Research Article

Association of CILP2 and ACE Gene Polymorphisms with Cardiovascular Risk Factors in Slovak Midlife Women

Lenka Luptáková, Dominika Benčová, Daniela Siváková, and Marta Cvičelová

Department of Anthropology, Faculty of Natural Sciences, Comenius University, Mlynska Dolina, 842 15 Bratislava, Slovakia

Correspondence should be addressed to Lenka Luptáková; luptakova@fns.uniba.sk

Received 29 April 2013; Revised 14 September 2013; Accepted 24 September 2013

Academic Editor: Susumu Minamisawa

Copyright © 2013 Lenka Luptáková et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

The aim of this study is to assess the association of two polymorphisms, the cartilage intermediate layer protein 2 (CILP2) G/T and angiotensin converting enzyme (ACE) I/D, with blood pressure and anthropometrical and biochemical parameters related to the development of cardiovascular disease. The entire study sample comprised 341 women ranging in age from 39 to 65 years. The CILP2 genotypes were determined by PCR-RFLP and the ACE genotypes by PCR. The Bonferroni pairwise comparisons showed the effect of the CILP2 genotype on high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), apolipoprotein B (apoB), apoB-to-apoA1 ratio, the total cholesterol (TC)-to-HDL-C ratio, non-HDL-C, and the LDL-C-to-HDL-C ratio ($P < 0.05$). Here, higher mean levels of HDL-C and lower mean levels of the remaining above mentioned lipid parameters were registered in the GT/TT genotype carriers than in GG carriers. Statistically significant association was identified between the ACE genotype and the following parameters: TC, LDL-C, and non-HDL-C ($P < 0.05$). The II genotype can lower serum level of TC ($B = 0.40$), LDL-C ($B = 0.37$), and non-HDL-C levels. The results of this study suggest that the minor T allele of CILP2 gene and I allele of ACE gene have a protective effect against elevated serum lipid and lipoprotein levels.

1. Introduction

Increased blood lipid and lipoprotein levels, low HDL cholesterol concentration, glucose intolerance, hypertension, and obesity have emerged as some of the most serious public health concerns in recent decades. These variables are closely related to a number of pathological disorders including cardiovascular disease (CVD). Although recent increases in CVD risk factors often reflect lifestyle changes, genetic factors also play a substantial role. Genome-wide association studies have revealed the association of DNA polymorphisms in both the CILP2 gene (cartilage intermediate layer protein) and the ACE gene (angiotensin converting enzyme) with CVD risk factors [1–3].

The CILP2 gene codes for a noncollagenous protein recently isolated from human articular cartilage. Kathiresan et al. [1] reported that an intergenic region between CILP2 and PBX4 (pre-B-cell leukaemia homeobox 4) located in chromosome 19p13 is associated with concentrations of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). The minor allele at SNP rs169996148 was associated with lower concentrations of both LDL-C and TG. In addition, Tai et al. [4] examined the association between this polymorphism and elevated high density lipoprotein cholesterol (HDL-C) levels in an Asian Malay population. In Slovakia, Rašlová et al. [5] identified an association between CILP2 allele and atherogenic index log (TG-to-HDL-C ratio) in Slovak women and FERHDL (cholesterol esterification rate in HDL plasma) in both genders. Genetic analysis has also highlighted a significant association between polymorphisms in the CILP gene and osteoarthritis progression [6].

Angiotensin converting enzyme (ACE) plays an important role in the pathophysiology of CVD. Although ACE is mainly localized in the endothelium of blood vessels, especially in the pulmonary circulation, it is also found in epithelial cells, in mononuclear blood vessels, and in macrophages [7]. ACE is a key enzyme in the body’s renin-angiotensin system (RAS), modulating the synthesis of angiotensin II and inactivation of bradykinin. The ACE gene has an insertion/deletion (I/D) polymorphism, with the D allele
Table 1: Anthropometrical, biochemical variables and blood pressure in Slovak women by menopausal status.

