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

Monika Rac[1], Grzegorz Kurzawski[2], Krzysztof Safranow[1], Michal Rac[3], Dagmara Sagasz-Tysiewicz[4], Andrzej Krzystolik[4], Wojciech Poncyljusz[5], Maria Olszewska[1], Grażyna Dawid[6], Dariusz Chlubek[1]

[1]Department of Biochemistry and Medical Chemistry, Pomeranian Medical University, Szczecin, Poland
[2]Department of Genetics and Pathomorphology, Pomeranian Medical University, Szczecin, Poland
[3]Department of Diagnostic Imaging and Interventional Radiology, Pomeranian Medical University, Szczecin, Poland
[4]Department of Cardiology, County Hospital, Szczecin, Poland
[5]Department of Interventional Radiology, Pomeranian Medical University, Szczecin, Poland
[6]Clinic of Pediatrics, Endocrinology, Diabetology, Metabolic Diseases and Cardiology of the Developmental Age, Pomeranian Medical University, Szczecin, Poland

Corresponding author:
Monika Rac PhD
Department of Biochemistry and Medical Chemistry
Pomeranian Medical University
72 Powstancow Wielkopolskich
70-111 Szczecin, Poland
Phone: +48 91 466 15 30
E-mail: carmon12@gmail.com

Submitted: 8 July 2012
Accepted: 10 October 2012

Arch Med Sci 2013; 9, 4: 640–650
DOI: 10.5114/aoms.2012.32619

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 $R_{V5(6)}$ and $R_{V5(6)} + S_{V1(2)}$ 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 (LVM) and end-diastolic diameter [7]. Thus, myocardial fatty acid metabolism is an independent predictor of left ventricular mass in hypertension 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

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

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 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/m^2]	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
β-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

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-

icant 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 (LVDF) 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 $R_{V1(2)}$ 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 $R_{V1(2)}$ 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 a shorter QTc V4 interval and more prevalent pseudonormal LVDF.

In the male subgroup (Table VII), similarly as in the whole study group, the IVS3-6 C allele was associated with higher hsCRP (2.71 ±2.94 mg/l vs. 1.70 ±3.00 mg/l, p = 0.044) and higher prevalence of impaired LVDF. Moreover, the IVS4-10 A allele was associated with older age of the first myocardial infarction, a lower tissue Doppler A' value and

Table III. Biochemical and clinical data and morphometric 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)	Value of p	GG (n = 93)	GA (n = 7)	Value of p	GG (n = 94)	GA (n = 6)	Value of p	AA (n = 96)	AT (n = 4)	Value of p
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Age of patients [years]	50.4 ±5.9	49.2 ±5.9	0.25	50.1 ±6.0	51.5 ±4.5	0.61	50.4 ±6.0	47.1 ±3.5	0.61	50.1 ±5.9	51.7 ±5.1	0.69
Gender [% males]	72%	84%	0.39	73%	86%	0.67	72%	100%	0.33	73%	100%	0.57
Past MI	67%	63%	0.79	66%	71%	1.00	66%	67%	1.00	69%	0%	0.012
Age of the first MI [years]	44.7 ±5.7	41.3 ±4.9	0.042	43.5 ±5.4	51.0 ±4.1	0.002	44.1 ±5.8	44.1 ±5.0	0.97	44.1 ±5.7	–	–
PTCA or CABG	88%	84%	0.71	86%	100%	0.59	86%	100%	0.58	88%	75%	0.43
History of hypertension	59%	68%	0.79	61%	57%	0.70	62%	50%	0.66	60%	75%	1.00
BMI [kg/m^2]	27.9 ±3.7	29.3 ±4.9	0.22	28.3 ±4.0	27.4 ±3.9	0.50	28.1 ±4.0	29.8 ±4.7	0.44	28.2 ±4.1	28.4 ±2.1	0.68

