Effect of deletion polymorphism of angiotensin converting enzyme gene on progression of diabetic nephropathy during inhibition of angiotensin converting enzyme: observational follow up study

Hans-Henrik Parving, Peter Jacobsen, Lise Tarnow, Peter Rossing, Laure Lecerf, Odette Poirier, François Cambien

Abstract

***Objective*—To evaluate the concept that an insertion/deletion polymorphism of the angiotensin converting enzyme gene predicts the therapeutic efficacy of inhibition of angiotensin converting enzyme on progression of diabetic nephropathy.**

***Design*—Observational follow up study of patients with insulin dependent diabetes and nephropathy who had been treated with captopril for a median of 7 years (range 3-9 years).**

***Setting*—Outpatient diabetic clinic in a tertiary referral centre.**

***Patients*—35 patients with insulin dependent diabetes and nephropathy were investigated during captopril treatment (median 75 mg/day (range 12.5 to 150 mg/day)) that was in many cases combined with a loop diuretic. 11 patients were homozygous for the deletion allele and 24 were heterozygous or homozygous for the insertion allele of the angiotensin converting enzyme gene.**

***Main outcome measures*—Albuminuria, arterial blood pressure, and glomerular filtration rate according to insertion/deletion polymorphism.**

***Results*—The two groups had comparable glomerular filtration rate, albuminuria, blood pressure, and haemoglobin A_{1c} concentration at baseline. Captopril induced nearly the same reduction in mean blood pressure in the two groups—to 103 (SD 5) mm Hg in the group with the deletion and 102 (8) mm Hg in the group with the insertion—and in geometric mean albumin excretion—573 (antilog SE 1.3) µg/min and 470 (1.2) µg/min, respectively. The rate of decline in glomerular filtration rate (linear regression of all glomerular filtration rate measurements during antihypertensive treatment) was significantly steeper in the group homozygous for the double deletion allele than in the other group (mean 5.7 (3.7) ml/min/year and 2.6 (2.8) ml/min/year, respectively; P = 0.01). Multiple linear regression analysis showed that haemoglobin A_{1c} concentration, albuminuria, and the double deletion genotype independently influenced the sustained rate of decline in glomerular filtration rate (R^2 (adjusted) = 0.51).**

***Conclusion*—The deletion polymorphism in the angiotensin converting enzyme gene reduces the long term beneficial effect of angiotensin converting enzyme inhibition on the progression of diabetic nephropathy in patients with insulin dependent diabetes.**

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Introduction

Increased synthesis of angiotensin II may play a part in the initiation and progression of diabetic nephropathy by affecting haemodynamic mechanisms and promoting growth of glomerular cells.[1,2] Angiotensin converting enzyme is of key importance in regulating systemic and glomerular circulation by converting angiotensin I into angiotensin II and inactivating bradykinin.[3] Pharmacological inhibitors of angiotensin converting enzyme have a beneficial effect on the initiation and progression of diabetic nephropathy.[4-9] However, the beneficial effect of such inhibition on the progression of diabetic nephropathy is highly variable and several so called progression promoters have been identified—namely, albuminuria, poor glycaemic control, and systemic blood pressure.[9,10] Recently, a deletion polymorphism in intron 16 of the angiotensin converting enzyme gene has been associated with accelerated deterioration in kidney function in patients with immunoglobulin A nephropathy.[11,12]

We examined the concept that the deletion polymorphism of the angiotensin converting enzyme gene predicts the therapeutic efficacy of angiotensin converting enzyme inhibition on deterioration in kidney function in insulin dependent diabetic patients suffering from diabetic nephropathy.

Patients and methods

We examined the records of all patients with albuminuria attending the outpatient clinic at Steno Diabetes Centre in 1993 who had insulin dependent diabetes mellitus and diabetic nephropathy[13] and had had their glomerular filtration rate measured during the same year. A total of 242 white patients over 18 years old were identified, and angiotensin converting enzyme genotyping was performed in 198 of them as described in detail previously.[14] Subjects were classed according to the presence (I) or absence (D) of a 287 base pair insertion in intron 16 of the angiotensin converting enzyme gene; subjects who were homozygous (II) or heterozygous (ID) for the insertion were grouped together and compared with those homozygous for the deletion (DD). We studied all previously untreated patients in whom treatment with an angiotensin converting enzyme inhibitor had been started and who had had their glomerular filtration rate measured yearly for at least three years during such treatment. Thirty five patients fulfilled these criteria and all gave fully informed consent (see table 1). The experimental design was approved by the local ethics committee.