| Parameter          | Total   | Premenopause | Postmenopause | P^μ |
|--------------------|---------|--------------|---------------|-----|
|                    | n = 341 | n = 194      | n = 147       |     |
| Age (years)^a      | 49.11 ± 5.61 | 45.78 ± 3.93 | 53.50 ± 4.33 | <0.001 |
| Weight (kg)^a      | 73.30 ± 15.22 | 71.92 ± 14.92 | 75.12 ± 15.46 | 0.786 |
| WC (cm)            | 85.79 ± 14.24 | 83.29 ± 14.63 | 89.08 ± 13.04 | 0.722 |
| HC (cm)^a          | 104.29 ± 14.24 | 102.85 ± 10.17 | 106.19 ± 11.26 | 0.804 |
| BMI (kg/m^2)^a     | 27.42 ± 5.56 | 26.59 ± 5.42 | 28.50 ± 5.57 | 0.307 |
| WHR                | 0.82 ± 0.08 | 0.81 ± 0.09 | 0.84 ± 0.07 | 0.602 |
| GMT ([μkat/L]^a     | 0.42 ± 0.43 | 0.34 ± 0.30 | 0.52 ± 0.54 | 0.022 |
| ALT ([μkat/L]^a     | 0.33 ± 0.20 | 0.29 ± 0.18 | 0.38 ± 0.22 | 0.030 |
| UA ([μmol/L]^a      | 256.00 ± 65.88 | 243.88 ± 64.09 | 272.00 ± 64.98 | 0.782 |
| Bilirubin ([μmol/L]^a | 8.88 ± 4.16 | 9.01 ± 4.30 | 8.72 ± 3.98 | 0.831 |
| Glucose ([mmol/L]^a | 5.01 ± 1.38 | 4.78 ± 0.69 | 5.23 ± 0.72 | 0.103 |
| HDL-C ([mmol/L]^a   | 1.56 ± 0.42 | 1.57 ± 0.43 | 1.53 ± 0.41 | 0.141 |
| LDL-C ([mmol/L]^a   | 3.25 ± 0.95 | 3.17 ± 0.85 | 3.37 ± 1.06 | 0.734 |
| apoA1 ([g/L]^a      | 1.71 ± 0.42 | 1.72 ± 0.51 | 1.69 ± 0.25 | 0.522 |
| apoB ([g/L]^a       | 0.94 ± 0.25 | 0.92 ± 0.24 | 0.97 ± 0.27 | 0.711 |
| apoB-to-apoA1       | 0.57 ± 0.18 | 0.55 ± 0.17 | 0.59 ± 0.20 | 0.537 |
| TC-to-HDL-C^a       | 3.73 ± 1.14 | 3.61 ± 1.08 | 3.88 ± 1.21 | 0.176 |
| non-HDL-C           | 3.89 ± 1.06 | 3.76 ± 0.94 | 4.07 ± 1.17 | 0.624 |
| LDL-C-to-HDL-C^a    | 2.26 ± 0.93 | 2.18 ± 0.88 | 2.37 ± 1.00 | 0.171 |
| log(TG-to-HDL-C)    | −0.09 ± 0.29 | −0.13 ± 0.29 | −0.05 ± 0.29 | 0.239 |
| sBP (mmHg)^a        | 123 ± 16.94 | 120 ± 15.55 | 127 ± 18.22 | 0.581 |
| dBP (mmHg)^a        | 79 ± 11.32 | 78 ± 10.16 | 80 ± 12.72 | 0.508 |

WC: waist circumference; HC: hip circumference; BMI: body mass index; WHR: waist-to-hip ratio; GMT: gamma glutamyl transpeptidase, ALT: alanine aminotransferase; UA: uric acid; TC: total cholesterol; TG: triglycerides, HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein; apoA1: apolipoprotein A1; apoB: apolipoprotein B; sBP: systolic blood pressure; dBP: diastolic blood pressure. Values represent mean ± SD. "^a" Not normally distributed parameters; "^μ" adjusted for age.

2. Subjects and Methods

This study is a part of cross-sectional survey conducted in Slovakia between 2009 and 2013 to analyze the effect of menopause on biomarkers of health in pre- and postmenopausal women. The entire study sample comprised 341 women ranging in age from 39 to 65 years (mean age = 49.11 ± 5.61). Of these, 259 participants provided all required data from the questionnaire and also anthropometrical, genetic, and biochemical data. The remainder (n = 82) failed to provide adequate information concerning at least one of these factors. Subjects were recruited from different localities in the western and middle parts of Slovakia via an invitation letter for the study circulated and distributed prior to data collection with the help of local medical doctors. Participants were then interviewed in a medical examination in the morning, and they were investigated with respect to their medical, anthropometrical, and life style aspects at local health centres. However, only selected variables were considered for the purpose of this paper. All participants gave written
Table 2: Anthropometrical, biochemical variables and blood pressure according to CILP2 genotypes in Slovak women.

| Parameter          | CILP2 genotype | Menopause status * CILP2 |       |
|--------------------|----------------|--------------------------|-------|
|                    |                |                          |       |
|                    | GG n = 299     | GT/TT n = 31              |       |
| Weight (kg)        | 73.35 ± 15.61  | 71.97 ± 12.05             | 0.303 | 0.583 |
| WC (cm)            | 85.66 ± 14.55  | 84.20 ± 11.42             | 0.479 | 0.489 |
| HC (cm)            | 104 ± 11.11    | 105 ± 8.66                | 0.012 | 0.911 |
| BMI (kg/m²)        | 27.47 ± 5.68   | 26.43 ± 4.56              | 1.203 | 0.274 |
| WHR                | 0.82 ± 0.08    | 0.80 ± 0.08               | 1.386 | 0.240 |
| GMT (µkat/L)       | 0.40 ± 0.40    | 0.47 ± 0.39               | 0.910 | 0.341 |
| ALT (µkat/L)       | 0.33 ± 0.20    | 0.37 ± 0.23               | 0.949 | 0.331 |
| UA (µmol/L)        | 255 ± 65.55    | 256 ± 67.38               | 0.000 | 0.996 |
| TC (mmol/L)        | 5.45 ± 1.05    | 5.10 ± 0.83               | 3.629 | 0.058 |
| TG (mmol/L)        | 1.42 ± 0.89    | 1.17 ± 0.47               | 2.733 | 0.099 |
| Bilirubin (µmol/L) | 8.82 ± 4.13    | 9.58 ± 4.47               | 0.963 | 0.327 |
| Glucose (mmol/L)   | 5.02 ± 1.41    | 4.79 ± 0.82               | 1.032 | 0.310 |
| HDL-C (mmol/L)     | 1.54 ± 0.40    | 1.76 ± 0.53               | 7.810 | 0.006 |
| LDL-C (mmol/L)     | 3.29 ± 0.96    | 2.84 ± 0.72               | 6.076 | 0.014 |
| apoA1 (g/L)        | 1.70 ± 0.43    | 1.77 ± 0.27               | 0.689 | 0.407 |
| apoB (g/L)         | 0.95 ± 0.26    | 0.80 ± 0.18               | 9.289 | 0.003 |
| apoB-to-apoA1      | 0.58 ± 0.18    | 0.47 ± 0.14               | 10.572| 0.001 |
| TC-to-HDL-C        | 3.77 ± 1.13    | 3.13 ± 0.93               | 8.601 | 0.004 |
| non-HDL-C          | 3.92 ± 1.07    | 3.38 ± 0.81               | 7.497 | 0.007 |
| LDL-C-to-HDL-C     | 2.29 ± 0.92    | 1.78 ± 0.77               | 8.454 | 0.004 |
| log(TG-to-HDL-C)   | −0.09 ± 0.29   | −0.18 ± 0.26              | 2.989 | 0.085 |
| sBP (mmHg)         | 122 ± 17.16    | 126 ± 16.07               | 0.956 | 0.329 |
| dBP (mmHg)         | 79 ± 11.54     | 79 ± 10.38                | 0.052 | 0.820 |

