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Paraoxonase 1 polymorphisms (L55M and Q192R) as a genetic marker of diabetic nephropathy in youths with type 1 diabetes

Polimorfizmy L55M i Q192R genu paraoksonazy jako markery genetyczne nefropatii cukrzycowej u młodzieży z cukrzycą typu 1

Ons Fekih[1, 2], Sonia Triki[1, 2], Jihen Rejeb[2], Fadoua Neffati[1, 2], Wahiba Douki[1], Asma Ommezzine[2], Slaheddine Chouchane [3], Mohamed Neji Guediche[3], Ali Bouslama[2], Mohamed Fadhel Najjar[1]

[1]Laboratory of Biochemistry and Toxicology, Monastir's University Hospital, Tunisia
[2]Biochemistry Department, LR 12 SP11, Sahloul's University Hospital, Sousse, Tunisia
[3]Paediatric Department, Monastir's University Hospital, Tunisia

Abstract

Introduction: Paraoxonase 1 (PON1) polymorphisms have been largely involved in diabetes complications. The aim of the study is to evaluate the effects of PON1 polymorphisms (L55M and Q192R) on diabetic nephropathy (DN).
Material and methods: The study involved 116 children and adolescents with type 1 diabetes (T1D) and 91 healthy subjects. Albumin excretion rate (AER) was determined by immunoturbidimetry. PON1 activity was measured by a spectrophotometric method, and genotyping of PON1 gene was assessed by multiplex PCR followed by RFLP.
Results: PON1 activity was inversely correlated to AER ($r = –0.245$, $p = 0.008$). A significant decrease ($p = 0.037$) in PON1 activity was shown between patients with nephropathy and those without (162 [57–618] vs. 316 [37–788] IU/L, respectively).
The distribution of AER was, for L55M polymorphism MM > LM > LL ($p = 0.002$), and for Q192R polymorphism QQ > QR > RR ($p < 0.001$). The opposite distribution was noted for PON 1 activity ($p < 0.001$). LMQQ and MMQQ haplotypes seem to increase AER ($p = 0.004$, $p = 0.003$, respectively) and to reduce PON1 activity ($p = 0.011$, $p = 0.052$, respectively) in youths with T1D. However, LLRR haplotype seems to have the opposite effect.
Conclusions: This study demonstrated that PON1 polymorphisms L55M and Q192R seem to be genetic markers involved in the development of DN in T1D. **(Endokrynol Pol 2017; 68 (1): 35–41)**

Key words: *paraoxonase 1 polymorphisms; type 1 diabetes; diabetic nephropathy; paraoxonase 1 activity*

Streszczenie

Wstęp: Polimorfizm genu paraoksonazy 1 (PON1) przyczynia się w dużym stopniu do występowania powikłań cukrzycy. Badanie przeprowadzono w celu oceny wpływu polimorfizmów genu PON1 (L55M i Q192R) na rozwój nefropatii cukrzycowej (diabetic nephropathy, DN).
Materiał i metody: W badaniu uczestniczyło 116 dzieci i młodzieży z cukrzycą typu 1 (type 1 diabetes, T1D) i 91 zdrowych osób. Zmierzono wydzielanie albumin z moczem (albumin excretion rate, AER), stosując metodę immunoturbidymetryczną. Aktywność PON1 określono metodą spektrofotometrii, a do genotypowania genu PON1 zastosowano metodę Multiplex PCR, a następnie RFLP.
Wyniki: Aktywność PON1 była ujemnie skorelowana z AER ($r = –0,45$; $p = 0,008$). Wykazano istotne zmniejszenie ($p = 0,037$) aktywności PON1 u chorych z nefropatią w porównaniu z osobami bez nefropatii (odpowiednio 162 [57–618] vs. 316 [37–788] j.m./l).
Rozkład AER był następujący: w przypadku polimorfizmu L55M — MM > LM > LL ($p = 0,002$), a w przypadku polimorfizmu Q192R — QQ > QR > RR ($p < 0,001$). Rozkład aktywności PON1 był odwrotny ($p < 0,001$). U młodzieży z T1D haplotypy LMQQ i MMQQ wpływały na zwiększenie AER (odpowiednio $p = 0,004$ i $p = 0,003$) i zmniejszenie aktywności PON1 (odpowiednio $p = 0,011$ i $p = 0,052$). Natomiast obecność haplotypu LLRR powodowała odwrotny efekt.
Wnioski: Badanie wykazało, że polimorfizmy L55M i Q192R są genetycznymi markerami uczestniczącymi w rozwój nefropatii cukrzycowej u chorych na T1D. **(Endokrynol Pol 2017; 68 (1): 35–41)**

