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PII: S0022-3999(14)00024-5
DOI: doi: 10.1016/j.jpsychores.2014.01.007
Reference: PSR 8767

To appear in: Journal of Psychosomatic Research

Received date: 23 August 2013
Revised date: 14 January 2014
Accepted date: 21 January 2014

Please cite this article as: Chumaeva Nadja, Hintsanen Mirka, Pulkki-Råback Laura, Jokela Markus, Juonala Markus, Lehtimäki Terho, Raitakari Olli T., Keltikangas-Järvinen Liisa, Interleukin-6 gene polymorphism, chronic stress and atherosclerosis, Journal of Psychosomatic Research (2014), doi: 10.1016/j.jpsychores.2014.01.007

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INTERLEUKIN-6 GENE POLYMORPHISM, CHRONIC STRESS AND ATHEROSCLEROSIS

Interleukin-6 -174G>C polymorphism, chronic stress and risk of early atherosclerosis in

The Cardiovascular Risk in Young Finns Study

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Abstract

Objective: Interleukin-6 protein has been suggested as a mediator connecting chronic stress and cardiovascular diseases. We investigated whether the functional G174C polymorphism (rs1800795) of interleukin-6 gene is associated with vital exhaustion, a measure of chronic stress, or with preclinical atherosclerosis.

Methods: Associations between the interleukin-6 -174G>C polymorphism, preclinical atherosclerosis, and vital exhaustion were examined in 1673 women and men aged 24-39 years participating in the Cardiovascular Risk in Young Finns study. Vital exhaustion was measured using Maastricht Questionnaire. Preclinical atherosclerosis was assessed by carotid intima-media thickness using ultrasound techniques. DNA was genotyped for the interleukin-6 -174G>C polymorphism (rs1800795).

Results: The GG genotype of the interleukin-6 -174G>C polymorphism was associated with higher vital exhaustion. Moreover, higher vital exhaustion was associated with greater intima-media thickness in men carrying G alleles, adjusted for cardiovascular risk factors.

Conclusion: Our findings support a role for the interleukin-6 -174G>C polymorphism in increased risk of atherosclerosis in individuals with chronic stress. In addition, individuals carrying the G allele of the interleukin-6 -174G>C polymorphism may be more prone to adverse effects of psychosocial stress.

Key words: atherosclerosis, chronic stress, gene polymorphism, interleukin-6
Introduction

Immune activity is a potential mechanism mediating the association between chronic psychological stress and cardiovascular diseases (CVD) [1]. Recently, genetic differences have been found to explain why some individuals cope with stress more effectively than others [2-4]. Coping with stress has been related for instance to genes regulating inflammatory processes, inflammatory cytokines [5] and levels of circulating interleukin-6 (IL-6) protein [6]. Interleukin-6 induces activation of the hypothalamic-pituitary-adrenocortical axis [7]; chronic cytokine elevation influences neuroendocrine function and neurotransmitter metabolism in the brain [5]. These processes are important part of the physiological stress reaction.

High level of IL-6 production is related to higher stress level [6]. Interleukin-6 plasma levels are partly regulated by the G174C polymorphism (rs1800795) of IL-6 gene [8]. The C allele of the IL-6 G174C polymorphism is related to lower IL-6 plasma levels whereas the G allele is associated with increased IL-6 plasma level [8]. This suggests that the G allele may be responsible for stress-related disease risk. Importantly, a recent study has identified a molecular pathway that is specific to the IL-6 -174G allele [9]. This pathway is triggered in the IL-6 -174G allele carriers by sympathetic nervous system induced activation of GATA1 transcriptional factor and results in IL-6 gene expression during periods of chronic sympathetic activation [9].