WC: waist circumference; HC: hip circumference; BMI: body mass index; WHR: waist-to-hip ratio; GMT: gamma glutamyl transpeptidase. ALT: alanine aminotransferase; UA: uric acid; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein; apoA1: apolipoprotein A1; apoB: apolipoprotein B; sBP: systolic blood pressure; dBP: diastolic blood pressure. Values represent mean ± SD. aAdjusted for age; badjusted for age, BMI, and WHR.

Informed consent for participation in the study and they were always accompanied to their local health centre by trained anthropologists.

Data concerning lifestyle habits including physical activity, smoking, and their health status and menstrual cycle characteristics were investigated by assisted questionnaire.

Women were divided according to their menopausal status (MS) into pre- and postmenopausal groups, in accordance with the WHO definition [15].

All anthropometrical parameters were measured by professional anthropologists and the same instruments were used for all subjects. Anthropometric measurements were taken using standard anthropometric technique [16]. Blood pressure was measured in the morning, in the sitting position using a mercury sphygmomanometer, during a medical examination.

Venous blood was collected after overnight fasting. The plasma was separated and biochemical analysis of gamma glutamyl transpeptidase (GMT), alanine aminotransferase (ALT), uric acid (UA), total cholesterol (TC), bilirubin (Bil), fasting blood glucose (Gl), total cholesterol (TC), TG, HDL-C, apolipoprotein Al (apoAl), and apolipoprotein B (apoB) was carried out by routine laboratory methods in the Department of Clinical Laboratories of the Bratislava Alpha Medical. The LDL-C levels were calculated using the Friedewald formula [17]. The atherogenic indices were calculated as follows: apoB-to-apoAl ratio, TC-to-HDL-C ratio, LDL-C-to-HDL-C ratio, log(TG-to-HDL-C ratio), and non-HDL-C as TC-HDL-C.

2.1. Genetic Analysis. Genomic DNA was extracted from peripheral blood samples using the SiMax Genomic DNA Extraction kit (Ecoli). PCR was used to detect the presence of the insertion (I) and deletion (D) alleles in intron 16 of the ACE gene, as previously described by Rigat et al. [18] and Danková et al. [19]. Genotyping of CILP2 polymorphism (rs16996148 variant near CILP/PBX4 genes) was carried out by PCR-RFLP, as described in Rašlová et al. [5]. PCR product (135 bp) was cleaved by restriction enzyme HinIII (Fermentas) and separated on 4% agarose gel (Super Fine...
HDL-C, and apoA1 and higher values for all other selected postmenopausal women had lower mean values for bilirubin, a common effect of menopause.

Two-way analysis of variance was used to analyze variables, with age, BMI, and waist-to-hip ratio (WHR), TC-to-HDL-C, and LDL-C-to-HDL-C not normally distributed and required logarithmic transformation. Only those variables that had values of P < 0.05 in the univariate analysis were included in the regression analysis as dependent variables. The values of age, BMI, WHR, TC-to-HDL-C, and LDL-C-to-HDL-C were not normally distributed and required logarithmic transformation. In addition, two-way analysis of variance was used to analyze a common effect of CILP2 and ACE polymorphisms as risk factors on the values of LDL-C, and non-HDL-C. All statistical computations were performed with the SPSS 17.0 software program (SPSS Inc., Chicago, IL). A P value of less than 0.05 was considered statistically significant.

### 3. Results

The mean values of anthropometric, biochemical characteristics, and blood pressure of women in each menopausal status are shown in Table 1. As expected, postmenopausal women had lower mean values for bilirubin, HDL-C, and apoA1 and higher values for all other selected variables than the premenopausal ones. However, after adjustment for age these differences remained significant only for liver enzymes GMT and ALT.