Słowa kluczowe: *polimorfizm paraoksonazy 1; cukrzyca typu 1; nefropatia cukrzycowa; aktywność paraoksonazy*

Abbreviations

AER — albumin excretion rate
BMI — body mass index
CAD — coronary artery disease
DN — diabetic nephropathy
HDL-c — high-density lipoprotein-cholesterol
LDL-c — low-density lipoprotein-cholesterol

Ons Fekih M.D., Laboratory of Biochemistry-Toxicology, Monastir University Hospital, 5000, Monastir, Tunisia, phone: 00 216 73 46 11 41, fax: 00 216 73 46 06 78, e-mail: onsfekihsallem@gmail.com

PCR — polymerase chain reaction
PON1 — paraoxonase 1
RFLP — restriction fragment length polymorphism
T1D — type 1 diabetes

Introduction

Type 1 diabetes (T1D) effects about 10% of all diabetes cases, and it represents a serious problem of health, due to the complications that can develop long-term [1]. Diabetic nephropathy (DN) is a major complication of diabetes and the most common cause of end-stage renal failure [2].

Paraoxonase 1 (PON1) is an HDL-associated lipolactonase preventing LDL and cell membrane oxidation, and is therefore considered to be atheroprotective [3, 4]. Thus, lower PON1 activity was observed in high oxidative stress diseases such as coronary heart disease, dyslipidaemia, inflammatory processes, and diabetes [5–8], and it has also been linked to kidney disease [1, 9, 10]. Furthermore, it has been reported that diabetic microangiopathy is genetically heterogeneous [11] and that oxidative stress pathway genes might be an important predictor for the development of diabetic complications [12]. The PON1 gene and its polymorphisms Q192R and L55M have been associated with increased risk of atherosclerosis [13–15]. These polymorphisms were identified in the coding region leading to different PON1 isoforms: substitution of leucine (L) to methionine (M) in position 55, and glycine (G) to arginine (R) in position 192 [4].

Despite the involvement of PON1 in different diseases, the potential relevance of PON1 for diabetes complications in children and adolescents with T1D has received relatively little attention [16–19].

Therefore, the purpose of this study is to investigate the effect of PON1 polymorphisms (L55M and Q192R) on diabetic nephropathy in a young Tunisian population with T1D.

Material and methods

Study population

Our study group consisted of 116 children and adolescents with type 1 diabetes from the paediatric department (mean age 13.97 ± 5.23 years, 72 males and 44 females), compared with 91 healthy children and adolescents without any evidence of diabetes, as a control group (mean age 12.59 ± 5.38 years, 52 males and 39 females). According to their medical files, the patients did not show any complication except for 26.7% of those youths who have DN (AER ≥ 30 mg/24 h).

We obtained parental consent for all patients and controls, as well as 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. Glucose was measured by an enzymatic method on an Integra 400™ (Roche Diagnostics, Mannheim, Germany) automaton, and HbA1C was determined by high-performance liquid chromatography on a G7™ (Tosch, Japan) automaton.

AER was determined by an immunoturbidimetry test (Integra 400™), in which 24-hour urine samples were collected. PON1 activity was measured by a kinetic method using paraoxon as substrate [20] on a Konelab 30™ automaton (Thermo Electron Corporation).

