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Office Blood Pressure, Heart Rate and A(-596)G Interleukin-6 Gene Polymorphism in Apparently Healthy Czech Middle-Aged Population

A. VAŠKŮ, M. SOUČEK[1], M. GOLDBERGOVÁ, J. VÁCHA

Institute of Pathological Physiology, Faculty of Medicine, Masaryk University and [1]First Clinic of Internal Medicine, St. Ann´s Faculty Hospital, Masaryk University, Brno, Czech Republic

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Summary

The aim of the study was to assess the association between promoter polymorphism [A(-596)G] in interleukin-6 gene and office systolic and diastolic blood pressures, and the heart rate (HR) in apparently healthy Czech subjects. Furthermore, we evaluated the possible influence of gender, BMI and smoking on these supposed associations. An age-matched (40-50 years) and gender-matched (F/M=81/89) sample of apparently healthy Czech subjects (n=170, F/M=81/89) without hypertension, other cardiovascular diseases or diabetes was examined. The A(-596)G *Il-6* gene polymorphism was detected by the PCR method. No differences in genotype distribution and/or allelic frequency was found between groups with lower systolic blood pressure (≤ 122 mm Hg) and higher systolic blood pressure (> 122 mm Hg). Similarly, no differences in the IL-6 polymorphism were found between lower (≤ 86 mm Hg) and higher (> 86 mm Hg) diastolic blood pressure groups. However, we proved a significant increase of genotypes AG+GG as well as the allele (-596)G in higher (>78 beats/min) heart rate group. The genotypes AG+GG represent significantly higher relative risk for higher HR frequency, especially in women. Among lean persons with a low heart rate frequency, fewer AG+GG genotypes were determined than among any other subjects. The genotypes AG+GG are more frequent in non-smoking persons with higher HR compared to non-smoking subjects with lower HR, especially in women. Gender, BMI and smoking substantially modify the distribution of A(-596)G *Il-6* gene polymorphism in apparently healthy persons with lower or higher heart rate.

Key words

Blood pressure • Heart rate • Interleukin 6 • Gene polymorphism • Healthy subjects • BMI • Smoking

Introduction

The role of inflammatory mediators such as cytokines is recently being discussed from the point of view of their possible contribution to the onset and/or progression of different symptoms including cardiovascular diseases (Sharma *et al*. 2000).

Interleukin-6 (IL-6) stimulates the central nervous system and activates the hypothalamus-pituitary-adrenal axis and the sympathetic nervous system (Besedovsky and Del Rey 1996). Circulating levels of IL-6 are largely regulated at the level of expression due to rapid plasma clearance of the cytokine. The transcription of IL-6 is closely regulated by coordinated transcriptional

factors binding at distinct sites of the promoter (Terry *et al*. 2000). Circulating levels of IL-6 correlate with systolic and diastolic blood pressures in healthy volunteers (Fernandez-Real *et al.* 2001, Chae *et al*. 2001). The positive correlation between serum levels of IL-6 and BMI was also demonstrated (Mohamed-Ali *et al*. 1997, Vgontzas *et al*. 1997).

IL-6 belongs to a family of puzzling, highly pleiotropic regulators exhibiting overlapping activities that include leukemia inhibitory factor (also called cholinergic differentiation factor), oncostatin, interleukin 11 and ciliar neutrophic factor (Muñoz-Fernandez and Fresno 1998). Therefore, *Il-6* gene polymorphisms could be associated with hemodynamic parameters such as blood pressure and/or heart rate. On the other hand, relations to obesity, gender and smoking could also be anticipated.

The aim of the present study was to assess the existence of association of promoter polymorphism in *Il-6* gene [A(-596)G] with office systolic and diastolic blood pressures as well as with the heart rate in a sample of apparently healthy middle-aged Czech subjects. Another task was to evaluate a possible influence of gender, BMI and smoking on these assumed associations.

Methods

All examined subjects were recruited by an experienced general practitioner according to the following criteria: age 40-50 years, absence of hypertension, other cardiovascular diseases and diabetes, without signs of acute or chronic diseases.

The office values of systolic and diastolic blood pressures and of the heart rate were measured according to recommended guidelines for the Czech population (Horký *et al*. 1998) in sitting subjects after 10 min of rest. Subjects seated with their arm bared, supported, and at heart level. The persons did not smoke and had not ingested caffeine within 30 min prior to the measurement. Their blood pressure was assessed with a calibrated aneroid manometer (JNC V 1993). Normotension was confirmed by ambulatory blood pressure monitoring performed on the same day (SpaceLab 90207, SpaceLabs International, Wokingham, England). An experienced nurse calculated the heart rate for one minute immediately after blood pressure measurement. All measurements were performed between 07:00-07:30 h.

