Association of CD36 gene polymorphisms with echo- and electrocardiographic parameters in patients with early onset coronary artery disease

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Abstract

Introduction: CD36 plays an important role in long-chain fatty acid homeostasis in skeletal muscle and the myocardium. CD36 deficiency may lead to reduced myocardial uptake of long-chain fatty acid. Therefore, different mutations of the CD36 gene may contribute to the clinical heterogeneity of cardiac hypertrophy.

Material and methods: The objective of the study was to investigate whether there is an association between the sequence changes in CD36 and echocardiographic and electrocardiographic parameters in Caucasian patients with early onset coronary artery disease. The study group comprised 100 patients. Electrocardiography and echocardiography were performed in all patients. Amplicons of exons 4 to 6 including fragments of introns were studied using the denaturing high-performance liquid chromatography technique.

Results: IVS3-6TC (rs3173798) heterozygotes had impaired left ventricle diastolic function. 573GA heterozygotes (rs5956) had higher frequency of pseudonormal left ventricular diastolic function and it was confirmed by the increase in wave A’ in the tissue Doppler. 591AT genotype was associated with borderline higher posterior wall end-diastolic thickness and lower E/A ratio. These results are consistent with electrocardiography parameters which could reflect left ventricular hypertrophy (higher RV5(6) and RV5(6) + SV1(2) parameters, depressed ST segments and tendency to longer Qtc II interval) in 591AT heterozygotes.

Conclusions: Detected variant alleles of CD36 may be associated with features of left ventricular hypertrophy and impaired diastolic function.

Key words: CD36, CAD, electrocardiography, echocardiography, left ventricular hypertrophy, diastolic function.
Introduction

The myocardium preferentially uses long-chain fatty acid (LCFA) as energy substrate [1]. In cardiomyocytes, the most important fatty acid transporter is fatty acid translocase referred to as CD36 (uptake about 70%) [2]. CD36 deficiency may lead to reduced myocardial uptake of LCFA. Therefore, different mutations of the CD36 gene may contribute to the clinical heterogeneity of hypertrophic cardiomyopathy detected using echocardiography [3]. In the Japanese population association of C478T substitution in the CD36 gene with significant reduction of the myocardial LCFA uptake was reported in patients with angina pectoris, myocardial infarction, hypertrophic cardiomyopathy, dilated cardiomyopathy, hypertension, aortic stenosis and mitral valve disease [4, 5]. Ma et al. [6] reported association of some CD36 polymorphisms (rs1984112, rs1761667, rs1527483, rs3840546, rs1049673) with increased plasma LCFA level in patients with coronary artery disease (CAD) and suggested that these alterations may be associated with a risk of CAD. In patients with dilated cardiomyopathy, fatty acid uptake and oxidation inversely correlate with left ventricular mass index (LVM) and end-diastolic diameter [7]. Thus, myocardial fatty acid metabolism is an independent predictor of left ventricular mass in hypertrophy and in left ventricular dysfunction [8, 9]. The animal model data strongly suggest that CD36 is a candidate gene for pleiotropic effects on LVM and factors related to metabolic syndrome in humans [10]. Heather et al. [11] observed higher left ventricular mass index (LVMI) in patients with the lowest CD36 protein levels in cardiac biopsy material taken from the ventricular apex and atria. The underlying mechanisms by which fatty acid utilization is decreased in cardiac hypertrophy are not fully understood. Moreover, in the myocardium, activation of AMPK (AMP-activated protein kinase) is essential for contraction-induced GLUT4 and CD36 translocation [12]. Glucose-induced CD36 up-regulation is associated with increased uptake of oxidized low-density lipoprotein (oxLDL) and increased oxidative damage, in various tissues including myocardium [13]. The interaction of oxLDL with CD36 triggers a signaling cascade that is necessary for oxLDL uptake and foam cell formation within the atherosclerotic plaque [14-16]. In our previous study [17] on Caucasian patients with CAD we found that the C allele of IVS3-6 T/C polymorphism (rs3173798) is associated with higher prevalence of obesity and diabetes, higher high-sensitivity C-reactive protein (hsCRP) and lower lipoprotein(a) (Lp(a)) serum concentrations and younger age of myocardial infarction. We also found that the A allele of IVS4-10 G/A polymorphism (rs3211892) is associated with older age of myocardial infarction and higher white blood cell count.