Vital exhaustion (VE) is considered a state of chronic stress characterized by physical exhaustion, unusual tiredness and increased irritability [10]; VE reflects decreased capacity of stress coping [11]. Vital exhaustion is potentially useful for early recognition of an increased risk of CVD, because it has been found to predict myocardial infarction [12], stroke [13], atherothrombotic events [14-16] and long-term risk for adverse cardiac events [17]. For example, high VE has been associated with a 2-fold risk of heart failure [18].
Studies have reported increased IL-6 plasma level associated with VE [19-21]. However, the elevation of IL-6 production showing in one immunological probe may not be adequate criteria for determining the role of IL-6 in stress and diseases conditions, because the increasing of IL-6 in plasma may be induced by many different immunological factors. The IL-6 -174G/C polymorphism is related to differences in the levels of circulating IL-6 protein in plasma, suggesting that there is genetic variation in response to inflammation and/or to stressful situations, and this level may be influenced by the IL-6 -174G/C promoter variants [8]. High levels of IL-6 production have been associated also with atherosclerosis [22]. However, studies on the IL-6 -174G/C polymorphism and atherosclerosis have reported conflicting results, some providing evidence for the GG genotype [23,24], and some for the CC genotype [25,26] as a risk factor for preclinical atherosclerosis. Part of these conflicting results might be related in yet undiscovered IL-6 gene variant (rs1800795) by environment interactions.

Although IL-6 production/genotype [27,28] and VE [29,30] both have been linked with indicators of cardiovascular risk, we are not aware of any studies assessing the role of both VE and the IL-6 -174G/C polymorphism in predicting atherosclerosis. Therefore, the purpose of the present study was 1) to examine associations between the IL-6 -174G/C polymorphism and preclinical atherosclerosis in Finnish middle-aged healthy adult population; 2) to investigate associations between the IL-6 -174G/C polymorphism and VE; and 3) to examine relationships between the IL-6 -174G/C polymorphism, VE and preclinical atherosclerosis, assessed by the carotid intima-media thickness (IMT). We expect to find the associations between VE and IMT in the G allele carriers, because the presence of the G allele has been suggested to be responsible for stress-related disease risk [8,9]. It is important to note that much of the associations between the IL-6 -174G/C polymorphism and IL-6 plasma concentration in relation to diseases risk have been found in stressful conditions.
Possible explanation of these associations has recently been suggested: selective participation of the IL-6 -174G allele in molecular mechanism triggering diseases-associated cytokine response has been found only in stress environment [9]. Subjects with the GG (or CG) genotypes have been associated with elevated IL-6 plasma level compared with the CC genotype subjects [8]. Based on these findings, lipid profiles have been studied in the joined CG+GG genotype group (the G allele carriers) and in the CC homozygotes separately [31]. The findings provide evidence about lipid abnormalities in subjects with the IL-6 -174G allele. The joined CG+GG subgroup has been used in stress research study examining the IL-6 -174G/C polymorphism and chronic stress (fatigue and depression) associations [32] as well as in the analyses of the IL-6 -174G/C polymorphism and atherosclerosis relations [33].

In many analyses the G allele has been found to show a dominant mode of action [8,32]. Thus, to examine relations between the -174G/C IL-6 gene polymorphism, VE and atherosclerosis, the CG and GG individuals were grouped together into the joined CG+GG subgroup. Sex differences are also expected, because the modulating influence of sex has been shown in the IL-6 -174G/C polymorphism effects on IL-6 expression [34]. Higher values of the IL-6 synthesis have been found in men as compared to women [34]. Significantly lower IL-6 concentration has been reported among pre-menopausal women than among men [35]. This can be explained by high circulating level of female sex hormones (e.g. estradiol) known to be a protective factors against CVD progression [36]. Estradiol has been found to inhibit cytokine gene expression (e.g., IL-6 gene expression: [37,38]) and, therefore, cytokine production. In addition, androgens may predispose males to atherosclerosis by increased expression of atherosclerosis-related genes [39]. Gender differences in the IL-6 expression [34] and in IL-6 concentration [35] may contribute to the differences in diseases risk. Based on these findings and on estradiol inhibitory action on IL-6
release [37,38] we can hypothesize that men would show increased risk of atherosclerosis as compared to women.

