Letters

Comment

– to: Pinizzotto M, Castillo E, Fiaux M, Temler E, Gaillard RC, Ruiz J (2001) Paraoxonase 2 polymorphisms are associated with diabetic nephropathy in Type II diabetes. Diabetologia 44: 104–107

To the Editor: Proteins encoded by paraoxonase genes *(PON1* and *PON2)* could modulate oxidative stress which is hypothesized to play an important part in the development of late diabetic complications [1]. In a recent article, Pinizzotto et al. examined the association of diabetic nephropathy with polymorphisms in these genes [2]. In that study, 152 patients with normoalbuminuria despite a long duration of Type II (non-insulin-dependent) diabetes mellitus were the group of controls, and 147 patients with Type II diabetes and microalbuminuria or macroalbuminuria were the group of cases. As we found in patients with Type I (insulin-dependent) diabetes mellitus [3], diabetic nephropathy was not associated with polymorphism in *PON1*. However, Pinizzotto et al. found it was associated with two polymorphisms in *PON2* that cause amino acid substitutions (Ala148Gly and Ser311Cys). In univariate analysis, the odds ratio for diabetic nephropathy for carriers of the Gly allele at codon 148 (Gly: Gly and Ala: Gly) relative to non-carriers (Ala: Ala) was 1.83 ($p = 0.012$). The odds ratio for carriers of the Cys variant at codon 311 was 2.0 ($p = 0.004$) [2].

To evaluate the role of the *PON2* polymorphisms in predisposition to diabetic nephropathy in Caucasians with Type I diabetes, we conducted a case-control study with 90% power to detect the odds ratio of 1.83 reported by Pinizzotto et al. [2]. Our study included 508 patients with Type I diabetes (241 controls and 267 cases). Control subjects had a urinary albumin-to-creatinine ratio less than 17 (mg/g) for men or less than 25 for women and at least 15 years diabetes duration. Cases had persistent proteinuria ($n = 164$) or end-stage renal disease ($n = 103$). Genomic DNA from cases and controls was amplified using polymerase chain reaction and previously published primers [4]. Genotypes were determined by an allelic-specific oligonucleotide method [5] with the following specific probes: 148-allele C-gaa gca gaa aat tct ct, 148-allele G-gaa gga gaa aat tct ct, 311-allele C-cta tct gag aag cct ac, and 311-allele G-cta tgt gag aag cct ac.

Frequencies of the Ala148Gly and Ser311Cys variants were similar to those described by Pinizzotto et al. (20.8% and 20.3%, respectively) [2] and genotype frequencies were con-

Table 1. Frequencies of PON2 polymorphisms that result in amino acid changes in patients with Type I diabetes according to diabetic nephropathy status[a]

	Controls $n = 241$	Cases $n = 267$	p
Codon 148			
Ala/Ala	61.8%	62.5%	0.87
Gly/Ala + Gly/Gly	38.2%	37.5%	
Codon 311			
Ser/Ser	66.0%	60.1%	0.16
Ser/Cys + Cys/Cys	34.0%	39.9%	

[a] More information about the study groups can be found in [6]

sistent with Hardy-Weinberg expectations (chi-square, $p > 0.05$). However, genotype distributions in controls and cases were not different. Carriers of the 148Gly variant were equally frequent in the controls and cases (38.2% and 37.5%, respectively; $p = 0.87$), and carriers of the 311Cys variant were also similarly frequent in controls and cases (34.0% and 39.9%; $p = 0.16$; Table 1).

In summary, our results in a well-powered case-control study do not indicate that *PON2* polymorphisms are associated with the development of diabetic nephropathy in Type I diabetes. The discrepant results reported by Pinizzotto et al. [2] are difficult to reconcile with our findings. One possibility is that *PON2* polymorphisms are associated with diabetic nephropathy exclusively in patients with Type II diabetes. Another possibility is that the positive associations were spurious, the results of population stratification. It is interesting that the frequency of carriers of the 148Gly allele among cases was similar in the study by Pinizzotto et al. and in our study (43.5% and 37.5%), while the frequency of this variant among controls was less frequent in their study than in ours (29.6 and 38.2%). Unfortunately, information about the selection of controls for their study is insufficient to evaluate possible causes for this difference. Replication of these findings in other populations of diabetic patients will be necessary to determine the role of the *PON2* in the predisposition of diabetic nephropathy.