The objective of this study was to investigate whether there is an association between the sequence changes in the CD36 gene region encoding the oxLDL- and fatty acid-binding domain and echocardiographic and electrocardiographic parameters in Caucasian patients with CAD.

Material and methods

The study group comprised 100 patients with early onset CAD, including 90 patients described in our previous study. The 74 men were no older than 50 years and the 26 women no older than 55 years. The patients were all Polish residents treated in the Department of Cardiology of the Regional Hospital in Szczecin (northwestern Poland) in 2007-2010. Consecutive, clinically stable patients with optimal pharmacological treatment and no acute coronary syndrome or revascularization procedures within the previous month were included in the study. Patients with hemodynamically significant congenital or acquired valvular heart disease, symptomatic heart failure (NYHA class > 1), serum creatinine > 3 mg/dl, type 1 diabetes mellitus, thyroid dysfunction (current hypo- or hyperthyroidism), or malignancy were excluded from the study. The criteria for CAD diagnosis included angiographically documented presence of at least one coronary lesion (> 40% diameter stenosis of the left main coronary artery or ≥ 50% stenosis of one of the three major epicardial arteries, or ≥ 70% stenosis of a branch) or a history of a revascularization procedure, or evidence of past myocardial infarction. These criteria allowed enrollment of patients with at least moderate coronary atherosclerosis. The study complies with the principles outlined in the Declaration of Helsinki and was approved by our institutional ethics committee. Informed consent was obtained from each patient.

The fasting blood sample was taken for DNA extraction, complete blood count, and measurements of serum glucose, lipid profile (total, high- and low-density lipoprotein cholesterol, and triglycerides), ApoA1, ApoB, and Lp(a). Each patient’s systolic and diastolic blood pressure were measured and body mass index was calculated. Standard protocol 12-lead electrocardiography (ECG) and echocardiography (Medison SA 9900) were performed in all patients. ECG criteria were used according to the cardiac standards applied in Poland [18]. Electrical axis deviation was diagnosed at < -30° or > +90°, ST depression at ≥ 1 mm. End-diastolic and end-systolic volumes assessed using the biplane Simpson’s method were used to calculate left ventricular ejection fraction (LVEF). Left ventricular mass was calculated using the Devereux equation [19]. Left ventricular mass index was calculated by dividing LVM by body surface area [20]. To evaluate left
ventricle diastolic function we used mitral flow pulse wave Doppler imaging with evaluation of E/A ratio and tissue Doppler imaging (TDI) with early diastolic mitral annular velocity measurement and evaluation of septal E’, A’ and E’/A’ ratio. As a criterion for normal diastolic function we used values of E/A 1-2.5 and E’/A’ > 1, impaired diastolic function: E/A < 1 and E’/A’ < 1, pseudonormal diastolic function: E/A 1-2 and E’/A’ < 1, restriction: E/A > 2.5 and E’/A’ > 1 [21]. None of the patients met the criteria for restriction.

Genomic DNA was isolated as previously described [22]. Amplicons of exons 4, 5 and 6 including fragments of introns were studied using the denaturing high-performance liquid chromatography (DHPLC) technique as previously described [23]. Exons 4-5 encode the oxLDL- and fatty acid-binding domain of CD36, which is crucial for lipoprotein uptake [24].