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)	Value of *p*	GG (*n* = 93)	GA (*n* = 7)	Value of *p*	GG (*n* = 94)	GA (*n* = 6)	Value of *p*	AA (*n* = 96)	AT (*n* = 4)	Value of *p*
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Left ventricular end-diastolic diameter [mm]	51.0 ±6.87	51.3 ±8.10	0.75	51.0 ±6.96	51.8 ±9.23	0.75	50.9 ±7.03	53.6 ±8.19	0.75	51.1 ±7.19	51.3 ±5.19	0.91
Left ventricular end-systolic diameter [mm]	36.1 ±7.51	37.3 ±7.90	0.47	36.2 ±7.43	37.8 ±9.52	0.98	36.2 ±7.55	38.5 ±8.17	0.98	6.28 ±7.71	37.8 ±2.22	0.47
Left ventricular end-diastolic volume [ml]	118 ±42.5	128 ±45.0	0.40	120 ±41.0	121 ±66.5	0.83	119 ±42.1	139 ±54.9	0.83	120 ±43.5	116 ±34.2	0.86
Left ventricular end-systolic volume [ml]	57.3 ±27.9	61.9 ±27.8	0.35	56.9 ±25.8	74.9 ±45.3	0.28	57.3 ±27.1	73.1 ±36.0	0.28	58.5 ±28.3	54.5 ±15.1	0.99
LVEF [%]	54.3 ±11.0	52.0 ±10.2	0.60	53.9 ±11.1	53.1 ±8.82	0.82	54.1 ±10.9	50.4 ±10.9	0.82	53.7 ±11.0	55.8 ±7.72	0.69
Aorta diameter [mm]	30.6 ±3.70	30.9 ±4.64	0.41	30.7 ±3.92	30.3 ±3.59	0.53	30.7 ±3.85	30.5 ±4.71	0.53	30.6 ±3.89	33.5 ±2.38	0.06
Left atrium diameter [mm]	38.6 ±5.61	39.2 ±6.39	0.66	38.5 ±5.76	39.2 ±5.84	0.92	38.3 ±5.54	42.3 ±7.78	0.92	38.7 ±5.80	35.3 ±3.10	0.25
Intraventricular septum end-diastolic thickness [mm]	11.6 ±1.90	11.8 ±2.18	0.91	11.6 ±2.00	11.9 ±1.34	0.75	11.6 ±1.97	11.8 ±1.48	0.75	11.6 ±1.96	12.3 ±1.50	0.53
Posterior wall end-diastolic thickness [mm]	11.4 ±2.17	12.2 ±1.76	0.15	11.6 ±2.15	10.9 ±1.33	0.37	11.6 ±2.13	10.7 ±1.73	0.37	11.5 ±2.13	13.0 ±0.82	0.08
LVMI [g/m^2]	182 ±63.3	174 ±55.5	0.68	180 ±62.0	183 ±60.2	0.83	179 ±62.3	193 ±52.3	0.83	179 ±62.2	201 ±45.7	0.31
E/A ratio	1.14 ±0.39	1.02 ±0.35	0.09	1.11 ±0.36	1.18 ±0.62	0.97	1.12 ±0.39	1.13 ±0.32	0.97	1.13 ±0.38	0.85 ±0.11	0.06
Tissue Doppler E' [cm/s]	9.08 ±2.19	8.53 ±1.67	0.19	9.06 ±2.03	7.81 ±2.70	0.29	8.97 ±2.15	8.93 ±1.20	0.96	8.99 ±2.06	8.33 ±3.13	0.42
Tissue Doppler A' [cm/s]	9.61 ± 2.01	10.0 ± 1.91	0.37	9.81 ± 2.00	8.20 ± 1.07	0.018	9.57 ± 1.96	11.1 ± 2.08	0.018	9.69 ± 2.01	9.58 ± 1.77	0.80
E'/A' ratio	1.00 ±0.35	0.88 ±0.29	0.16	0.98 ±0.34	0.97 ±0.36	0.75	0.99 ±0.35	0.82 ±0.15	0.31	0.98 ±0.33	0.95 ±0.61	0.39

Table IV. Cont.