All physiological investigations were carried out 4 to 18 (median 15) times during 3 to 9 (8) years of angiotensin converting enzyme inhibition in the DD group and 3 to 16 (9) times during 3 to 9 (7) years of angiotensin converting enzyme inhibition in the ID/II group, respectively. Glomerular filtration rate was measured in the morning after a single injection of edetic acid labelled with chromium-51 (3.7 MBq).[15]

Urinary albumin concentration was determined by radioimmunoassay during the four hour clearance period and from all 24 hour urine collections made at home (about four times a year).[16] Blood pressure was measured with a standard clinical mercury sphygmomanometer (cuff 25 cm × 12 cm) on the right arm.

From venous blood samples haemoglobin A_{1c} concentration was determined by high performance liquid chromatography (DIAMAT Analyzer, Bio-Rad, Richmond, California). Serum creatinine concentration

Table 1—*Clinical data at baseline on 35 patients with insulin dependent diabetes and diabetic nephropathy according to insertion (I)/deletion (D) polymorphism of angiotensin converting enzyme gene. Values are means (SD) unless stated otherwise*

	DD genotype	II and ID genotypes	P value	Mean difference (95% confidence interval) between DD and II+ID genotypes
No of men/women	9/2	14/10	0.32	
Age at onset of diabetes (years)	11 (6)	13 (7)	0.28	−3 (−8 to 2)
Duration of diabetes at onset of nephropathy (years)	17 (10)	16 (6)	0.60	1 (−4 to 7)
Serum creatinine (μmol/l)	81 (29)	85 (30)	0.73	−4 (−28 to 20)
No of patients with retinopathy:				
Simplex	2	8	0.48	
Proliferative	9	16	0.71	
Insulin dose (IU/kg/day)	0.6 (0.1)	0.6 (0.2)	0.52	0.0 (−0.2 to 0.1)
Body mass index (kg/m^2)	24.2 (2.5)	23.7 (3.4)	0.62	0.6 (−1.8 to 2.9)
Antihypertensive treatment during study (mg/day):				
Captopril	76 (23)	65 (36)	0.25	11 (−13 to 35)
Frusemide	220 (210)	130 (80)	0.11	91 (−60 to 250)
Nifedipine	40	40	1.00	0
No of patients taking:				
Frusemide	10	18	0.32	
Nifedipine	5	4	0.08	

was assessed by a kinetic Jaffé method. Serum cholesterol concentrations were measured by a conventional laboratory technique. Retinopathy was assessed by fundus photography after pupillary dilatation.

STATISTICAL ANALYSIS

Values are given as means (SD), but values for urinary albumin excretion are expressed as geometric means (antilog SE) owing to the skewed distribution. Baseline data on haemoglobin A_{1c} concentration, albuminuria, and arterial blood pressure are based on all values during the six months preceding the start of angiotensin converting enzyme inhibition. Analysis of the three above mentioned variables during angiotensin converting enzyme inhibition was based on all measurements.

The initial rate of decline in glomerular filtration rate was determined from baseline to the first examination after the start of angiotensin converting enzyme inhibition a mean of 3 months (range 3-24) later in the group with the deletion polymorphism and a mean of 6 (3-21) months later in the others. Linear regression analysis was used to assess the sustained rate of decline in glomerular filtration rate from all values of glomerular filtration rate in each patient during angiotensin converting enzyme inhibition. For normally distributed variables, including logarithmically transformed values of urinary albumin excretion, groups were compared by an unpaired Student's *t* test. For non-normally distributed continuous variables groups were compared by the Mann-Whitney U test. A χ^2 test was used for comparison between groups of non-continuous variables. Multivariate regression analysis of putative progression promoters was performed with backward selection. The R^2 value is adjusted for the number of variables introduced into the model. A P value (two sided) of <0.05 was considered to be significant. All calculations were performed with a commercially available program, Statgraphics (Manugistics, Rockville, Massachusetts).