### Table I. List of CD36 sequence alterations detected by DHPLC in 100 early CAD patients

| CD36 exon/intron | DNA sequence alteration | Deduced protein sequence alteration | Genotype frequency | Minor allele frequency | HWE test Value of p |
|------------------|-------------------------|-------------------------------------|--------------------|-----------------------|-------------------|
| Intron 3         | IVS3-6 T/C              | –                                   | 81% TT, 19% TC     | 9.5%                  | 0.59              |
| Intron 4         | IVS4-10 G/A             | –                                   | 93% GG, 7% GA     | 3.5%                  | 1                 |
| Exon 6           | G573A                   | Pro191Pro                           | 94% GG, 6% GA     | 3.0%                  | 1                 |
| Exon 6           | A591T                   | Thr197Thr                           | 96% AA, 4% AT     | 2.0%                  | 1                 |

Data are given as mean ± SD or percentage of patients. MAP – mean arterial pressure, BMI – body mass index, MI – myocardial infarction, PTCA – percutaneous transluminal coronary angioplasty, CABG – coronary artery bypass grafting, ACEI – angiotensin 1 converting enzyme inhibitors, ARB – angiotensin 2 receptor blockers

Moreover, the alternative splicing of exons 4-6 maintains the reading frame seen in the CD36 variant cDNA and gives rise to isoforms of CD36 [25]. Polymerase chain reaction products with alterations detected by DHPLC were bidirectionally sequenced using the Applied Biosystems Dye-terminator Cycle Sequencing Ready Reaction kit, according to the manufacturer’s protocol. Semi-automated sequence analysis was performed using a 373A DNA fragment analyzer (Applied Biosystems, Foster City, CA).

### Statistical analysis

Differences between subgroups of patients classified according to the CD36 genotype were tested with the Mann-Whitney U test for quantitative variables and Fisher’s exact test for qualitative variables. The consistency of genotype distribution with Hardy-Weinberg equilibrium was assessed using the exact test.

### Results

Changes detected by DHPLC included two single nucleotide substitutions in introns (IVS3-6 T/C – rs3173798 and IVS4-10 G/A – rs3211892) and two synonymous polymorphisms in exon 6 (G573A – rs5956 and A591T) listed in Table I. Genotype distributions were consistent with the Hardy-Weinberg equilibrium for all sequence changes.

Clinical characteristics of the study group are presented in Table II. Clinical data of patients stratified by the CD36 genotype are presented in Table III. In the subgroup of patients with past myocardial infarction IVS3-6 TC genotype was associated with significantly younger age of this event (p = 0.042). In contrast, IVS4-10 GA heterozygotes had significantly older age of the first myocardial infarction than wild-type GG homozygotes. None of four A591T AT heterozygotes had past myocardial infarction (p = 0.012).

The biochemical parameters in the extended group of 100 patients were similar to our previous study [17]: hsCRP was significantly higher (2.73 ± 2.75 mg/l vs. 1.60 ± 2.65 mg/l, p = 0.016), but Lp(a) was significantly lower (21.39 ± 30.88 mg/dl vs. 44.80 ± 51.92 mg/dl, p = 0.044) in the IVS3-6 TC heterozygotes than in the TT patients, while exon 6 G573A genotype was associated with slightly (39.50 ± 9.65 mg/dl vs. 48.94 ± 11.42 mg/dl, p = 0.056) lower HDL cholesterol concentrations. No other signif-

### Table II. Characteristics of the study group (n = 100)

| Parameter                        | Value          |
|----------------------------------|----------------|
| Gender [% males]                 | 74             |
| Age of patients [years]          | 49.9 ±5.91     |
| MAP [mm Hg]                      | 93.8 ±9.4      |
| BMI [kg/m²]                      | 28.1 ±4.0      |
| History of hypertension [%]      | 66             |
| Past MI [%]                      | 70             |
| Age of the first MI [years]      | 44.0 ±5.6      |
| Current smoking [%]              | 15             |
| Past smoking [%]                 | 89             |
| Past PTCA [%]                    | 71             |
| Past CABG [%]                    | 37             |
| ACEI [%]                         | 80             |
| ARB [%]                          | 17             |
| ß-Blockers [%]                   | 88             |
| Diuretics [%]                    | 31             |
| Calcium channel blockers [%]     | 18             |
| Statins [%]                      | 96             |

Data are given as mean ± SD or percentage of patients. MAP – mean arterial pressure, BMI – body mass index, MI – myocardial infarction, PTCA – percutaneous transluminal coronary angioplasty, CABG – coronary artery bypass grafting, ACEI – angiotensin 1 converting enzyme inhibitors, ARB – angiotensin 2 receptor blockers
significant associations of CD36 genotype with biochemical parameters were found (data not shown).

