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CCR5-Δ32 polymorphism is a genetic risk factor associated with dyslipidemia in patients with type 1 diabetes

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ABSTRACT

Aim: In the currently available literature there are no works investigating the correlation between *CCR5*-Δ32 polymorphism and dyslipidemia in children with type 1 diabetes mellitus (T1D). Therefore, we have decided to explore the potential role played by this polymorphic locus in the incidence of dyslipidemia as an important risk factor for cardiovascular disease (CVD) in patients with T1D.

Methods: A total of 380 patients with T1D were selected. Patients were divided into two groups: 180 patients with diabetic dyslipidemia and 200 controls without dyslipidemia. Characterization of CCR5-Δ32 genotypes was analyzed by polymerase chain reaction. Logistic regression model was used to examine the association between *CCR5*-Δ32 polymorphism and dyslipidemia.

Results: When participants were analyzed according to *CCR5*-Δ32 polymorphism, Δ32 carriers presented higher levels of: HbA1c ($p < 0.001$), fasting plasma glucose ($p < 0.001$), LDL ($p = 0.02$) as well as TG ($p = 0.01$) and lower levels of HDL ($p = 0.01$) than noncarriers. Moreover, the minor allele Δ32 was more frequent in dyslipidemic subjects than controls ($p < 0.001$) and conferred an increased individual risk for dyslipidemia (OR = 2.327; 95% CI = 11.241–4.365; $p = 0.009$).

Conclusions: The findings of our study suggest that the *CCR5*-Δ32 polymorphism is associated with elevated plasma lipid levels and the Δ32 allele increases the risk of dyslipidemia in patients with T1D. Identification of the functional variant underlying these associations may potentially lead to the development of a novel and adjunctive approach for the treatment of dyslipidemia and CVD.

1. Introduction

Type 1 diabetes mellitus (T1D) is characterized by the autoimmune destruction of pancreatic β-cells, induced by T lymphocytes and inflammatory cytokines. Long-standing hyperglycemia and the low-grade inflammation that underlies the course of T1D play critical role in the development of micro- and macrovascular diabetic complications.

Dyslipidemia is one of the major contributory factors for macrovascular complications in T1D [1]. Patients with T1D presented a 10-fold increased risk for developing cardiovascular disease (CVD) as compared with general population, and cardiovascular events remain the leading cause of mortality in this population [2]. Although macrovascular complications of T1D rarely manifest prior to puberty [3], it is recognized that atherosclerosis begins in childhood in association with dyslipidemia [4]. Moreover, experimental and clinical studies have demonstrated the deleterious effects of dyslipidemia on the microvasculature and progression of diabetic microvascular complications [5].

Atherosclerosis is characterized by the formation of arterial lesions comprising lipids, fibrous elements, and immune cell infiltrates [6]. Leukocyte recruitment into the vessel wall is a key step in atherosclerotic lesion formation, and the interaction of CCL5/RANTES with CCR5 is known to be pivotal in this process [7]. Mounting evidence suggests that CCR5 plays an important role in the development and progression of atherosclerosis in dyslipidemic murine models. Studies using an antagonist of the CCR5 or a recombinant RANTES receptor antagonist have demonstrated their capacity to reduce the atherosclerotic burden and the systemic secretion of proinflammatory Th1 cytokines [8,9].

A polymorphism in *CCR5*, a 32-nucleotide deletion known as *CCR5*-Δ32, exists at allele frequencies of typically 10–20% heterozygous and 1% homozygous carriers in Caucasian population [10]. In individuals

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homozygous for *CCR5*-Δ32, the receptor is not present on a cell surface and in heterozygous individuals, the CCR5 surface expression is reduced by 20% to 30% relative to the wild-type concentrations [11]. Consistent with observations from a murine model regarding CCR5 and atherosclerosis, the *CCR5*-Δ32 genotype has also been found to be protective against cardiovascular disease risk in several studies [12–14]. However, other studies have demonstrated no relationship with cardiovascular events [15–17] or an increased risk associated with Δ32 allele and an earlier age of onset of acute myocardial infarction in women [18].

