002, People's Republic of China

[2] Department of Obstetrics and Gynecology, Shanghai Ninth People's Hospital & School of Medicine, Shanghai Jiaotong University, Shanghai 200011, People's Republic of China

Introduction

Endometrial adenocarcinoma (EAC) is the most frequently diagnosed gynecologic cancer, representing 6 % of all cancer cases in women in America [1]. It is more common in the developed world and is the most common cancer of the female reproductive tract in developed countries. Asia contained 41 % of the world's EAC diagnosis in 2012, whereas Northern Europe, Eastern Europe, and North America together comprised 48 % of diagnosis [2]. In recent years, the incidence of EAC has risen.

Interleukin-6 (IL-6), a pro-inflammatory cytokine, is a multifunctional protein principally for inflammatory modulation [3]. Human IL-6 gene is constituted of five exons and four introns that map to the short arm of chromosome 7 (7p21) [4]. Two biallelic polymorphisms of IL-6 gene are at positions of −174 and −572 in its promoter region [5, 6]. Plenty of previous studies indicate that IL-6 promoter polymorphisms (−174G/C and -572G/C) are associated with various cancers risks [7, 8]. So it is supposed that IL-6 gene polymorphisms at positions of −174 and −572 may also increase the risks of gynecological malignancies, including EAC [9, 10]. Therefore, we aim to evaluate the association of IL-6 gene polymorphisms at positions of −174 and −572 and predisposition of specific EAC in a Chinese population.

Materials and Methods

Study Population

EAC patients (n = 464) and healthy controls (n = 487) were recruited in this hospital-based case-control study between February 2011 and October 2013 for stratification analysis.

Healthy female controls were recruited when they were attending a clinic for routine examination. All cases were histopathologically confirmed as primary EAC independently by two gynecologic pathologists. For the cases, clinical and pathological information was extracted including subtypes, International Federation of Gynecology and Obstetrics (FIGO) stage, tumor size (the largest tumor diameter of the primary tumor), pelvic lymph node metastasis (PLNM), lympho-vascular space invasion (LVSI), depth of myometrial invasion (DMI), stromal estrogen receptor expression (ERE) and progesterone receptor expression (PRE). All individuals were interviewed by trained nurse-interviewers using a structured questionnaire that detailed their age, body mass index (BMI) and menopausal status. The Ethical Committee of the IPMCH approved the study protocols. And all participants also provided written informed consents according to the Declaration of Helsinki.

DNA Extraction and Genotyping

The commercially available Qiagen kit (QIAGEN Inc., Valencia, CA, USA) was used to extract DNA from peripheral blood leukocytes. A single base pair (bp) polymorphism at −174 and −572 in the promoter region of the IL-6 gene was analyzed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The polymorphic region containing the NlaIII restriction site at position −174 base pairs, as previously described [11]. The polymorphic region containing the BsrBI restriction site at position −572 base pairs from the transcription start site was amplified as well. The amplified fragments were analyzed by 4 % agarose gel electrophoresis to test genotype distribution (Fig. 1).

Statistical Analysis

The allele and genotype frequencies of IL-6 gene in patients were compared to healthy controls' by Chi-squared test. The Hardy-Weinberg equilibrium was tested for goodness-of-fit of chi-square test with one degree of freedom to compare the observed genotype frequencies among the subjects with the expected genotype frequencies. The SPSS software was applied to execute all statistical analysis. Comparisons between groups were done with χ^2 test (nominal data) or Student *t*-test (interval data). $P < 0.05$ was considered to be statistically significant.

Results

Characteristics of Participants

Characteristics of EAC cases and healthy controls were showed in Table 1. No significant differences were found between the EAC cases and healthy controls on age, body mass index (BMI), and menopausal status (Table 1). Other information about EAC patients was also provided.