DNA analysis

Blood was drawn from the peripheral veins in K_3-EDTA tubes, and genomic DNA was prepared from leucocytes by guanidium chloride extraction, lysis by ammonium acetate, and ethanol precipitation.

Determination of PON1 polymorphism was achieved by a multiplex polymerase chain reaction according to Motti et al. [21] followed by a restriction digestion using HinfI. The fragments obtained were electrophoresed in a 4% agarose gel. The sizes of fragments were estimated in comparison to previously known size markers.

Statistical analysis

SPSS 18.0 was used for data analysis. All results were expressed as mean ± standard deviation for quantitative variables with normal distribution and as median (min–max) for non-Gaussian distribution.

The student-t test and Mann-Whitney-test were carried out to compare the quantitative variables, respectively, for Gaussian distribution and for non-Gaussian distribution. To determine genotypic and allelic frequencies the $\chi 2$ test was used. The Kruskall-Wallis-test was used to test the differences in parameters between genotypes and haplotypes. For the study of correlations, we used the correlation coefficient of Spearman rank. $p < 0.05$ was considered as statistically significant.

Results

The clinical characteristics of the study groups are shown in Table I. No difference was observed between patients and control group in mean age, BMI, or PON1 activity.

Table I. *Demographic and biological details of the study groups*
Tabela I. *Dane demograficzne i cechy biologiczne w badanej populacji*

Characteristics	Control population (n = 91)	T1D population (n = 116)	p
Age (years)	12.59 ± 5.38	13.97 ± 5.23	0.088
Gender (M/F)	1.33	1.63	0.474
BMI [kg/m2]	20.6(15.3–27.7)	20.5 (10.7–30.7)	0.756
Age of onset (years)	–	6.5 (1.0–23.0)	–
Duration (years)	–	7.0 (1.0–18.0)	–
Fasting glucose [mmol/L]	3.5 (2.0–5.5)	9.9 (2.0–26.2)	< 0.001
HbA1c (%)	5.9 (4.7–7.2)	9.9 (5.9–16.8)	< 0.001
PON1 activity [IU/L]	298 (55–1147)	302 (37–788)	0.817
AER [mg/24 h]	4.6 (1.1–30.1) *	12.8 (0.8–153.2) **	< 0.001

* n=76, **n = 110; BMI — body mass index

We noted a statistically significant increase in fasting glucose, HbA1c, and AER in patients.

PON1 activity was inversely correlated to AER (r = –0.245, p = 0.008).

A significant decrease (p = 0.037) in PON1 activity was shown between patients with nephropathy and those without nephropathy (162 (57–618) *vs.* 316 (37–788) IU/L, respectively).

The genotypic and allelic distribution of PON1 polymorphism showed no significant differences in patients and controls (Table II).

AER and PON1 activity values according to PON1 L55M and Q192R polymorphisms are defined in Table III.

We found that AER increased significantly in the T1D group, according to genotypes in the order LL < LM < MM and RR < QR < QQ (p = 0.002, p < 0.001, respectively). We noted a significant increase in AER in patients compared to controls in all genotypes except for RR.

Conversely to AER, PON1 activity increased significantly in youths with diabetes and in the control group following the order of genotypes: MM < LM < LL and QQ < QR < RR (p < 0.001).

We also observed a significant decrease of this activity in patients with QQ genotype compared to the control group (163 [81–356] *vs.* 112 [57–555] IU/L, p < 0.001).

The combined effects of PON1, L55M, and Q192R polymorphisms are summarised in Table IV. We noted a significant lower frequency of LMQQ haplotype (p = 0.037) with T1D patients compared to the controls.

We noted a significant increase of AER and a significant decrease of PON1 activity in patients with LMQQ haplotype (p = 0.004, p = 0.011) and with MMQQ haplotype (p = 0.003, p = 0.052).