Recent results have shown that the *CCR5*-Δ32 polymorphism is associated with elevated plasma concentration of high-density lipoprotein cholesterol (HDL) and reduced plasma triglycerides (TG) in a population with pre-existing CVD [11]. On the other hand, maraviroc that mimics the effect of the Δ32 allele by blocking CCR5, was not associated with elevations in total cholesterol (TC), LDL (low-density lipoprotein cholesterol) and TG levels in dysplipidemic HIV-infected patients [19].

To the best of our knowledge, there are no previous studies reporting the potential associations of the *CCR5*-Δ32 polymorphism with lipid levels as well as dyslipidemia in patients with T1D. Therefore, in the present study, we have examined the possible connection of this polymorphism with the lipid parameters in T1D dyslipidemic and non-dyslipidemic patients.

2. Materials and methods

2.1. Subjects

The study group included a 380 Caucasoid patients with diagnosed type 1 diabetes recruited from the Chair and Clinics of Pediatrics, Diabetology and Endocrinology, Medical University of Gdańsk. Mean age of patients was 15.5 ± 3.2 years. The diagnosis of type 1 diabetes was based on the American Diabetes Association criteria [20]. Patients with coexisting hypothyroidism, renal, hepatic and autoimmune diseases were excluded from the study. All patients were treated with humanized insulin at doses of 0.87 ± 0.2 U/kg. At the time of sampling, blood glucose level, glycated hemoglobin (HbA1c), fasting plasma glucose, lipid levels (TC, HDL and TG) along with biochemical measurement of renal function were monitored.

Patients were divided into two groups: 180 patients with diabetic dyslipidemia and 200 diabetic controls without dyslipidemia. All of the patients with diabetes-related dyslipidemia were newly diagnosed and previously did not receive lipid-lowering drugs before including into the study.

Dyslipidemia was defined by the presence of one or more abnormal serum lipid concentrations: TC ≥ 5.17 mmol/l (200 mg/dl); HDL < 1.03 mmol/l (40 mg/dl); LDL ≥ 2.6 mmol/l (100 mg/dl); TG ≥ 1.69 mmol/l (150 mg/dl) [21]. Further analysis were performed after controlling for age and pubertal stage to avoid differences in lipid values [22].

The study was conducted according to the principles of the Declaration of Helsinki. Written informed consent was obtained from every subjects. The study protocol was approved by the Ethics Committee of the Medical University of Gdańsk.

2.2. Medical examinations

Systolic and diastolic blood pressures (SBP and DBP, resp.) were measured using automatic 24 h ambulatory blood pressure monitoring (ABPM) by the Holter method. All the average values of the blood pressure were expressed in the centyle charts.

Renal function was evaluated by eGFR (estimated glomerular filtration rate), which was estimated by using the Schwartz et al. [23] formula: eGFR (ml/min/1.73 m^2) = k × (height (cm)/serum creatinine (mg/dL)). The value of k was 0.55 for children and adolescent girls and 0.7 for adolescent boys.

2.3. Methods

Venous blood samples were withdrawn after 12–14 h overnight fasting. Plasma TC, TG, and HDL concentrations were measured in an independent, ISO certified laboratory. LDL was estimated by the Friedewald equation [24].

Genomic DNA from all subjects was isolated from EDTA-stabilized blood using the EXTRACTME DNA BLOOD kit (Blirt, Poland). DNA was stored at −20 °C untill the time of use. Characterization of *CCR5*-Δ32 genotypes (rs333) was analyzed as previously described [25].

2.4. Statistical analysis

The results were analyzed using Statistica, ver. 12 (StatSoft, Inc., USA). Conformation of the allele frequencies to the Hardy-Weinberg equilibrium proportions was tested by the χ^2 test. Differences between groups were analyzed by ANOVA, followed by Bonferroni's test for normally distributed values or the Kruskal–Wallis test for nonparametric values and by the χ^2 Pearson test for dichotomous variables. The genotypes and allele frequencies of the *CCR5*-Δ32 polymorphism were compared using Fisher's exact test. The level of significance was set at $p \leq 0.05$. The study's power was calculated *post hoc* using the Genetic Association Study (GAS) Power Calculator online tool based in the algorithm from the CaTS power calculator for two-stage association studies [26]. Logistic regression model was used to examine the association between *CCR5*-Δ32 polymorphism and dyslipidemia. Odds ratio (OR) and 95% confident interval (95% CI) were calculated.