Parameter	IVS3-6 T/C			IVS4-10 G/A			Exon 6 G573A			Exon 6 A591T		
	TT (n = 81)	TC (n = 19)	Value of p	GG (n = 93)	GA (n = 7)	Value of p	GG (n = 94)	GA (n = 6)	Value of p	AA (n = 96)	AT (n = 4)	Value of p
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
LVDF												
Normal	44%	16%	0.033	37%	57%	0.42	39%	17%	0.63	40%	0%	0.29
Impaired	49%	79%	0.037	57%	43%	0.70	57%	50%	1.00	55%	75%	0.63
Pseudonormal	7%	5%	1.00	6%	0%	1.00	4%	33%	0.041	5%	25%	0.23
Right ventricular end-diastolic diameter [mm]	32.8 ±5.67	32.9 ±5.85	0.56	32.9 ±5.73	32.6 ±5.32	0.89	32.9 ±5.75	31.7 ±4.79	0.89	32.8 ±5.73	34.8 ±4.57	0.41
Right ventricular mean systolic pressure [mm Hg]	21.2 ±5.91	24.5 ±6.92	0.10	21.5 ±5.89	26.4 ±8.38	0.12	21.9 ±6.24	21.5 ±6.48	0.12	21.8 ±6.29	23.3 ±4.99	0.63
Presence of pericardial fluid	6%	0%	0.58	3%	29%	0.038	4%	17%	0.29	5%	0%	1.00

LVEF – left ventricular ejection fraction, LVMI – left ventricular mass index, LVDF – left ventricular diastolic function

higher prevalence of detectable pericardial fluid. 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 $R_{V5(6)}$ and $R_{V5(6)}$ + $S_{V1(2)}$ 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

Table V. Electrocardiographic 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)	Value of *p*	GG (*n* = 93)	GA (*n* = 7)	Value of *p*	GG (*n* = 94)	GA (*n* = 6)	Value of *p*	AA (*n* = 96)	AT (*n* = 4)	Value of *p*
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Heart rate [1/min]	70.0 ±12.0	74.4 ±12.9	0.21	70.9 ±11.6	64.0 ±13.0	0.28	70.7 ±12.5	74.3 ±7.12	0.20	70.7 ±12.1	75.8 ±15.8	0.62
PQ interval [s]	0.19 ±0.11	0.16 ±0.01	0.24	0.19 ±0.11	0.17 ±0.04	0.75	0.19 ±0.11	0.16 ±0.02	0.83	0.19 ±0.10	0.16 ±0.05	0.76
QRS II width [s]	0.082 ±0.020	0.079 ±0.023	0.47	0.081 ±0.019	0.085 ±0.033	0.87	0.082 ±0.021	0.077 ±0.018	0.67	0.081 ±0.020	0.096 ±0.022	0.23
QRS V5 width [s]	0.085 ±0.041	0.078 ±0.021	0.59	0.083 ±0.038	0.090 ±0.034	0.46	0.084 ±0.039	0.77 ±0.022	0.80	0.083 ±0.038	0.085 ±0.024	0.61
$R_{V5(6)}$ amplitude [mm]	12.2 ±6.07	13.0 ±6.52	0.58	12.3 ±6.18	12.4 ±5.97	0.86	12.3 ±6.13	13.3 ±6.62	0.70	12.0 ±6.04	18.8 ±5.12	0.019
$S_{V1(2)}$ amplitude [mm]	8.51 ±4.45	10.2 ±5.17	0.14	8.92 ±4.68	7.50 ±3.56	0.85	8.75 ±4.67	9.92 ±3.90	0.39	8.66 ±4.56	12.3 ±5.06	0.13
$R_{V1(2)}$ amplitude [mm]	2.46 ±2.26	3.21 ±3.41	0.46	2.48 ±2.48	4.50 ±2.76	0.043	2.73 ±2.58	1.00 ±0.89	0.045	2.65 ±2.58	1.88 ±0.85	0.89
$S_{V5(6)}$ amplitude [mm]	3.10 ±3.22	2.50 ±3.25	0.40	3.08 ±3.29	1.50 ±1.38	0.23	3.06 ±3.29	1.75 ±1.54	0.39	3.08 ±3.25	0.63 ±0.95	0.021
$R_{V1(2)} + S_{V5(6)}$ amplitude [mm]	5.53 ±4.07	5.71 ±5.01	0.82	5.54 ±4.28	6.00 ±4.09	0.77	5.76 ±4.31	2.75 ±1.72	0.06	5.70 ±4.29	2.50 ±1.29	0.07
$R_{V5(6)} + S_{V1(2)}$ amplitude [mm]	20.5 ±8.27	22.6 ±8.99	0.23	21.1 ±8.34	18.9 ±9.60	0.78	20.8 ±8.55	22.4 ±6.38	0.78	20.5 ±8.30	31.0 ±1.41	0.005
QTc II interval [s]	0.41 ±0.04	0.40 ±0.04	0.92	0.41 ±0.04	0.40 ±0.06	0.49	0.41 ±0.04	0.37 ±0.03	0.048	0.40 ±0.04	0.44 ±0.04	0.079
QTc V4 interval [s]	0.41 ±0.04	0.40 ±0.04	0.49	0.41 ±0.03	0.41 ±0.05	0.59	0.41 ±0.04	0.37 ±0.03	0.01	0.41 ±0.04	0.43 ±0.03	0.31
Electrical axis deviation	4%	16%	0.08	4%	29%	0.055	6%	0%	1.00	6%	0%	1.00
ECG criteria of past myocardial infarction	57%	53%	0.62	55%	71%	0.70	55%	67%	1.00	58%	0%	0.035
ST depression	32%	42%	0.60	33%	43%	0.70	34%	33%	1.00	31%	100%	0.013

