

Interleukin-6 -634C>G polymorphism in hypertensive patients with and without left ventricular hypertrophy

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Abstract. There is an accumulating body of evidence indicating that inflammation plays a pivotal role in the pathogenesis of cardiovascular disease. Interleukin-6 (IL-6) is a pleiotropic cytokine secreted by many cells of the immune system, cardiovascular components and adipose tissue, and functions as a mediator of inflammatory response with both pro- and anti-inflammatory properties. Circulating levels of IL-6 differ greatly between individuals due to both genetic and environmental factors. The *IL-6* -634C>G polymorphism is common in eastern Asian populations. The aim of the present study was to investigate the association of this polymorphism with essential hypertension (EH) and left ventricular hypertrophy (LVH) in 440 subjects (246 EH patients and 194 controls) from a Han Chinese population. In this study, *IL-6* -634C>G genotypes were identified by polymerase chain reaction and restriction digestion in all study participants, and left ventricular mass was assessed by 2-mode echocardiography in 178 untreated EH patients. There was no significant difference in either genotype distribution ($p=0.9528$) or allele frequency ($p=0.7775$) between the EH and control groups. In addition, the -634C>G polymorphism had no effect on blood pressure in either the controls or the untreated EH patients. No significant differences in genotype distribution ($p=0.7998$) or allele frequency distribution ($p=0.5468$) were found between EH patients with and without LVH. Moreover, the echocardiographic parameters were not statistically different between the CC and CG+GG genotypes. These findings suggest that there is no association of the *IL-6* -634C>G polymorphism and EH with LVH in EH patients.

Introduction

Essential hypertension (EH) is a complex disorder in which multiple genetic factors account for 40% of blood pressure variability, and gene expression is altered by environmental and host factors (1,2). An accumulating body of evidence indicate that inflammation plays a pivotal role in the pathogenesis of cardiovascular disease (3,4). Studies have shown that increased levels of interleukin-6 (IL-6) are associated with a variety of disease states, including high blood pressure (5-7).

IL-6 is a pleiotropic cytokine of 23.7 kDa secreted by many cells of the immune system, cardiovascular components and adipose tissue, and functions as a mediator of inflammatory response with both pro- and anti-inflammatory properties (8). Circulating levels of IL-6 differ greatly between individuals (8,9), due to genetic and environmental factors (10). The human *IL-6* gene is located at chromosome 7p21 and contains 5 exons. Three single nucleotide polymorphisms (SNPs) in the *IL-6* promoter region (-597G>A, -634C>G and -174G>C) have been reported to influence IL-6 transcription, and -174G>C was found to be in tight linkage disequilibrium with -597G>A (11,12). An association between the -174G>C polymorphism and systolic blood pressure (SBP) as well as diastolic blood pressure (DBP) was found in middle-aged healthy Caucasian men (13). However, the -174G>C polymorphism is recognized with only low allele frequency in eastern Asian populations (6,14-16).

The development of left ventricular hypertrophy (LVH) in hypertensive patients has a negative prognostic impact and is associated with cardiovascular morbidity and mortality (17,18). Increased prevalence of LVH was found in hypertensive patients with higher cytokine levels (19), and the *IL-6* -174G>C polymorphism was associated with high blood pressure and LVH in hemodialysis patients (20). To date, however, the relationship between the *IL-6* -634C>G polymorphism and LVH in hypertensive patients has not been investigated.

Based on these findings, we carried out a case-control study of the *IL-6* -634C>G (rs1800796) polymorphism to examine its putative association with EH in a Han Chinese population. We also assessed the association of this polymorphism with LVH.