3. Results

3.1. Clinical and biochemical characteristics of dyslipidemic and control subjects.

Patients enrolled in the present study were divided into two groups: 180 patients with diabetic dyslipidemia and 200 diabetic controls without dyslipidemia. The clinical and biochemical characteristics of both groups are summarized in Table 1. There were no significant differences for estimated glomerular filtration rate, systolic and diastolic blood pressure between dyslipidemic and control subjects. The age ($p = 0.002$), duration of diabetes ($p < 0.001$), BMI ($p = 0.01$), HbA1c ($p < 0.001$) and fasting plasma glucose levels ($p < 0.001$) were higher in the individuals with dyslipidemia compared with controls.

Table 1
Clinical and biochemical characteristics of dyslipidemic and control subjects.

	Control	Dyslipidemic	*p*
N	200	180	–
Sex (Male/Female)	95/105	77/103	0.35*
Age (years)	15.3 ± 3.3	16.3 ± 3.0	**0.002**
Duration of diabetes (years)	6.6 ± 3.5	8.0 ± 3.9	**< 0.001**
BMI (kg/m^2)	18.8 ± 3.0	19.6 ± 3.3	**0.01**
HbA1c (mmol/mol)	67.9 ± 18.6	92.1 ± 25.5	**< 0.001**
Fasting plasma glucose (mmol/l)	7.70 ± 1.67	8.92 ± 3.6	**< 0.001**
eGFR (ml/min/1.73 m^2)	129 ± 23	133 ± 25	0.11
Systolic blood pressure (mmHg)	115 ± 8	116 ± 7	0.20
Diastolic blood pressure (mmHg)	72 ± 7	73 ± 6	0.14

N, number of patients.
Differences were calculated by the ANOVA test. Data are presented as arithmetic mean ± standard deviation (SD).
p – the comparison between analyzed genotypes: wild-type *CCR5* and deletion of 32 bp.
Bold *p* values indicate that the differences are statistically significant.
* χ^2 Pearson test.

Table 2
CCR5-Δ32 genotypes and selected clinical and biochemical characteristics in T1D patients.

Parameter	*CCR5*		*p*
	wt/wt	wt/Δ32 and Δ32/Δ32	
N (%)	331 (87.1)	49 (12.9)	–
Age (years)	15.4 ± 3.2	16.1 ± 2.5	0.11
Duration of diabetes (years)	6.6 ± 3.2	7.2 ± 2.9	0.18
BMI (kg/m^2)	18.9 ± 2.7	19.1 ± 3.1	0.61
HbA1c (mmol/mol)	71.0 ± 21.2	79.5 ± 19.4	**0.006**
Fasting plasma glucose (mmol/l)	7.70 ± 1.82	9.41 ± 3.21	**< 0.001**
eGFR (ml/min/1.73 m^2)	131 ± 21	135 ± 19	0.17
Systolic blood pressure (mmHg)	115 ± 7	114 ± 6	0.31
Diastolic blood pressure (mmHg)	72 ± 6	73 ± 7	0.25

N, number of patients.
Differences were calculated by the ANOVA test. Data are presented as arithmetic mean ± standard deviation (SD).
p – the comparison between analyzed genotypes: wild-type *CCR5* and deletion of 32 bp.
Bold *p* values indicate that the differences are statistically significant.

3.2. CCR5 genotypes and clinical characteristics of patients

The selected clinical characteristics of diabetic patients differing in the *CCR5*-Δ32 polymorphism are presented in Table 2. Subjects with different *CCR5*-Δ32 genotypes did not reveal significant differences in: age, duration of diabetes, eGFR and values of systolic and diastolic blood pressure. However, we have noticed that HbA1c ($p = 0.006$) and fasting plasma glucose levels ($p < 0.001$) were the highest in patients with 32-bp deletion and the lowest in non-carriers.