The genotype distribution and allele frequencies of the CILP2 gene polymorphism in the entire sample fell within the Hardy-Weinberg equilibrium ($\chi^2 = 8.01, df = 1, P < 0.005$). The CILP2 genotype and allele frequencies were as follows: GG = 90.6% ($n = 299$), GT = 9.1% ($n = 30$), TT = 0.3% ($n = 1$). The genotype distribution and allele frequencies of the ACE gene polymorphism in the entire sample did not fall within the Hardy-Weinberg equilibrium ($\chi^2 = 8.01, df = 1, P < 0.005$). The ACE genotype and allele frequencies were as follows: DD = 35.4% ($n = 111$), ID = 41.4% ($n = 130$), II = 23.2% ($n = 73$) D allele = 56%, and I allele = 44%. To address the association and impact of the CILP2 polymorphism on CVD risk factors, we evaluated the mean values of anthropometrical and biochemical parameters on each genotype and tested the significance of differences between GG and GT/TT genotypes by ANCOVA. A statistically significant impact of particular genotypes on the investigated parameters (Table 2) was evident in the following variables: HDL-C ($P = 0.007$), LDL-C ($P = 0.016$), apoB ($0.004$), apoB-to-apoA1 ratio ($P = 0.002$), TC-to-HDL-C ratio ($P = 0.005$), non-HDL-C ($P = 0.009$), and LDL-C-to-HDL-C ratio ($P = 0.006$), even after adding the age, WHR, and BMI as confounding factors (Table 2). Here, higher mean levels of HDL-C and lower mean levels of the other investigated lipid parameters were registered in the GT/TT genotype carriers than in the GG carriers. Further, we tested the common effect of menopausal status and CILP2 on lipid parameters. However, the two-way analysis of variance did not reveal a statistically significant interaction between these two risk factors and their common effect on lipids ($P > 0.05$). In addition, the Bonferroni pairwise comparisons shown in
Table 4: Anthropometrical, biochemical variables and blood pressure according to ACE genotypes in Slovak women.

| Parameter | ACE genotypes |  |  | Menopausal status *ACE |  |  |  |
|-----------|---------------|---|---|-----------------------|---|---|---|
|           | DD | ID | II | DD versus ID versus II | II versus ID/DD |  |  |
| n = 111 | n = 130 | n = 73 |  |  |  |  |  |
| Weight (kg) | 73.30 ± 15.91 | 72.86 ± 15.27 | 73.61 ± 15.10 | 0.062 | 0.940 | 0.024 | 0.877 |
| WC (cm) | 84.36 ± 13.84 | 85.78 ± 14.95 | 87.38 ± 14.35 | 0.697 | 0.499 | 1.018 | 0.314 |
| HC (cm) | 105 ± 11.54 | 104 ± 10.30 | 105 ± 11.20 | 0.549 | 0.578 | 0.347 | 0.556 |
| BMI (kg/m²) | 27.24 ± 5.85 | 27.41 ± 5.56 | 27.56 ± 5.34 | 0.021 | 0.979 | 0.027 | 0.868 |
| WHR | 0.80 ± 0.07 | 0.82 ± 0.09 | 0.83 ± 0.08 | 2.422 | 0.090 | 1.162 | 0.282 |
| GMT (µkat/L) | 0.41 ± 0.38 | 0.39 ± 0.35 | 0.42 ± 0.51 | 1.018 | 0.080 | 0.121 | 0.729 |
| ALT (µkat/L) | 0.34 ± 0.22 | 0.33 ± 0.20 | 0.30 ± 0.17 | 1.109 | 0.331 | 1.990 | 0.159 |
| UA (µmol/L) | 257 ± 69.37 | 254 ± 64.61 | 258 ± 66.42 | 0.096 | 0.908 | 0.030 | 0.860 |
| Bilirubin (µmol/L) | 8.62 ± 4.06 | 9.38 ± 4.80 | 8.53 ± 3.33 | 0.135 | 0.873 | 0.121 | 0.729 |
| Glucose (mmol/L) | 5.02 ± 1.38 | 4.98 ± 1.56 | 5.01 ± 1.00 | 0.070 | 0.933 | 0.008 | 0.928 |
| HDL-C (mmol/L) | 1.57 ± 0.41 | 1.60 ± 0.46 | 1.54 ± 0.38 | 0.393 | 0.676 | 0.480 | 0.489 |
| LDL-C (mmol/L) | 3.37 ± 0.99 | 3.33 ± 0.95 | 3.03 ± 0.83 | 2.619 | 0.075 | 5.011 | 0.027 |
| apoA1 (g/L) | 1.73 ± 0.26 | 1.75 ± 0.59 | 1.62 ± 0.24 | 1.855 | 0.158 | 3.646 | 0.057 |
| apoB (g/L) | 0.95 ± 0.25 | 0.95 ± 0.28 | 0.89 ± 0.20 | 1.603 | 0.203 | 3.172 | 0.076 |
| apoB-to-apoA1 | 0.57 ± 0.19 | 0.57 ± 0.18 | 0.56 ± 0.17 | 0.019 | 0.981 | 0.035 | 0.851 |
| TC-to-HDL-C | 3.74 ± 1.14 | 3.69 ± 1.12 | 3.58 ± 1.10 | 0.548 | 0.579 | 0.981 | 0.323 |
| non-HDL-C | 3.94 ± 1.09 | 3.93 ± 1.06 | 3.66 ± 0.96 | 2.268 | 0.105 | 4.549 | 0.023 |
| LDL-C-to-HDL-C | 2.26 ± 0.95 | 2.25 ± 0.88 | 2.12 ± 0.90 | 0.660 | 0.517 | 1.319 | 0.252 |
| log(TG-to-HDL-C) | −0.08 ± 0.29 | −0.12 ± 0.30 | −0.10 ± 0.29 | 0.492 | 0.612 | 0.004 | 0.949 |
| sBP (mmHg) | 123 ± 17.77 | 123 ± 18.71 | 121.03 ± 13.65 | 0.781 | 0.459 | 1.509 | 0.220 |
| dBP (mmHg) | 79 ± 9.41 | 79 ± 11.20 | 78.04 ± 14.56 | 0.260 | 0.771 | 0.451 | 0.503 |