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Materials and methods

Study subjects. A total of 246 patients with EH (including 178 untreated hypertensive subjects) were eligible for this study. The subjects were enrolled at the Fourth People's Hospital of Wuxi and Fourth Affiliated Hospital of Soochow University (Wuxi, China). Hypertension was diagnosed if blood pressure measurements on at least three separate occasions were ≥ 140 mmHg for SBP and/or ≥ 90 mmHg for DBP, or when the participant was taking antihypertensive agents. Subjects with secondary hypertension, diabetes mellitus, valvular disease or apparent ischemic heart disease were excluded. The controls were 194 age-, gender- and BMI-matched healthy individuals. The controls had no history of hypertension or diabetes mellitus. Normotension was defined as SBP < 140 mmHg, DBP < 90 mmHg and lack of current antihypertensive drug treatment. We also excluded from the control group those subjects whose first-degree relatives had hypertension. All study participants were unrelated residents of Han nationality from Wuxi (in the south of China). No participants included in the sample admitted to any regular alcohol intake. There was also no history of cigarette smoking among these subjects. The study was approved by the Medical Ethics Committee of the Fourth People's Hospital of Wuxi, and written informed consent was obtained from all participants.

Evaluation of LVH. Two-dimensional-controlled M-mode echocardiograms were recorded with each untreated hypertensive subject in the partial left decubitus position after a resting period of at least 10 min. According to the criteria set by the American Society of Echocardiography (21), the following parameters relative to the left ventricle were obtained in a blinded fashion, each as an average of at least three measurements: i) left ventricular end-diastolic diameter (LVEDD), ii) left ventricular end-systolic diameter (LVESD), iii) left ventricular diastolic posterior wall thickness (LVDWPT), iv) interventricular septum thickness (IST) and ejection fraction (EF) measured according to the Teicholz method. Left ventricular mass was determined by the Devereux and Reichek formula (22), and the obtained value was divided by the body surface area in order to calculate the left ventricular mass index (LVMI). LVH was diagnosed if the LVMI exceeded 100 g/m² in women and 131 g/m² in men (23). The relative wall thickness (RWT) was measured using the standardized formula (24): $2 \times \text{LVDWPT}/\text{LVEDD}$.

Genetic analysis. Genomic DNA was extracted from peripheral blood leukocytes by the salting-out method (25), with minimal modifications. Sequence amplification was performed using polymerase chain reaction (PCR). The primers were 5'-AGTGGGCTGAAGCAGGTGA-3' (sense) and 5'-CTTTGTTGGAGGGTGAGGG-3' (antisense). This set encompasses the region of interest in the IL-6 promoter and generates a 617-bp product. The reaction volume was 50 μ l in each well of a 96-well plate, with final reaction component concentrations of 0.4 mM for each of the four dNTPs, 2.0 mM MgCl₂, 200 μ M for each of the primers, 0.1 mg/l for genomic DNA and 1.25 units of *Taq* polymerase. The PCR program consisted of initial denaturation at 94°C for 5 min and 40 cycles of denaturation at 94°C for 30 sec, annealing at

55°C for 30 sec, extension at 72°C for 1 min and a final extension at 72°C for 10 min. Amplification products (15 μ l) were digested with 2 units *Bsr*BI at 37°C for 6 h to detect allele C (617 bp) and allele G (131 + 486 bp). The size of the digestion products was then determined by electrophoresis on 2% agarose gel stained with ethidium bromide, with positive and negative controls used to ensure accuracy.

Statistical analysis. All continuous variables are expressed as the mean and standard deviation (SD). The student's t-test was used to compare continuous variables between two groups. Genotypes and allele frequencies were obtained by direct count. Differences in the distribution of alleles and genotypes between the groups and deviations from Hardy-Weinberg equilibrium were assessed by the χ^2 test. All significant tests were two-tailed and were considered statistically significant at $p < 0.05$. SPSS for Windows version 11.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical analyses.

Results

The clinical characteristics of the participants enrolled in the study are depicted in Table I. There were no significant differences in gender, age or BMI between the hypertensive patients and the controls. In this study, 178 untreated hypertensive patients were divided into LVH(+) and LVH(-) groups according to the echocardiographic diagnosis. The two groups had no significant differences in gender, age or BMI, or in SBP or DBP (Table II).

