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Synergistic effect of angiotensin II type 1 receptor genotype and poor glycaemic control on risk of nephropathy in IDDM

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Summary We investigated the contribution of polymorphisms in the angiotensin II type 1 receptor gene (*AGTR1*) to renal complications in an inception cohort of 152 insulin-dependent diabetic (IDDM) patients examined 15–21 years after diabetes onset. This nested case-control study included 79 normoalbuminuric control subjects and 73 cases with evidence of nephropathy ranging from microalbuminuria to overt proteinuria. Subjects were genotyped for two *AGTR1* polymorphisms ($T^{573}\rightarrow C$ and $A^{1166}\rightarrow C$), and an adjacent CA repeat microsatellite. Allele C^{1166} and the 140 bp allele of the microsatellite were more frequent among nephropathy cases than normoalbuminuric control subjects (0.322 vs 0.247, and 0.618 vs 0.521, respectively), but these differences were not statistically significant. Although not significant by themselves, the *AGTR1* polymorphisms contributed significantly to the risk of diabetic nephropathy when accompanied by poor glycaemic control. Among patients with frequent severe hyperglycaemia during the first decade of diabetes, the relative risk of nephropathy among allele C^{1166} carriers was 12.1 (95 % CI: 3.7–39.8), whereas it was only 1.4 (95 % CI: 0.6–3.5) among allele A^{1166} homozygotes. The difference between relative risks was highly significant ($\chi^2 = 8.25$, $p = 0.004$ with 1 *df*). A similar pattern of higher risk of microalbuminuria, specifically among those carriers of allele C^{1166} who had poor glycaemic control was also found in an independent study of a cross-sectional sample of 551 IDDM individuals, although the effect was smaller in magnitude. We conclude that DNA sequence differences in the *AGTR1* gene may modify the noxious effects of hyperglycaemia on the kidney. Allele C^{1166} carriers might especially benefit from nephropathy prevention programmes. [Diabetologia (1997) 40: 1293–1299]

Keywords Insulin-dependent diabetes mellitus, diabetic nephropathy, angiotensin II receptor, DNA polymorphisms, genetics.

Less than half of the patients with insulin-dependent diabetes mellitus (IDDM) develop diabetic nephropathy, which represents the major predictor of morbidity and premature mortality among these individuals [1, 2]. Why this complication develops in only a subset of IDDM patients is not known. Poor glycaemic control has been recognized as a major determinant of renal complications in IDDM [3, 4], but other factors, unrelated to hyperglycaemia, also appear to be operating. The recent finding of familial clustering of diabetic nephropathy suggests that renal complications in IDDM are the result of an interaction between the diabetic milieu and nephropathy predisposing genetic factors [5–7].

The search for the identity of the genes underlying predisposition to nephropathy is a very active area of research [8]. Since several studies have shown that familial predisposition to essential hypertension is associated with increased risk of diabetic nephropathy [9–11], most of the candidate genes have been selected

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Abbreviations: IDDM, insulin-dependent diabetes mellitus; ACE, angiotensin converting enzyme; AER, albumin excretion rate; ACR, albumin creatinine ratio; DGGE, denaturing gradient gel electrophoresis

from among loci involved in the regulation of blood pressure. The genes coding for the renin-angiotensin system have attracted special attention because angiotensin II, beside increasing systemic blood pressure, has several intrarenal effects that may act as powerful co-factors in the development of glomerular alterations. These effects include both alterations of renal haemodynamics, such as an increase in intraglomerular pressure, and direct stimulation of mesangial cell proliferation and matrix production [12, 13]. Thus, genetic variability in angiotensin II generation and/or action may contribute to variability in susceptibility to diabetic nephropathy. Further support for this hypothesis has been provided by clinical studies showing that inhibition of angiotensin II generation by angiotensin converting enzyme (ACE) inhibitors retards the progression of diabetic nephropathy [14].