Table II. *Genotypic and allelic distribution of PON1 L55M and Q192R polymorphisms in control and T1D populations*
Tabela II. *Dystrybucja genotypów i alleli polimorfizmów L55M i Q192R genu PON1 w grupie kontrolnej i w grupie chorych na cukrzycę typu 1*

	Control population (n = 91)	T1D population (n = 116)	p
PON1 L55M polymorphism			
Genotype			
LL	31.9% (n = 29)	28.4 % (n = 33)	0.354
LM	45.1% (n = 41)	41.3 % (n = 48)	0.355
MM	23.1% (n = 21)	26.7 % (n = 35)	0.201
Allele frequency			
L	0.55	0.49	0.414
M	0.45	0.51	0.414
PON1 Q192R polymorphism			
Genotype			
QQ	22.0% (n = 20)	19.8 % (n = 23)	0.383
QR	68.1% (n = 62)	65.5 % (n = 76)	0.381
RR	9.9% (n = 9)	14.7 % (n = 17)	0.230
Allele frequency			
Q	0.56	0.52	0.415
R	0.44	0.48	0.401

Lower AER value and higher PON1 activity were observed for LLRR haplotype in the T1D group (3.20 [0.80–6.00] mg/24 h and 692 [105–778] IU/L, respectively).

We noted that the frequency of having an AER ≥ 30 mg/24 h was significantly higher (p < 0,001) in patients

Table III. *PON1 activities and microalbuminuria according to PON1 L55M and Q192R genotypes in control and in T1D populations*

Tabela III. *Aktywność PON1 i mikroalbuminuria w zależności od genotypu L55M i Q192R genu PON1 w grupie kontrolnej i w grupie chorych na cukrzycę typu 1*

Genotypes	PON1 activity (IU/L)			Microalbuminuria (mg/24 h)		
	Control population (n = 91)	T1D population (n = 116)	p	Control population (n =71)	T1D population (n = 110)	P*
LL	460 [55–672] (n = 29)	367 [100–778] (n = 33)	0.352	2.87 [1.30–9.10] (n = 18)	9 [0.80–54.30] (n = 30)	0.002
LM	267 [63–1147] (n = 41)	324 [37–788] (n = 48)	0.749	4.9 [1.10–30.12] (n = 37)	12.2 [2.6–153.20] (n = 47)	< 0.001
MM	135 [93–350] (n = 21)	120 [57–641] (n = 35)	0.754	3.65 [2.30–7.90] (n = 16)	20.10 [5.20–59.40] (n = 33)	< 0.001
p**	< 0.001	< 0.001		0.223	0.002	
QQ	163 [81–356] (n = 20)	112 [57–555] (n = 23)	< 0.001	5.2 [2.40–8.40] (n = 14)	25.8 [7.60–59.40] (n = 21)	< 0.001
QR	335 [55–1147] (n = 62)	309 [37–788] (n = 76)	0.597	4.3 [1.10–30.12] (n = 51)	12.25 [2.60–153.20] (n = 74)	< 0.001
RR	513 [110–672] (n = 9)	618 [143–788] (n = 17)	0.312	4.01 [1.30–6.40] (n = 6)	4.3 [0.80–31.10] (n = 15)	0.815
p***	< 0.001	< 0.001		0.434	< 0.001	

Table IV. *Haplotype frequencies, PON1 activities, and microalbuminuria in control and in T1D populations*

Tabela IV. *Częstość występowania haplotypów, aktywność PON1 i mikroalbuminuria w grupie kontrolnej i w grupie chorych na cukrzycę typu 1*