IL-6-174 G/C Polymorphisms and EAC

Patients with EAC had a significantly higher frequency of IL-6 -174 CC genotype (OR =1.56, 95% CI =1.07–2.23; $P = 0.03$) and IL-6 -174 C allele (OR =1.22, 95% CI =1.03–1.46; $P = 0.04$) than healthy controls (Table 2). When stratifying by the FIGO stage, patients with III-IV EAC had a

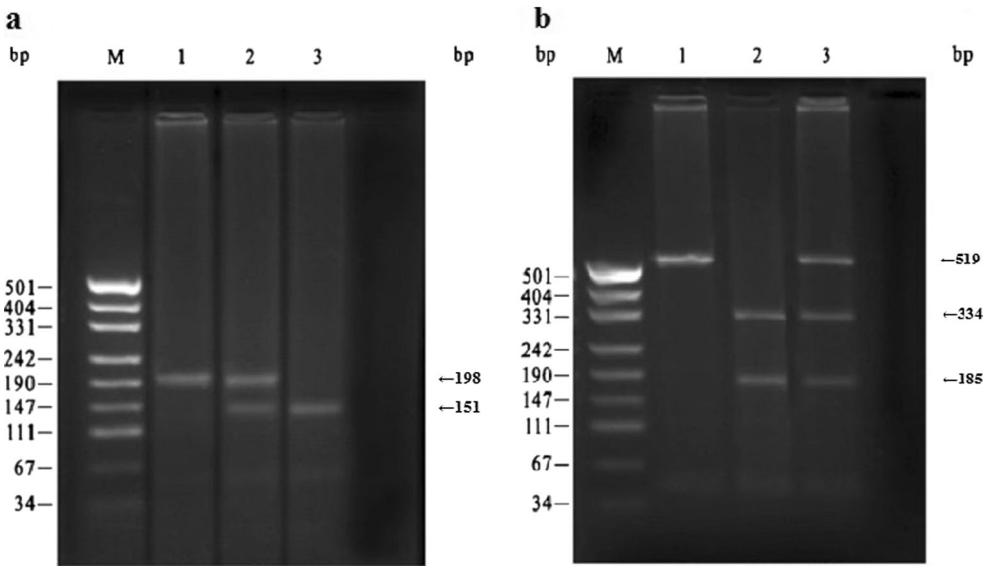

Fig. 1 Electrophoresis of the PCR products for IL-6 -174 (**a**) and -572 (**b**) polymorphisms on agarose gel. For IL-6 -174 (**a**), lane M: DNA marker; lane 1: GG (198 bp); lane 2: GC (198 and 151 bp); lane 3: CC (151 bp). For IL-6 -572 (**b**), lane M: DNA marker; lane 1: CC (519 bp); lane 2: GG (334 and 185 bp); lane 3: GC genotype (519, 334 and 185 bp)

Table 1 Distribution of characteristics of EAC cases

Parameters	Controls	EAC cases	*P* value
Number of subjects	487	464	
Age (years)	55.0 ± 10.3	55.4 ± 9.7	0.96
BMI, kg/m^2 (%)			
<25	371(76.2)	363(78.2)	0.89
≥25	116(23.8)	112(21.6)	
Menopausal status (%)			
Premenopausal	351(72.1)	338(72.8)	0.84
Postmenopausal	136(27.9)	126(27.2)	
Subtypes (%)			
Type I		403(86.9)	
Type II		61(13.1)	
FIGO stage (%)			
I		217(46.8)	
II		162(34.9)	
III-IV		85(18.3)	
Tumor size, cm (%)			
<4		285(61.4)	
≥4		179(38.6)	
PLNM (%)			
Negative		341(73.5)	
Positive		123(26.5)	
LVSI (%)			
Negative		265(57.1)	
Positive		199(42.9)	
DMI (%)			
Negative		203(43.8)	
Positive		261(56.2)	
ERE (%)			
Negative		312(67.2)	
Positive		152(32.8)	
PRE (%)			
Negative		322(69.4)	
Positive		142(30.6)	

FIGO International federation of gynecology and obstetrics, *CIN* Cervical intraepithelial neoplasia, *PLNM* Pelvic lymph node metastasis, *LVSI* Lympho-vascular space invasion, *DMI* Depth of myometrial invasion, *ERE* Estrogen receptor expression, *PRE* Progesterone receptor expression

significantly higher frequency of IL-6-174 CC genotype (OR =1.66, 95%CI =1.06–2.58; P = 0.02) (Table 3).

IL-6-572 G/C Polymorphisms and EAC

Patients with EAC had a significantly higher frequency of IL-6-572 CC genotype (OR =1.93, 95%CI =1.17–3.15; P = 0.01) than healthy controls. When stratifying by the menopausal status, subtypes, FIGO stage, tumor size, PLNM, LVSI, DMI, ERE and PRE of EAC, no obvious statistical association was found (No listed).

