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Polymorphism of *CD36* gene, carbohydrate metabolism and plasma CD36 concentration in obese children. A preliminary study*

Polimorfizm genu *CD36* a gospodarka węglowodanowa i osoczowe stężenie CD36 u dzieci otyłych. Doniesienie wstępne

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A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Introduction: CD36 may play an important role in removal of oxidized LDLs from plasma, protein glycation, the pathogenesis of insulin resistance, type 2 diabetes, and diabetic micro- and macroangiopathy. Some reports have pointed to decreased expression of macrophages in association with mutations of the *CD36* gene in hyperglycemic and obese subjects. The aim of the study was to search for an association between *CD36* gene polymorphism and carbohydrate metabolism disturbances or variability of plasma soluble CD36 concentrations in obese children.

Material/Methods: The study included 60 children aged 10 to 15 years: 30 with (study group) and 30 without (control group) obesity. Each patient's glycated hemoglobin, weight, height, waist and hip circumference, and systolic and diastolic blood pressure were measured, BMI, WHR and MAP were calculated, and oral glucose tolerance test was performed with glucose and insulin concentration measurements. Amplicons of exons 4–6 of *CD36* were studied using DHPLC technique. The PCR products with alterations were bidirectionally sequenced. Plasma concentrations of human antigen CD36 was measured using a commercially available enzyme-linked immunosorbent assay (ELISA).

Results: We found two intronic alterations: IVS3-6 T/C (rs3173798) and IVS4-10 G/A (rs3211892), one non-synonymous substitution: G367A (Glu123Lys, rs183461468) in exon 5 and two synonymous transitions in exon 6: G573A (Pro191Pro, rs5956) and A591T (Thr197Thr, rs141680676). There were no significant differences in any biochemical or morphometric parameters between genotype groups.

Discussion: The polymorphisms of the studied fragment of *CD36* are not associated with carbohydrate metabolism disturbances or the variability of plasma soluble CD36 concentrations in obese children, but further research is necessary to assess their functional implications.

Key words: ***CD36*** • **obesity** • **diabetes risk factors**

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Streszczenie

Wprowadzenie: Receptor CD36 to błonowa glikoproteina uczestnicząca w usuwaniu oxLDL (utlenionych cząstek LDL) z osocza, wiązaniu produktów glikacji białek, patogenezie insulinooporności, cukrzycy typu 2, mikro- i makroangiopatii cukrzycowej. Ekspresja receptora CD36 na makrofagach zwiększa się u pacjentów z hiperglikemią i genetycznie uwarunkowaną otyłością. Jego rola w rozwoju cukrzycy typu 2 i insulinooporności jest niejednoznaczna. Celem pracy było stwierdzenie czy u dzieci z otyłością polimorfizm genu *CD36* wiąże się z zaburzeniami gospodarki węglowodanowej oraz zmiennością stężenia CD36 w osoczu.

Materiał/Metody: Badaniami objęto 60 dzieci w wieku 10–15 lat: 30 z masą ciała >97 centyla i 30 z prawidłową masą ciała. Wykonano pomiary hemoglobiny glikowanej, wysokości, masy ciała, obwodu talii i bioder oraz RR. Obliczono BMI, WHR oraz MAP. Wykonano DTTG z pomiarem stężenia insuliny. Amplikony eksonów 4–6 *CD36* z przyległymi intronami analizowano metodą dHPLC. Produkty PCR z wykrytymi zmianami były sekwencjonowane. Osoczowe stężenie CD36 oznaczono z użyciem testu ELISA.

Wyniki: Zidentyfikowano dwie intronowe zmiany: IVS3-6 T/C (rs3173798) i IVS4-10 G/A (rs3211892), niesynonimiczną substytucję G367A (Glu123Lys, rs183461468) w eksonie 5 oraz dwie synonimiczne zmiany w eksonie 6: G573A (Pro191Pro, rs5956) i A591T (Thr197Thr, rs141680676). Nie stwierdzono istotnych statystycznie różnic pomiędzy badanymi grupami genotypowymi w żadnym z morfometrycznych i biochemicznych parametrów.

