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Metabolic control, *ApoE* genotypes, and dyslipidemia in children, adolescents and young adults with type 1 diabetes

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ABSTRACT

Background and aims: Limited data is available on the factors influencing the lipid profiles and the prevalence of dyslipidemia in children, adolescents and young adults with type 1 diabetes. We aimed at assessing the influences of metabolic control and *ApoE* genotypes on lipid profiles and the prevalence of dyslipidemia in children, adolescents and young adults with type 1 diabetes.
Methods: Children, adolescents and young adults with type 1 diabetes from our nationwide cohort attending the annual check-up were prospectively included. Data on metabolic control and expanded lipid profiles were collected, and *ApoE* genotyping performed. Test for homoscedasticity of continuous variables was followed by ANOVA and Welch's ANOVA tests, and Chi-square test was used for categorical variables with Kruskal-Wallis test as a control.
Results: 467 patients were included in the data analysis: 226 female (48.4%), mean age 14.71 ± 5.09 years and diabetes duration 6.74 ± 4.54 years. Mean HbA1c was 61 ± 5 mmoL/mol (7.71 ± 1.22%), with no gender-related differences. Females had higher mean total cholesterol ($p < 0.001$), LDL-C ($p = 0.005$), HDL-C ($p < 0.001$), non-HDL-C ($p = 0.003$), and ApoB levels ($p < 0.001$). 26.3% of participants had LDL-C levels above the type 1 diabetes LDL-C-goal of 2.6 mmoL/L, and 19.5% had elevated/borderline-elevated lipoprotein(a) (Lp(a)). HbA1c levels were positively related to higher levels of LDL-C ($p = 0.0070$) and Lp(a) ($p = 0.0020$). Participants with *ApoE4(e3/e4)* allele had higher levels of LDL-C ($p = 0.010$), independently of HbA1c.
Conclusions: Females and subjects with suboptimal metabolic control had more adverse lipid profiles. *ApoE4(e3/e4)* alleles were associated with significantly higher LDL-C levels, independently of HbA1c.

1. Introduction

Type 1 diabetes is a major risk factor for a premature cardiovascular disease (CVD) [1]. Clinical manifestations of CVD are extremely rare in childhood, but the atherosclerosis process starts already in the first years of life and is significantly accelerated by type 1 diabetes [2,3]. People with type 1 diabetes have a substantially increased risk of developing coronary artery disease (CAD) and a 20-40- times higher risk of dying of CVD by the age of 40 years as compared to healthy individuals [4,5]. Hyperglycemia is considered the primary mediator of atherosclerosis in type 1 diabetes, where for each % absolute raise in glycated hemoglobin (HbA1c) the future CVD events relative risk is increased by 7% [1,6]. Even individuals with type 1 diabetes with HbA1c levels below 6.9% (≤52 mmoL/mol) have doubled CVD mortality risk as compared to healthy individuals, and the risk was shown to be substantially higher in women [4,7,8].

Dyslipidemia is considered one of the most important CVD risk factors in people with diabetes, while possibly severely undertreated [9]. People with type 1 diabetes and good metabolic control have similar lipid profiles as healthy population, but it is not clear whether their lipids' composition is more atherogenic [4,10,11]. On the other hand, sub-optimal metabolic control could lead to

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diabetic dyslipidemia, which is characterized by high levels of low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG), and low levels of high-density lipoprotein cholesterol (HDL-C) [11,12]. Stringent lipid goals are set already for children and adolescents with diabetes by ISPAD and ADA guidelines, to reduce the CVD risk, recommending LDL-C and TG levels to be below 2.6 and 1.7 mmoL/L, respectively, and HDL-C levels above 1.1 mmoL/L [13–16].

Apolipoprotein E (*ApoE*) genetic variants (E2, E3, E4) importantly influence lipid metabolism and dyslipidemia development, also modifying the CVD risk [17,18]. However, limited data are available on the influence of the *ApoE* genotypes on the lipid profiles in type 1 diabetes patients and on their possible clinical role.

Our entire childhood-onset type 1 diabetes population is prospectively included in a nation-wide, type 1 diabetes registry, demonstrating reasonable overall metabolic control in recent years [19,20]. Furthermore, we currently conduct a nation-wide universal cholesterol screening in pre-school children to detect those with familial hypercholesterolemia (FH) and include them prospectively in a national FH registry [21]. In this study, we aimed at assessing the influences of the metabolic control and *ApoE* genotypes on the lipid profiles and the prevalence of dyslipidemia in children, adolescents and young adults with type 1 diabetes.

2. Patients and methods

2.1. Design and participants

From February 2015 to February 2016, we prospectively included, nation-wide, all children, adolescents and young adults with type 1 diabetes attending the annual expanded check-up visit. Besides regular clinical assessment, we obtained their expanded lipid profile, reviewed the data on type 1 diabetes characteristics and its management, and performed the *ApoE* genotyping. The study was approved by the National Medical Ethics Committee and conducted in line with the principles of the last revision of Declaration of Helsinki. All participating subjects or their parents, as applicable, signed the Informed Consent form.

The main inclusion criteria were: age between 2 and 25 years; clinical diagnosis of type 1 diabetes for at least one year; current use of insulin; no other illness apart from stable celiac disease or thyroiditis; not regularly using any other medications besides insulin. Excluded were patients with genetically confirmed FH. Altogether, 484 subjects performed the annual check-up visit including expanded lipid profiling and *ApoE* genotyping. No reference data were available from a comparable group of healthy controls.