3.3. CCR5-Δ32 genotypes and lipid parameters in T1D patients without dyslipidemia

Table 3 describes the association between *CCR5*-Δ32 genotypes and serum lipid levels in T1D patients without dyslipidemia. There were no differences in total serum cholesterol levels between individuals with distinct *CCR5* genotype. However, Δ32 carriers had higher levels of LDL ($p = 0.02$) and TG ($p = 0.01$) and lower levels of HDL ($p = 0.01$) than noncarriers.

3.4. Distribution of CCR5-Δ32 genotype and allele frequencies in T1D patients with and without dyslipidemia

The group of patients with type 1 diabetes was analyzed with regard to dyslipidemia. Genotype distributions are shown in Table 4. None deviated significantly from Hardy-Weinberg equilibrium. The frequency of Δ32 allele was higher in a group with dyslipidemia in comparison to diabetic subjects without this complication (0.094 vs. 0.042; $p < 0.001$). A multivariable logistic regression analysis adjusted for age, duration of diabetes, BMI, HbA1c and fasting plasma glucose levels revealed that *CCR5*-Δ32 polymorphism was significantly associated with dyslipidemia, with the minor Δ32 allele representing a risk for these conditions. The risk of dyslipidemia in patient carriers of the 32-bp deletion was nearly two and a half times higher than for non-carriers (OR = 2.327; 95% CI = 1.214–4.365; $p = 0.009$). Based on the observed prevalence of *CCR5*-Δ32 in our population, this study had more than 80% power to detect a relative risk of dyslipidemia between carriers and non-carriers with a significance of $p = 0.05$.

Table 3
CCR5-Δ32 genotypes and lipid parameters in T1D patients without dyslipidemia.

Parameter	*CCR5*		*p*
	wt/wt	wt/Δ32 and Δ32/Δ32	
Total cholesterol	4.50 ± 0.78	4.66 ± 0.82	0.15
HDL	1.45 ± 0.37	1.32 ± 0.32	**0.01**
LDL	2.54 ± 0.58	2.73 ± 0.59	**0.02**
Triglycerides	1.02 ± 0.63	1.24 ± 0.59	**0.01**

All the values are in mmol/l.
Differences were calculated by the ANOVA test. Data are presented as arithmetic mean ± standard deviation (SD).
p – the comparison between analyzed genotypes: wild-type *CCR5* and deletion of 32 bp.
Bold *p* values indicate that the differences are statistically significant.

4. Discussion

Recent large meta-analysis confirmed the protective relation between *CCR5*-Δ32 and T1D [27]. However, it is suggested that the Δ32 allele is a risk factor for the development of retinopathy [25], nephropathy [28] and concomitant celiac disease or autoimmune thyroiditis in patients with T1D [29]. In the currently available literature there are no works investigating the correlation between *CCR5*-Δ32 polymorphism and dyslipidemia in children with T1D. Therefore, we have decided to explore the potential role played by this polymorphic locus in the incidence of dyslipidemia as an important risk factor for CVD development in patients with T1D.

Schwab et al. [30] have demonstrated that lipid profiles in children and adolescents with T1D are primarily influenced by 4 factors; age, gender, BMI, and HbA1c levels which is consistent with our observation. On the other hand, it is well known that dyslipidemia is a complex trait caused by multiple environmental and genetic factors and their interactions. Many literatures data suggest that about 40–60% of the variation in serum lipid profiles is genetically determined [31].

In the current study, we have shown the association between *CCR5*-Δ32 genotypes and serum lipid levels in T1D patients without dyslipidemia. There were no differences between genotypes and total serum cholesterol levels. However, each lipoprotein class is heterogeneous in size and density, and subtle changes in these parameters may contribute to atherosclerosis. Moreover, we have found that Δ32 carriers had higher levels of LDL as well as TG and lower levels of HDL than noncarriers. Elevated LDL levels during adolescence may contribute to fatty streaks and future lesions formation and CVD development. Even among T1D adults with better lipid profile than non-diabetic adults, higher LDL as well as TG and lower HDL still contribute to CVD risk, and thus are important targets for therapy [32]. Lipid levels in T1D have also been associated with cardiac and vascular abnormalities, suggesting direct effect of lipids on cardiovascular function. Previous studies on the relation between CCR5 deficiency and lipid levels have produced divergent data [11,19]. Despite these differences, controversy still remains regarding the association between *CCR5*-Δ32 polymorphism and lipid metabolism.