WC: waist circumference; HC: hip circumference; BMI: body mass index; WHR: waist-to-hip ratio; GMT: gamma glutamyl transpeptidase, ALT: alanine aminotransferase; UA: uric acid; TC: total cholesterol; TG: triglycerides, HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein; apoA1: apolipoprotein A1; apoB: apolipoprotein B; sBP: systolic blood pressure; dBP: diastolic blood pressure. Values represent mean ± SD. aAdjusted for age; bAdjusted for age, BMI, and WHR.

Table 3 confirmed the effect of CILP2 genotype on the above mentioned parameters (P < 0.05).

Table 4 shows differences in the mean values of particular variables between the II, ID, and DD genotypes of the ACE gene tested for significance by ANCOVA models. There was a statistically significant association between genotype and the following parameters: TC (P = 0.035), LDL-C (P = 0.027), and non-HDL-C (P = 0.023). The DD and ID carriers had significantly higher TC, LDL-C, and non-HDL-C levels than the II genotype carriers, even after adjustment for age, BMI, and WHR. In addition, we tested the common effect of menopause status and ACE on lipid parameters. However, the two-way analysis of variance did not reveal a statistically significant interaction between these two risk factors and their common effect on TC, LDL-C, and non-HDL-C.

A stepwise regression analysis was used to test the independent impact of CILP2 and ACE gene polymorphisms and other considered risk factors on the lipid and lipoprotein parameters (Table 6). The regression analysis confirmed the effect of ACE genotype on the TC, LDL-C, and non-HDL-C, as previously detected in ANCOVA models. Here, the II genotype and ID/DD genotype groups were compared and positive B coefficient was determined, indicating that the II genotype can lower serum levels of TC (B = 0.40), LDL-C (B = 0.37), and non-HDL-C (B = 0.41), respectively. In the same table, the stepwise regression analysis also confirmed the effect of CILP2 genotypes (GT/TT versus GG) on LDL-C, non-HDL-C, HDL-C, apoB, and three atherogenic indices (apoB-to-apoA1, TC-to-HDL-C, and LDL-C-to-HDL-C). The positive values of estimated B coefficient indicated the lowering effect of the minor T allele on all lipid parameters.
Table 5: Bonferroni pairwise comparisons between ACE genotypes and lipid levels.

| Parameter | ACE genotype | Estimated marginal mean | SE | 95% CI | Mean difference | SE | P | 95% CI for difference |
|-----------|--------------|-------------------------|----|--------|----------------|----|----|----------------------|
| LDL-C     | II           | 3.02                    | 0.11 | 2.79  | 3.24           | II versus ID/DD | −0.29 | 0.13 | 0.027 | −0.55 | −0.03 |
|           | DD/ID       | 3.31                    | 0.06 | 3.18  | 3.43           | II versus ID/DD | −0.29 | 0.14 | 0.035 | −0.56 | −0.02 |
| TC        | II           | 5.21                    | 0.12 | 4.98  | 5.45           | II versus ID/DD | −0.29 | 0.14 | 0.035 | −0.56 | −0.02 |
|           | DD/ID       | 5.50                    | 0.07 | 5.37  | 5.63           | II versus ID/DD | −0.33 | 0.14 | 0.023 | −0.60 | −0.05 |
| non-HDL-C | II           | 3.62                    | 0.12 | 3.38  | 3.87           | II versus ID/DD | −0.33 | 0.14 | 0.023 | −0.60 | −0.05 |
|           | DD/ID       | 3.95                    | 0.07 | 3.81  | 4.08           | II versus ID/DD | −0.33 | 0.14 | 0.023 | −0.60 | −0.05 |

Based on estimated marginal means.
Adjustment for multiple comparisons: Bonferroni.

Figure 1: Association between ACE/CILP2 genotypes and LDL cholesterol in Slovak women.

Figure 2: Association between ACE/CILP2 genotypes and nonHDL cholesterol.