The genes of the renin-angiotensin system include renin (*REN*), angiotensinogen (*AGT*), angiotensin-converting enzyme (*ACE*), and at least two types of angiotensin II receptors (*AGTR1*, the subtype through which most of angiotensin II actions are exerted, and *AGTR2*, whose function is uncertain [15]. While only one report has been published on the *REN* gene, showing a lack of association with diabetic nephropathy [16], several groups have investigated the relationships between *AGT* and *ACE* genes and renal complications with inconsistent results [8, 17–24]. Regardless of the controversies, however, it is clear that neither of these loci has an effect on the risk of nephropathy large enough to account for the observed familial clustering of renal complications in IDDM.

We have investigated the association between nephropathy in IDDM and three polymorphisms in the *AGTR1* gene, one of which (A^{1166}→C) has been previously found to be weakly associated with essential hypertension in the general population [25]. Since hyperglycaemia is the best described and, probably, the most important predictor of diabetic nephropathy, we have also studied whether these polymorphisms may influence susceptibility to renal complications in IDDM by magnifying the effect of poor glycaemic control on the risk of nephropathy.

Subjects, materials and methods

Study population. Individuals selected for this study were the participants in a nested case-control study of determinants of late diabetic complications that was conducted between 1986 and 1988 in a well-defined cohort of patients who had had IDDM for 15 to 21 years [11]. DNA was still available in 1994–1995 for 152 (94%) of the 162 participants; 79 of these were normoalbuminuric (referred to as normoalbuminuria group), and 73 had a variable degree of diabetic nephropathy (nephropathy group). The selection and evaluation of these patients has been described in detail previously [11]. The evaluation of renal status included measurements of fasting serum creatinine, and albumin excretion rate (AER) during a 3-h timed urine collection performed in the morning. Normoalbuminuria was defined as an AER under 30 μg/min. Overt proteinuria was defined as an AER 250 μg/min or more including patients on haemodialysis or with renal transplant. Microalbuminuria was defined as the range between normoalbuminuria and overt proteinuria. Patients were considered hypertensive if they were on antihypertensive drugs, or the average of two supine measurements (standard sphygmomanometer) of systolic or diastolic blood pressure was equal to or greater than 140 or 90 mmHg, respectively. Parental history of hypertension was ascertained by questionnaires mailed to the parents of patients and was considered positive if at least one parent had hypertension diagnosed and treated before age 60 years. An index of the frequency of hyperglycaemia during the first 12 years of IDDM was computed for each patient from clinical records as previously described [10]. The index is the percentage of the total number of blood glucose values (in mg/dl), recorded during visits to the Joslin Clinic before 1981 that exceeded the following criteria: 180 fasting; 240 less than 1.5 h after eating; 220 1.5 to 2.4 h after eating; 200 2.5 to 3.4 h after eating; and 180 3.5 h or more after eating. Table 1 summarizes the clinical characteristics of these individuals.

Table 1. Clinical characteristics of study patients according to renal status when examined 15 to 21 years after diagnosis of juvenile-onset IDDM

	Normoalbuminuria	Nephropathy
n (male/female)	79 (38/41)	73 (35/38)
Age at IDDM diagnosis (years)	12 ± 5	11 ± 4
Age at examination (years)	30 ± 5	29 ± 4
Albumin excretion rate (μg/min)	8 ± 7	1558 ± 2561
Microalbuminuria (*n*)	–	32
Overt proteinuria (*n*)	–	41
Impaired renal function (*n*)	–	16
Systolic blood pressure (mm Hg)	118 ± 14	134 ± 21[a]
Diastolic blood pressure (mm Hg)	78 ± 11	87 ± 14[a]
Antihypertensive drugs (%)	1	37
Parental hypertension (%)	49	59
Index of hyperglycaemia (%)	39 ± 19	54 ± 19[a]
HbA_1 (%)	10.9 ± 1.9	12.0 ± 2.0[a]