Results

Demographic and clinical data were comparable between patients in the two groups (table 1). The initial decline in glomerular filtration rate (ml/min/month) was more pronounced in 10 patients homozygous for the deletion polymorphism than in 19 patients homozygous or heterozygous for the insertion (mean difference 2.4 (95% confidence interval 4.6 to 0.2), P = 0.02). The initial decline in mean arterial blood pressure also tended to be larger in those with the double deletion (13 (11) *v* 7 (8), P = 0.10). However, no significant correlation was found between the initial drop in blood pressure and glomerular filtration rate. The mean ratio of initial and baseline albuminuria was nearly identical (0.65 (0.34 to 1.24) in those with the double deletion and 0.59 (0.40 to 0.88) in the others).

Table 2—*Changes in haemoglobin A_{1c} concentration, arterial blood pressure, albuminuria, and glomerular filtration rate before and during long term inhibition of angiotensin converting enzyme for diabetic nephropathy according to insertion (I)/deletion (D) polymorphism of angiotensin converting enzyme gene. Values are means (SD) unless stated otherwise*

	Baseline					Angiotensin converting enzyme inhibition			
Genotype	Period of investigation (years)	Haemoglobin A_{1c} (%)	Arterial blood pressure (mm Hg)	Albuminuria (μg/min)*	Glomerular filtration rate (ml/min/1.73 m^2)	Haemoglobin A_{1c} (%)	Arterial blood pressure (mm Hg)	Albuminuria (μg/min)*	Sustained decrease in glomerular filtration rate (ml/min/year)
DD (n = 11)	7 (2)	9.7 (1.8)	146/94 (14/8)	908 (1.3)	100 (21)†	9.7 (1.3)	138/85 (11/5)	573 (1.3)	5.7 (3.7)‡
II + ID (n = 24)	6 (2)	9.0 (1.2)	142/90 (16/9)	756 (1.2)	95 (29)†	9.5 (1.2)	137/84 (14/7)	470 (1.2)	2.6 (2.8)‡
Mean difference (95% confidence interval) in DD and II + ID genotypes	1 (−0.3 to 3)	0.7 (−0.4 to 1.9)	4 (−7 to 16)/ 4 (−3 to 10)	1.2 (0.6 to 2.3)	5 (−14 to 25)	0.3 (−0.6 to 1.2)§	1 (−9 to 11)/ 2 (−3 to 7)§	1.0 (0.5 to 1.8)	3.1 (0.8 to 5.4)

*Geometric mean (antilog SE) and mean ratio (95% confidence interval) indicated.
†Ten patients in DD group, 19 in ID + II group.
‡ P<0.01 for DD *v* ID + II group.
§Mean difference between changes (95% confidence interval) in DD and II + ID genotype.

Key messages

- Glycaemic control, albuminuria, and the deletion polymorphism of the angiotensin converting enzyme gene independently influence the decline in kidney function in diabetic nephropathy or, in other words, act as progression promoters
- Determination of the insertion/deletion polymorphism of the angiotensin converting enzyme gene can identify patients with accelerated progression in diabetic nephropathy
- Inhibition of angiotensin converting enzyme frequently combined with diuretics reduces blood pressure, arrests the progressive rise in albuminuria, and reduces the rate of decline in glomerular filtration rate in diabetic nephropathy
- The deletion polymorphism in the angiotensin converting enzyme gene reduces the long term beneficial effect of angiotensin converting enzyme inhibition on progression of diabetic nephropathy
- Patients with diabetic nephropathy who are homozygous for the deletion polymorphism should be offered earlier and more aggressive antihypertensive treatment with angiotensin converting enzyme inhibitors in addition to strict glycaemic control

A direct correlation between the initial and the sustained decline in glomerular filtration rate was observed ($r = 0.46$, $P = 0.01$). An initial decline in glomerular filtration rate of 1 ml/min/month corresponds to an enhanced sustained glomerular filtration rate reduction of 0.57 ml/min/year (R^2 = 22%).