4. Discussion

In this study, we determined a profound impact of the CILP2 gene on HDL-C, LDL-C, apoB, non-HDL-C levels, and three atherogenic indices in Slovak women. Only scanty and inconsistent information exists so far on associating this polymorphism with blood lipids. A relationship between CILP2 gene polymorphism and TG and LDL-C concentrations was documented in European population, where the minor T allele was associated with lower concentrations of TG and LDL-C [1]. According to J´aromi et al. [20], the relation of the CILP2 gene to lipid metabolism is not yet discovered. The observations on the TG-lowering association were not replicated in Japanese population [21], in Hungarian population [20], or in the 40-years-old Slovak population [5]. Our study also failed to replicate the association between CILP2 and TG concentrations. However, our results indicated that the minor T allele was associated with lower LDL-C, apoB, and atherogenic indices and higher HDL-C levels. In addition, Tai et al. [4] conducted a cross-sectional study which examined the relationship between CILP2 gene polymorphism; blood lipid levels, and CVD prevalence in...
Table 6: Regression analysis of selected confounder effects on lipid, and lipoprotein levels, and atherogenic indices in Slovak women.

| Dependent variable | Independent variable | B     | SE    | Beta  | P      | 95.0% CI for B | Collinearity statistics tolerance |
|--------------------|----------------------|-------|-------|-------|--------|----------------|----------------------------------|
| TC                 | ln age               | 1.72  | 0.52  | 0.89  | 0.008  | 0.70 - 2.73    | 0.99                             |
|                    | Current smoker       | 0.32  | 0.09  | 0.20  | 0.008  | 0.14 - 0.50    | 0.98                             |
|                    | ACE: II versus ID/DD | 0.40  | 0.14  | 0.16  | 0.005  | 0.12 - 0.69    | 0.98                             |
| n = 292            |                      |       |       |       |        |                |                                  |
| LDL-C              | ln age               | 1.25  | 0.51  | 0.15  | 0.016  | 0.24 - 2.26    | 0.99                             |
|                    | Current smoker       | 0.32  | 0.09  | 0.20  | 0.008  | 0.14 - 0.50    | 0.98                             |
|                    | ACE: II versus ID/DD | 0.40  | 0.14  | 0.16  | 0.005  | 0.12 - 0.69    | 0.98                             |
| n = 259            |                      |       |       |       |        |                |                                  |
| non-HDL-C          | ln age               | 1.52  | 0.59  | 0.16  | 0.010  | 0.37 - 2.67    | 0.91                             |
|                    | WHR                  | 2.39  | 0.84  | 0.17  | 0.005  | 0.73 - 4.05    | 0.91                             |
|                    | Current smoker       | 0.33  | 0.10  | 0.20  | 0.001  | 0.14 - 0.53    | 0.98                             |
|                    | ACE: II versus ID/DD | 0.41  | 0.16  | 0.16  | 0.008  | 0.11 - 0.72    | 0.97                             |
|                    | CILP2: GT/TT versus GG | 0.52  | 0.22  | 0.14  | 0.018  | 0.09 - 0.94    | 1.00                             |
| n = 259            |                      |       |       |       |        |                |                                  |
| HDL-C              | ln age               | 0.49  | 0.20  | 0.14  | 0.017  | 0.09 - 0.89    | 0.92                             |
|                    | ln BMI               | −0.59 | 0.14  | −0.28 | <0.001 | −0.86 - −0.32  | 0.69                             |
|                    | WHR                  | −0.89 | 0.35  | −0.17 | 0.010  | −1.57 - −0.21  | 0.67                             |
|                    | CILP2: GT/TT versus GG | −0.21 | 0.08  | −0.14 | 0.009  | −0.36 - −0.05  | 0.99                             |
| n = 282            |                      |       |       |       |        |                |                                  |
| apoB               | ln BMI               | 0.26  | 0.08  | 0.19  | 0.001  | 0.10 - 0.41    | 0.99                             |
|                    | Current smoker       | 0.05  | 0.02  | 0.12  | 0.038  | 0.00 - 0.09    | 1.00                             |
|                    | CILP2: GT/TT versus GG | 0.14  | 0.05  | 0.15  | 0.011  | 0.03 - 0.24    | 1.00                             |
| n = 282            |                      |       |       |       |        |                |                                  |
| apoB-to-apoA1      | ln BMI               | 0.23  | 0.05  | 0.25  | <0.001 | 0.13 - 0.34    | 0.99                             |
|                    | Current smoker       | 0.04  | 0.02  | 0.13  | 0.026  | 0.00 - 0.07    | 1.00                             |
|                    | CILP2: GT/TT versus GG | 0.11  | 0.04  | 0.16  | 0.004  | 0.03 - 0.18    | 1.00                             |
| n = 282            |                      |       |       |       |        |                |                                  |
| ln TC-to-HDL-C     | ln BMI               | 0.37  | 0.10  | 0.24  | <0.001 | 0.17 - 0.57    | 0.70                             |
|                    | WHR                  | 0.71  | 0.25  | 0.18  | 0.005  | 0.21 - 1.20    | 0.70                             |
|                    | Current smoker       | 0.06  | 0.03  | 0.13  | 0.022  | 0.01 - 0.11    | 1.00                             |
|                    | CILP2: GT/TT versus GG | 0.17  | 0.06  | 0.16  | 0.004  | 0.06 - 0.29    | 1.00                             |
| n = 282            |                      |       |       |       |        |                |                                  |
| ln LDL-C-to-HDL-C  | ln BMI               | 0.61  | 0.12  | 0.28  | <0.001 | 0.37 - 0.85    | 0.99                             |
|                    | Current smoker       | 0.08  | 0.04  | 0.13  | 0.024  | 0.01 - 0.15    | 1.00                             |
|                    | CILP2: GT/TT versus GG | 0.25  | 0.08  | 0.17  | 0.003  | 0.08 - 0.42    | 1.00                             |
| n = 282            |                      |       |       |       |        |                |                                  |

\( R^2 = 0.085, \) adjusted \( R^2 = 0.075, \) SE = 1.008

Excluded variables: menopausal status, BMI, WHR, former sport activities, and recent sport activities, current smoker.