Left ventricular hypertrophy was assessed using both echo and ECG examinations to facilitate comparisons with previous studies, though it is evident that echo criteria of both LV and RV hypertrophy are more sensitive and specific than ECG criteria. The results of echocardiography of patients stratified by the CD36 genotype are presented in Table IV. Left ventricle diastolic function (LVDVF) was impaired more frequently in the IVS3-6 TC heterozygotes than in the TT homozygotes. The IVS4-10 G/A genotype was associated with a significantly lower tissue Doppler A’ value and higher prevalence of pericardial fluid. Left ventricular diastolic function was pseudonormal more frequently and the tissue Doppler A’ value was significantly higher in 573GA heterozygotes. Furthermore, we found a tendency ($p = 0.06$) to larger aorta diameter and lower E/A ratio in 591AT heterozygotes than in wild-type homozygotes.

The results of electrocardiography of patients stratified by the CD36 genotype are presented in Table V. IVS4-10 GA heterozygotes had significantly higher $R_{V5(6)}$ amplitude and were also characterized by higher prevalence ($p = 0.055$) of cardiac axis deviation. The exon 6 573GA genotype was associated with significantly lower $R_{V2}$ amplitude and shorter QT interval corrected by Bazett formula in leads II and V4. The $R_{V5(6)}$ and $R_{V5(6)} + S_{V1(2)}$ amplitudes were significantly higher, while $S_{V5(6)}$ amplitude was significantly lower ($p = 0.021$) in 591AT heterozygotes than in the 591AA patients. All 591AT heterozygotes and only 31% of 591AA homozygotes had depressed ST segments ($p = 0.013$). There were no significant associations between genotypes and blood pressure, prevalence of inferior, anterior, posterior or lateral ECG localization of myocardial infarction and arrhythmia (data not shown).

The statistically significant associations were recalculated in the subgroup of 70 patients with past myocardial infarction (Table VI) and in the subgroup of 74 males (Table VII). In the subgroup with past myocardial infarction (Table VI) the IVS3-6 C allele was associated with higher hsCRP (3.17 ±2.95 mg/l vs. 1.51 ±2.80 mg/l, $p = 0.004$), similarly as in the whole study group. Moreover, the C allele was associated with higher prevalence of cardiac axis deviation. The IVS4-10 A’ allele was associated with a lower tissue Doppler A value and the 573 A allele was associated with older age of the first myocardial infarction, a lower tissue Doppler A’ value and
Table IV. Echocardiographic parameters of 100 early CAD patients stratified by the CD36 genotype