Subjects were divided into six groups according to lower or higher values of blood pressures and heart rate. The arbitrary values for the division were taken from the calculated value of median for the sample of subjects as 122 mm Hg for systolic blood pressure, 86 mm Hg for diastolic blood pressure and 78 beats/min for heart rate (HR). Thus, groups with a similar number of subjects were obtained. Genotype distribution and/or allelic frequency of A(-596)G *Il-6* gene polymorphism were compared between blood pressure and heart rate groups. BMI (below and above 25 kg/m^2), smoking status (smoker/non-smoker) and gender were taken for further analyses.

The study was approved by the Committee for Ethics of Medical Experiments on Human Subjects, Faculty of Medicine, Masaryk University, Brno (no. 64/93, 1993). A signed, informed consent of the examined subjects was obtained beforehand.

Genotype identification

Genomic DNA was isolated from peripheral leukocytes by a standard technique using proteinase K. The A(-596)G IL-6 polymorphism was detected by PCR using sense 5´-GGAGTCACACACTCCACCTG-3´ and antisense 5´-AAGCAGAACCACTCTTCCTTTACTT-3´ primers (reaction: first cycle 95 °C /5:00 min, 57 °C/1:00 min, 72 °C/1:30 min; 35-times 95 °C /1:30 min, 57 °C /1:00 min, 72 °C/1:30 min; one cycle 95 °C/1:30 min, 57 °C/1:00 min, 72 °C/7:00 min) to amplify a 418 bp product. Digestion with FokI [GGATG(N)$_9$↓], New England BioLabs) at 37 °C for 3-4 h provided fragments of 418 bp for the GG genotype, 418+352+66 bp for the GA genotype and 352+66 bp for the AA genotype of polymorphism as detected by gel electrophoresis with ethidium bromide (Fig. 1).

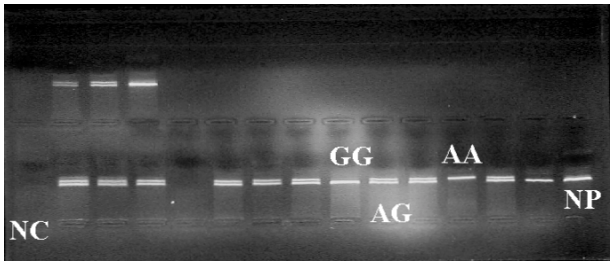

Fig 1. *Genotypes of A(-596)G Il-6 gene polymorphism. NC – negative control, AA, AG, GG – genotypes, NP – non-cleaved product.*

Statistics

The differences in genotype and allelic distributions as well as consistency of genotype

distribution with Hardy-Weinberg equilibrium were tested using the χ^2 test. Holm´s test was used when appropriate to prevent problem of multiple comparisons. P-values corrected by Holm´s test are presented as Pcorr. The program package Statistica v. 6.0 (Statsoft Inc., Tulsa, USA) was used.

Results

Demographic data of the evaluated group of healthy subjects are presented in Table 1. Non-significantly more men-smokers than women-smokers were observed (49/40 vs. 36/45, P=0.11). Significantly more men than women were found with BMI above 25 kg/m^2 (60/29 vs. 35/46, P=0.001).

Table 1. Demographic data.

Subjects **N=170 , F=81 , M=89**	**Median**	**Range**
SBP (mm Hg)	122	95-160
DBP (mm Hg)	86	62-110
HR (beats/min)	78	54-114
BMI (kg m^{-2})	25	19-39
Smokers / non-smokers: 85/85		
Females: 36/45		
Males: 49/40		
Daily number of cigarettes	14	1-30
Duration of smoking (years)	20	1-34
Age (years)	45	41-52

No significant differences in genotype and/or allelic distribution for the A(-596)G *Il-6* gene polymorphism were found between men and women.

The values of blood pressures were indistinguishable for single A(-596)G *Il-6* genotypes (systolic blood pressure: AA: median=124 mm Hg, range 100-150 mm Hg, AG: 122 mm Hg, 95-160 mm Hg, GG: 124 mm Hg, 95-155 mm Hg; diastolic blood pressure: AA: median=86 mm Hg, range 68-100 mm Hg, AG: 88 mm Hg, 62-124 mm Hg, GG: 86 mm Hg, 62-102 mm Hg). No significant differences in genotype and/or allelic distribution for the polymorphism were found when the subjects were divided according to systolic and/or diastolic blood pressure values (Tables 2 and 3). The polymorphism was observed to be consistent with Hardy-Weinberg equilibrium in all compared groups.