Data are means ± SD.
[a] $p < 0.001$

Additional study subjects. To confirm some of the findings in the main study population, the influence of glycaemic control on the relationship between the *AGTR1* A^{1166}→C polymorphism and the risk of microalbuminuria was investigated in a cross-sectional sample of 551 IDDM patients from a study of the natural history of microalbuminuria that is being conducted at the Joslin Clinic. The selection and evaluation of patients for this study has been described in detail previously [4]. In brief, all patients enrolled in this study ($n = 1613$) are Caucasian residents of Massachusetts and had IDDM onset before age 41 years. They came to the Joslin Clinic soon after the diagnosis of IDDM (on average 3 years) and have remained under the care of the clinic ever since. The evaluation of renal status was based on measurements of the albumin to creatinine ratio (ACR) in multiple random urine samples collected at the time of clinic visits during 1991–1993 [4]. Normoalbuminuria was defined as an ACR less than 17 μg/mg for men and less than 25 μg/mg for women (1.9 and 2.8 mg/mmol, respectively). Overt proteinuria was defined as an ACR greater than 250 mg/mg for men or greater than 355 for women (28 and 40 mg/

mmol, respectively) or a reagent strip reading 2 + or more. Microalbuminuria was defined as an ACR in the range between normoalbuminuria and overt proteinuria. Classification of a patient's renal status was based on a consensus of two out of three determinations. Of the 1613 patients screened, 1117 were normoalbuminuric, 295 had microalbuminuria, and 201 overt proteinuria [4]. We selected 410 normoalbuminuric and 141 microalbuminuric individuals for the present study. While the index of hyperglycaemia during the first 12 years of diabetes was not available for this additional study group, haemoglobin A_1 measurements during the 2 years preceding the determination of nephropathy (1990–1991) were available, and the geometric mean of these measurements was used as an index of long term glycaemic control, as previously described [4]. Since the values of recent haemoglobin A_1 measurements no longer correlate well with long term glycaemic control once overt proteinuria is established, patients with persistent proteinuria were excluded from the study [4].

DNA analysis. The main study population was genotyped for three polymorphisms in the *AGTR1* gene and adjacent regions: $T^{573}\rightarrow C$ – a conservative substitution in the *AGTR1* coding region [24], $A^{1166}\rightarrow C$-located in the *AGTR1* 3' untranslated region [25, 26], and a CA repeat microsatellite placed about 15 kb 3' of the *AGTR1* gene [27]. The 551 additional study subjects were genotyped for the $A^{1166}\rightarrow C$ polymorphism only.

$T^{573}\rightarrow C$ genotypes were determined by PCR amplification of genomic DNA followed by denaturing gradient gel electrophoresis (DGGE). A 236 bp fragment including *AGTR1* cDNA position 573 was amplified by PCR using a forward primer (5'-CCTGGCTATTGTTCACCC-3') and a reverse primer (5'-**CGCCGC**AACCCAGTATATTTTTGG-3'). The latter carried a 6 bp 'GC-clamp' at the 5' end (in bold face) to facilitate DGGE analysis. PCR was performed on 0.5 μg of DNA in 50 μl containing TRIS HCl 10 mmol/l pH 8.3, KCl 50 mmol/l, $MgCl_2$ 1.5 mmol/l, gelatin 0.001 %, each dNTP 0.2 mmol/l, each primer 0.25 mmol/l, Taq polymerase (Amplitaq, Perkin Elmer, Norwalk, Conn., USA) 25 U/ml, for 30 cycles (60 s at 95 °, 60 s at 52 °, 60 s at 72 °) in a Perkin Elmer Thermal Cycler 480. DGGE was performed according to a previously described protocol [28] in an 8 % polyacrylamide gel with a linear gradient of denaturants from 10 to 50 % (100 % = 7 mol/l urea in 40 % formamide) in 1 × Tris-Acetate-EDTA (TAE) buffer at a constant temperature of 60 ° at 10 V/cm for 4.5 h. Under these conditions allele T^{573} is recognized as a band stopping at 31 % of denaturant concentration, allele C^{573} as a band stopping at 32 %.