Methods

Participants

The subjects were participating in the on-going Cardiovascular Risk in Young Finns study, a population-based study investigating risk factors for CVD in children, adolescents and young adults in Finland [40]. Since 1980, the Young Finns study (n = 3596 at the baseline in 1980) has been monitoring the development of risk factors for coronary heart disease at intervals of 3 or 5 years. In 2001, cardiovascular risk factors in 2109 participants were examined and ultrasound measurements taken, including the carotid IMT measurements for assessment of preclinical atherosclerosis. Previous attrition analyses have shown that the subjects excluded from the follow-up (in 2001) were younger and a greater proportion of men were excluded [41]. In age-adjusted analyses, no significant differences between excluded and included participants were found for low-density lipoprotein (LDL) and high-density lipoprotein (HDL), triglycerides, body-mass index (BMI), systolic blood pressure (SBP) and diastolic blood pressure (DBP) [41]. In 2001, the sample of 2228 subjects was genotyped for the IL-6 gene. Stress assessment (measurements of VE level) was also conducted in 2001; valid VE questionnaires were obtained from 2080 subjects. In the present study, 1673 participants (798 women and 695 men) had complete data on ultrasound measurements, genetic data and VE. The study has cross-sectional design; all data were assessed in 2001, when the participants were 24-39 years old. All subjects gave written informed consent. The Young Finns study was approved by the local ethics committees in all five centres (Helsinki, Turku, Tampere, Kuopio and Oulu). The study procedure was conducted in accordance with the Declaration of Helsinki.
Measures

Ultrasound imaging: measurements of the carotid intima-media thickness, IMT

Ultrasound studies of the carotid arteries were performed using Sequoia 512 ultrasound mainframes (Acuson, Mountain View, CA, USA) with 13.0 MHz linear array transducer, as previously described [42]. To assess intra-individual reproducibility of ultrasound measurements, 57 subjects were re-examined 3 months after the initial visit (2.5% random sample). Carotid IMT was measured on the posterior (far) wall of the left carotid artery. At least four measurements were taken approximately 10 mm proximal to the bifurcation to derive mean carotid IMT. The between-visit coefficient of variation of IMT measurements was 6.4% and the intra-observer coefficient of variation was 3.4% [42].

IL-6 -174G>C genotyping

Genomic DNA was extracted from whole blood using a commercially available kit (Qiagen Inc., Hilden, Germany). Interleukin-6 -174G>C genotyping (rs1800795) was performed by employing the 5'-nuclease assay and fluorogenic allele-specific TaqMan probes and primers [43]. PCR was performed in 384-well plates in a total volume of 5 μl, using ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) with the following conditions: 2 min at 52 ºC, 10 min at 95 ºC followed by 40 cycles of 95 ºC for 15 sec and 60 ºC for 1 min. PCR reactions for the rs1800795 contained genomic DNA, 1×Universal PCR Master Mix, 900 nM of each primer and 200 nM of each TaqMan probe. The forward and reverse amplification primer sequences were: 5'-GACGACCTAAGCTGCACTTTTC-3' and 5'-GGCTGATTGGAAACCTTATTAAGATTG-3', respectively. The used probe sequences for the identification of rs1800795 polymorphism were as follows: 5’-VIC-
CCTTTAGCATGCAAGAC -3’ and 5’-6-FAM-CTTTAGCATGCAAGAC-3’. The gDNA and master mix were pipetted on 384-plates by an automated TECAN Freedom EVO-100 robot (Tecan Schweiz AG, Männedorf, Switzerland). For quality analysis random duplicates, empty controls (water) and known control samples were run in parallel with unknown DNA samples.

**Vital exhaustion**

Vital exhaustion was assessed with the Maastricht Questionnaire (MQ), a 21-item checklist of symptoms of exhaustion [44]. It has been specially developed to assess feelings of exhaustion and consists of questions asking about symptoms of VE state: increased irritability, unusual fatigue, loss of energy and feelings of demoralization, e.g., “Do you ever wake up with a feeling of exhaustion and fatigue? Do little things irritate you more lately then used do? Do you feel you want to give up trying? Do you lately feel more listless than before?” The significant associations between the MQ items and myocardial infarction have been shown [44]. Each of the items was rated on a three-point scale, ranging from 0 to 2. The response alternatives were: “no” = 0, “I cannot say” = 1 and “yes” = 2. The mean score of all the items was used to index the level of VE. Cronbach’s alpha was 0.92, indicating good reliability. The MQ has been designed for self-application; the questionnaire was sent to the participants to be completed at home. Those participants who had 50% or more missing values in VE items were excluded.