Glycaemic control, systemic blood pressure, albuminuria, and glomerular filtration rate were comparable at baseline in the two groups (table 2). Table 2 also shows the variables during angiotensin converting enzyme inhibition lasting from 3 to 9 years. Patients homozygous for the deletion tended to receive a higher dose of captopril and frusemide and a greater proportion of them were treated with nifedipine (5 (45%) *v* 4 (17%), respectively). The mean ratio of sustained and baseline urinary albumin values was nearly identical in all patients (0.63 (0.40 to 1.00) in 11 patients with the double deletion and 0.65 (0.40 to 1.06) in 24 patients with the insertion). The mean difference of change in haemoglobin A_{1c} concentration between those with the double deletion and those with the insertion was 0.3 (–0.6 to 1.2), in arterial blood pressure 1(–9 to 11)/2(–3 to 7) mm Hg, albuminuria ratio 1.0 (0.5 to 1.8), and sustained decrease in glomerular filtration rate (ml/min/year) 3.1 (0.8 to 5.4). Furthermore, a multiple linear regression analysis showed that haemoglobin A_{1c} concentration, albuminuria, and the DD genotype of the angiotensin converting enzyme gene independently influenced the sustained rate of decline in glomerular filtration rate (R^2 (adjusted) = 0.51). Serum cholesterol concentration remained nearly identical in the two groups during the whole study period (6.0 (1.2) and 5.9 (1.0) mmol/l).

Discussion

Our longitudinal study of kidney function in patients with insulin dependent diabetes and diabetic nephropathy showed an accelerated initial and sustained loss of glomerular filtration rate during angiotensin converting enzyme inhibition in patients homozygous for the deletion polymorphism of the angiotensin converting enzyme gene. We found a direct correlation between the initial loss and the decline in glomerular filtration rate during maintenance treatment with angiotensin converting enzyme inhibitors. Finally, glycaemic control and albuminuria during the whole treatment period and the DD genotype independently influenced the sustained rate of decline in glomerular filtration rate or, in other words, acted as progression promoters.

The genotype distribution of the insertion/deletion polymorphism in the present study was similar to that found in a larger cohort[14](DD, 31% *v* 32%; ID, 49% *v* 48%; and II, 20% *v* 20%, respectively). The method of measuring glomerular filtration rate that we used is accurate and precise,[17] and we took repeated measurements over at least three years, thus fulfilling the requirements for obtaining a valid determination of the rate of decline in glomerular filtration rate.[18]

Originally, Yoshida *et al* suggested that the deletion polymorphism in the angiotensin converting enzyme gene is a risk factor for the progression of IgA nephropathy.[11] Recently, the data have been confirmed and extended to include various non-diabetic nephropathies.[12 19]

The natural course of diabetic nephropathy is characterised by a progressive increase in systemic blood pressure and albuminuria and an average decline in glomerular filtration rate of 10-15 ml/min/year.[2 20] Treatment to lower blood pressure induces a faster initial and slower subsequent decline in glomerular filtration rate in diabetic nephropathy.[7 8-10 13] Our long term study found that treatment with angiotensin converting enzyme inhibitors often combined with diuretics reduces arterial blood pressure, arrests the progressive increase in albuminuria, and reduces the rate of decline in glomerular filtration rate in diabetic nephropathy; this is in agreement with previous studies.[7-10 13] Differences in well established risk factors for progression of diabetic nephropathy, such as arterial blood pressure, glycaemic control, albuminuria, and serum cholesterol concentration,[2 10 21] could not account for the observed difference in the sustained rate of decline in glomerular filtration rate between the patients with the double deletion and those with the insertion polymorphism. It should be recalled that patients' sex had no impact on the progression of diabetic nephropathy.[22] These findings and the multiple linear regression analysis suggest that the deletion polymorphism of the angiotensin converting enzyme gene independently influences the progression of diabetic nephropathy. A possible explanation for the deleterious effect of the deletion polymorphism on kidney function may be increased angiotensin II formation[23] or resistance to long term angiotensin converting enzyme inhibition, or both. This is corroborated by the fact that the dose and the number of different antihypertensive drugs tended to be higher in the patients with the double deletion polymorphism. From a therapeutic point of view, our study suggests that patients with diabetic nephropathy who are homozygous for the D allele should be offered earlier and more aggressive antihypertensive treatment with angiotensin converting enzyme inhibitors. A better option may be angiotensin II receptor blockade combined with strict glycaemic control.