\( R^2 = 0.086, \) adjusted \( R^2 = 0.071, \) SE = 0.924

Excluded variables: menopausal status, BMI, WHR, former sport activities, and recent sport activities.

\( R^2 = 0.136, \) adjusted \( R^2 = 0.119, \) SE = 1.009

Excluded variables: menopausal status, BMI, WHR, former sport activities, and recent sport activities, current smoker.

\( R^2 = 0.075, \) adjusted \( R^2 = 0.065, \) SE = 0.249

Excluded variables: age, menopausal status, WHR, former sport activities, and recent sport activities.

\( R^2 = 0.106, \) adjusted \( R^2 = 0.096, \) SE = 0.172

Excluded variables: age, menopausal status, WHR, former sport activities, and recent sport activities.

\( R^2 = 0.182, \) adjusted \( R^2 = 0.170, \) SE = 0.273

Excluded variables: age, menopausal status, WHR, former sport activities, and recent sport activities.

\( R^2 = 0.124, \) adjusted \( R^2 = 0.115, \) SE = 0.395

Excluded variables: age, menopausal status, WHR, former sport activities, and recent sport activities.
the Singaporean population ranging from 40 to 80 years of age. They found an association of the CILP2 (T allele) with elevated HDL-C ($P = 0.005$) and lower LDL-C ($P = 0.048$) levels. Contrary to this finding, Zhuang et al. [22] did not observe a significant relationship between the CILP2 gene and the serum lipid profile in the Japanese population. However, they investigated a lower frequency of T allele in patients with ischemic heart disease and 33% lower risk of the disease prevalence. Yan et al. [23] reported that the levels of TC, HDL-C, LDL-C, apoA1, and apoB in Han population (China) were associated with the CILP2 genotypes in males but not in females. The inconsistent results in the above-mentioned association studies could be caused by the different investigated populations and ethnic groups. Their exposure to different lifestyles and environments could modify the effect of these genetic variations on blood lipids. Different sample sizes could also play a role in the various findings.

When evaluating the impact of ACE I/D gene polymorphism on anthropometrical and biochemical parameters, we identified a statistically significant relationship with TC, LDL-C, and non-HDL-C in Slovak women. The DD/DD genotype carriers exhibited a worse lipid profile than the II carriers. Contrary to this finding, Cubrilo-Turek et al. [24] did not reveal statistically significant differences between the ACE DD/ID/II groups; the serum lipid, and apolipoprotein concentrations in Croatian menopausal women.

We have considered common influence of menopausal status and ACE I/D polymorphism on lipid parameters due to the fact that according to Proudl er et al. [25] the serum ACE activity is modifiable, at least in part, by circulating levels of oestrogen and progestagen, which are levels that vary during menopausal transition. However, this effect was not confirmed in our study.

In addition, the findings in this study showed a lack of association between the ACE genotype and blood pressure, and this is consistent with the previous studies in the Slovak population [19, 26].

In accordance with other studies [27–29], we found no evidence to suggest that the three ACE genotypes differ in BMI or WHR values. Moreover, Ryan et al. [30] suggest that total body fat mass, visceral and subcutaneous abdominal fat areas, plasma lipid levels, and systolic and diastolic blood pressures were not influenced by the ACE genotype in Caucasian and Afro-American women. Although, Bienertova-Vasku et al. [31] reported that the ACE I/D polymorphism did not express a prediction role on any of the investigated parameters of BMI, total body fat, total body water, waist circumference, hip circumference, WHR, and total body fat in Czech population, Das et al. [32] found that combined APOE*4/4 and ACE DD genotypes had significant associations with elevated blood pressure, lipid abnormalities, and metabolic syndrome in adult Asian Indians.

5. Conclusion

The results of this study indicate that the minor T allele of the CILP2 gene and the I allele of the ACE gene have a protective effect against elevated blood lipid and lipoprotein levels.

Acknowledgments

The authors wish to thank Dr. Pavel Blažiček for biochemical analysis and Dr. Ladislava Wsólóva for statistical advice. This study was supported by the Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic (VEGA 1/0247/09, 1/0493/13).