**Health-related characteristics and clinical measurements**

For the determination of serum lipoprotein levels, venous blood samples were drawn after an overnight fast. All measurements of lipid levels were performed in duplicate in the same laboratory. LDL-cholesterol concentration was calculated by the Friedewald formula
Standardized enzymatic methods were used for measuring levels of triglycerides and HDL-cholesterol. Details of the methods have been reported elsewhere [46]. Serum insulin was measured by microparticle enzyme immunoassay kit (Abbott Laboratories, Diagnostic Division, Dainabot, Japan). Body-mass index was calculated as participants' weight in kilograms divided by the square of their height in meters. Blood pressure was measured with a random-zero sphygmomanometer. Average of three measurements was used in the analysis.

Health-related characteristics included smoking status (daily smoking), alcohol consumption [how often beer, wine, or spirits was used at least six portions at a time (one portion equals 12 g): ranging from 1=once a year or never to 6=at least twice a week], and physical activity (an index formed of five variables describing intensity, frequency, and average duration of physical activity, hours/week of intensive physical activity, and participation in structured sports). The physical activity index (PAI) was calculated [47]; high scores on PAI indicate high physical activity.

Statistical analyses

The IL-6 -174G>C polymorphism was coded as follows: 0 = CC, 1 = CG and 2 = GG genotype. Chi-square tests were performed to test for deviations from Hardy-Weinberg equilibrium. Differences between the original sample and current study sample were analyzed using $\chi^2$-test and t-test. Linear regression analyses were used to examine the relationships between the IL-6 -174G>C polymorphism, VE and carotid IMT. First, the IL-6 gene polymorphism was used as independent variable and VE was the dependent variable; second, the IL-6 gene polymorphism was independent variable and the carotid IMT was the dependent variable. These analyses were conducted in the whole sample and in women and men separately. In the CC and the CG+GG genotype subgroups, IMT was used as the dependent variable. As the first step, IMT was regressed on VE and age. In the second step,
lifestyle factors (smoking status, alcohol consumption and physical activity) were added into the model; in the third step, age, plasma insulin, LDL- and HDL-cholestersols, triglycerides, BMI, SBP and DBP were controlled for. Finally, a fully adjusted model (Step 4) included age, lifestyle factors and clinical measurements. All the statistical analyses were performed using the PASW 18.0 software.

Results

Sample attrition

Differences between included and excluded participants at the 2001 follow-up were relatively small. Participants excluded from the current sample were more likely to be men (60.6% of the excluded participants were men vs. 39.4% of the included participants were men; p < 0.001), slightly younger (mean ages in 2001: 31.26 vs. 31.65, p = 0.018), were physically less active (means 9.41 vs. 9.64, p = 0.024), and consumed more alcohol (means 2.65 vs. 2.52, p = 0.031) than participants included in the current study. The excluded participants had lower HDL-cholesterol level (means 1.25 vs. 1.31, p < 0.001), higher triglycerides level (means 1.42 vs. 1.27, p < 0.001), higher BMI (means 25.23 vs. 24.85, p = 0.030) and borderline significantly higher VE level (means 0.47 vs. 0.42, p = 0.05) than the included participants. There were no significant differences between the excluded and the included participants in IMT (means 0.58 vs. 0.58, p = 0.769) and in the IL-6 -174 genotype (p = 0.194).

Characteristics of the study participants

Table 1 presents the descriptive statistics of the sample. Genotype and allele proportions for the IL-6 -174G>C polymorphism were in Hardy-Weinberg equilibrium (p > 0.05). IMT and VE scores were normally distributed.
Table 2 shows differences between the CC and the CG+GG IL-6 genotype subgroups. Individuals carrying the G allele had higher VE and HDL-cholesterol levels than those carrying no G alleles; individuals with the CC genotype showed higher level of physical activity. There were no differences between IMT in the CC genotype and the CG+GG genotype groups.

Associations between VE and the IL-6 -174G>C polymorphism

Positive association between VE and the IL-6 -174G/C polymorphism was found for the whole sample ($\beta = 0.059$, $p = 0.015$, $N = 1673$) (Fig. 1).