In the present study we observed that the incidence of Δ32 allele is higher in a group with dyslipidemia in comparison to T1D controls. A multivariable logistic regression analysis adjusted for age, duration of diabetes, BMI, HbA1c and fasting plasma glucose levels revealed that *CCR5*-Δ32 polymorphism was significantly associated with dyslipidemia. The risk of dyslipidemia for patient carriers of the 32-bp deletion was two and a half times higher than for non-carriers. It is well known that dyslipidemia is pathophysiological condition that perpetuate endothelial damage, which in turn compromises vasorelaxation and increases lipid permeability of the cell and a wide range of different vasculopathies.

Inflammation is a key component that regulates lipid metabolism and leads to changes in lipid homeostasis. Multiple cytokines are likely to affect the metabolism of cholesterol or TG through several pathways

Table 4
Distribution of *CCR5*-Δ32 genotype and allele frequencies in T1D patients with and without dyslipidemia.

CCR5 genotypes	Total subjects						
	No dyslipidemia (*N* = 200)		Dyslipidemia (*N* = 180)		Fisher's exact test	OR	95% CI
	N	%	*N*	%			
wt/wt	183	91.5	148	82.2	***p* < 0.001***	2.327	1.241–4.365
wt/Δ32	17	8.5	30	16.7			
Δ32/Δ32	0	0	2	1.1			
Δ32 frequency	0.042		0.094				

N, number of patients.
OR – odds ratio.
95% CI – 95% confidence interval.
Bold *p* values indicate that the differences are statistically significant.
* Significance between T1D patients with and without dyslipidemia.

[33]. We have previously found that the Δ32 allele is associated with enhanced inflammatory response. The Δ32 carriers had higher serum levels of inflammatory markers (CRP, TNF-α), adhesion molecules (VCAM-1, ICAM-1, ICAM-3) and CCR5 ligand (MCP-1) than non-carriers [25]. The acceleration of the inflammatory process by the Δ32 allele may result in alterations in lipid metabolism, although the underlying mechanism remains to be elucidated.

Dyslipidemia is a pathological condition that causes damage of endothelium by triggering cell proliferation, apoptosis, vascular remodeling and increased cellular permeability. These along with increased expression of adhesion molecules that bind monocytes and T lymphocytes create a vicious cocktail of pathophysiological factors. Moreover, natural chemokine ligand of CCR5, RANTES have been reported to be present in human atherosclerotic plaques [34] and so $CCR5^+$ T lymphocytes and monocytes are trapped in the atherosclerotic lesions where they exert pathological effect. The presence of dysfunctional CCR5 chemokine receptor can modulate this process – such hypothesis is supported by a cohort study data reporting a lower incidence of cardiovascular events in *CCR5*-Δ32-carrying patients [11]. On the other hand, CCR5 deficiency seems to have impact on the function of suppressive Treg lymphocytes. Guasti et al. [35] showed that Treg cells seem to be over-stimulated in the early pre-clinical phase of atherosclerosis and a relationship exists between their frequency and circulating lipids. Moreover, depletion of regulatory T cells promotes hypercholesterolemia and atherosclerosis in murine model [36].

In conclusion, the findings of our studies suggest that the *CCR5*-Δ32 polymorphism is associated with elevated plasma lipid levels and the Δ32 allele increases the risk of dyslipidemia in patients with T1D. Therefore, the understanding of the association of this genetic variant and dyslipidemia in children and adolescents may have important implications for future development and the pathogenesis of atherosclerosis and CVD at adulthood.

A number of limitations of the present study need to be addressed. Firstly, despite we have detected the association of *CCR5*-Δ32 polymorphism and dyslipidemia in this study, there are still many unmeasured genetic and environmental factors. Secondly, all of the patients with diabetes-related dyslipidemia were newly diagnosed and in some cases the diet may contribute to the lipoprotein phenotype in these patients. Therefore, further studies on a larger sample size, especially with the consideration of genetic substructure in populations, are needed to confirm our findings.

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Conflict of interest

The authors declare that they have no conflict of interest.

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