Genotyping of $A^{1166}\rightarrow C$ was carried out by PCR followed by *DdeI* digestions and agarose electrophoresis as previously described [26]. The CA microsatellite was genotyped by [γ-^{32}P]ATP labelled PCR followed by denaturing polyacrylamide gel electrophoresis as described by Davies et al. [27]. A total of eight alleles were detected in our population. Genotype distributions determined with these methods did not significantly depart from Hardy-Weinberg equilibrium in any of the study groups.

Statistical analysis. Allele frequencies were computed from genotype frequencies. The distribution of genotypes and alleles were compared between study groups by chi-square tests [29]. Haplotype frequencies were estimated by gene counting as previously described [30]. As a descriptive measure of association between genotypes and outcomes, odds ratios were calculated along with 95 % confidence intervals [29]. Odds ratios were compared by the Breslow-Day test. Logistic regression analysis was used to assess the independent contributions of risk factors to the development of diabetic nephropathy.

Results

AGTR1 polymorphisms and diabetic nephropathy. Only small differences in allele and genotype distributions were observed between cases and control subjects for all three polymorphisms (Table 2). Allele C^{1166}, which had previously been found to be weakly associated with essential hypertension [25], was not significantly more frequent in the nephropathy group than among normoalbuminuric individuals (0.322 vs 0.247, $\chi^2 = 2.1$, $p = 0.15$ with 1 *df*) (Table 2). Similarly, the nephropathy risk among C^{1166} allele carriers (heterozygotes and C^{1166} homozygotes) was not significantly higher than that of A^{1166} homozygotes (Table 2). Similar results were obtained when IDDM individuals with microalbuminuria and overt proteinuria or end-stage renal failure were considered separately (data not shown). The major allele of the AGTR1 microsatellite (# 4, 140 bp, indicated as A_1 in Table 2) showed a weak, non-significant association with nephropathy (0.618 in cases vs 0.521 in control subjects, $\chi^2 = 2.8$, $p = 0.24$ with 2 *df*). In order to consider these polymorphisms simultaneously, we used gene counting methods to estimate the distribution of haplotypes defined by the two polymorphisms in cases and control subjects. Carriers of the haplotype defined by allele C^{1166} and microsatellite allele #4 had a 2.8-fold risk of diabetic nephropathy relative to carriers of other genotypes (95 % CI: 1.4–5.8). While this comparison has a *p*-value of .0.004, the weight given to this finding must be tempered by the number of comparisons made; for example, there were eight possible pairings of alleles of the microsatellite with the apparent risk allele C^{1166}. Therefore, from Bonferroni's inequality, one may argue that the *p*-value is closer to 0.032. No significant association with arterial hypertension could be found for any of the *AGTR1* polymorphisms within each renal group (data not shown).

Interaction between AGTR1 polymorphisms and poor glycaemic control. We also asked whether *AGTR1* polymorphisms, despite a weak association with renal complications in the total group, might contribute importantly to susceptibility to diabetic nephropathy in the presence of poor glycaemic control. Individuals whose index of severe hyperglycaemia was above the median for the overall population had a 3.5-fold risk of diabetic nephropathy relative to individuals whose index of hyperglycaemia was below the median (95 % CI: 1.7–3.5) (Table 3). The magnitude of this risk, however, depended strongly on the $A^{1166}\rightarrow C$ genotype. Among carriers of the allele C^{1166} the nephropathy risk associated with an index of hyperglycaemia above the median was 12.1 (3.7–39.8), while it was only 1.4 (0.6–3.5) among non-carriers (i.e. allele A^{1166} homozygotes) (Table 3). The difference between the odds ratios was highly

Table 2. Comparison of genotype and allele frequencies for $T^{573}\rightarrow C$, $A^{1166}\rightarrow C$, and *AGTR1* microsatellite in IDDM subjects with and without nephropathy

