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Diabetes & Metabolism 35 (2009) 37–42

Original article

Association of the *ENPP1* K121Q polymorphism with type 2 diabetes and obesity in the Moroccan population

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Received 7 February 2008; received in revised form 10 June 2008; accepted 24 June 2008
Available online 28 November 2008

Abstract

Aim. – The ectonucleotide pyrophosphatase/phosphodiesterase 1 enzyme (*ENPP1*), which downregulates insulin signaling by inhibiting insulin-receptor tyrosine kinase activity, is encoded by the *ENPP1* gene. A common functional *ENPP1* K121Q polymorphism has been suggested to contribute to insulin resistance, obesity and type 2 diabetes (T2D) in various ethnic groups. For this reason, we assessed the association between the *ENPP1* K121Q polymorphism in T2D and obesity phenotypes in the Moroccan population.

Methods. – Using LightCycler® technology, we genotyped the *ENPP1* K121Q polymorphism in 503 subjects with T2D and 412 normoglycaemic individuals.

Results. – There was no evidence of an association between *ENPP1* K121Q and T2D in either an additive ($P = 0.99$) or recessive mode of inheritance ($P = 0.47$). However, the Q121 variant was significantly more frequent in obese than in non-obese subjects after adjusting for age, gender and T2D status. We observed genetic heterogeneity between obese and non-obese T2D patients ($P = 0.02$). The K121Q polymorphism was associated with T2D in the presence of obesity in both additive (1.55 [95% CI 1.16–2.07]; $P = 0.003$) and recessive (2.31 [95% CI 1.34–3.97]; $P = 0.002$) modes of inheritance.

Conclusion. – Although there was no evidence of an association between the *ENPP1* K121Q variant and the general phenotype of T2D, we did find an association with adult obesity and T2D. The Q121 allele frequency in Morocco is 37.3%, placing it between European Caucasians (15%) and Black Africans (79%). This study is the first to report an association between K121Q and metabolic diseases in the Moroccan population.

Résumé

Association du polymorphisme K121Q du gène *ENPP1* avec le diabète de type 2 et l'obésité dans la population marocaine.

Objectif. – Le gène codant l'enzyme ecto-nucléotide pyrophosphatase/phosphodiestérase 1 (*ENPP1*) est un inhibiteur du récepteur de l'insuline. Le polymorphisme fonctionnel *ENPP1* K121Q constitue un facteur de risque pour la résistance à l'insuline, l'obésité et le diabète de type 2 (DT2). L'objectif de cette étude était d'évaluer l'association du polymorphisme K121Q avec le DT2 et l'obésité dans la population marocaine.

Méthodes. – Cinq cents trois patients diabétiques (DT2) et 412 sujets normoglycémiques (témoins) ont été génotypés pour le polymorphisme K121Q à l'aide de la technologie LightCycler®.

Résultats. – Aucune association significative n'a été mise en évidence entre le polymorphisme K121Q et le risque de DT2 sous le modèle additif ($P = 0{,}99$) ou récessif ($P = 0{,}47$). Cependant, le variant Q121 était significativement plus fréquent chez les obèses après ajustement pour l'âge, le sexe et le statut DT2. Une hétérogénéité génétique a été observée entre les DT2 obèses et les DT2 non obèses ($P = 0{,}02$). Le rapport de côte (*odds ratio*) pour le DT2 en présence d'obésité, après ajustement pour l'âge et le sexe, était de 1,55 [IC à 95 % 1,16–2,07] ($P = 0{,}003$) sous le modèle additif et de 2,31 [IC à 95 % 1,34–3,97] ($P = 0{,}002$) sous le modèle récessif.

Conclusion. – Cette étude démontre qu'il n'y a pas d'association entre le variant *ENPP1* K121Q avec le phénotype général de DT2 dans cet échantillon de la population marocaine. Cependant, une association significative est observée avec l'obésité et le DT2 en présence d'obésité.

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doi:10.1016/j.diabet.2008.06.005

La fréquence allélique du variant Q121 au Maroc est de 37,3 %, une valeur intermédiaire entre celle observée dans les populations européennes (15 %) et africaines (79 %). Cette étude démontre pour la première fois l'association entre le polymorphisme K121Q et des anomalies métaboliques dans la population marocaine.

Keywords: *ENPP1* K121Q polymorphism; Type 2 diabetes; Obesity; Morocco

Mots clés : Polymorphisme K121Q *ENPP1* ; Diabète de type 2 ; Obésité ; Maroc

1. Abbreviations

ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1 enzyme
T2D	type 2 diabetes
BMI	body mass index
WHR	waist–hip ratio
OR	odds ratio
SNP	single nucleotide polymorphism

2. Introduction

The *ENPP1* gene, also known as PC-1 (plasma cell-1), is located on chromosome 6 (6q22-q23) and encodes for an inhibitor of insulin-signaling tyrosine kinase activity [1]. Consequently, *ENPP1* has been proposed as a candidate gene for insulin resistance, obesity and T2D susceptibility [2]. A functional non-synonymous polymorphism in exon 4 of the *ENPP1* gene (K121Q) has already been associated with insulin resistance in healthy Italian subjects [3].