| Parameter                          | IVS3-6 T/C | IVS4-10 G/A | Exon 6 G573A | Exon 6 A591T |
|-----------------------------------|-----------|-------------|--------------|-------------|
|                                   | TT (n = 81) | TC (n = 19) | GG (n = 93) | GA (n = 7)  |
|                                   | Mean ± SD  | Mean ± SD   | Mean ± SD   | Mean ± SD   |
|                                   | Value of p |             | Value of p  | Value of p  |
| Left ventricular end-diastolic    | 51.0 ± 6.87 | 51.3 ± 10.0 | 0.75        | 51.8 ± 9.23 |
| diameter [mm]                     |            |             | 0.75        |             |
|                                   |            |             | Value of p  |             |
|                                     | 50.9 ± 7.03 | 53.6 ± 8.19 | 0.75        |             |
| Left ventricular end-systolic      | 36.1 ± 7.51 | 37.3 ± 7.90 | 0.47        | 37.8 ± 9.52 |
| diameter [mm]                      |            |             | 0.98        |             |
|                                     |            |             | Value of p  |             |
|                                     | 36.2 ± 7.55 | 38.5 ± 8.17 | 0.98        |             |
| Left ventricular end-diastolic     | 118 ± 42.5 | 128 ± 45.0  | 0.40        | 121 ± 66.5  |
| volume [ml]                        |            |             | 0.83        |             |
|                                     |            |             | Value of p  |             |
|                                     | 119 ± 42.1 | 139 ± 54.9  | 0.83        |             |
| Left ventricular end-systolic      | 57.3 ± 27.9 | 619 ± 27.8  | 0.35        | 74.9 ± 45.3 |
| volume [ml]                        |            |             | 0.28        |             |
|                                     |            |             | Value of p  |             |
|                                     | 57.3 ± 27.1 | 73.1 ± 36.0 | 0.28        |             |
| LVEF [%]                           | 54.3 ± 11.0 | 52.0 ± 10.2 | 0.60        | 53.1 ± 11.1 |
| Aorta diameter [mm]                | 30.6 ± 3.70 | 30.9 ± 4.64 | 0.41        | 30.3 ± 3.59 |
| Left atrium diameter [mm]          | 38.6 ± 5.61 | 39.2 ± 6.39 | 0.66        | 39.2 ± 5.84 |
| Intraventricular septum end-diastolic thickness [mm] | 11.6 ± 190 | 11.8 ± 2.18 | 0.91        | 11.9 ± 2.15 |
| Posterior wall end-diastolic       | 11.4 ± 2.17 | 12.2 ± 1.76 | 0.15        | 10.9 ± 1.33 |
| thickness [mm]                     |            |             | 0.37        |             |
| LVMI [g/m^2]                       | 182 ± 63.3 | 174 ± 55.5  | 0.68        | 183 ± 60.2  |
| E/A ratio                          | 1.14 ± 0.39 | 1.02 ± 0.35 | 0.09        | 1.18 ± 0.62 |
| Tissue Doppler E' [cm/s]           | 9.06 ± 2.19 | 8.53 ± 1.67 | 0.19        | 7.81 ± 2.70 |
| Tissue Doppler A' [cm/s]           | 9.61 ± 2.01 | 10.0 ± 1.91 | 0.37        | 8.20 ± 1.07 |
| E/A' ratio                         | 1.00 ± 0.35 | 0.88 ± 0.29 | 0.16        | 0.98 ± 0.34 |

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The 573 A allele was associated with a shorter QTc V4 interval and higher prevalence of pseudonormal LVDF. Associations of electrocardiography parameters with the 591 T allele were similar in the male subgroup as in the whole study group since all four 591AT heterozygotes were males.

**Discussion**

In the previous report [17] we described associations between cardiovascular risk factors and CD36 gene polymorphisms in exons 4-5. In the current study we additionally analyzed exon 6. In intron fragments adjacent to the tested exons, we found the presence of IVS3-6 T/C (rs3173798) and IVS4-10 G/A (rs3211892) polymorphisms. The sequence alterations in exon 6 were synonymous substitutions G573A (Pro191Pro, rs5956) and A591T (Thr197Thr). The functional effects of these polymorphisms have not been elucidated so far.

Associations of various genes with early onset CAD have been studied recently [26, 27]. The results of a genome-wide association study (GWAS) for presence of CAD [28, 29] showed no associations with the CD36 region

Vasan et al. [30] identified no associations between LVMI and single nucleotide polymorphisms (SNPs) at the region of chromosome 7 where CD36 is located in Caucasians. The GWAS data for LVMI [31] showed borderline association of rs10499859 within the CD36 gene with left ventricular hypertrophy. According to the HapMap database (HapMap Data Phase III/Release 2 Feb 09) [32] rs10499859 (substitution A/G in 5`UTR) is in weak linkage disequilibrium with rs5956 ($D' = 1, r^2 = 0.053$, LOD = 2.37), rs3173798 ($D' = 1, r^2 = 0.039$, LOD = 1.59) and rs3211892 ($D' = 1, r^2 = 0.017$, LOD = 0.82). Moreover, Hall et al. [33] reported that rs1761663 (SNP situated in intron 1 of the CD36 gene) correlated with lower LVMI (measured either by ECG as RV5SV(6) and RV5SV(6) + SVocardiovascular parameters or echo as left ventricular mass calculated with the formula proposed by Devereux [19]) in patients diagnosed with essential hypertension, suggesting a potentially protective role against these cardiovascular risk factors. The rs1761663 variant did not indicate any predicted effect on splicing or transcription factor binding. According to the HapMap database (Phase III/Release 2) rs1761663 is in weak linkage disequilibrium with rs5956 ($D' = 1, r^2 = 0.046$, LOD = 2.17), rs3173798 ($D' = 1, r^2 = 0.045$, LOD = 1.89) and rs3211892 ($D' = 1, r^2 = 0.055$, LOD = 2.02). We did not observe an association between analyzed CD36 polymorphisms and LVMI in CAD patients.