	$T^{573}\rightarrow C$		$A^{1166}\rightarrow C$		*AGTR1* microsatellite	
	Normoalbuminuria	Nephropathy	Normoalbuminuria	Nephropathy	Normoalbuminuria	Nephropathy
Genotypes						
A_1/A_1	18 (23.4)	12 (17.6)	47 (59.5)	35 (48.0)	17 (23.9)	23 (33.8)
A_1/A_2	37 (48.0)	35 (51.5)	25 (31.7)	29 (39.7)	15 (21.1)	13 (19.1)
A_2/A_2	22 (28.6)	21 (30.9)	7 (8.9)	9 (12.3)	0 (0.0)	1 (1.5)
A_1/A_3	–	–	–	–	25 (35.2)	25 (36.8)
A_2/A_3	–	–	–	–	6 (8.4)	3 (4.4)
A_3/A_3	–	–	–	–	8 (11.3)	3 (4.4)
Significance	$\chi^2 = 0.7, p = 0.70$[b]		$\chi^2 = 2.1, p = 0.36$[b]		$\chi^2 = 3.6, p = 0.46$[c]	
Alleles[a]						
A_1	0.474	0.434	0.753	0.678	0.521	0.618
A_2	0.526	0.566	0.247	0.322	0.148	0.132
A_3	–	–	–	–	0.331	0.250
Significance	$\chi^2 = 0.5, p = 0.49$[d]		$\chi^2 = 2.1, p = 0.15$[d]		$\chi^2 = 0.24$[b]	
Odds ratios (vs A_1/A_1)						
A_1/A_2	1.4 (0.6–3.4)		1.6 (0.8–3.1)			
$A_2/A2$	1.4 (0.6–3.7)		1.7 (0.6–5.1)			

Genotype frequencies are given as counts (%). Allele frequencies are given as proportions. Due to failure of PCR amplification, $T^{573}\rightarrow C$ genotypes were not available for two normoalbuminuric and five nephropathic individuals; microsatellite genotypes were not available for eight normoalbuminuric and five nephropathic subjects. [a] A_1 corresponds to alleles T^{573} and A^{1166}, A_2 to alleles C^{573} and C^{1166}. For the *AGTR1* microsatellite, A_1 corresponds to the major allele (#4, 140 bp), A_2 to the second most frequent allele (#3, 142 bp), and A_3 to all the other six minor alleles grouped together. [b] 2 *df*; [c] 4 *df* (after pooling genotypes A_2/A_2 and A_2/A_3, both having expected counts less than 5); [d] 1 *df*

Table 3. Index of hyperglycaemia during first 12 years of diabetes and risk of nephropathy among carriers and non-carriers of *AGTR1* allele C^{1166}

	All		C^{1166} Non-carriers		C^{1166} carriers	
	Normoalbuminuria	Nephropathy	Normoalbuminuria	Nephropathy	Normoalbuminuria	Nephropathy
Index of hyperglycaemia[a]						
Below median	47	23	25	16	22	7
Above median	26	44	19	17	7	27
Odds ratio	3.5		1.4		12.1[b]	
95 % Confidence interval	(1.7–6.9)		(0.6–3.5)		(3.7–39.8)	

[a] Omitted are 12 individuals (six cases and six control subjects) for whom the index of hyperglycaemia was not available.
[b] $\chi^2 = 8.25, p = 0.004$ with 1 *df* for the difference between odds ratios among C^{1166} carriers and non-carriers (Breslow-Day test)

significant ($\chi^2 = 8.25$, $p = 0.004$ with 1 *df*). A similar risk difference was observed when carrier status for haplotype C^{1166}-microsatellite allele #4 rather than the whole allele C^{1166} was used to subdivide individuals (data not shown). Since the AGTR1 gene is involved in the control of blood pressure and increased blood pressure in parents has been shown to be a determinant of nephropathy in IDDM [9, 10, 11], we examined whether this effect of allele C^{1166} may be due to an increased prevalence of parental hypertension among carriers. No association, however, was found between allele C^{1166} and presence of hypertension in parents within each renal group. Further, the effect of allele C^{1166} on the risk of nephropathy associated with poor glycaemic control was present and similar among IDDM individuals with and without a parental history of hypertension, indicating that the allele C^{1166} and parental history of hypertension contribute independently to the risk of nephropathy (data not shown).