have been published so far. In our study, IVS3-6TC heterozygotes had impaired left ventricular diastolic function (LVDF) more frequently than wild-type homozygotes. We also observed higher frequency of pseudonormal LVDF 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 CD 36 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 $R_{V5(6)}$ and $R_{V5(6)} + S_{V1(2)}$ 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 $R_{V1(2)}$, which may suggest right ventricular hypertrophy, while 573GA heterozygotes had significantly lower $R_{V1(2)}$ and 591AT heterozygotes had significantly lower $S_{V5(6)}$, 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 VI. Parameters of the subgroup of 70 early CAD patients with past myocardial infarction stratified by the *CD36* genotype. All patients in this subgroup had exon 6 591AA 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 G573A			
	TT ($n = 58$)	TC ($n = 12$)	Value of p	GG ($n = 65$)	GA ($n = 5$)	Value of p	GG ($n = 66$)	GA ($n = 4$)		Value of p
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		
$R_{V1(2)}$ amplitude [mm]	2.56 ±2.57	3.54 ±4.06	0.53	2.62 ±2.91	4.75 ±2.53	0.053	2.90 ±2.96	0.75 ±0.96		0.053
QTc II interval [s]	0.41 ±0.05	0.38 ±0.03	0.18	0.40 ±0.04	0.41 ±0.08	1.00	0.41 ±0.04	0.36 ±0.02		0.052
QTc V4 interval [s]	0.42 ±0.03	0.39 ±0.04	0.06	0.41 ±0.03	0.42 ±0.06	0.73	0.42 ±0.04	0.37 ±0.02		0.017
Tissue Doppler A' [cm/s]	9.87 ±2.11	10.3 ±1.62	0.36	10.1 ±2.01	8.08 ±0.73	0.0074	9.84 ±1.95	11.5 ±2.56		0.27
Electrical axis deviation	4%	25%	0.047	5%	40%	0.058	8%	0%		1.00
Pseudonormal LVDF	7%	0%	0.58	7%	0%	1.00	3%	50%		0.018
Impaired LVDF	46%	83%	0.052	54%	40%	0.64	53%	50%		1.00