Haplotypes	Control population			T1D population			p	p*	p**
	n (%) (n = 91)	PON1 activity (IU/L) (n = 91)	Microalbuminuria (mg/24h) (n = 71)	n (%) (n = 116)	PON1 Activity (IU/L) (n = 116)	Microalbuminuria (mg/24h) (n = 110)			
LL QQ	4 (4.4)	309 [253–356]	7.1 (n = 1)	2 (1.7)	375 [350–401]	4.62 [7.60–21.65] (n = 2)	0.202	0.165	0.221
LL QR	17 (18.7)	418 [55–554]	2.62 [1.40–9.10] (n = 12)	20 (17.2)	297 [100–427]	14.30 [3,0–54.30] (n = 19)	0.399	0315	< 0.001
LL RR	8 (8.8)	552 [110–672]	4.6 [1.30–6.40] (n = 5)	12 (10.3)	692 [105–778]	3.20 [0.80–6.00] (n = 9)	0.384	0.114	0.317
LM QQ	11 (12.1)	177 [81–263]	5 [2.40–8.40] (n = 9)	5 (4.3)	115 [112–117]	17.40 [8.20–43.00] (n = 5)	0.037	0.011	0.004
LM QR	27 (31.9)	346 [63–1147]	4.8 [1.10–30.12] (n = 27)	39 (33.6)	340 [37–788]	12.05 [2.60–153.20] (n = 38)	0.399	0.774	< 0.001
LM RR	1 (1.1)	317 [317 –317]	3.43 (n =1)	4 (3.4)	277 [143–485]	18.30 [4.30–31.10] (n = 4)	0.213	1.000	0.157
MM QQ	5 (5.5)	140 [113–1098]	5 [2.52–7.0 0] (n = 4)	14 (12.1)	101 [57–157]	27.20 [14.20–59.40] (n = 13)	0.095	0.052	0.003
MM QR	16 (17.6)	140 [93–350]	3.6 [2.3–7.90] (n = 12)	18 (15.5)	289 [72–565]	12.55 [6.65–35.30] (n = 18)	0.380	0.227	< 0.001
MM RR	0 (0)	_	(n = 0)	2 (1.7)	494 [347–641]	6.95 [5.20–8.70] (n = 2)	0.171	_	_

p: χ^2 test between haplotype frequencies in control and T1D populations; p*: χ^2 test between PON1 activity in control and T1D populations with the same haplotype;

p**: χ^2 test between microalbuminuria in control and T1D populations with the same haplotype

with LMQQ haplotype and in those with MMQQ haplotype compared to patients with LLRR haplotype.

Discussion

Epidemiological and family studies have suggested the existence of a genetic susceptibility to DN [22]. Genes that encode proteins involved in the attenuation of oxidative stress have been proposed as candidates because an increase in oxidative stress seems to be a mechanism that contributes to the development of DN [23]. Thus, we suggest that the PON1 gene could be considered among these candidate genes.

Low PON1 activity has been consistently linked to an increased risk of cardiovascular disease [24, 25], which is the leading cause of mortality of patients with T1D [26, 27].

The present study showed no significant difference in PON1 activity among children and adolescents with T1D. This can be partly explained by the young age of this population in which the majority has not had any complications yet.

Many other studies suggested that PON1 activity was decreased in T1D groups compared to control groups [17, 19, 28, 29].

A significant decrease in PON1 activity was noted for patients with nephropathy compared to those without. This result confirmed many other studies that reported that PON1 activity is lower in renal failure patients and that it is involved in the development of DN [10, 26, 30, 31].

Moreover, it has been reported that PON1 plays an important role in maintaining normal kidney homeostasis. Indeed, PON1 is a hydrolase that participates in homocysteine metabolism and is carried in the circulation on high-density lipoprotein. Thus, deficiency in this enzyme is toxic to kidney function because it impairs metabolic pathways that are important for normal kidney homeostasis [10]. It has also been reported that PON1 activity is involved in microvascular complications of diabetes [1, 32, 33].

In the current study, we found no significant differences in genotypic and allelic frequencies of PON1, L55M, and Q192R polymorphisms, although we noted that M and R alleles as well as MM and RR genotypes were more common in children with T1D than in controls. Our findings are in disagreement with those of Mackness et al. and Flekač et al., who reported that M allele and MM genotype were much more common in subjects with diabetes than in controls, while R allele and RR genotype were more common in controls [28, 34].