Table III summarizes the distribution of the IL-6 -634C>G genotypes and allele frequencies in all the groups. Genotype distribution among the subjects was in Hardy-Weinberg equilibrium in both the control group ($\chi^2=1.1094$, $p=0.2922$) and the EH group ($\chi^2=1.1506$, $p=0.2834$). All subgroups in the EH group were also within Hardy-Weinberg proportions ($p > 0.05$). The distribution of the IL-6 -634C>G genotypes (CC, CG and GG) was 59.28, 37.11 and 3.61% for the controls, and 58.13, 37.80 and 4.07% for the EH subjects, respectively. The derived allele frequencies for the C and G alleles were 77.84 and 22.16% in the control subjects, and 77.03 and 22.97% in the EH subjects, respectively. There were no significant differences in either genotype frequency distribution ($p=0.9528$) or allele frequency distribution ($p=0.7775$) between these two groups, which suggested that the -634C>G polymorphism of the IL-6 gene was not significantly associated with EH. The distribution of genotype frequency in the LVH(+) group showed no statistical difference compared to that of the controls ($p=0.9629$). In addition, there were no significant differences in either genotype distribution ($\chi^2=0.4467$, $p=0.7998$) or allele frequency distribution ($\chi^2=0.3631$, $p=0.5468$) between the LVH(+) and the LVH(-) groups. Moreover, the association between the IL-6 -634C>G genotypes and hypertension was not significant in either men or women (Table IV).

The effects of the different genotypes on blood pressure are shown in Table V. Since the numbers of individuals with the GG genotype were small, the carriers of the G allele (CG + GG) were pooled into one group. There were no significant differences in SBP or DBP between the two genotypes in either the control or untreated EH groups. We also evaluated the relationship between the IL-6 -634C>G gene polymorphism

Table I. Clinical characteristics of hypertensive patients and control subjects.

Characteristics	Controls (n=194)	EH (n=246)	p-value
Gender (% male)	57.73	55.69	0.6680
Age (years)	53.76±10.04	52.34±9.89	0.1382
BMI (kg/m ²)	23.97±3.04	24.33±3.46	0.2539
SBP (mmHg)	115.39±15.31	159.87±22.53	0.0000
DBP (mmHg)	74.32±8.54	101.61±9.68	0.0000

EH, essential hypertension; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table II. Clinical characteristics of the LVH group as compared to the group without LVH in untreated hypertensive patients.

Characteristics	LVH(-) (n=110)	LVH(+) (n=68)	p-value
Gender (% male)	56.36	55.88	0.9499
Age (years)	52.89±10.17	52.09±9.42	0.6007
BMI (kg/m ²)	24.24±3.31	24.46±3.98	0.6908
SBP (mmHg)	161.94±22.76	163.27±23.14	0.7071
DBP (mmHg)	102.37±10.34	103.15±10.87	0.6322

LVH, left ventricular hypertrophy; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Table III. Distribution of the *IL-6* -634C>G genotypes and alleles in the controls, hypertensive patients and hypertensive subgroups.

Groups	Genotype frequency (%)			p-value ^a	Allele frequency (%)		p-value ^a
	CC	CG	GG		C	G	
Controls (n=194)	115 (59.28)	72 (37.11)	7 (3.61)		77.84	22.16	
EH total (n=246)	143 (58.13)	93 (37.80)	10 (4.07)	0.9528	77.03	22.97	0.7775
EH treated (n=68)	40 (58.82)	25 (36.77)	3 (4.41)	0.9567	77.21	22.79	0.8795
EH untreated (n=178)	103 (57.87)	68 (38.20)	7 (3.93)	0.9575	76.97	23.03	0.7771
LVH(+) (n=68)	41 (60.29)	25 (36.77)	2 (2.94)	0.9629	78.68	21.32	0.8383
LVH(-) (n=110)	62 (56.36)	43 (39.09)	5 (4.55)	0.8474	75.91	24.09	0.5868

EH, essential hypertension; LVH, left ventricular hypertrophy. ^aCompared to controls.

and echocardiographic parameters in untreated hypertensive patients (Table VI). The echocardiographic parameters were not statistically different between the CC and CG + GG genotypes.