No data analyzing the association between variation in the human CD36 gene and other echocardiographic parameters in any subjects...
| Parameter                          | IVS3-6 T/C   | IVS4-10 G/A  | Exon 6 G573A | Exon 6 A591T |
|-----------------------------------|--------------|--------------|--------------|--------------|
|                                   | TT (n = 81)  | TC (n = 19)  | GG (n = 93)  | GA (n = 7)   |
| Heart rate [1/min]                | Mean ± SD    | Value of p   | Mean ± SD    | Value of p   |
|                                   | 70.0 ±12.0   | 70.9 ±11.6   | 70.7 ±12.5   | 70.7 ±12.1   |
|                                   | 74.4 ±12.9   | 64.0 ±13.0   | 74.3 ±7.12   | 75.8 ±15.8   |
|                                   | 0.21         | 0.28         | 0.20         | 0.62         |
| PQ interval [s]                   | 0.19 ±0.11   | 0.19 ±0.11   | 0.19 ±0.11   | 0.19 ±0.10   |
|                                   | 0.16 ±0.01   | 0.17 ±0.04   | 0.16 ±0.02   | 0.16 ±0.05   |
|                                   | 0.24         | 0.75         | 0.83         | 0.76         |
| QRS II width [s]                  | 0.082 ±0.020 | 0.081 ±0.019 | 0.082 ±0.021 | 0.081 ±0.020 |
|                                   | 0.079 ±0.023 | 0.085 ±0.033 | 0.077 ±0.018 | 0.096 ±0.022 |
|                                   | 0.47         | 0.87         | 0.67         | 0.23         |
| QRS V5 width [s]                  | 0.085 ±0.041 | 0.083 ±0.038 | 0.084 ±0.039 | 0.083 ±0.038 |
|                                   | 0.078 ±0.021 | 0.090 ±0.034 | 0.077 ±0.022 | 0.085 ±0.024 |
|                                   | 0.59         | 0.46         | 0.80         | 0.61         |
| Rv5(6) amplitude [mm]             | 12.2 ±6.07   | 12.3 ±6.18   | 12.3 ±6.13   | 12.0 ±6.04   |
|                                   | 13.0 ±6.52   | 12.4 ±5.97   | 13.3 ±6.62   | 18.8 ±5.12   |
|                                   | 0.58         | 0.86         | 0.70         | 0.019        |
| Rv1(2) amplitude [mm]             | 8.51 ±4.45   | 8.92 ±4.68   | 8.75 ±4.67   | 8.66 ±4.56   |
|                                   | 10.2 ±5.17   | 7.50 ±3.56   | 9.92 ±3.90   | 12.3 ±5.06   |
|                                   | 0.14         | 0.85         | 0.39         | 0.13         |
| Sv1(2) amplitude [mm]             | 2.46 ±2.26   | 2.48 ±2.48   | 2.73 ±2.58   | 2.65 ±2.58   |
|                                   | 3.21 ±3.41   | 4.50 ±2.76   | 100 ±0.89    | 1.88 ±0.85   |
|                                   | 0.46         | 0.043        | 0.045        | 0.89         |
| Rv5(6) amplitude [mm]             | 3.10 ±3.22   | 3.08 ±3.29   | 3.06 ±3.29   | 3.08 ±3.25   |
|                                   | 2.50 ±3.25   | 1.50 ±1.38   | 1.75 ±1.54   | 0.63 ±0.95   |
|                                   | 0.40         | 0.23         | 0.39         | 0.021        |
| Rv1(2) + Sv5(6) amplitude [mm]    | 5.53 ±4.07   | 5.54 ±4.28   | 5.76 ±4.31   | 5.70 ±4.29   |
|                                   | 5.71 ±5.01   | 6.00 ±4.09   | 2.75 ±1.72   | 2.50 ±1.29   |
|                                   | 0.82         | 0.77         | 0.06         | 0.07         |
| Rv5(6) + Sv1(2) amplitude [mm]    | 20.5 ±8.27   | 21.1 ±8.34   | 20.8 ±8.55   | 20.5 ±8.30   |
|                                   | 22.6 ±8.99   | 18.9 ±9.60   | 22.4 ±6.38   | 31.0 ±1.41   |
|                                   | 0.23         | 0.78         | 0.78         | 0.005        |
| QTc II interval [s]               | 0.41 ±0.04   | 0.41 ±0.04   | 0.41 ±0.04   | 0.40 ±0.04   |
|                                   | 0.40 ±0.04   | 0.40 ±0.06   | 0.37 ±0.03   | 0.44 ±0.04   |
|                                   | 0.92         | 0.49         | 0.048        | 0.079        |
| QTc V4 interval [s]               | 0.41 ±0.04   | 0.41 ±0.03   | 0.41 ±0.05   | 0.41 ±0.04   |
|                                   | 0.40 ±0.04   | 0.41 ±0.03   | 0.37 ±0.03   | 0.43 ±0.03   |
|                                   | 0.49         | 0.59         | 0.01         | 0.31         |
| Electrical axis deviation         | 4%           | 4%           | 6%           | 6%           |
|                                   | 16%          | 29%          | 0%           | 0%           |
|                                   | 0.08         | 0.055        | 1.00         | 1.00         |
|                                   | 0%           | 6%           | 0%           | 0%           |
| ECG criteria of past myocardial infarction | 57%          | 53%          | 55%          | 58%          |
|                                   | 33%          | 71%          | 67%          | 0%           |
|                                   | 0.62         | 0.70         | 1.00         | 31%          |
| ST depression                     | 32%          | 33%          | 34%          | 33%          |
|                                   | 42%          | 43%          | 34%          | 33%          |
|                                   | 0.60         | 0.70         | 1.00         | 100%         |
|                                   | 33%          | 67%          | 0%           | 0.035        |
have been published so far. In our study, IVS3-6TC heterozygotes had impaired left ventricular diastolic function (LVEDP) more frequently than wild-type homozygotes. We also observed higher frequency of pseudonormal LVEDP in 573GA heterozygotes and their left ventricular diastolic dysfunction was confirmed by the increase in wave A’ in the tissue Doppler. However, the E/A’ ratio was not significantly associated with IVS3-6TC and G573A polymorphisms.