References

[1] S. Kathiresan, O. Melander, C. Guiducci et al., “Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans,” Nature Genetics, vol. 40, no. 2, pp. 189–197, 2008.
[2] T. Sipahi, M. Budak, S. Şen, A. Ay, and S. Şener, “Association between ACE gene insertion (I)/deletion (D) polymorphism and primary hypertension in Turkish patients of Trakya region,” Biotechnology and Biotechnological Equipment, vol. 20, no. 2, pp. 104–108, 2006.
[3] S. Fialat, E. Szigethy, G. Széles, R. Tóth, and R. Ádány, “Insertion/deletion polymorphism of angiotensin-1 converting enzyme is associated with metabolic syndrome in Hungarian adults,” Journal of the Renin-Angiotensin-Aldosterone System, vol. 12, no. 4, pp. 531–538, 2011.
[4] E. S. Tai, X. L. Sim, T. H. Ong et al., “Polymorphisms at newly identified lipid-associated loci are associated with blood lipids and cardiovascular disease in an Asian Malay population,” Journal of Lipid Research, vol. 50, no. 3, pp. 514–520, 2009.
[5] K. Rašlová, M. Dobiašová, A. J. Hubáček et al., “Association of metabolic and genetic factors with cholesterol esterification rate in HDL plasma and atherogenic index of plasma in a 40 years old Slovak population,” Physiological Research, vol. 60, no. 5, pp. 785–795, 2011.
[6] A. M. Valdes, D. J. Hart, K. A. Jones et al., “Association study of candidate genes for the prevalence and progression of knee osteoarthritis,” Arthritis and Rheumatism, vol. 50, no. 8, pp. 2497–2507, 2004.
[7] F. Cambien, “The angiotensin-converting enzyme (ACE) genetic polymorphism: its relationship with plasma ACE level and myocardial infarction,” Clinical Genetics, vol. 46, no. 1, pp. 94–101, 1994.
[8] B. Rigat, C. Hubert, F. Alhenc-Gelas, F. Cambien, P. Corvol, and F. Soubrier, “An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels,” The Journal of Clinical Investigation, vol. 86, no. 4, pp. 1343–1346, 1990.
[9] A. H. J. Danser, M. A. D. H. Schalekamp, W. A. Bax et al., "Angiotensin-converting enzyme in the human heart: effect of the deletion/insertion polymorphism," Circulation, vol. 92, no. 6, pp. 1387–1388, 1995.

[10] B. Petelin, D. Petrović, M. Zorc, and I. Keber, “Deletion/insertion polymorphism in the angiotensin—converting enzyme gene as a risk factor in the Slovenian patients with coronary heart disease,” Pflügers Arch—European Journal of Physiology, vol. 439, no. 7, pp. R40–R41, 2000.

[11] F. Cambien, O. Poirier, L. Leercr et al., “Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction,” Nature, vol. 359, no. 6396, pp. 641–644, 1992.

[12] P. Strazzullo, R. Iacone, L. Iacoviello et al., “Genetic variation in the renin-angiotensin system and abdominal adiposity in men: the olivetti prospective heart study,” Annals of Internal Medicine, vol. 138, no. 1, pp. 17–23, 2003.

[13] Badaruddoza and N. Sudhir, “No evidence for association between ACE gene insertion (I)/deletion (D) polymorphism and hypertension in North Indian Punjabi population,” International Journal of Human Genetics, vol. 12, pp. 179–185, 2012.

[14] M. Zajc-Petranović, T. Skarić-Jurić, N. Smolej-Narančić et al., “Angiotensin converting enzyme deletion allele is beneficial for the longevity for Europeans,” Age, vol. 34, no. 3, pp. 583–595, 2012.

[15] World Health Organization, Research on the Menopause in the 1990s: Report of a WHO Scientific Group, WHO Technical Report Series, no. 866, World Health Organization, 1996.

[16] R. Knussmann, Anthropologie. Band I: Wesen und Methoden der Anthropologie, Gustav Fischer, Stuttgart, Germany, 1988.

[17] W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, “Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge,” Clinical Chemistry, vol. 18, no. 6, pp. 499–502, 1972.

[18] B. Rigat, C. Hubert, P. Corvol, and F. Soubrier, “PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCPI) (dipeptidyl carboxypeptidase I),” Nucleic Acids Research, vol. 20, no. 6, p. 1433, 1992.

[19] Z. Danková, D. Siváková, L. Luptákova, and P. Blažíček, “Association of ACE, (I/D) polymorphism with metabolic syndrome and hypertension in two ethnic groups in Slovakia,” Anthropologischer Anzeiger, vol. 67, pp. 305–316, 2009.

[20] L. Járomi, V. Csöngei, N. Polgár et al., “Triglyceride level-influencing functional variants of the ANGPTL3, CILP2, and TRIB1 loci in ischemic stroke,” NeuroMolecular Medicine, vol. 13, no. 3, pp. 179–186, 2011.

[21] K. Nakayama, T. Bayasgalan, K. Yamanaka et al., “Large scale replication analysis of loci associated with lipid concentrations in a Japanese population,” Journal of Medical Genetics, vol. 46, pp. 370–374, 2009.

[22] K. Zhuang, W. Zhang, X. Zhang, F. Wu, and L. Cheng, “Effects of SNPs at newly identified lipids loci on blood lipid levels and risk of coronary heart disease in Chinese Han population: a case control study,” Journal of Huazhong University of Science and Technology. Medical Sciences, vol. 31, no. 4, pp. 452–456, 2011.

[23] T.-T. Yan, R.-X. Yin, Q. Li et al., “Sex-specific association of rs16996148 SNP in the NCAN/CILP2/PBX4 and serum lipid levels in the Mulao and Han populations,” Lipids in Health and Disease, vol. 10, article 248, 2011.