This association remains significant after adjustment for age and sex: $\beta = 0.058$, $p = 0.016$, $N = 1673$. However, when women and men were analysed separately, VE was only marginally significantly associated with the IL-6 -174G/C polymorphism both in unadjusted (women: $\beta = 0.055$, $p = 0.083$, $N = 978$; men: $\beta = 0.063$, $p = 0.095$, $N = 695$) and in age-adjusted models (women: $\beta = 0.055$, $p = 0.086$, $N = 978$; men: $\beta = 0.067$, $p = 0.079$, $N = 695$).

Associations between IMT and the IL-6 -174G>C polymorphism

There were no associations between IMT and the IL-6 -174G>C polymorphism in the whole sample in unadjusted ($\beta = 0.005$, $p = 0.847$, $N = 1673$) and in age and sex adjusted models ($\beta = 0.009$, $p = 0.685$, $N = 1673$). No associations between IMT and the IL-6 -174G>C polymorphism were found among women or men both in unadjusted (women: $\beta =$
0.03, p = 0.356, N = 978; men: β = -0.022, p = 0.571, N = 695) and in age adjusted models (women: β = 0.026, p = 0.396, N = 978; men: β = -0.007, p = 0.835, N = 695).

**Associations between VE and IMT**

Vital exhaustion was not associated with IMT in the total sample (β = 0.012, p = 0.637, N = 1673) nor in women (β = -0.01, p = 0.765, N = 978). In men, VE was positively associated with IMT (β = 0.083, p = 0.029, N = 695). However, in age adjusted model VE was not associated with IMT in women (β = -0.020, p = 0.509, N = 978) nor in men (β = 0.059, p = 0.100, N = 695). Controlling for age and sex in the whole sample also produced non-significant results: β = 0.014, p = 0.541, N = 1673.

**Associations between VE and IMT in IL-6 genotype subgroups**

Table 3 presents results of linear regression analyses on associations between VE and IMT in IL-6 genotype subgroups.

(Insert Table 3 about here)

Controlling for age and health-related characteristics showed no significant associations among women or men in both genotype subgroups. In the model, adjusted for age and clinical measurements higher VE was associated with higher IMT (p = 0.022) in men with the joined CG+GG genotype. Fully-adjusted model including age, clinical measurements and health-related characteristics also showed positive associations between VE and IMT in men with the IL-6 -174CG or GG genotype (p = 0.019). No associations were found in women or in participants with the IL-6 -174CC genotype.

**Discussion**
No studies have examined the association between IL-6 genetic factors and VE. Our present study is the first one showing association between VE and the IL-6 -174G/C polymorphism: higher level of VE was associated with the GG genotype. This result is in line with our preliminary hypothesis and with the suggestion that the IL-6 -174G allele may be responsible on stress-related IL-6 response [8]. Our findings may have high importance because of recently found molecular mechanism according to which socio-environmental stress may influence IL-6 gene expression especially in IL-6 -174G allele carriers [9]. In line with our findings, depressive symptoms have been related to increased mortality risk among homozygous IL-6 174G allele carriers, but not among homozygous C allele carriers [9]. In addition, higher levels of cancer-related fatigue have been found among the IL-6 -174GG genotype individuals [48]. However, fatigue is only a part of VE.

Because sex can modulate effect of the IL-6 -174G/C polymorphism on IL-6 expression [34], the investigations in sex subgroups may be important. In our study, positive association between higher VE and higher IMT was found among men with the joined CG+GG genotype after adjusting for cardiovascular risk factors. In line with our findings, the -174 IL-6G/C polymorphism has previously been associated with the risk factors and markers of subclinical atherosclerosis only in men [49,50]. Thus, serum total cholesterol and LDL-cholesterol were higher in the IL-6 -174GG, than in the GC or CC groups [50]. The genetic effect size of the IL-6 -174G>C polymorphism on markers of atherosclerosis was not significant in women [49,50]. Our results are also in line with the recent findings found in sub-populations: an increased risk of atherosclerotic events in overweight IL-6 -174GG carriers with rheumatoid arthritis [51]; and higher coronary heart disease risk in heart disease patients with the IL-6 -174CG+GG genotype after taking medications [52]. The IL-6 -174GG genotype patients with rheumatoid arthritis have demonstrated more severe endothelial
dysfunction – an index of subclinical atherosclerosis [42] – than the CG or the CC IL-6 -174 genotype patients [53].