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References

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The author's reply

To the Editor: We read with interest the negative study of Canani et al. regarding the association between *PON2* polymorphisms and diabetic nephropathy in patients with Type I (insulin-dependent) diabetes mellitus, as well as the related letter. We have a few observations about their conclusions. As suggested by the authors [1], the results of association studies could be discordant because of the vulnerability of case-control studies to various biases and phenotype definitions. This is partly illustrated by the discordant results of these two case-control studies. First, our study, was carried out on patients with Type II (non-insulin-dependent) diabetes mellitus [2].

Second, the definition of diabetic nephropathy is very different: cases had persistent proteinuria or end-stage renal disease in the study of Type I diabetic patients and in our study, cases had either micro-albuminuria (83% of the cases) or persistent proteinuria. Third, we observed that the susceptibility to diabetic nephropathy was enhanced by the degree of obesity. Unfortunately, information about the body mass index is not available in the study of Canani et al. The interaction between the body mass index and the risk of diabetic nephropathy-associated *PON2* polymorphisms in Type II diabetic patients could be the most important message of our study. Fourth, population stratification seems very unlikely because classical risk factors associated with diabetic nephropathy are present in our study (Table 1). Furthermore, microvascular and macrovascular complications in diabetes mellitus are both related to endothelial dysfunction. The definitions of these diabetic vascular complications remain controversial. The absence of consensus concerning definitions for these phenotypes is well illustrated by the recent article regarding the value of albumin excretion rate as predictor of diabetic nephropathy [3]. Initial studies have shown an approximate 80% rate of progression from microalbuminuria to proteinuria in Type I diabetic patients. More recent studies have observed only a 30 to 45% progression to proteinuria over 10 years and about a 40% progression during the same period from normoalbuminuria to macroalbuminuria. Similar findings have been reported in Type II diabetes mellitus. Microalbuminuria seems to be more a marker of endothelial dysfunction than a risk factor for diabetic nephropathy. This marker is associated with macrovascular, and micro-vascular diabetic complications. Our observations suggest that *PON2* gene polymorphisms could be more closely related to endothelial dysfunction than to diabetic nephropathy and that PON cluster play a part in the oxidative stress pathway.

In conclusion, the discordant results between these two case-control studies could be explained by the different definitions of the diabetic nephropathy and the type of diabetes rather than a selection bias. Replication of these findings on other populations of Type II diabetic patients with the same definition for diabetic nephropathy is required to determine whether the *PON2* gene is a susceptibility factor for endothelial dysfunction or micro-albuminuria.

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Table 1. Clinical and biological characteristics of Type II diabetic patients with and without nephropathy

	Nephropathy + (n = 147)	Nephropathy – (n = 152)	p value
Age (years)*	63.0 (61.0–65.0)	56.0 (53.0–58.0)	0.0001
Diabetes duration (years)*	11.0 (8.1–15.0)	5. 2 (5.0–5.5)	0.0001
Male sex (%)	70.5	53.6	0.003
Smoker (%)	48.3	42.8	NS
HBP (%)	72.2	46.0	0.001
BMI (kg/m^2)*	29.1 (28.4–29.8)	28.0 (27.1–29.1)	0.0834
HbA1$_c$ (%)*	8.30 (7.90–8.73)	7.35 (7.10–7.74)	0.0001
HDL-chol (mmol/l)*	1.00 (0.94–1.08)	1.13 (1.07–1.20)	0.0022
Triglycerides (mmol/l)*	2.07 (1.90–2.27)	1.59 (1.47–1.80)	0.0003
LDL-chol.*	3.14 (2.83–3.34)	3.21 (2.96–3.44)	0.109

NS > 0.1

* Findings given as median (95% CI)