The values of heart rate were found to be more different according to the genotype (AA: median= 72 beats/min, range 60-90 beats/min, AG: 78 beats/min, 54-114 beats/min, GG: 80 beats/min, 54-96 beats/min). Significant differences were found both in the genotype (P=0.006, Pcorr=0.02) and the allele distribution (P=0.01, Pcorr=0.03) of the polymorphism between two groups with different heart rate (lower with ≤ 78 beats/min and higher with > 78 beats/min, Table 4). Hardy-Weinberg disequilibrium was found in the group with higher heart rate (P=0.02). The subjects with genotypes AG+GG carry higher relative risk for higher HR frequency (OR=4.27, 95 % confidential interval 1.66-10.98, P=0.001), especially in women (OR=7.80, 95 % confidential interval 1.63-37.33, P=0.004).

Table 2. Systolic blood pressure (SBP) and *Il-6* gene polymorphism.

	A(-596)G *Il-6* gene polymorphism						
Genotypes and alleles	**AA**	**AG**	**GG**	**P**	**A**	**G**	**P**
Subjects with SBP ≤122 mm Hg (N=87)	15	52	20	0.464	0.47	0.53	0.844
Subjects with SBP >122 mm Hg (N=83)	19	42	22		0.48	0.52	

Table 3. Diastolic blood pressure (DBP) and *Il-6* gene polymorphism.

	A(-596)G *Il-6* gene polymorphism						
Genotypes and alleles	**AA**	**AG**	**GG**	**P**	**A**	**G**	**P**
Subjects with DBP ≤86 mm Hg (N=85)	19	44	22	0.623	0.48	0.52	0.828
Subjects with DBP >86 mm Hg (N=85)	15	50	20		0.47	0.53	

When the two heart rate groups were further divided according to the BMI (BMI < 25 kg/m^2 and BMI ≥ 25 kg/m^2, Table 5), a significant increase of genotypes AG+GG was proved in the groups of lean subjects with higher heart rate compared to lean subjects with lower heart rate (P=0.003, Pcorr=0.02). Relative risk of the genotypes in lean subjects for higher HR is 14.93, 95 % confidential interval 1.86-120.12, P=0.001). Furthermore, the allele G(-596) of *Il-6* gene was more frequent in all other subjects compared to the lean subjects with low heart rate frequency (P=0.01, Pcorr=0.05). Relative risk for subjects with genotypes AG+GG for obesity and lower HR and for higher HR is 3.14, 95 % confidential interval 1.43-6.89, P=0.004.

When the groups with higher and lower HR were alternatively divided according to the smoking habits (Table 6), a significant increase in allelic frequency of the allele G(-596) of *Il-6* gene polymorphism was proved between the groups of non-smokers (P=0.01, Pcorr=0.05). The difference was significant only in women (P=0.002, Pcorr=0.008), but not in men. Among non-smoking women with higher HR, the genotype AA was not found at all (AA=0, AG=12, GG=9).

Taken together, we proved a significant increase of genotypes AG+GG as well as of allele G(-596) in higher (> 78 beats/min) HR groups. The genotype AG+GG carriers exhibited a significantly higher relative risk for higher HR frequency, especially in women. The lean persons with low heart rate frequency have fewer AG+GG genotypes than any other subjects. The genotypes AG+GG are more frequent in non-smoking persons with higher HR compared to non-smoking subjects with lower HR, especially in women. Gender, BMI and smoking substantially modify different distribution of A(-596)G *Il-6* gene polymorphism in apparently healthy middle-aged persons with lower and higher heart rate.

Table 4. Heart rate and *Il-6* gene polymorphism.

	A(-596)G *Il-6* gene polymorphism						
Genotypes and alleles	**AA**	**AG**	**GG**	**P**	**A**	**G**	**P**
Subjects with HR ≤ 78/min (N=99)	28	50	21	0.006 Pcorr=0.02	0.54	0.46	0.01 Pcorr=0.03
Subjects with HR > 78min (N=71)	6	44	21		0.39	0.61	

Table 5. Heart rate, BMI and *Il-6* gene polymorphism.