Most of the cardiovascular risk factors increasing the risk of atherosclerosis have been found to be more harmful to men than to women [54]. In addition, men are more influenced by stress and have less stress coping potential [55]. By contrast, young and middle-aged women are highly protected by female sex hormones against harmful action of the most of cardiovascular risk factors [36]. In our previous study, interactions of VE and cardiac autonomic reactivity after acute stress on IMT have been found only in men [29]. In addition, high VE and low vascular elasticity interaction on greater IMTs have also been obtained in men [30]. Summarizing previous findings and our results, it can be hypothesized that the presence of the G allele and especially the -174GG IL-6 genotype may predispose individuals to increased vulnerability to stress and, therefore to increased risk of stress-related immune mediated diseases.

In the present study, no direct associations between the IL-6 -174G>C polymorphism and IMT were found in the whole sample, nor in men or women. The absence of these associations is not in accordance with our preliminary suggestion, but it is in line with the previous contradictory findings on the relations between the IL-6 -174G/C polymorphism and preclinical atherosclerosis [23-26]. Reasons for conflicting results are unclear. It has been suggested that the IL-6 gene polymorphism effect depends on the surrounding genes [56]. Several polymorphisms determine vascular and heart diseases and main effects may be masked by interactions between the genes [56]. Gene x environment interactions may also partly explain conflicting findings [9]. Several inflammatory genes are involved in overlapping metabolic, biochemical and immunological pathways leading to atherosclerosis development; and many risk factors may influence the processes directly or via participation in cross talk interactions between the pathways [57]. The IL-6 protein may
be involved in these processes; moreover, it has complex transcriptional regulation [9,56] and multifactorial physiological properties [58]. For these reasons, the role of one single nucleotide polymorphism in disease-associated risk should not be overestimated.

**Methodological considerations**

Although our original data was randomly selected from the Finnish population, there has been some systematic selection of the participants during the follow-up examinations. The present data was somewhat over-represented by females and participants with better socioeconomic position, in other words, our data was slightly selected towards better health and wealth. If anything, this may have truncated variance in our main variables (VE and IMT), thereby possibly under-estimating true associations.

Our results cannot be generalized to older individuals with more advanced stage of atherosclerosis because the present analyses were conducted in participants aged 24 to 39. Our study focused on young and middle-aged adults, for whom the first stages of atherosclerosis are present but clinical symptoms of carotid artery disease are not yet manifested. The present study has cross-sectional nature, and we cannot make statements regarding causality of associations or the atherosclerosis progress.

**Conclusions**

The present study has been found positive associations between the IL-6 -174G/C polymorphism and VE. We can suggest that genetic differences may explain different possibilities to cope with stress among different individuals, that is, vulnerability to VE may depend on genotype. In addition, positive association between VE and IMT found in men with the IL-6 -174CG+GG genotype, when cardiovascular risk factors were controlled for, suggests that the G allele may be responsible for higher level of diseases risk. Our results
imply that the detrimental effects of IL-6 polymorphism on atherosclerosis may be dependent on the chronic stress levels the individual is exposed to. Furthermore, our findings suggest that VE may only be detrimental for the cardiovascular health of those who have pre-existing genetic risk. This kind of group differences may explain some of the contradictions in previous findings.

The findings of our study on the role of individual difference in VE and associated cardiovascular outcomes may be useful for preventive medicine programs aiming to improve health of young adults. As a practical implication, our findings enhance understanding on individual differences in the cardiovascular health risks that are related to exhaustion. A higher risk of CVD associated with chronic stress may be of importance in the planning of future preventive programs. Stress reduction strategies and early intervention programs aimed at reducing symptoms of exhaustion may enhance cardiovascular health in the long run, especially in genetically susceptible individuals.

**Competing interests**

The authors declare that they have no competing interests.