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 G573A			Exon 6 A591T		
	TT (*n* = 58)	TC (*n* = 16)	Value of *p*	GG (*n* = 68)	GA (*n* = 6)	Value of *p*	GG (*n* = 68)	GA (*n* = 6)	Value of *p*	AA (*n* = 70)	AT (*n* = 4)	Value of *p*
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Past MI	72%	63%	0.54	71%	67%	1.00	71%	67%	1.00	74%	0%	0.006
Age of the first MI [years]	44.3 ±5.90	43.1 ±2.69	0.24	43.52±5.19	50.2 ±4.43	0.015	44.1 ±5.50	44.1 ±4.95	0.96	44.1 ±5.41	–	–
$R_{V5(6)}$ amplitude [mm]	12.8 ±6.04	13.5 ±6.72	0.59	13.0 ±6.18	12.5 ±6.53	0.89	12.9 ±6.17	13.3 ±6.62	0.91	12.6 ±6.07	18.8 ±5.12	0.032
$S_{V1(2)}$ amplitude [mm]	2.77 ±2.50	3.34 ±3.63	0.76	2.75 ±2.74	4.90 ±2.88	0.087	3.09 ±2.84	1.00 ±0.89	0.024	2.97 ±2.86	1.88 ±0.85	0.67
$S_{V5(6)}$ amplitude [mm]	2.83 ±3.16	1.84 ±1.18	0.53	2.68 ±2.92	1.60 ±1.52	0.48	2.68 ±2.94	1.75 ±1.54	0.53	2.72 ±2.88	0.63 ±0.95	0.028
$R_{V5(6)} + S_{V1(2)}$ amplitude [mm]	21.5 ±8.42	23.5 ±9.10	0.24	22.3 ±8.38	18.3 ±10.4	0.45	21.9 ±8.78	22.4 ±6.38	0.91	21.4 ±8.50	31.0 ±1.41	0.007
QTc II interval [s]	0.41 ±0.04	0.40 ±0.04	0.64	0.40 ±0.04	0.41 ±0.07	0.89	0.41 ±0.04	0.37 ±0.03	0.053	0.40 ±0.04	0.44 ±0.04	0.072
QTc V4 interval [s]	0.41 ±0.04	0.40 ±0.04	0.67	0.41 ±0.04	0.41 ±0.06	1.00	0.41 ±0.04	0.37 ±0.03	0.016	0.41 ±0.04	0.43 ±0.03	0.26
Tissue Doppler A' [cm/s]	9.70 ±1.97	10.3 ±1.96	0.29	9.97 ±1.98	8.40 ±1.02	0.032	9.71 ±1.93	11.1 ±2.08	0.17	9.84 ±1.98	9.58 ±1.77	0.91
ECG criteria of past myocardial infarction	59%	56%	0.77	57%	67%	1.00	57%	67%	1.00	61%	0%	0.021
ST depression	28%	38%	0.76	29%	33%	1.00	29%	33%	1.00	26%	100%	0.0085
LVDF												
Normal	45%	13%	0.020	36%	50%	0.67	38%	17%	0.40	39%	0%	0.29
Impaired	50%	81%	0.044	58%	50%	0.69	59%	50%	0.69	57%	75%	0.63
Pseudonormal	5%	6%	1.00	6%	0%	1.00	3%	33%	0.033	4%	25%	0.21
Presence of pericardial fluid	5%	0%	1.00	1.5%	33%	0.018	3%	17%	0.24	4%	0%	1.00

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.

Acknowledgments

This study was supported by the Ministry of Science and Higher Education, grant no. 2 P05D 002 30.