To confirm the significant interaction between poor glycaemic control and $A^{1166}\rightarrow C$ genotype, we investigated an additional sample of 551 IDDM patients from a cross-sectional study conducted at the Joslin Clinic [4]. The clinical characteristics of these subjects are illustrated in Table 4. In this population, allele C^{1166} frequency was 0.266 in the normoalbuminuria and 0.282 in the microalbuminuria group ($\chi^2 = 0.25$, $p = 0.62$ with 1 *df*). No significant association with hypertension was detected within each renal group (data not shown). Since we do not have data to calculate an index of hyperglycaemia during the first 12 years of diabetes, the mean glycated haemoglobin level in the years 1990–1991 was used as an index of long term glycaemic control, as described in Methods [4]. The risk of microalbuminuria rose

Table 4. Clinical characteristics of patients from the natural history of microalbuminuria study according to renal status

	Normoal-buminuria	Microal-buminuria
n (male/female)	410 (209/201)	141 (71/70)
Age at IDDM diagnosis (years)	17 ± 7	15 ± 7
Age at examination (years)	29 ± 7	31 ± 7
Albumin/creatinine ratio (μg/mg)	12 ± 4	90 ± 74
Systolic blood pressure (mm Hg)	125 ± 15	133 ± 16[a]
Diastolic blood pressure (mm Hg)	70 ± 11	76 ± 9[a]
Antihypertensive drugs (%)	4	24[a]
HbA_1 (%)	10.3 ± 1.8	10.9 ± 2.0[a]

Data are means ± SD.
[a] $p < 0.001$
HbA_1 represents the geometric mean of HbA_1 values during 1988 and 1990–1991

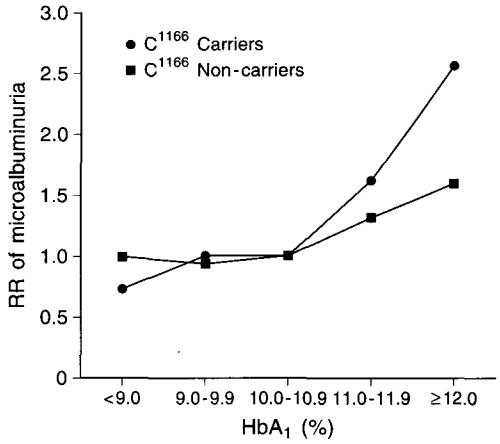

Fig. 1. Relation between mean haemoglobin A_1 values and risk of microalbuminuria among carriers (–▲–) and non-carriers (--■--) of *AGTR1* allele C^{1166} in the additional study population. Odds ratios were calculated taking the allele C^{1166} non-carriers with haemoglobin A_1 lower than 9 % as the reference group. The 95 % confidence intervals for odds ratios were respectively 0.3–1.9, 0.4–2.5, 0.4–2.7, 0.7–4.0, and 1.0–6.4 for the five haemoglobin A_1 classes among carriers of allele C^{1166}, and 0.4–2.3, 0.4–2.4, 0.5–3.3, and 0.7–3.8 for the four upper classes among non-carriers

with increasing glycated haemoglobin values regardless of *AGTR1* genotype (Fig. 1) [4]. Above a glycated haemoglobin of 11 %, however, the risk of microalbuminuria increased more steeply in carriers than in non-carriers of allele C^{1166} (Fig. 1). Among carriers, poor glycaemic control (glycated haemoglobin value of 12 %) carried a 2.6-fold risk of microalbuminuria (C.I.:1.02–6.4); whereas, among non-carriers, the relative risk was only 1.6 (C.I.:0.7–3.8). Thus, this additional data set seems to show the same modifying effect of *AGTR1* allele C^{1166} on the relationship between poor glycaemic control and the risk of nephropathy, but the magnitude of the interaction in a cross-sectional study design is less strong and is not statistically significant with this sample size.