**Acknowledgements**

This study was supported by the Academy of Finland (grant numbers: 123621 LP-R, 258578 MH), Wihuri Research Foundation (NC), Emil Aaltonen Foundation (MH), Ella and Georg Ehrnrooth Foundation (MH), Research Funds of the University of Helsinki (MH), Signe and Ane Gyllenberg Foundation (MH) and Finnish Foundation for Cardiovascular Research (MH).

The Young Finns Study has been financially supported by the Academy of Finland: grants 126925, 121584, 124282, 129378, 117797, and 41071; the Social Insurance Institution...
of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds, Juho Vainio Foundation, Paavo Nurmi Foundation, Finnish Foundation of Cardiovascular Research and Finnish Cultural Foundation, Sigrid Juselius Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation.
References


Table 1

Characteristics of the study participants (N = 1673): differences between women and men

<table>
<thead>
<tr>
<th>Variable</th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
<td>N</td>
<td></td>
</tr>
<tr>
<td>Age, years (24-39)</td>
<td>31.56</td>
<td>5.04</td>
<td>798</td>
<td>31.78</td>
<td>5.09</td>
<td>695</td>
<td>0.371</td>
</tr>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.57</td>
<td>0.08</td>
<td>978</td>
<td>0.59</td>
<td>0.10</td>
<td>695</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vital exhaustion, VE</td>
<td>0.47</td>
<td>0.39</td>
<td>978</td>
<td>0.38</td>
<td>0.34</td>
<td>695</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.16</td>
<td>0.77</td>
<td>977</td>
<td>3.46</td>
<td>0.92</td>
<td>678</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.40</td>
<td>0.31</td>
<td>978</td>
<td>1.17</td>
<td>0.28</td>
<td>678</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerids (mmol/l)</td>
<td>1.17</td>
<td>0.58</td>
<td>977</td>
<td>1.42</td>
<td>0.73</td>
<td>678</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>7.89</td>
<td>6.59</td>
<td>582</td>
<td>7.72</td>
<td>5.02</td>
<td>472</td>
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<tr>
<td>Systolic BP (mm Hg)</td>
<td>117.4</td>
<td>13.17</td>
<td>580</td>
<td>116.44</td>
<td>13.09</td>
<td>470</td>
<td>0.242</td>
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<tr>
<td>Diastolic BP (mm Hg)</td>
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<td>10.65</td>
<td>580</td>
<td>70.40</td>
<td>11.02</td>
<td>470</td>
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</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.83</td>
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<td>24.89</td>
<td>4.09</td>
<td>541</td>
<td>0.822</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.19</td>
<td>0.39</td>
<td>959</td>
<td>1.26</td>
<td>0.44</td>
<td>680</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol intake (1-6)</td>
<td>2.10</td>
<td>1.17</td>
<td>964</td>
<td>3.12</td>
<td>1.39</td>
<td>688</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Physical activity (5-16)</td>
<td>9.60</td>
<td>2.10</td>
<td>916</td>
<td>9.69</td>
<td>2.52</td>
<td>658</td>
<td>0.473</td>
</tr>
<tr>
<td><strong>IL-6 gene polymorphism</strong></td>
<td></td>
<td></td>
<td></td>
<td>N = 978</td>
<td></td>
<td>N = 695</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>471</td>
<td>(28.16%)</td>
<td></td>
<td>270</td>
<td>(27.61%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CG</td>
<td>861</td>
<td>(51.46%)</td>
<td></td>
<td>508</td>
<td>(51.94%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>341</td>
<td>(20.38%)</td>
<td></td>
<td>200</td>
<td>(20.45%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: IL-6, interleukin-6; IMT, intima-media thickness; HDL, high density lipoprotein; LDL, low density lipoprotein; BP, blood pressure; BMI, body-mass index; VE, vital exhaustion; SD, standard deviation. P-values refer to the mean differences between men and women.
### Table 2
Characteristics of the study participants: differences between the CC and the CG+GG -174IL-6 genotype groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>CC</th>
<th>CG+GG</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (24-39)</td>
<td>31.56</td>
<td>31.69</td>
<td>0.645</td>
</tr>
<tr>
<td>Carotid IMT (mm)</td>
<td>0.58</td>
<td>0.58</td>
<td>0.475</td>
</tr>
<tr>
<td>Vital exhaustion, VE</td>
<td>0.38</td>
<td>0.43</td>
<td>0.010</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.27</td>
<td>3.29</td>
<td>0.731</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.28</td>
<td>1.32</td>
<td>0.048</td>
</tr>
<tr>
<td>Triglycerides (mmol/l)</td>
<td>1.30</td>
<td>1.26</td>
<td>0.330</td>
</tr>
<tr>
<td>Insulin (mU/l)</td>
<td>7.64</td>
<td>7.89</td>
<td>0.537</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
<td>116.25</td>
<td>117.25</td>
<td>0.261</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
<td>69.93</td>
<td>71.28</td>
<td>0.067</td>
</tr>
<tr>
<td>BMI (kg/m^2)</td>
<td>25.02</td>
<td>24.79</td>
<td>0.391</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.21</td>
<td>1.22</td>
<td>0.467</td>
</tr>
<tr>
<td>Alcohol intake (1-6)</td>
<td>2.50</td>
<td>2.53</td>
<td>0.704</td>
</tr>
<tr>
<td>Physical activity (5-16)</td>
<td>9.85</td>
<td>9.55</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Note: IMT, intima-media thickness; HDL, high density lipoprotein, LDL, low density lipoprotein, BP, blood pressure; BMI, body-mass index; VE, vital exhaustion; SD, standard deviation.