As described in the literature about different populations [5, 28, 35–37], we noted that PON1 activity reached its maximum for LL and RR genotypes and its minimum for MM and QQ genotypes in patients and in controls. We therefore concluded that LL and RR genotypes may be protective against oxidative damage of lipoprotein by decreasing peroxidation of LDL by HDL. In addition, the high prevalence of MM and QQ genotypes in the population with diabetes was associated with poor glycaemic control, resulting in non-enzymatic glycation and the acceleration of oxidative stress [34].

AER values, according to PON1 polymorphisms, showed the opposite distribution to PON1 activity in a T1D population. Consequently, we noticed that MM and QQ genotypes were associated with a lower PON1 activity and a higher AER in patients, which increases the risk of nephropathy among them and consequently contributes to acceleration of atherosclerosis in the long term. In contrast to LL and RR genotypes, we observed increased PON1 activity and decreased AER, so these genotypes can be protective against DN in this T1D population.

Jenkins et al. [38] found that QQ genotype was associated with AER (≥ 40 mg/24 h) in people with diabetes. However, another study suggested that LL genotype was associated with an excess of AER in T1D, which increased cardiovascular risk among them [14]. Kao et al. [39] noted that the risk of developing AER is higher for patients with LM and with RR genotype compared to other genotypes.

However, a meta-analysis study [40] indicated that PON1Q192R as well as PON2 gene polymorphisms may not confer a major genetic risk to DN or diabetic retinopathy and highlighted results for the risk of association of PON155L with diabetic retinopathy and not with nephropathy. Although studies in this context have been variable and sometimes contradictory, it must be noted that there were differences in terms of sample size, selection bias, age, ethnic distribution, and environmental background in the study groups involved.

Almost no studies have investigated the combined effect of PON1, L55M, and Q192R polymorphisms in youths with T1D. In the current study, the distribution of PON1 haplotypes showed a significant lower frequency of LMQQ haplotype ($p = 0.037$). Another study in a healthy Iranian population showed that LMQR haplotype was more frequent [35].

The combined effects of PON1 polymorphisms revealed much lower PON1 activity and higher AER values in patients with LMQQ and MMQQ haplotypes. This can be explained by the cumulative effect found for every genotype for which we noted that LM, MM, and QQ genotypes reduced PON1 activity and increased AER in youths with T1D. We also noted that LLRR haplotype was associated with the highest PON1 activity and the lowest AER in a T1D population, which could be more protective to lipid peroxidation and to

microvascular complications of diabetes. This result confirmed the isolated effect of each polymorphism for which we noted a significant increase in PON1 activity for LL and RR genotypes. Our results are in agreement with those found in other populations with high risk of cardiovascular diseases [35, 41, 42].

Ozkök et al. [43] demonstrated that LLRR haplotypes were associated with coronary artery disease (CAD) in a Turkish population. However, Arca et al. [44] indicated a lack of combined effects of PON1, L55M, and Q192R polymorphisms on CAD risk. Shemesh et al. [45] also suggested that there was no combined effect of PON55-PON192 polymorphisms in renal diseases.

Conclusions

In conclusion, this study among children and adolescents with T1D showed a significant inverse association of PON1 activity with AER. Combined effects of PON1 polymorphisms showed that LMQQ and MMQQ haplotypes seem to reduce PON1 activity and increase AER in patients. This is largely in relationship with the severity of complications that youths with T1D can develop long term. However, LLRR haplotype seems to be protective against DN in this population. Therefore, we conclude that PON1 polymorphisms (L55M and Q192R) seem to be genetic markers involved in the development of DN in T1D. These results could have important clinical significance for the prevention and prognosis of DN in T1D by improving PON1 activity to prevent the development of DN to a renal failure stage and also by increasing monitoring for high genetic risk patients. Additional studies with enlarged sample size are required for a better evaluation of the role of PON1 haplotypes, which could bring better insight into the association of PON1 polymorphisms with diabetes complications.

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