References

1. Sack MN, Rader TA, Park S, Bastin J, McCune SA, Kelly DP. Fatty acid oxidation enzyme gene expression is downregulated in the failing heart. Circulation 1996; 94: 2837-42.
2. Schwenk RW, Luiken JJ, Bonen A, Glatz JF. Regulation of sarcolemmal glucose and fatty acid transporters in cardiac disease. Cardiovasc Res 2008; 79: 249-58.
3. Tanaka T, Sohmiya K, Kawamura K. Is CD36 deficiency an etiology of hereditary hypertrophic cardiomyopathy? J Mol Cell Cardiol 1997; 29: 121-7.
4. Kintaka T, Tanaka T, Imai M, Adachi I, Narabayashi I, Kitaura Y. CD36 genotype and long-chain fatty acid uptake in the heart. Circ J 2002; 66: 819-25.
5. Tanaka T, Nakata T, Oka T, et al. Defect in human myocardial long-chain fatty acid uptake is caused by FAT/CD36 mutations. J Lipid Res 2001; 42: 751-9.
6. Ma X, Bacci S, Mlynarski W, et al. A common haplotype at the CD36 locus is associated with high free fatty acid levels and increased cardiovascular risk in Caucasians. Hum Mol Genet 2004; 13: 2197-205.
7. Neglia D, De Caterina A, Marraccini P, et al. Impaired myocardial metabolic reserve and substrate selection flexibility during stress in patients with idiopathic dilated cardiomyopathy. Am J Physiol Heart Circ Physiol 2007; 293: 3270-8.
8. de las Fuentes L, Herrero P, Peterson LR, et al. Myocardial fatty acid metabolism: independent predictor of left ventricular mass in hypertensive heart disease. Hypertension 2003; 41: 83-7.
9. Mayosi BM, Keavney B, Kardos A, et al. Electrocardiographic measures of left ventricular hypertrophy show greater heritability than echocardiographic left ventricular mass. Eur Heart J 2002; 23: 1963-71.
10. Koonen DP, Febbraio M, Bonnet S, et al. CD36 expression contributes to age-induced cardiomyopathy in mice. Circulation 2007; 116: 2139-47.
11. Heather LC, Howell NJ, Emmanuel Y, et al. Changes in cardiac substrate transporters and metabolic proteins mirror the metabolic shift in patients with aortic stenosis. PLoS One 2011; 6: 26326.
12. Habets DD, Coumans WA, El Hasnaoui M, et al. Crucial role for LKB1 to AMPKalpha2 axis in the regulation of CD36-mediated long-chain fatty acid uptake into cardiomyocytes. Biochim Biophys Acta 2009; 1791: 212-9.
13. Farhangkhoee H, Khan ZA, Barbin Y, Chakrabarti S. Glucose-induced up-regulation of CD36 mediates oxidative stress and microvascular endothelial cell dysfunction. Diabetologia 2005; 48: 1401-10.
14. Silverstein RL, Li W, Park YM, Rahaman SO. Mechanisms of cell signaling by the scavenger receptor CD36: implications in atherosclerosis and thrombosis. Trans Am Clin Climatol Assoc 2010; 121: 206-20.
15. Calvo D, Gómez-Coronado D, Suárez Y, Lasunción MA, Vega MA. Human CD36 is a high affinity receptor for the native lipoproteins HDL, LDL, and VLDL. J Lipid Res 1998; 39: 777-88.
16. Zimman A, Podrez EA. Regulation of platelet function by class B scavenger receptors in hyperlipidemia. Arterioscler Thromb Vasc Biol 2010; 30: 2350-6.
17. Rać ME, Suchy J, Kurzawski G, et al. Polymorphism of the CD36 gene and cardiovascular risk factors in patients with coronary artery disease manifested at a young age. Biochem Genet 2012; 50: 103-11.
18. Zalecenia dotyczące stosowania rozpoznań elektrokardiograficznych [Polish]. Kardiol Pol 2010; 68 supl. IV.
19. Devereux RB, Alonso DR, Lutas EM, et al. Echocardiographic assessment of left ventricular hypertrophy: comparison to necropsy findings. Am J Cardiol 1986; 57: 450-8.
20. de Simone G, Daniels SR, Devereux RB, et al. Left ventricular mass and body size in normotensive children and adults: assessment of allometric relations and impact of overweight. J Am Coll Cardiol 1992; 20: 1251-60.
21. Szczeklik A, Tendera M. Kardiologia [Polish]. Medycyna Praktyczna, Krakow 2010; 2: 718.
22. Lahiri DK, Schnabel B. DNA isolation by a rapid method from human blood samples: effects of MgCl2, EDTA, storage time, and temperature on DNA yield and quality. Biochem Genet 1993; 31: 321-8.
23. Rać ME, Suchy J, Kurzawski G, et al. Analysis of human CD36 gene sequence alterations in the oxidized low-density lipoprotein-binding region using denaturing high-performance liquid chromatography. Genet Test Mol Biomarkers 2010; 14: 551-7.
24. Rać ME, Safranow K, Poncyljusz W. Molecular basis of human CD36 gene mutations. Mol Med 2007; 13: 288-96.
25. Tang Y, Taylor KT, Sobieski DA, Medved ES, Lipsky RH. Identification of a human CD36 isoform produced by exon skipping. Conservation of exon organization and pre-mRNA splicing patterns with a CD36 gene family member, CLA-1. J Biol Chem 1994; 269: 6011-5.