Discussion

This study provided an evidence for a role of genetic factors in the development of EAC. According to present researches about the EAC and gene polymorphism, there were an overwhelming number of findings indicating the polymorphism of target genes may make differences to EAC, such as *RAD51*, MDM2, XRCC1, etc. It is credible to come up with a presumption that IL-6 promoter polymorphisms play a dangerous role in EAC.

IL-6 promoter polymorphisms were also associated with various cancers risks. A case-control study inferred that IL-6 Asp358Ala (A/C) polymorphism was associated with cholangiocarcinoma risk [12]. And IL-6 -174 CC genotype was associated with increased renal cancer risk [13] and bladder cancer risk [14]. While a case-control study from Italy reported that IL-6-174G > C polymorphism might be related to onset and progression of neuroblastoma [15] and it might be a carcinogenetic factor in the diffuse type gastric adenocarcinoma [16]. Meanwhile, as for IL-6-572C > G polymorphism, it was reported to influenced susceptibility of HBV-related hepatocellular carcinoma according to Tang S's case research [17].

However, it is still unclear what the association or molecular mechanism between IL-6 promoter polymorphisms (−174G/C and -572G/C) and EAC risk. It is well-known that host immune response and chronic inflammation play critical roles on preventing the progression of EAC [18]. IL-6, an important pre-inflammatory cytokine, is a multifunctional protein principally involved in the genesis and maintenance of the inflammatory response [2, 19]. Substantially, high levels of IL-6 in the microenvironment promoted tumor angiogenesis and the development of EAC [20]. IL-6 regulated the mcl-1 expression via a PI 3-K/Akt-dependent pathway that might facilitate the oncogenesis of human EAC by modulating the apoptosis threshold [21, 22]. IL-6-174G/C polymorphism could also affect the transcription rate of a reporter gene in transient transfection studies, which was associated with the different IL-6 responses to stressful stimuli.

In conclusion, the CC genotype of IL-6 gene polymorphisms at positions of −174 and −572 might confer a high risk of EAC. This was hospital-based case-control study, so the selection bias was unavoidable and the subjects might not be representative for general population. These results should be noted that the population was only from China, so it may not permit extrapolation of results to other ethnic groups.

Table 2 Genotype and allele frequencies of IL-6 gene polymorphisms among EAC cases

Genotypes	Cases (%)	Controls (%)	OR (95%CI)	*P* value
−174 GG	138(29.7)	169(34.7)	1.00(Reference)	
−174 GC	223(48.1)	242(49.7)	1.13(0.85–1.47)	0.45
−174 CC	103(22.2)	76(15.6)	1.56(1.07–2.23)	0.03
−174 G allele frequency	514(55.4)	586(60.2)	1.00(Reference)	
−174 C allele frequency	414(44.6)	388(39.8)	1.22(1.03–1.46)	0.04
−572 GG	268(57.8)	290(59.5)	1.00(Reference)	
−572 GC	153(33.0)	171(35.1)	0.94(0.72–1.23)	0.68
−572 CC	43(9.3)	26(5.3)	1.93(1.17–3.15)	0.01
−572 G allele frequency	685(73.8)	751(77.1)	1.00(Reference)	
−572 C allele frequency	243(26.2)	223(22.9)	1.19(0.96–1.46)	0.08