A recent meta-analysis of 30 studies suggested that the Q allele of the K121Q non-synonymous SNP is associated with T2D in the European population in a recessive model [4]. In that report, McAteer et al. also demonstrated that the BMI strongly modulates the effect of K121Q on T2D risk. However, the case for obesity is uncertain: although a reproducible association has been documented between K121Q and childhood obesity [5,6], more controversial results have been reported for adult obesity [5,7–10].

An interpopulation frequency of the *ENPP1* Q121 allele that varies, depending on ethnicity and geographical location, was reported by Keshavarz et al. [11]. In European Caucasians, the Q121 prevalence ranged from 10% in Finns [12] to 17.8% in Sicilians [13]. In the Dominican Republic, a population with a mixed genetic background, the allele's frequency was 54.2% [14], and an even higher Q121 allele frequency (79%) was reported in African-Americans [8].

Until now, there have been scanty data available on the genetics of T2D in the Moroccan population, except for our recent study confirming the association of the *TCF7L2* rs7903146 T allele with T2D in the Northern European population [15]. In Morocco, recent environmental and behavioral changes, such as the adoption of new eating habits, a sedentary lifestyle, and stress linked to urbanization and poor working conditions have contributed to an increase in the incidence of T2D and obesity [16–18]. Therefore, with this cross-sectional study, we aimed to determine whether or not the *ENPP1* K121Q variant is associated with T2D and obesity phenotypes in the Moroccan population.

3. Research design and methods

3.1. Subjects

Patients with T2D (153 men and 350 women) were recruited from the T2D registries of diabetes associations and health centers in three different regions (Fez, Sale and Taounate). The diagnosis of diabetes mellitus was made according to American Diabetes Association criteria [19] or if the patient was taking medication for diabetes. The non-diabetic control subjects comprised 412 volunteers (127 men and 285 women), recruited from an unselected population undergoing a routine health check-up at the same health centers. Inclusion criteria were: age greater than or equal to 40 years; no history of a diagnosis of diabetes; no diabetes in first-degree relatives; and fasting plasma glucose is smaller than 1.11 g/l.

Information regarding age, type of diabetes, duration of diabetes and type of treatment was completed on a data collection sheet. Weight and height were measured for all participants, and were recorded to the nearest kilogram (kg) and centimeter (cm), respectively. BMI was calculated as weight divided by the height squared (kg/m^2). WHR was defined as the ratio between the circumferences of the waist to the hip. Obesity was defined by values of BMI $\geq 30\,kg/m^2$, according to the recommendations of the World Health Organization [20].

The study protocol was approved by the Moroccan Ministry of Health. Informed consent was obtained from each study participant according to the guidelines of the Helsinki Convention.

3.2. Genotyping

Genotyping of K121Q was performed using the LightCycler® 480 technology based on hybridization probes labeled with fluorescent dyes (Roche). The PCR primers were designed by Primer Express and optimized according to the manufacturer's protocol. For the SNP, a total of 10% of the samples were re-genotyped for quality control, and there was 100% concordance of genotypes.

3.3. Statistical methods

Tests for deviation from the Hardy–Weinberg equilibrium and for associations were performed with the De Finetti programme (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). SPSS 14.1 software (SPSS Inc., Chicago, IL, USA) was used for all

Table 1
Study participants' characteristics relating to diabetes and obesity.

	T2D			Normoglycaemic		
	All ($n=503$)	Obese ($n=157$)	Non-obese ($n=346$)	All ($n=412$)	Obese ($n=102$)	Non-obese ($n=310$)
Male (%)	30.4	16.6	36.7	30.8	15.4	36.5
Age (years)	57.6 ± 11.2**	55.6 ± 10.5	58.7 ± 11.4	55.2 ± 12.3	52.3 ± 10.5	56.1 ± 12.7
BMI (kg/m^2)	28.02 ± 4.73*	33.5 ± 2.9	25.5 ± 2.9	27.18 ± 5.32	34.21 ± 4.46	24.87 ± 3.08
WHR	0.92 ± 0.07***	0.92 ± 0.06	0.92 ± 0.07	0.91 ± 0.06	0.91 ± 0.07	0.91 ± 0.06
Family history of diabetes (%)	60.4	63.1	59.2	–	–	–
Diabetes duration (years)	6.4 ± 6.0	5.4 ± 5.4	6.9 ± 6.2	–	–	–
Fasting plasma glucose (g/l)	–	–	–	0.92 ± 0.11	0.91 ± 0.12	0.92 ± 0.11

*$P=0.01$ vs controls; **$P=0.001$ vs controls; ***$P=0.006$ vs controls.
BMI: body mass index; WHR: waist–hip ratio; T2D: type 2 diabetes.

statistical analyses. Student's t test was applied for mean comparisons of continuous traits, and the chi-square test (χ^2) applied for binary traits. Logistic-regression analysis was performed to assess the effect of the K121Q polymorphism on T2D and obesity after adjustment for covariates that were established risk factors for diabetes and obesity. Statistical significance was considered to be a P value <0.05.