Discussion

In this study, we investigated the *AGTR1* gene as a candidate locus for susceptibility to diabetic nephropathy. In a cohort of 152 IDDM individuals examined between 15 and 21 years after diabetes onset, we could not find any significant association between diabetic nephropathy and *AGTR1* allele C^{1166}. Although allele C^{1166} by itself did not contribute significantly to renal complications, we observed a powerful, significant interaction between this allele and poor glycaemic control in determining the risk of diabetic nephropathy. Among carriers of allele C^{1166}, the relative risk of renal complications associated with frequent severe hyperglycaemia in the first decade of diabetes was almost ten times that observed among A^{1166} homozygotes (12.1 vs 1.4). A similar pattern of interaction between allele C^{1166} and poor glycaemic control was observed in a cross-sectional sample of 551 IDDM individuals currently attending the Joslin Clinic. Hyperglycaemia was associated with higher risk of nephropathy among C^{1166} carriers than non-carriers, although the estimate of this magnifying effect in carriers of this allele was much smaller in this study design and not detectable with this sample size.

Our findings suggest that IDDM individuals carrying the AGTR1 allele C^{1166} are particularly susceptible to the damage caused by hyperglycaemia and would especially benefit from good glycaemic control. While our findings are striking, association studies are subject to error [31], and this interaction between poor glycaemic control and the *AGTR1* allele C^{1166} must be confirmed in other populations and corroborated by cellular studies, before allele C^{1166} carriers are preferentially selected for intensified treatment programmes. There are several reasons, however, for confidence in the validity of the present findings. First, our study population was a sample from a well-defined inception cohort of IDDM patients who were ascertained regardless of their attendance at the Joslin Clinic after diabetes diagnosis. All individuals were Caucasian and resided in Massachusetts. Thus, biases in patient selection or the presence of population stratification, are unlikely. A second consideration is that the *p*-value for the difference between odds ratios was small ($p = 0.004$), making it also unlikely that these findings were a type I error. Finally, findings consistent with this interpretation were obtained in a second, independent sample of IDDM patients. While the interaction between allele C^{1166} and the effect of hyperglycaemia on the risk of nephropathy was not statistically significant in the second sample, this was likely due to the less powerful study design, a cross-sectional sample from which patients with advanced nephropathy were excluded

due to the unavailability of a suitable long term measure of glycaemic control [4].

The *AGTR1* gene spans 47 kb on chromosome 3q22 and consists of five exons, four of which are untranslated and alternatively spliced [32]. The open reading frame is entirely contained in exon 5, and codes for a 41 kDa seven-transmembrane domain protein which binds angiotensin II and mediates most of its actions on the kidney and vessels [15]. The $A^{1166}\rightarrow C$ transversion is located at the 5' end of the 3' untranslated region on the same exon as the open reading frame [25, 26] and is not known to have any biological function. Therefore, we hypothesize that the observed synergism with glycaemic control might be due to linkage disequilibrium between allele C^{1166} and other sequence differences, as yet unidentified but possibly located in regulatory regions. These polymorphisms may increase AGTR1 expression, enhance angiotensin II action, and ultimately increase kidney susceptibility to the effect of hyperglycaemia through abnormalities of systemic or renal haemodynamics, or by altering the function of renal cells [33, 34]. Since both angiotensin II and excess glucose activate protein kinase C, the interaction between poor glycaemic control and the renin angiotensin system might take place along this signal transduction pathway [35, 36].

In conclusion, our data provide preliminary evidence that genetic variability at the *AGTR1* locus potentiates the effect of poor glycaemic control on the risk of diabetic nephropathy. If confirmed, these results may provide a basis for identifying IDDM individuals who would benefit most from good glycaemic control. Studying the cellular pathways linking *AGTR1* variability to the effects of hyperglycaemia may also lead to new insights in the cellular mechanisms by which diabetes causes glomerular alterations. Knowledge of these mechanisms might suggest additional strategies for preventing diabetic nephropathy and its burden of morbidity and mortality among patients with IDDM.

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