As regards the association of ECG parameters with CD36 polymorphisms, Kamiya et al. [34] observed ST depression more than 0.5 mm during a treadmill exercise test in one patient with type I CD36 deficiency (neither platelet nor monocyte expression of the CD36 molecule). Coronary angiography identified spontaneous spasm of the proximal right coronary artery, and right coronary obstruction was improved from 90% to about 50% stenosis after intracoronary administration of nitroglycerin. Myocardial FFA uptake was absent and high blood pressure, plasma triglyceride, fasting plasma glucose and low high-density lipoprotein cholesterol concentrations were observed. On this basis, the authors suggested a possible association between type I CD36 deficiency and metabolic syndrome and vasospastic angina.

No other data analyzing an association between variation in the CD36 gene and ECG parameters have been published so far. The results of GWAS for ECG parameters (duration of QRS, PR and QT interval) [35–37] also showed no associations with the CD36 region. In our study, the 591AT genotype was associated with higher RV5(6) and RV5(6) + SV1(2) parameters, which could reflect left ventricular hypertrophy. These results are consistent with the tendency to higher posterior wall end-diastolic thickness and lower E/A ratio in 591AT heterozygotes. Moreover, all 591AT heterozygotes had depressed ST segments (mainly in lead V5), and a tendency to a longer QTc II interval was observed, which may be associated with features of left ventricular hypertrophy, too. IVS4-10 GA heterozygotes had significantly higher RV5(6), which may suggest right ventricular hypertrophy, while 573GA heterozygotes had significantly lower RV5(6) and 591AT heterozygotes had significantly lower RV5(6), which may suggest lower right ventricular mass. These results seem to be accidental, because they are not reflected by echocardiography. The 573GA genotype was also associated with a significantly shorter QT interval.