Table 3 Stratification analysis of IL-6-174G/C polymorphisms in EAC cases

Parameters	Caese	GG			GC			CC		
		n (%)	OR (95%CI)	*P* value	n (%)	OR (95%CI)	*P* value	n (%)	OR (95%CI)	*P* value
Menopausal status	464	139(30.0)	1(Reference)		226(48.7)	1(Reference)		99(21.3)	1(Reference)	
Premenopausal	338	107(31.7)	1.04(0.78–1.33)	0.81	158(46.7)	0.97(0.77–1.20)	0.87	73(21.6)	1.06(0.74–1.45)	0.95
Postmenopausal	126	32(25.4)	0.93(0.64–1.36)	0.57	68(54.0)	1.13(0.82–1.53)	0.54	26(20.6)	0.92(0.55–1.43)	0.86
Subtypes (%)	464	140(30.2)	1(Reference)		226(48.7)	1(Reference)		98(21.1)	1(Reference)	
Type I	403	124(30.8)	1.03(0.77–1.32)	0.90	192(47.6)	0.96(0.78–1.21)	0.86	87(21.6)	1.01(0.72–1.47)	0.91
Type II	61	16(26.2)	0.84(0.58–1.33)	0.74	34(55.7)	1.15(0.81–1.62)	0.41	11(18.0)	0.83(0.49–1.32)	0.66
FIGO stage	464	143(30.8)	1(Reference)		223(48.1)	1(Reference)		98(21.1)	1(Reference)	
I	217	69(31.8)	1.04(0.77–1.42)	0.92	110(50.7)	1.04(0.79–1.38)	0.78	38(17.5)	0.72(0.52–1.31)	0.22
II	162	50(30.9)	1.01(0.72–1.40)	0.98	82(50.6)	1.03(0.78–1.36)	0.76	30(18.5)	0.78(0.57–1.37)	0.31
III-IV	85	24(28.2)	0.91(0.56–1.42)	0.86	31(36.5)	0.81(0.53–1.27)	0.27	30(35.3)	1.66(1.06–2.58)	0.02
Tumor size, cm	464	143(30.8)	1(Reference)		226(48.7)	1(Reference)		95(20.5)	1(Reference)	
< 4	285	91(31.9)	1.03(0.79–1.36)	0.82	139(48.8)	1.01(0.79–1.29)	0.99	55(19.3)	0.88(0.67–1.34)	0.72
≥ 4	179	52(29.1)	0.96(0.70–1.34)	0.91	87(48.6)	1.00(0.76–1.32)	0.99	40(22.3)	1.11(0.79–1.62)	0.68
PLNM	464	143(30.8)	1(Reference)		226(48.7)	1(Reference)		95(20.5)	1(Reference)	
Negative	341	104(30.5)	0.99(0.74–1.31)	0.95	169(49.6)	1.03(0.83–1.29)	0.89	68(19.8)	0.98(0.71–1.35)	0.98
Positive	123	39(31.7)	1.04(0.72–1.53)	0.89	57(46.3)	0.92(0.68–1.31)	0.77	27(22.0)	1.08(0.69–1.68)	0.81
LVSI	464	143(30.8)	1(Reference)		253(48.8)	1(Reference)		105(20.3)	1(Reference)	
Negative	265	79(29.8)	0.98(0.73–1.31)	0.88	143(48.0)	0.98(0.77–1.26)	0.89	65(21.8)	1.08(0.77–1.51)	0.67
Positive	199	64(32.2)	1.03(0.75–1.42)	0.86	110(50.0)	1.02(0.78–1.35)	0.87	40(18.2)	0.90(0.60–1.33)	0.59
DMI	518	160(30.9)	1(Reference)		226(48.7)	1(Reference)		95(20.5)	1(Reference)	
≤ 50 %	230	70(30.4)	0.98(0.73–1.31)	0.94	127(48.0)	0.98(0.68–1.25)	0.89	59(22.2)	1.04(0.77–1.51)	0.67
> 50 %	288	90(31.3)	1.04(0.75–1.42)	0.71	99(49.7)	1.02(0.77–1.26)	0.76	36(18.1)	0.93(0.63–1.33)	0.58
ERE	464	143(30.8)	1(Reference)		226(48.7)	1(Reference)		95(20.5)	1(Reference)	
Negative	312	95(30.4)	1.01(0.77–1.32)	0.98	156(50.0)	1.04(0.80–1.31)	0.84	61(19.6)	0.95(0.67–1.34)	0.81
Positive	152	48(31.6)	1.05(0.73–1.45)	0.91	70(46.1)	0.84(0.68–1.28)	0.70	34(22.4)	1.10(0.74–1.65)	0.65
PRE	464	143(30.8)	1(Reference)		226(48.7)	1(Reference)		95(20.5)	1(Reference)	
Negative	322	98(30.4)	0.98(0.74–1.31)	0.98	155(48.1)	0.99(0.77–1.27)	0.95	69(21.4)	1.06(0.76–1.46)	0.76
Positive	142	45(31.7)	1.04(0.71–1.52)	0.84	71(50.0)	1.35(0.81–2.25)	0.25	26(18.3)	0.88(0.56–1.40)	0.61

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Conflict of Interest Statement The authors declare that they have no conflict of interests.

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