Discussion

We studied genetic variations in the promoter region of *IL-6*, an upstream factor influencing *IL-6* transcription. No significant difference was observed in either genotype frequency distribution ($p=0.9528$) or in allele frequency distribution ($p=0.7775$) between the control and EH groups, suggesting that the -634C>G polymorphism of the *IL-6* gene is not significantly associated with EH. Additionally, no association was found between the *IL-6* -634C>G polymorphism and blood

pressure. The present findings are in agreement with existing reports from multiple studies conducted in Chinese and other populations (6,15,16,26,27). Although the mechanism remains unclear, studies have shown that the influence of the *IL-6* polymorphism on blood pressure differs according to gender (13,14). Thus, we further investigated the genotype and allele frequency distribution and blood pressure values according to genotype by gender. No positive results were found.

IL-6 plays an important role in the regulation of blood pressure by stimulating the sympathetic nervous system, enhancing angiotensinogen expression, inducing vessel wall collagen synthesis, and increasing fibrinogen and blood viscosity (28). Therefore, it is possible that high levels of *IL-6* may affect vascular compliance and blood pressure over time. Among the three SNPs (-597G>A, -634C>G and -174G>C)

Table IV. Distribution of the *IL-6* -634C>G genotype in the controls, hypertensive patients and hypertensive subgroups by gender.

Groups	Genotypes frequencies (%)		p-value ^a
	CC	CG + GG	
Controls (n=194)	115 (59.28)	79 (40.72)	
Male (n=112)	67 (59.82)	45 (40.18)	
Female (n=82)	48 (58.54)	34 (41.46)	
EH total (n=246)	143 (58.13)	103 (41.87)	
Male (n=138)	82 (59.42)	56 (40.58)	0.9487
Female (n=108)	61 (56.48)	47 (43.52)	0.7766
EH treated (n=68)	40 (58.82)	28 (41.18)	
Male (n=38)	23 (60.53)	15 (39.47)	0.9389
Female (n=30)	17 (56.67)	13 (43.33)	0.8591
EH untreated (n=178)	103 (57.87)	75 (42.13)	
Male (n=100)	59 (59.00)	41 (41.00)	0.9032
Female (n=78)	44 (56.41)	34 (43.59)	0.7857
LVH(+) (n=68)	41 (60.29)	27 (39.71)	
Male (n=38)	24 (63.16)	14 (36.84)	0.7160
Female (n=30)	17 (56.67)	13 (43.33)	0.8591
LVH(-) (n=110)	62 (56.36)	48 (43.64)	
Male (n=62)	35 (56.45)	27 (43.55)	0.6656
Female (n=48)	27 (56.25)	21 (43.75)	0.7990

EH, essential hypertension; LVH, left ventricular hypertrophy. ^aCompared to controls by gender.

Table V. Means (\pm SD) of SBP and DBP values according to the *IL-6* -634C>G genotype in the controls and untreated hypertensive patients.

