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Original Articles

Can paraoxonase 1 polymorphisms (L55 M and Q192 R) protect children with type 1 diabetes against lipid abnormalities?

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BACKGROUND: Only a few studies have focused on the possible modulatory role of paraoxonase 1 (PON1) polymorphisms in lipid profiles, especially in children and in adolescents with type 1 diabetes (T1D).

OBJECTIVE: We propose to study the association between PON1 polymorphisms (PON1-55 and PON1-192) and a lipid profile in a young Tunisian population with T1D.

METHODS: The study compared 122 children and adolescents with T1D with 97 controls. Genomic DNA was collected from 116 patients and 91 controls. Lipid parameters were determined by automated methods. PON1 activity was measured by a spectrophotometric method and genotyping of the PON1 gene was assessed by multiplex polymerase chain reaction followed by restriction fragment-length polymorphism.

RESULTS: A significant increase in total cholesterol, high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol (LDL-C), apolipoprotein B (ApoB), and lipoprotein (a) (Lp(a)) and a significant decrease in apolipoprotein A1 (ApoA1), ApoA1/ApoB ratio, and PON1 activity/HDL-C ratio were observed in children with T1D compared with controls. In the LLQR haplotype, the group with diabetes showed significantly higher values of total cholesterol, LDL-C, apoB, Lp(a), and apoA1/apoB ratio compared with the control group. Those with diabetes with the LLQQ haplotype showed a significant decrease in LDL-C and Lp(a) compared with controls ($P < .0001$).

CONCLUSION: PON1 polymorphisms (PON1-55 and PON1-192) seem to be involved in the altering the lipid profile in T1D. The LLQR haplotype provided an atherogenic lipid profile in children with T1D compared with controls. LLQQ haplotype seemed to have a protective effect against the increase in LDL-C and Lp(a) that are heavily involved in the development of cardiovascular diseases.

Conflict of interest: The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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Paraoxonase 1 (PON1) is a serum high-density lipoprotein-bound enzyme with an antioxidant function, which plays an important role in lipid metabolism preventing low-density lipoproteins from oxidative modifications.[1,2] Besides its protection against lipid oxidation, paraoxonase's candidacy for an antiatherogenic role is further supported by the increased risk of atherosclerosis associated with PON1 polymorphisms.[3–8] The *PON* gene clusters maps to human chromosome 7q21- 22, and several polymorphisms in the promoter and coding regions have been identified.[3,9–11] It contains 2 coding region polymorphisms leading 2 different PON1 isoforms: 1 at position 192 (glutamine [Q] to arginine [R] substitution) and the second at position 55 (leucine [L] to methionine [M] substitution).[12]

PON1 activity is reduced in high oxidative stress diseases such as coronary heart disease, dyslipidemia, inflammatory processes, diabetes, and certain neuropathies.[13,14]

Type 1 diabetes (T1D) mellitus accounts for only 5% to 10% of all cases of diabetes but represents an important health problem, because this disorder begins early in life and leads to long-term complications.[13] Patients with T1D present with lipid disorders that are mostly qualitative abnormalities of lipoproteins that can promote atherogenesis. Decreased PON1 activity is associated with the increased lipid peroxidation; this might be a factor for determining predisposition to complications of diabetes mellitus.[12]

Considering the important role of PON1 polymorphisms in the genetic susceptibility for cardiovascular diseases and the little attention of the potential relevance of PON to T1D, especially in children and adolescents, we propose to study the association between PON1 polymorphisms (PON1-55 and PON1-192) and lipid profiles in a young population with T1D.

Materials and methods

Study population

Our study group consisted of 122 children and adolescents with T1D from the pediatric department, (mean age 14.01 ± 5.30 years, 76 males and 46 females), compared with 97 healthy children and adolescents without any evidence of diabetes as the control group (mean age 12.86 ± 5.41 years, 54 males and 43 females). The genetic study included 116 patients and 91 controls.

We had parental consent for all patients and controls and the permission of the ethics committee of the University Hospital of Monastir.

Laboratory measurements

Venous blood samples were drawn in lithium heparinate tubes after a 12 hours of fasting. All assays were performed in the biochemistry and toxicology laboratory.

HbA1C was determined by high-performance liquid chromatography on a Tosoh G7 (Tosoh, Japan) automaton.

Glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein -cholesterol (LDL-C) were determined by enzymatic methods on an Integra 400 automaton (Roche Diagnostics, Mannheim, Germany).

Apolipoproteins A_1 and B (Apo A_1 and ApoB) and lipoprotein (a) (Lp(a)) were measured by immunoturbidimetry on an Integra 400.

PON1 activity was measured via the kinetic method at 405 nm using paraoxon as a substrate.[15]

DNA analysis

Blood was drawn from the peripheral veins in K_3-EDTA tubes; genomic DNA was isolated from leukocytes by the salting-out method[16] using guanidinium chloride.

Determination of PON1 polymorphisms was achieved by multiplex polymerase chain reaction using the following flanking primers previously described:

F: 5′-GAGTGATGTATAGCCCCAGTTTC-3′ and R: 5′-AGTCCATTAGGCAGTATCTC CG-3′ for PON1- L55 M polymorphism and F: 5′-TTGAATGATATTGTTGCTGTG GGACC.

TGAG-3′ and R: 5′-CGACCACGCTAAACCCAAATA CATCTCCCAGAA-3′ for PON1-Q192 R polymorphism.[17]

Table 1 Demographic and biological details of the study groups

Characteristics	Control population (n = 97)	Population with diabetes (n = 122)	*P*
Age (y)	12.9 ± 5.4	14.0 ± 5.3	.109
Sex ratio	1.25	1.65	–
BMI (kg/m^2)	20.5 ± 2.5	20.3 ± 3.3	.428
Age of onset (y)	–	7.0 ± 4.3	–
Duration (y)	–	6.8 ± 4.4	–
Fasting glucose (mmol/L)	**3.7 ± 0.7**	**10.8 ± 5.9**	**<.001**
HbA1c (%)	**5.9 ± 0.4**	**10.3 ± 2.1**	**<.001**
TC (mmol/L)	**3.29 ± 0.70**	**3.93 ± 0.78**	**<.001**
TG (mmol/L)	0.81 ± 0.51	0.72 ± 0.32	.120
HDL-C (mmol/L)	**1.23 ± 0.44**	**1.44 ± 0.34**	**<.001**
LDL-C (mmol/L)	**1.80 ± 0.52**	**2.30 ± 0.68**	**<.001**
HDL-C/LDL-C	0.74 ± 0.46	0.68 ± 0.26	.205
Apo A1 (g/L)	**1.42 ± 0.38**	**1.32 ± 0.21**	**<.02**
ApoB (g/L)	**0.58 ± 0.16**	**0.64 ± 0.20**	**<.02**
Apo A1/Apo B	**2.57 ± 0.90**	**2.24 ± 0.80**	**<.01**
Lp(a) (g/L)	**0.26 ± 0.26**	**0.41 ± 0.45**	**<.01**
PON1 activity (U/L)	334 ± 220*	330 ± 198	.563
PON1 activity/HDL-c (U/mmol)	**288 ± 206***	**239 ± 157**	**.048**

ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; HDL-C, high-density lipoprotein-cholesterol; Lp(a), lipoprotein (a); LDL-C, low-density lipoprotein-cholesterol; PON1, paraoxonase 1; TC, total cholesterol; TG, triglycerides.

The bold values indicate statistical significance between control and patients.

*n = 95.