2.2. Data collection, methods and definitions

The data for the study population were collected from the prospective, national, childhood-onset type 1 diabetes registry as follows: (1) demographic and clinical data (age, gender, type 1 diabetes duration, weight, height, BMI, and systolic and diastolic blood pressure (sBP, dBP), also expressed as standard deviation scores (SDS)); (2) data on the characteristics of type 1 diabetes management (type of insulin therapy (multi-daily injections (MDI) or continuous subcutaneous insulin infusion (CSII)); total insulin dose per day, insulin requirement (IR), insulin sensitivity (IS), insulin/carbohydrate ratio (INS:COHs), number of insulin injections and blood glucose measurements (SMBG) per day, sensor use, blood glucose (BG) and HbA1c levels at the time of lipid profiling, and means of the amount of COHs per day. In addition to the data collected from the registry, expanded lipid profile (total cholesterol (TC), LDL-C, HDL-C, TG, apolipoprotein A1 (ApoA1), apolipoprotein B (ApoB), ApoB/ApoA1 ratio, lipoprotein(a) (Lp(a)) and *ApoE* genotyping were performed.

The expanded lipid panel was analyzed with an automated analyzer Advia 1800 (Siemens Healthcare, Erlangen, Germany), using the direct enzymatic colorimetric method; LDL-C was determined using the Friedewald equation.

In line with the ADA guidelines, lipid levels were considered as normal if: TC < 4.4 mmoL/L, LDL-C <2.6 mmoL/L, HDL-C >1.2 mmoL/L, TG < 0.8 mmoL/L for children <9 years of age and <1 mmoL/L if > 9 years of age, TG/HDL-C <1.5, non-HDL-C <3.1 mmoL/L, ApoA1 >1.2 g/L, ApoB <0.9 g/L, ApoB/ApoA1 <0.75, and Lp(a) < 300 mg/L [15]. Lipid levels were considered as elevated/lowered if: TC > 5.2 mmoL/L, LDL-C >3.4 mmoL/L, TG > 1.1 mmoL/L for children <9 years of age and >1.5 mmoL/L if > 9 years of age, HDL-C <1 mmoL/L, TG/HDL-C >2, non-HDL-C >3.8 mmoL/L, ApoA1 <1.15 g/L, ApoB >1.1 g/L, ApoB/ApoA1 >1, and Lp(a) > 500 mg/L. The lipid levels in-between the cut-offs were considered borderline.

The HbA1c level was determined with an immunochemical method using the Siemens DCA Vantage® Analyzer (Siemens Healthcare GmbH, Erlangen, Germany). Subjects with HbA1c ≤ 7.5% (≤59 mmoL/mol) were considered to have a target metabolic control as indicated in the ISPAD Guidelines [14].

ApoE genotyping was performed using TaqMan genotyping assay (Applied Biosystems, Foster City, CA, US). For detection of *ApoE* c.334T > C (ref SNP ID: rs429358) we used assay ID:C_3084793_20 and for c.472C > T (ref SNP ID: rs7412) we used assay ID:C_904973_10.

The c.334T > C (rs429358) and c.472C > T (rs7412) polymorphisms in *ApoE* gene determine 3 major alleles, e2, e3, and e4, which combine into 6 corresponding genotypes: e2/e2, e2/e3, e2/e4, e3/e3, e3/e4, and e4/e4. The alleles combinations encode 3 isoforms: *ApoE2* (*e2/e2* or *e2/e3*), *ApoE3* (*e2/e4* or *e3/e3*) and *ApoE4* (*e3/e4* or *e4/e4*). *ApoE3* isoform is considered the normal and the most common isoform without any particular influence on lipid metabolism, while *ApoE2* and *ApoE4* seem to be dysfunctional isoforms [17].

2.3. Statistical analysis

A linear model of all independent variables against each dependent variable was performed. A p-value <0.05 indicated significance of the model with given coefficient estimates. Because most of the variables were not likely relevant, optimizations were performed - the linear model was controlled for variables with known influence on lipids profile (gender, age, onset age, disease duration, type of therapy, sensor use, BMI). The Shapiro-Wilk test of normality was performed on the continuous dependent variables to assess their normal distribution in the population. If the distribution of the variable was normal, the Student's t-test (when we compared two groups) or one-way ANOVA (when we compared more than two groups) were used. Otherwise, Mann-Whitney U test or Kruskal-Wallis H test were performed.

3. Results

3.1. Study population characteristics

467 (out of 484) subjects met the criteria to be included in the final study analysis; 226 females (48.4%), a mean age of 14.71 ± 5.09 years, and type 1 diabetes duration of 6.74 ± 4.54 years.

The mean HbA1c was 7.71 ± 1.22% (61 ± 5 mmoL/mol), while the median HbA1c value was 7.5 ± 5.0% (58 ± 31 mmoL/mol); 115 (24.6%) subjects had optimal metabolic control (HbA1c <7% (<53 mmoL/mol)), while 151 (32.3%) had inadequate metabolic control (HbA1c ≥ 8% (≥64 mmoL/mol)). The mean HbA1c in females (7.71 ± 1.1%; 61 ± 5 mmoL/mol) did not differ from that in