26. Sakowicz A, Fendler W, Lelonek M, Pietrucha T. Genetic variability and the risk of myocardial infarction in Poles under 45 years of age. Arch Med Sci 2010; 6: 160-7.
27. Gorący J, Gorący I, Kaczmarczyk M, et al. VAMP-8 gene variant is associated with increased risk of early myocardial infarction. Arch Med Sci 2011; 7: 440-3.
28. Prins BP, Lagou V, Asselbergs FW, Snieder H, Fu J. Genetics of coronary artery disease: genome-wide association studies and beyond. Atherosclerosis 2012; 222: 1-10.
29. Samani NJ, Erdmann J, Hall AS, et al. Genomewide association analysis of coronary artery disease. N Engl J Med 2007; 357: 443-53.
30. Vasan RS, Glazer NL, Felix JF, et al. Genetic variants associated with cardiac structure and function: a meta-analysis and replication of genome-wide association data. JAMA 2009; 302: 168-78.
31. Arnett DK, Li N, Tang W, et al. Genome-wide association study identifies single-nucleotide polymorphism in KCNB1 associated with left ventricular mass in humans: the HyperGEN Study. BMC Med Genet 2009; 10: 43-51.
32. HapMap Homepage. www.hapmap.org. Last access: 01.07.2012
33. Hall D, Mayosi BM, Rahman TJ, Avery PJ, Watkins HC, Keavney B. Common variation in the CD36 (fatty acid translocase) gene is associated with left-ventricular mass. J Hypertens 2011; 29: 690-5.
34. Kamiya M, Nakagomi A, Tokita Y, et al. Type I CD36 deficiency associated with metabolic syndrome and vasospastic angina: a case report. J Cardiol 2006; 48: 41-4.
35. Kolder IC, Tanck MW, Bezzina CR. Common genetic variation modulating cardiac ECG parameters and susceptibility to sudden cardiac death. J Mol Cell Cardiol 2012; 52: 620-9.
36. Huang H, Chanda P, Alonso A, Bader JS, Arking DE. Gene-based tests of association. PLoS Genet 2011; 7: e1002177.
37. Nolte IM, Wallace C, Newhouse SJ, et al. Common genetic variation near the phospholamban gene is associated with cardiac repolarisation: meta-analysis of three genome-wide association studies. PLoS One 2009; 4: e6138.
38. Rać M, Safranow K, Rać M, et al. CD36 gene is associated with thickness of atheromatous plaque and ankle-brachial index in patients with early coronary artery disease. Kard Pol 2012; 70: 918-23.
39. Alexopoulos N, Raggi P. Calcification in atherosclerosis. Nat Rev Cardiol 2009; 6: 681-8.