Dyskusja: Brak jest związku polimorfizmów badanego fragmentu *CD36* z zaburzeniami gospodarki węglowodanowej oraz zmiennością stężenia CD36 w osoczu dzieci otyłych. Jednakże ze względu na stosunkowo małą liczebność grup oraz brak danych co do funkcjonalnych efektów badanych polimorfizmów konieczne są dalsze badania.

Słowa kluczowe: ***CD36*** **• otyłość • czynniki ryzyka cukrzycy**

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Introduction

Diabetes in adults increases the risk of coronary heart disease and cardiovascular complications [1]. Also, insulin resistance in patients with normoglycemia or impaired glucose tolerance is a risk factor for atherosclerosis [9]. Recent studies indicate the involvement of CD36 protein in the pathogenesis of insulin resistance and type 2 diabetes and diabetic micro-and macrovasculopathy [4,5,10,24]. The CD36 receptor is a membrane glycoprotein present on the surface of many cells. It participates in the removal of oxidized LDL (oxLDL) in plasma and the binding protein glycation products [18,20,23]. It was found that glycation of LDL particles more strongly than their oxidation increases the expression of CD36 receptor, oxLDL uptake and accumulation of cholesterol in macrophages. It therefore appears that glycation of LDL particles in diabetes initiates foam cell formation and accelerated atherosclerosis [14]. Some researchers have found that monocytes in subjects with hyperglycemia have increased expression of the CD36 receptor in comparison to those with normal glucose concentration [22]. In addition, patients with mutations in the gene encoding CD36 are more likely to have type 2 diabetes [6]. CD36 receptor expression in macrophages in patients with diabetes is directly proportional to blood glucose level [8]. Increased expression of the CD36 receptor on macrophages was found in genetically determined obesity, which is associated with insulin resistance. This is probably a consequence of disturbances of insulin signaling in these cells [2,17]. On the other hand, in the Japanese population a CD36-receptor defect was not found to be associated with the occurrence of impaired glucose tolerance, insulin resistance, or diabetes [25]. It is possible that in the future plasma circulating soluble CD36 receptor could be added to non-classical cardiovascular risk factors [11]. However, its role in the development of insulin resistance and type 2 diabetes is not clearly understood, and needs further investigations. The available literature contains no reports on the importance of the CD36 receptor in obese children. Thus, for a fuller understanding of the role of CD36 in glucose metabolism pathogenesis, further study is required. The aim of the present study was to search for an association between *CD36* gene polymorphism and carbohydrate metabolism disturbances

or the variability of plasma soluble CD36 concentrations in obese children.

Material and Methods

The study included 60 Caucasian children (34 girls and 26 boys) aged 10–15 years: 30 with overweight or obesity (study group) and 30 with normal mass (control group) [12,21]. All patients were treated at the Independent Laboratory of Propedeutics in Pediatrics of Pomeranian Medical University in Szczecin (northwestern Poland) in 2008–2010. The patients were Polish residents. 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. Patients with endocrine or chronic diseases were excluded from the study.

There were no significant differences between the study and control groups as regards age (11.9±3.0 and 12.7±2.1 years, p=0.16; 11.9±3.0 and 12.7±2.1 years, respectively, p=0.16) or gender (male 11 and 15, respectively, p=0.20). Each patient's weight, height, waist and hip circumference, and systolic and diastolic blood pressure were measured. The body mass index (BMI), waist-to-hip ratio (WHR), and mean arterial pressure (MAP) were calculated. A fasting blood sample (7 mL) was taken for glycated hemoglobin measurement and DNA extraction. Genomic DNA was isolated as previously described [13]. Moreover, an oral glucose tolerance test (1.75 g glucose/kg body mass, max. dose 75 g) was performed with insulin measurement.

Amplicons of exons 4–6 (region encoding the oxLDL domain) including fragments of introns were studied using denaturing high-performance liquid chromatography (DHPLC) technique as previously described [19]. The PCR 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). Plasma concentrations of human antigen CD36 (Platelet Membrane Glycoprotein IV) were measured using the commercially available enzyme-linked immunosorbent assay (ELISA) kits (EIAab, Wuhan EIAab Science Co., Ltd., China) according to the manufacturer's protocol.