Groups	Genotype		p-value
Controls (n=194)	CC (n=115)	CG + GG (n=79)	
SBP (mmHg)	115.20 \pm 15.28	115.67 \pm 15.54	0.8346
DBP (mmHg)	74.89 \pm 8.85	73.50 \pm 8.24	0.2705
Male (n=112)	CC (n=67)	CG + GG (n=45)	
SBP (mmHg)	115.45 \pm 15.33	115.40 \pm 15.36	0.9865
DBP (mmHg)	74.38 \pm 8.78	74.20 \pm 8.47	0.9143
Female (n=82)	CC (n=48)	CG + GG (n=34)	
SBP (mmHg)	115.19 \pm 15.15	115.55 \pm 14.88	0.9152
DBP (mmHg)	74.26 \pm 8.54	74.45 \pm 8.82	0.9222
EH untreated (n=178)	CC (n=103)	CG + GG (n=75)	
SBP (mmHg)	161.84 \pm 22.58	163.28 \pm 24.31	0.6847
DBP (mmHg)	102.51 \pm 10.45	102.88 \pm 10.59	0.8169
Male (n=100)	CC (n=59)	CG + GG (n=41)	
SBP (mmHg)	162.32 \pm 23.14	163.37 \pm 24.44	0.8278
DBP (mmHg)	102.55 \pm 10.49	102.31 \pm 10.37	0.9102
Female (n=78)	CC (n=44)	CG + GG (n=34)	
SBP (mmHg)	162.27 \pm 23.06	161.79 \pm 22.89	0.9274
DBP (mmHg)	102.47 \pm 10.26	103.57 \pm 10.83	0.6480

SBP, systolic blood pressure; DBP, diastolic blood pressure.

of the *IL-6* promoter region reported to influence *IL-6* transcription (29), -174G>C is in tight linkage disequilibrium

with -597G>A and is commonly detected with a prevalence of approximately 40% in Caucasians (14,29,30), but is rare in

Table VI. Echocardiographic parameters according to the *IL-6* -634C>G genotype in the untreated hypertensive patients.

	CC (n=103)	CG + GG (n=75)	p-value
LVEDD (mm)	48.01±3.89	48.81±4.06	0.1852
LVESD (mm)	30.12±0.95	29.88±0.92	0.0935
LVDPW (mm)	10.24±0.97	10.32±1.01	0.5940
IST (mm)	10.93±1.13	11.15±1.18	0.2097
EF (%)	0.62±0.14	0.63±0.14	0.6385
LVMI (g/m ²)	142.37±9.16	144.21±9.78	0.2001
RWT	0.44±0.05	0.43±0.04	0.1544

LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVDPW, left ventricular diastolic posterior wall thickness; IST, interventricular septum thickness; EF, ejection fraction; LVMI, left ventricular mass index; RWT, relative wall thickness.

eastern Asian populations (6,14-16). By contrast, -634C>G has been recognized with a prevalence of only approximately 5% in Caucasians (13,31), while being found at a prevalence of 75% in eastern Asian populations (6,32). In the present study, however, no association was detected between the -634C>G genotype and EH. The negative results could be explained in a number of ways. First, EH is not a single entity; rather, it is likely to result from the interaction between environmental factors and a genetic predisposition to many polygenic quantitative traits acting in concert in various combinations in different individuals (26). The contribution of the -634C>G polymorphism to EH may therefore be so small that our study simply lacked the power to detect it. Second, the pattern of phenotype and genotype association is far more complex than ever envisioned in studies on the pathogenesis of EH. It is often the case in common polygenic diseases that genetic heterogeneity masks an association otherwise apparent between the phenotype and the genotype. Finally, the actual functional mutation for EH may be present in or around the *IL-6* gene, and may as yet be undiscovered. In fact, an *in vitro* study found that the -634C>G allele was not associated with *IL-6* production by leukocytes after lipopolysaccharide stimulation (33). More powerful genetic methods, such as affected-sib pair analysis or family-linkage studies, and/or population studies in different ethnic backgrounds, are necessary to clarify this issue.

LVH is the most powerful independent risk factor for morbidity and mortality in hypertensive patients (17,18). Studies have suggested that *IL-6* may induce a cardiac hypertrophic phenotype by activation of a gp130 receptor (34,35). *IL-6* also directly mediates hypertrophic remodeling associated with hypertension (35,36). Most recently, Melendez *et al* (37) demonstrated the ability of *IL-6* to induce a pattern of myocardial remodeling consistent with that occurring in the heart of hypertensive SD rats, including concentric hypertrophy, fibrosis and diastolic dysfunction. In addition, overexpression of *IL-6* was confirmed in the injured myocardium with hypertrophy in patients who died of acute myocardial infarction (AMI) within 7 days after onset (38). The expression of *IL-6* in the myocardium under AMI appears to be associated with the pathogenesis of cardiac hypertrophy.

