Comment

To the Editor: Regarding Dr. Kahn's recent review article [1] and his consideration of the prevention of Type 2 diabetes mellitus, I think that such an issue deserves a strict analysis of its feasibility from a public health perspective. It must be emphasised that the prevention of Type 2 diabetes mellitus has been proven only in high-risk individuals. The ascertainment of this category is not routinely done in most westernised countries. On the contrary, overt Type 2 diabetes mellitus is currently diagnosed when a considerable part of its natural history has evolved and its complications are frequently present. So, its prevention will have to rely upon a distinct health strategy directed to detect and follow high-risk individuals, that is to say, completely asymptomatic individuals. In a cost-conscious era this issue poses a considerable financial threat. First you need a public health system that works properly, something that not every developed country has. Even with this premise, it can be imagined how difficult it will be to gain

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—to: Rippin JD, Patel A, Belyaev ND, Gill GV, Barnett AH, Bain SC (2003) Nitric oxide synthase gene polymorphisms and diabetic nephropathy. *Diabetologia* 46:426–428

To the Editor: In recent years numerous studies have tested associations between various candidate genes and the development or progression of diabetic kidney disease in Type 1 and Type 2 diabetic patients in different populations.

We read with interest the recent paper by Rippin et al. [1], showing the results of a search for associations between molecular markers in the genes encoding for inducible and constitutive endothelial nitric-oxide synthase (*iNOS*, *eNOS*) and the presence of overt nephropathy in a large group of Type 1 diabetic patients. The rationale for the study was previous observations linking endothelial dysfunction with cardiovascular disease and diabetic nephropathy, as well as the conflicting results of smaller previous studies on that subject. In the study of Rippin et al. genotype or allele frequencies of both polymorphisms (*iNOS* +/- and *eNOS* a/b, a 27-base pairs insertion/deletion in intron 4, which affects plasma nitric oxide concentrations [2]) in patients with overt nephropathy, were not different from those in a normoalbuminuria group. Such an observation, obtained in what appears to be an adequately powered study, practically excludes the role of the polymorphisms examined in the development of nephropathy in Type 1 diabetes.

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enough priority to detect and follow asymptomatic individuals at high risk for Type 2 diabetes mellitus, given that priority has not been assigned to detect timely Type 2 diabetes mellitus. Conceivably, it is very difficult to prevent a disease that is not even properly detected when it is already present, simply because the task of rescuing high-risk but asymptomatic individuals can be simply unaffordable. Only in this context the prevention of Type 2 diabetes mellitus can be viewed from a comprehensive point of view.

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On the other hand, it has also been documented that hereditary predisposition to progressive renal damage exists, and that such a predisposition is probably independent of underlying causes of kidney disease [3, 4]. Despite a few studies addressing the issue, the molecular basis of this phenomenon is still poorly understood.

While nitric oxide was previously found to be fundamental for the regulation of local renal haemodynamics, diuresis and natriuresis, gene polymorphisms affecting its generation are possible candidates in the search for molecular mechanisms of hereditary predisposition to the development of end-stage renal damage [5, 6].

In 1999 we collected DNA from 247 family trios consisting of patients with chronic renal disease, and both their parents [7]. Of these patients, 157 were receiving dialysis (haemodialysis or peritoneal dialysis), while 90 were being treated conservatively, but had creatinine clearance lower than 30 ml/min. The causes of chronic renal disease were: glomerulonephritis in 120 patients, interstitial nephritis (including chronic pyelonephritis) in 80 patients, and diabetic nephropathy in 47 Type 1 diabetic patients. The subjects of the study gave written informed consent, the study protocol has been approved by the Silesian School of Medicine Ethics Committee and was carried out in accordance with the Declaration of Helsinki.

In 217 of the trios, where good quality DNA was available for the affected patient and both parents, we determined *eNOS* a/b genotypes. Allele transmission from heterozygous parents to affected offspring was compared using the transmission/disequilibrium test (TDT) [8]. Of 434 parents 125 were heterozygous for the *eNOS* a/b polymorphism. Transmission of *eNOS* a/b alleles did not differ significantly from the random transmission of 50%/50% expected if no association exists between the examined marker and phenotype. The numbers of transmitted a/b alleles were 57/68 (46%/54%), (the χ^2 value was 0.96, the *p* value was 0.32) suggesting no association between the examined marker, and the presence of chronic renal

failure. The limited number of trios included in our study did not allow us to do further stratification analyses (e.g. by cause of kidney disease). However, allele transmission did not differ from random after stratification by gender (data not shown).

In summary, Rippin et al. observed no association between the *eNOS* *a/b* polymorphism and the presence of overt nephropathy in Type 1 diabetic patients in a case-control study. We now present data from a family-based study showing no association between this marker and the development of chronic renal failure in patients with various causes of chronic renal disease. According to our observation, the hypothesis that the *eNOS* polymorphism represents a molecular mechanism leading to chronic renal failure, independent of causes, is highly unlikely. On the other hand, a minor role of this marker in particular subsets of renal patients with different causes of kidney disease cannot be excluded on the basis of our observations.

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–to: Poa NR, Cooper GJS, Edgar PF: Amylin gene promoter mutations predispose to Type 2 diabetes in New Zealand Maori. *Diabetologia* 46: 574–578

Keywords Amylin mutations, IAPP mutations, Type 2 diabetes, ethnic background.

To the Editor: Poa et al. [1] screened for amylin gene mutations in New Zealand Maori and identified two different mutations in the promoter region of the amylin gene (–215 T/G and –132 G/A) and a missense mutation in exon 3 (Q10R), with a prevalence of 5.4%, 0.76% and 0.76%, respectively, in Type 2 diabetic Maori patients. The authors suggest that amylin is likely to be a susceptibility gene for Type 2 diabetes in this population, which has a high prevalence of diabetes.

We agree with the authors on the potential role of the amylin gene in the development of Type 2 diabetes. However, because the authors omitted any reference to the –132 G/A muta-

tion in their article, we would also like to remark the importance of this mutation. The presence of this mutation in Spanish subjects was described by our group [2] and corroborated by another group in a British cohort [3] of Caucasian origin. The aim of our study was to investigate the presence of the amylin gene mutations in a group of 186 Spanish Type 2 diabetic patients and 130 non-diabetic subjects. We detected a single heterozygous mutation consisting of a G/A substitution at position –132 bp in 18 patients with Type 2 diabetes and in two (2) control subjects. The frequency of the G/A genotype was higher in diabetic patients, than in controls (9.7% vs 1.5%, $p < 0.005$, odds ratio: 6.85, 95% CI: 1.56–30.08). Furthermore, studies of a family cohort (14 first-degree relatives belonging to eight families) detected the presence of the mutation in nine relatives, two of which were identified to be homozygous and having disturbed glucose metabolism.

A similar screening approach was carried out on British subjects, where the –132 G/A mutation was detected in 5.3% of the diabetic patients, compared to 3.2% of the non-diabetic control group [3]. The same mutation has been reported in 4.1% of a Type 2 diabetic Danish cohort, compared with 7.1% non-diabetic controls [4]. Unlike our findings, no difference in the frequency of the –132 G/A variant was detected between these two groups, probably due to differences in the genetic background of the British and Danish cohorts.

Due to the high prevalence of this mutation in our study group (this mutation is located in the enhancer domain close to transcription-factor DNA-binding motifs), we hypothesised that this polymorphism confers increased IAPP promoter activity and could result in an increased expression and peptide se-