Differences between subgroups of patients classified according to the intron 3 polymorphism (IVS3-6 T/C) and exon 6 (G573A) were tested with the Mann-Whitney test for quantitative variables and Fisher's exact test for qualitative variables.

Results

Changes detected by DHPLC comprised 2 single nucleotide substitutions in introns (IVS3-6 T/C – rs3173798 and IVS4-10 G/A – rs3211892) and 2 synonymous polymorphisms in exon 6 (G573A – rs5956 and A591T – rs141680676). IVS4-10 G/A alteration was detected in one case in both groups and A591T (Thr197Thr) in two cases in the control group. Moreover, a non-synonymous substitution G367A (Glu123Lys – rs183461468) was detected in case in exon 5. Due to low statistical power of the single polymorphisms (IVS4-10 G/A, A591T, G367A) we analyzed statistically only the associations with IVS3-6 T/C and G573A genotypes. The IVS3-6C allele frequency in the whole children population (10.8%) was similar to that described earlier in the Caucasian populations (6.2% to 11.2%), according to the NCBI dbSNP database. The 573A allele frequency (5.8%) was slightly higher than that described in Caucasians (4.2–4.5%) according to the dbSNP database. Genotype distributions were consistent with the Hardy-Weinberg equilibrium for all sequence changes (p=1).

There were no significant differences between the study and control groups as regards genotype frequency (p=0.74 for IVS3-6 T/C and p=0.34 for G573A). But we identified significant differences between these two groups in CD36 plasma concentration (p=0.02 for IVS3-6 T/C and p=0.05 for G573G). There were no significant differences between the genotype subgroups in terms of any of the clinical, morphometric or biochemical analyzed parameters (Table 1). We found only a tendency (p=0.06) to higher fasting insulin level in IVS3-6 TC heterozygotes than in wild-type homozygotes.

Discussion

No data have been published so far that would suggest an association between variation in the *CD36* gene and carbohydrate metabolism disturbances or plasma soluble CD36 concentrations in obese children. Available studies on adults did not analyze changes in the sequence presented in this paper. Leprêtre et al. [16] observed no association between the IVS4-10 G/A alteration and type 2 diabetes in the Caucasian population. There was found a low serum adiponectin level and the associated insulin resistance accompanied by A (-178) C alteration in the promoter of *CD36* [16]. In addition there was found an association of low plasma concentrations of adiponectin with a rare nonsense mutation T1079G in exon 10, leading to premature termination of translation and the formation of nonfunctional CD36 receptor protein in patients with type 2 diabetes, including in the Caucasian population [17]. Other authors have often (44%) found in Caucasian adults with type 2 diabetes the C (-3489) T (rs1527479) alteration in the promoter of *CD36*. The proportion of TT genotype within the study group was 26.5% [3]. By contrast 539AC deletion in exon 6, resulting in a reading frame shift, was indicated as the *CD36* gene mutation often associated with the presence of type 2 diabetes in the Japanese population [7].

This report is an attempt to draw attention to the possibility of association of *CD36* gene polymorphism with impaired glucose metabolism and the variability of plasma concentrations of the protein CD36 in obese children, which, in contrast to adults, has not been studied by researchers. It seems reasonable to continue the research and its extension to a larger group of patients to be able to draw better conclusions for the pediatric population.

Our results suggest no association of the analyzed fragment *CD36* polymorphism with impaired glucose metabolism or the variability of plasma concentrations of CD36 protein in obese children. However, due to the relatively small group size and the lack of data regarding the functional effects of polymorphisms studied, further research is needed.