Several studies have investigated the influence of genetic background on the variability in left ventricular mass in

humans, and the heritability of this trait has been estimated to be between 30 and 70% in different populations (39). Familial studies have shown that LVH in hypertension shows an association with genetic predisposition, but the exact genes responsible for hypertrophy are unknown (40).

To the best of our knowledge, the present study is the first to investigate the relationship between the *IL-6* -634C>G polymorphism and LVH in hypertensive patients. Since LVH regresses with antihypertensive therapy and the regression of LVH improves prognosis, we compared a LVH(+) group to a LVH(-) group in untreated EH patients. No significant differences in either genotype or allele frequency distribution were found between these two groups. Moreover, the echocardiographic parameters were not statistically different between the CC and CG + GG genotypes. The results suggest that the *IL-6* -634C>G polymorphism is not significantly associated with LVH in EH patients. This is not surprising, since the aetiology of LVH is multifactorial. In addition, the effect of *IL-6* on collagen synthesis by isolated cardiac fibroblasts has been inconsistent (41,42). Siwik *et al* (41) found that *IL-6* resulted in a modest decrease in collagen synthesis, together with increased matrix metalloproteinase activity in neonatal cardiac fibroblasts. In another report, the soluble *IL-6* receptor was found to be essential, in combination with *IL-6*, to the production of increased collagen concentrations by isolated cardiac fibroblasts, and played a role in mediating a phenotypic conversion to myofibroblasts (37). Previous clinical studies on the relationship between LVH in EH patients and inflammatory markers are limited and controversial (19,43). In a small pilot study with 35 EH patients, hypertensive patients with elevated LV mass did not consistently exhibit elevated cytokine levels compared to those with normal LV mass (43). Recently, Rosello-Lleti *et al* (19) assessed the association of different cytokine levels with LVH in 251 asymptomatic hypertensive patients. EH patients with LVH had higher inflammatory cytokine levels than the group without hypertrophy; additionally, the prevalence of LVH was increased in the group of patients with higher cytokine levels. However, regression analysis showed that soluble tumor necrosis factor receptor 1 (sTNF-R1) was an independent predictor of LVH and LNMI. It is of note that the EH patients in this study were receiving conventional therapy for their disease, and it is known that several kinds of drugs reduce cytokine levels (44).

Human linkage studies have attempted to associate polymorphisms of IL-6 with LVH. Patel *et al* (45) found that the TNF- α -308G>A polymorphism, but not IL-6 -174G>C polymorphism, was associated with greater LVMI and a younger age at clinical diagnosis in patients with hypertrophic cardiomyopathy. Losito *et al* (20) examined the IL-6 -174G>C polymorphism in a cohort of 161 patients with end-stage renal disease (ESRD) treated by hemodialysis, showing that individuals with the GC + CC genotype had a higher DBP and LVMI than those with GG homozygotes. They also found that the prevalence of LVH in the former group was higher than in the latter. However, the etiology of LVH may be quite different between ESRD and EH patients. Furthermore, as previously described, the -174C allele is extremely rare and the -634C allele is common in eastern Asian populations, whereas in Caucasians the -174C allele is relatively frequent and the -634C allele is less frequent.

In conclusion, our data did not demonstrate an association of the IL-6 -634C>G polymorphism and EH with LVH in Han Chinese EH patients. Given the inherent limitations of case-control studies and the complex nature of genetic susceptibility for chronic degenerative diseases, more powerful genetic methods in different ethnic backgrounds and gene-environment interactions status are necessary to clarify this issue.

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