4. Results

The clinical data obtained in this study are shown in Table 1. There were significant differences in the distributions of age, BMI and WHR between the T2D and control groups. The mean age of the diabetic group (57.6 ± 11.2 years) was slightly higher ($P=0.001$) than that of the control group (55.2 ± 12.3 years). BMI and WHR means were significantly higher in T2D patients than in controls ($P \leq 0.01$), and the obesity distribution was significantly higher in T2D patients than in controls ($P<0.01$). Gender did not differ significantly between the two groups, and two-third of the participants were women.

Allele frequency of the Q121 variant was 37.3%. First, we evaluated the association of the *ENPP1* K121Q variant with T2D and with obesity in the case-control Moroccan study (Table 2). As the second step, we divided each group of T2D patients and controls into two subgroups according to obesity status, and evaluated the eventual associations (Table 3). Genotype distributions of the *ENPP1* K121Q did not significantly deviate from the Hardy–Weinberg equilibrium ($P>0.2$).

Logistic-regression analyses for T2D risk, including age, gender and BMI as covariates and an additive or a recessive gene effect (the most plausible for *ENPP1* K121Q polymorphism, according to the literature), could find no statistical significance ($P=0.99$ with the additive model; $P=0.47$ with the recessive model; Table 2).

However, assessing the *ENPP1* K121Q association with obesity in the same cohort did give significant results. After adjusting for age, gender and T2D status, the OR for obesity were 1.36 (95% CI 1.09–1.21; $P=0.006$) for the additive model and 1.91 (95% CI 1.27–2.85; $P=0.002$) for the recessive model (Table 2). In this model of logistic regression, the effect of T2D status was statistically significant ($P=0.01$), which pointed to the association of K121Q with obesity in both diabetic patients and normoglycaemic subjects.

Genotype distribution differed significantly between obese T2D ($n=157$) and non-obese T2D ($n=346$) patients, in both additive ($P=0.003$) and recessive ($P=0.002$) models (Table 3). In the normoglycaemic subgroup (controls), the Q121Q genotype was not associated with obesity (Table 3).

On testing the association between the K121Q polymorphism and diabesity, and after adjusting for age and gender, a nominal trend of association was observed between T2D in the presence of obesity and the K121Q genotype in the additive model (OR = 1.29 [0.99–1.67]; $P=0.057$; Table 3).

Table 2
Case-control association analyses for the *ENPP1* K121Q polymorphism and T2D and obesity.

	Genotype (%)			Allele (%)		Additive model		Recessive model	
	KK	KQ	QQ	K	Q	OR (95% CI)	P value	OR (95% CI)	P value
T2D	194 (38.6)	240 (47.7)	69 (13.7)	628 (62.4)	378 (37.6)	0.99[a] (0.83–1.21)	0.99	0.87[a] (0.60–1.27)	0.47
Normoglycaemic	168 (40.8)	183 (44.4)	61 (14.8)	519 (63)	305 (37)				
Obese	93 (35.9)	116 (44.8)	50 (19.3)	302 (58.3)	216 (41.7)	1.36[b] (1.09–1.68)	0.006	**1.91**[b] (1.27–2.85)	0.002
Non-obese	269 (41.0)	307 (46.8)	80 (12.2)	845 (64.4)	467 (35.6)				

Data are expressed as the number of subjects with each genotype and number of alleles (frequency in %).
OR: odds ratio; CI: confidence interval; T2D: type 2 diabetes.
[a] Adjusted for age, gender and body mass index.
[b] Adjusted for age, gender and T2D status.

Table 3
Case-control association analyses for the *ENPP1* K121Q polymorphism and diabesity and obesity.