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Dyschromatopsia (number 97) and rectal bleeding

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We report three cases of delayed presentation of colorectal disease in colour blind men due to a failure to recognise bleeding as a symptom of their pathology. In all three individuals bleeding was recognised by the spouse and had been misinterpreted as loose motion by the patients. Dyschromatopsia is a common condition; it is important to identify rectal bleeding early in colour blind individuals so as to diagnose and treat the source of the bleeding.

Case reports

Case 1—A 43 year old colour blind man was seen in clinic with painless rectal bleeding which had been noted by his spouse. It had been interpreted as loose motion and had been present for three months. Examination of the abdomen and rectum were unremarkable, as was rigid sigmoidoscopy. A barium enema showed a tumour in the mid sigmoid colon. A sigmoid colectomy was performed and histology revealed a Dukes's C1 adenocarcinoma.

Case 2—A 48 year old man was admitted on the emergency intake with passage of clotted blood rectally together with mild colicky abdominal pain on the left side. The patient was colour blind and had had symptoms for two weeks but had interpreted the blood as diarrhoea. His wife had noted blood on the toilet pan and contacted the general practitioner. Examination was unremarkable apart from mild tenderness in the left iliac fossa, and rectal examination together with proctoscopy and sigmoidoscopy were normal. A barium enema showed diverticular disease of the sigmoid colon. His bleeding settled with conservative management.

Case 3—A 36 year old man presented to the outpatient clinic with painless fresh rectal bleeding, which had been noticed on the toilet pan by his wife. The length of the history was indeterminable and there were no other symptoms suggesting colorectal disease. His history included dyschromatopsia. Examination of the abdomen and rectum was unremarkable, as was sigmoidoscopy. Proctoscopy showed second degree haemorrhoids, which were banded with successful control of bleeding.

Comment

In these three cases of rectal bleeding, presentation was delayed because the subjects were colour blind. In all cases the bleeding was first noted by the spouse and had not been correctly identified as bleeding by the patient because of his defective colour vision.

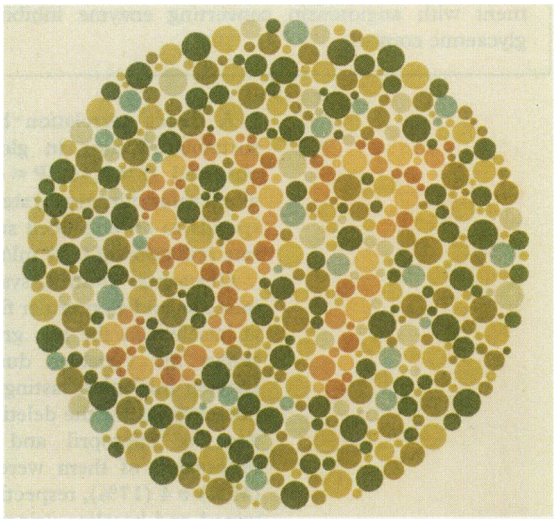

Fig 1—*People with dyschromatopsia are unable to identify the 97, which can be read by people with normal vision*

Incomplete colour blindness or dyschromatopsia is an X linked disorder which affects up to 8% of males and 0.4% of females.[1] It usually manifests as defective perception of red and green and is related to the presence of abnormal cone photopigments.

Colour blindness can be confirmed with the use of Ishihara charts (fig 1). In this example a patient with normal vision reads 97 but a patient with dyschromatopsia is unable to see any number.

A literature search revealed only a single comment relating to rectal bleeding and colour blindness. This was a letter by an American proctologist warning of the potential for missing both rectal bleeding and haematuria and encouraging the use of confirmation by spouse if bleeding was suspected.[2]

We recommend a very low threshold for the investigation of colorectal symptoms in colour blind people, and in particular those with a family history of colorectal disease, as presentation may be delayed owing to a failure to correctly identify bleeding.

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We thank Isshin-Kai Foundation, which holds the copyright of the Ishihara charts, for allowing us to reproduce plate 12 of the 38 plate Ishihara charts. We must emphasise that this reproduced plate should not be used for testing purposes.

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