P-values refer to the mean differences between the CC and the CG+GG genotype groups.
Table 3

Standardized Regression Coefficients ($\beta$) of VE on IMT in subgroups

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\beta$</td>
<td>t-value</td>
<td>p-value</td>
<td>$\beta$</td>
</tr>
<tr>
<td></td>
<td>$R^2$</td>
<td></td>
<td>$R^2$</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.03</td>
<td>0.52</td>
<td>0.600</td>
<td>0.00</td>
</tr>
<tr>
<td>Adjusted for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1.</td>
<td>-0.04</td>
<td>-0.23</td>
<td>0.817</td>
<td>0.11</td>
</tr>
<tr>
<td>Step 2.</td>
<td>-0.03</td>
<td>-0.45</td>
<td>0.654</td>
<td>0.13</td>
</tr>
<tr>
<td>Step 3.</td>
<td>-0.01</td>
<td>-0.83</td>
<td>0.934</td>
<td>0.12</td>
</tr>
<tr>
<td>Step 4.</td>
<td>0.00</td>
<td>0.05</td>
<td>0.959</td>
<td>0.16</td>
</tr>
<tr>
<td>CG + GG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unadjusted</td>
<td>-0.02</td>
<td>-0.64</td>
<td>0.521</td>
<td>0.00</td>
</tr>
<tr>
<td>Adjusted for:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Step 1.</td>
<td>-0.02</td>
<td>-0.64</td>
<td>0.522</td>
<td>0.10</td>
</tr>
<tr>
<td>Step 2.</td>
<td>-0.03</td>
<td>-0.85</td>
<td>0.396</td>
<td>0.11</td>
</tr>
<tr>
<td>Step 3.</td>
<td>-0.07</td>
<td>-1.40</td>
<td>0.163</td>
<td>0.13</td>
</tr>
<tr>
<td>Step 4.</td>
<td>-0.08</td>
<td>-1.68</td>
<td>0.093</td>
<td>0.14</td>
</tr>
</tbody>
</table>

Adjusted for: - Age in Step 1;
- Age, smoking status, alcohol consumption, and physical activity in Step 2;
- Age, LDL-cholesterol, HDL-cholesterol, triglycerides, plasma insulin, systolic/diastolic blood pressure, and BMI in Step 3;
- Age, LDL-cholesterol, HDL-cholesterol, triglycerides, plasma insulin, systolic/diastolic blood pressure, BMI, smoking status, alcohol consumption, and physical activity in Step 4.
Fig. 1.

![Graph showing the relationship between IL-6 174 genotype and vitality exhaustion. P-values and statistical significance are indicated.](image)
Figures captions

Fig. 1. Differences in vital exhaustion between genotype groups.

Note: IL-6, interleukin-6.

ns – non-significant.