	Genotype (%)			Allele (%)		Additive model		Recessive model	
	KK	KQ	QQ	K	Q	OR (95% CI)	*P* value	OR (95% CI)	*P* value
T2D obese	52 (33.1)	74 (47.1)	31 (19.7)	178 (56.7)	136 (43.3)	1.55 (1.16–2.07)	0.003	2.31 (1.34–3.97)	0.002
T2D non-obese	142 (41)	166 (48)	38 (11)	450 (65)	242 (35)				
Normoglycaemic obese	41 (40.2)	42 (41.2)	19 (18.6)	124 (60.8)	80 (39.2)	1.48 (0.80–2.75)	0.21	1.14 (0.82–1.58)	0.43
Normoglycaemic non-obese	127 (41)	141 (45.5)	42 (13.5)	395 (63.7)	225 (36.3)				
T2D obese	52 (33.1)	74 (47.1)	31 (19.7)	178 (56.7)	136 (43.3)	1.29 (0.99–1.67)	0.057	1.42 (0.87–2.30)	0.16
Normoglycaemic	168 (40.8)	183 (44.4)	61 (14.8)	519 (63)	305 (37)				

Data are expressed as the number of subjects with each genotype and number of alleles (frequency in %); all associations have been adjusted for age and gender.
OR: odds ratio; CI: confidence interval; T2D: type 2 diabetes.

5. Discussion

In the present case-control study, we evaluated the effects of the *ENPP1* 121Q variant on T2D, diabesity and obesity in a Moroccan population, and found no evidence of an association between the *ENPP1* K121Q polymorphism and T2D. Nevertheless, we were able to detect nominal evidence of an association between *ENPP1* 121Q, obesity and diabesity. We also showed that the Q121 variant is harbored at an intermediate frequency in our population compared with individuals of European or African descent.

The *ENPP1* K121Q polymorphism was significantly associated with a genetic susceptibility for T2D in a meta-analysis conducted by Abate et al. [21], and further recent studies have confirmed this finding [22,23]. In contrast, no significant association has been found in other studies [8,9,11,12,24], including the present one. Given the rather modest role of ENPP1 on T2D risk, it is likely that our study is not sufficiently powered to allow any definitive conclusions to be drawn for the Moroccan population. Obesity is strongly associated with insulin resistance in both normoglycaemic and T2D individuals [25–27], and the Q121 allele may increase insulin resistance in the Moroccan population. In the recent large-scale meta-analysis performed for the *ENPP1* consortium, McAteer et al. found that BMI modulates the association between *ENPP1* Q121 and T2D risk in a recessive model [4]. This finding is concordant with the nominal association with T2D in the presence of obesity found in our study, and also described by other authors for T2D risk [28–30] and, more recently, by Stolerman et al. [31] for glycaemic traits.

Our data confirm a possible role of the K121Q polymorphism in obesity susceptibility. The *ENPP1* gene has pleiotropic effects, but the mechanisms by which it might modulate BMI are unknown [2]. Meyre et al. reported a deleterious effect of the Q121 variant on the risk of obesity in both case-control and prospective studies [5,30]. Similarly, Barosso et al. have reported an increased BMI in British adults who were homozygous for the Q121 variant [32]. However, a contentious effect of this variant on obesity has been shown in Danish subjects by Grarup et al. [33]. In contrast, the Q121 variant has been associated with a lower BMI in both Caucasians [7,10] and African-Americans [7]. It was hypothesized that the higher BMI in Q121 carriers could be due to the fact that individuals carrying this variant might develop insulin resistance in the brain, where insulin has potent anorectic actions [34], resulting in weight gain. On the other hand, the reported peripheral insulin resistance—a possible consequence of impaired insulin-mediated lipid storage in adipocytes—is associated with a lower risk of weight gain [35]. Thus, the reduced BMI in Q121 carriers might be due to the deleterious effect of this polymorphism on peripheral insulin resistance.

K121Q frequency varies according to ethnicity and geographical location. In Caucasians, the frequency of the Q121 allele ranges from 10 to 17.8% [5,12,13,33,36–39], whereas the allele frequency in Japanese and Chinese populations is relatively low (10.5 and 9.8%, respectively) [11,24]. Furthermore, higher frequencies of the Q121 allele have been reported

in African-American (78.5%) [22] and Dominican-Republic populations (54.2%) [14].

In the present study, we showed that the Q121 allele frequency (conferring risk of diabesity) in the Moroccan population is 37.3%, a frequency that lies between those of Caucasian and African populations. Our study is the first to report the frequency of the K121Q variant that is associated with diabesity in Morocco. Further studies using large-scale cohort studies are required to ascertain the possible involvement of the *ENPP1* K121Q variant in the pathogenesis of insulin resistance or T2D and its complications in the Moroccan population.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

This work was partly supported by the Moroccan–French convention CNRST-CNRS. We would like to thank the Ministry of Health for authorizing this study. We also wish to thank the regional delegations of the Ministry of Health in Fez, Taounate and Sale, and the technical recruitment staff—in particular, Benslimane Abdelillah, Cheba Mohamed, Barhaila M'hamed, Janati Idrissi Azzedine, Bennis Youssef, El Hayani Fatima and Moustachfa Khalid. We are indebted to all of the individuals and diabetes associations in Fez, Taounate and Sale for their participation in this study.

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