In our previous study [17], we reported that the IVS3-6C allele of CD36 was associated with cardiovascular risk factors such as high hsCRP, BMI and diabetes type 2. Moreover, the IVS3-6C allele was associated with younger and IVS3-10A with older age of myocardial infarction. The current study sug-
Table VII. Parameters of the subgroup of 74 early CAD males stratified by the CD36 genotype. Only parameters associated with CD36 genotype in the whole group ($p < 0.06$) were analyzed

| Parameter                                      | IVS3-6 T/C | IVS4-10 G/A | Exon 6 GS73A | Exon 6 AS91T |
|------------------------------------------------|-----------|-------------|--------------|-------------|
| Past MI                                        | $72\%$    | $63\%$     | $1.00$       | $74\%$      |
| Age of the first MI [years]                    | $44.3 \pm 5.90$ | $43.1 \pm 2.69$ | $1.90$       | $1.90$      |
| $R_{V5(6)}$ amplitude [mm]                     | $12.8 \pm 6.04$ | $13.5 \pm 6.72$ | $0.90$       | $0.90$      |
| $S_{V5(2)}$ amplitude [mm]                     | $2.73 \pm 2.25$ | $2.57 \pm 2.74$ | $0.89$       | $0.90$      |
| $S_{V5(2)}$ amplitude [mm]                     | $2.83 \pm 3.16$ | $1.84 \pm 1.18$ | $0.90$       | $0.90$      |
| $R_{V5(6)} + S_{V5(2)}$ amplitude [mm]        | $21.5 \pm 8.42$ | $23.5 \pm 9.10$ | $0.90$       | $0.90$      |
| QTc II interval [s]                            | $0.41 \pm 0.04$ | $0.40 \pm 0.04$ | $0.90$       | $0.90$      |
| QTc V4 interval [s]                            | $0.41 \pm 0.04$ | $0.40 \pm 0.04$ | $0.90$       | $0.90$      |
| Tissue Doppler A' [cm/s]                       | $9.70 \pm 1.97$ | $10.3 \pm 1.96$ | $0.90$       | $0.90$      |
| ECG criteria of past myocardial infarction     | $59\%$    | $56\%$     | $1.00$       | $61\%$      |
| ST depression                                  | $28\%$    | $38\%$     | $1.00$       | $26\%$      |
| LVDF                                           | $45\%$    | $36\%$     | $0.67$       | $0.40$      |
| Impaired                                       | $50\%$    | $81\%$     | $0.69$       | $0.69$      |
| Pseudonormal                                   | $5\%$     | $6\%$      | $1.00$       | $0.033$     |
| Presence of pericardial fluid                  | $5\%$     | $0\%$      | $0.018$      | $0.24$      |
gests that the IVS3-6C allele is associated with impaired LVDF.

In another study [38] we reported that two exon 6 variants (G573A or A591T) have similar functional implications for atheromatous plaque formation at common carotid artery bifurcation. The density of plaque was significantly lower in patients with these alterations. Density of plaque reflects its calcification and lower plaque calcification is associated with plaque instability [39]. Furthermore, the 591T allele was associated with a low ankle-brachial index, which is a cardiovascular risk factor. The current study suggests that the 591T allele is associated with some features of left ventricular hypertrophy and the 573A allele with pseudonormal LVDF. However, it should be noted that the statistical power for associations with these rare alleles in exon 6 is low and the results need confirmation in further research.

This is the first study which demonstrates an association between CD36 variants and echocardiographic or electrocardiographic parameters in early onset CAD cases. In conclusion, the presented data suggest that variant alleles of the CD36 gene may be associated with features of left ventricular hypertrophy and impaired diastolic function in patients with early onset coronary artery disease.

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