		A(-596)G *Il-6* gene polymorphism						
Genotypes and alleles		**AA**	**AG**	**GG**	**P**	**A**	**G**	**P**
1	*Subjects with HR ≤ 78/min BMI < 25 kg/m^2*	16	22	8	1:3 P=0.003 Pcorr=0.02	0.59	0.41	1:3 P=0.097
2	*Subjects with HR ≤ 78/min BMI ≥ 25 kg/m^2*	12	28	13	2:4 P=0.172	0.49	0.51	2:4 P=0.065
3	*Subjects with HR > 78/min BMI < 25 kg/m^2*	1	24	4	1: (2+3+4) P=0.01 Pcorr=0.05	0.45	0.55	1: (2+3+4) P=0.01 Pcorr=0.05
4	*Subjects with HR > 78/min BMI ≥ 25 kg/m^2*	5	20	17		0.36	0.64	

Table 6. Heart rate, smoking and *Il-6* gene polymorphism

	Genotypes and alleles	A(-597)G *Il-6* gene polymorphism AA	AG	GG	P	A	G	P
1	*Subjects with HR ≤78/min smokers*	13	24	11	1:3 P=0.165	0.52	0.48	1:3 P=0.253
2	*Subjects with HR ≤78/min non-smokers*	15	26	10	2:4 P=0.020	0.54	0.46	2:4 P=0.012 Pcorr=0.05
3	*Subjects with HR >78/min smokers*	4	24	9		0.43	0.57	
4	*Subjects with HR >78/min non-smokers*	2	20	12		0.35	0.65	

Discussion

The -174C allele of *Il-6* gene promoter, which is known to be closely linked with the A(-596) allele (Brull *et al*. 2001, Georges *et al*. 2001, Villuendas *et al*. 2002), was associated with higher systolic blood pressure as well as with higher risk of coronary heart disease in 2751 middle-aged healthy U.K. men (Humphries *et al.* 2001). This allele was associated with a susceptibility to myocardial infarction, especially with events occurring in smaller coronary artery lesions (Georges *et al.* 2001). So far, we have not found any studies on relations between *Il-6* gene polymorphisms and heart rate.

An accelerated resting heart rate represents an independent predictor of cardiovascular mortality, but only in men (Benetos *et al*. 1999). Parasympathetic modulation of heart rate appeared to be greater in younger women than in younger men (Fagard 2001). Women seem to have greater heart rate reactivity than men (Adan and Sanchez-Turet 2001). The more pronounced vagal influence in cardiac regulation in middle-aged women and the gender-different influence of heart rate and metabolic factors on heart rate variability may help to explain the lower susceptibility of women to cardiac arrhythmias (Kuch *et al.* 2001). Fagard and coworkers (1999, 2001) reported that age and gender, but not BMI and smoking, significantly influence the heart rate and various components of short-term heart rate variability. Heart rate reactivity evaluated during performance of auditory and visual vigilance tasks and a working memory task were found to be influenced by smoking and gender, but only at certain times of the day, especially at the earliest and latest hours and during the post-lunch period.

The diastolic function of the left ventricle was found to be significantly reduced in obese patients independently of hemodynamic factors (such as 24-h blood pressure and heart rate) or the left ventricle geometry (Grandi *et al.* 2000). In 100 000 French subjects, the relationship of resting heart rate with total cholesterol was proved, but only in men. In healthy men, not in women, the heart rate is linked to plasma leptin concentrations, which are significantly correlated with BMI in men (Narkiewicz *et al*. 2001).

Heart rate in the rest is significantly higher in smokers vs. non-smokers in all race/gender groups except of black men (Gidding *et al.* 1995). Smoking was found to have opposite effects on heart rate in men and women (Morcet *et al*. 1999). Stress-reducing effects of smoking seem to be transient, situationally specific, partly gender-dependent and dissociated from the effects of smoking on cardiovascular arousal (Perkins *et al.* 1992). Smoking acutely impairs glucose tolerance and insulin sensitivity, enhances serum cholesterol and triglyceride levels and raises blood pressure and heart rate (Frati *et al*. 1996). Insulin sensitivity correlates with cardiac sympathovagal balance in men, but not in women (Flanagan *et al*. 1999).

Our results including *Il-6* genotype distribution do not contradict most results presented above. The allele G(-596) of *Il-6* gene is associated with higher office heart rate at rest in apparently healthy Czech subjects. The association of this genotype with the heart rate is partly modified by overweight in men and by smoking in women.

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Reprint requests

Anna Vašků, M.D., PhD., Institute of Pathological Physiology, Faculty of Medicine, Masaryk University, Komenského nám. 2, 662 43 Brno, Czech Republic, e-mail: avasku@med.muni.cz.