Table 1. Clinical, morphometric and biochemical, parameters of obese and normal-weight children stratified by IVS3-6 T/C and G573A *CD36* genotypes

CD36 genotype	IVS3-6 T/C (control group)			IVS3-6 T/C (study group)			exon 6 G573A (control group)			exon 6 G573A (study group)		
	TT (n = 23)	TC (n=7)	p-value	TT (n=24)	TC (n=6)	p-value	GG (n=26)	GA (n=4)	p-value	GG (n=27)	GA (n=3)	p-value
Age (years)	10.8±3.3	11.1±2.5	0.67	13.5±2.2	12.8±2.6	0.51	10.8±3.2	11.3±3.0	0.86	13.4±2.2	12.5±2.5	0.56
Gender (% males)	35%	43%	0.68	42%	50%	1.00	35%	50%	0.37	44%	100%	0.22
Weight (kg)	43.6±13.5	43.8±13.0	0.78	76.6±14.2	87.4±25.4	0.33	43.5±13.6	45.0±10.1	0.52	79.5±16.5	72.7±24.9	0.66
Waist (cm)	72.5±10.2	69.1±6.92	0.38	97.1±10.4	108±14.4	0.16	71.5±9.94	71.5±2.29	0.83	100±12.1	93.8±10.0	0.39
Hip (cm)	81.8±10.3	81.7±8.12	0.92	103±8.07	108±15.8	0.53	81.8±10.1	81.3±4.16	0.97	104±10.2	101±10.9	0.57
BMI (kg/m^2)	20.6±2.93	19.5±2.58	0.20	28.7±3.57	33±6.54	0.14	20.3±2.97	20.0±1.71	0.66	29.8±4.74	27.6±2.15	0.61
WHR	0.89±0.08	0.85±0.06	0.31	0.94±0.07	1.00±0.04	0.17	0.88±0.08	0.88±0.07	0.97	0.96±0.07	0.93±0.03	0.22
Systolic BP (mmHg)	110±10	102±9	0.09	122±13	133±15	0.13	108±10	118±4	0.26	125±13	120±20	0.66
Diastolic BP (mmHg)	67±12	58±11	0.67	76±12	87±14	0.11	64±12	75±7	0.22	79±13	67±18	0.22
HR (1/min)	82±14	73±13	0.22	80±12	73±10	0.17	79±15	82±9	0.61	80±12.3	70±3	0.11
MAP (mmHg)	81.6±10.2	72.7±9.90	0.09	91.2±11.9	102±14.4	0.13	78.9±10.6	89.2±5.89	0.18	94.5±12.3	84.4±18.4	0.39
Fasting glucose (mg/dL)	83.7±6.30	87.9±0.18	0.18	85.5±6.91	92.3±18.7	0.89	84.0±6.29	90.5±6.31	0.10	86.5±10.2	88.3±6.95	0.52
OGTT 60 min	97.7±32.3	86.8±4.56.	0.78	127±35.1	150±5.16	0.67	97.7±32.3	86.8±14.2	0.40	132±32.2	150±5.16.	0.67
OGTT 120 min	96.7±27.0	103±0.11.2	0.83	112±36.0	108±11.5	0.89	96.7±27.0	130±25.0	0.50	111±31.5	108±11.5	0.89
Fasting insulin (μU/mL)	8.27±5.17	13.7±8.72	0.06	17.2±8.50	46.2±51.7	0.14	9.19±4.93	13.7±15.9	0.80	22.7±25.1	17.6±4.88	0.81
insulin 60 min	42.0±32.7	41.6±27.9.	0.93	162±97.8	194±67.6	0.83	42.0±32.7	41.6±3.61	1.00	172±87.4	194±67.6	0.83
insulin 120 min	46.9±32.5	26.7±25.4	0.68	187±222	89.5±25.3	0.67	46.9±32.5	26.7±3.42	0.82	157±188	89.5±25.3	0.67
HbA1c (%)	5.15±1.31	5.51±0.24	0.70	5.57±0.25	5.51±0.18	0.72	5.21±1.19	5.57±0.19	0.35	5.56±0.25	5.63±0.11	0.48
CD36 (μg/mL)	20.4±12.9	30.0±25.7	0.18	16.8±11.0	11.7±3.8	0.49	23.5±17.9	17.8±6.5	0.80	16.1±10.6	13.0±5.9	0.87

Data are given as mean ±SD of patients with the indicated genotype. OGTT = oral glucose tolerance test; HbA1c = glycated hemoglobin; CD36 = soluble CD36 protein in plasma; BMI = body mass index; WHR = waist-to-hip ratio; HR = heart rate; MAP = mean arterial pressure.

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