European Journal of Endocrinology (2006) **154** 715–721 ISSN 0804-4643

CLINICAL STUDY

An insulin-like growth factor-I gene polymorphism modifies the risk of microalbuminuria in subjects with an abnormal glucose tolerance

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Abstract

Objective: Microalbuminuria (MA) is related to cardiovascular disease both in diabetic patients and non-diabetic subjects.
Design: We investigated whether a polymorphism near the promoter region of the IGF-I gene was related to the development of MA.
Methods: For this study, 1069 participants of the Rotterdam study were selected (440 participants with an abnormal glucose tolerance (AGT), 220 participants with type 2 diabetes and 254 subjects with pre-diabetes, and 595 subjects with a normal glucose tolerance (NGT).
Results: 787 subjects were carriers of the wild type IGF-I genotype (73.6%) and 282 subjects were variant carriers (26.4%) of this IGF-I gene polymorphism. Compared to subjects with NGT the risk for microalbuminuria was higher (Odds Ratio (OR): 3.1 (95% CI: 1.2–7.7); $P = 0.02$) in variant carriers with AGT than in carriers of the wild type of this IGF-I gene polymorphism (OR: 2.2 (95% CI: 1.2–4.0); $P = 0.009$). Compared with wild type carriers with AGT, the relative risk for MA was unadjusted and non-significantly increased in variant carriers with AGT (1.6; 95% CI: 0.8–2.9). However, after adjustment for possible confounding factors (age, gender, mean blood pressure, fasting insulin, fasting glucose and smoking) this risk became significant (OR: RR 2.1; 95% CI:1.1–4.4; $P = 0.04$).
Conclusions: In subjects with AGT, a higher risk for MA was observed in variant carriers than in carriers of the wild type genotype of this IGF-I gene polymorphism. Since MA is primarily associated with cardiovascular disease in subjects with AGT, our study suggests that variant carriers have a higher risk for cardiovascular disease than carriers of the wild type when they develop an AGT.

European Journal of Endocrinology **154** 715–721

Introduction

The presence of microalbuminuria (MA) may be a marker of generalized vascular endothelial dysfunction reflecting generalized vascular disease (1, 2). Development of MA is associated with glycaemic control, blood pressure, smoking, and male gender (3, 4). MA may precede type 2 diabetes, occurring in parallel with the metabolic syndrome and its components, obesity and hypertension (1). MA is also considered to be a marker for diabetic nephropathy in type 2 diabetes (5–7).

The insulin-like growth factor-I (IGF-I) system exerts multiple physiologic effects on the vasculature through both endocrine and autocrine/paracrine mechanisms (8). Both macrovessel and microvessel endothelial cells express IGF-I receptor (IGF-IR; 9). The expression of IGF-I in endothelial cells is low and might reflect mainly IGF-I sequestered from serum (10). IGF-I has been also implicated in the development of cardiovascular disease (8, 11–13).

IGF-I has been further implicated in the development of diabetic nephropathy (14, 15). IGF-I bioactivity is regulated by genetic and non-genetic factors like growth hormone, nutrition and insulin (16). In humans, a cytosine–adenine $(CA)_n$ polymorphism in the promoter region of the IGF-I gene has been identified (17). Studies of other genes have suggested that polymorphic CA repeats in the promoter region of a gene, affects transcription activity of a gene (18). This polymorphism has thus the potential to influence directly the expression of IGF-I in the body (19).

Circulating IGF-I levels are often used as a substitute for tissue IGF-I bioactivity due to the lack of *in vivo* methods to measure the latter. However, determinations of circulating total IGF-I levels by radioimmunoassays may be especially problematic in pathological conditions, due to interferences of IGF-binding proteins. As a consequence, circulating IGF-I levels may not adequately reflect the IGF-I bioactivity. An alternative approach may be genetic

DOI: 10.1530/eje.1.02144
Online version via www.eje-online.org

association studies. In these studies, genetic variants can be treated as risk factors that cannot be influenced by secondary factors such as hypertension or hyperglycemia, unless these phenotypes are in the causal pathway. This opens the opportunity to characterize on a genetic basis, individuals who are at risk for MA. The aim of the present study was to investigate whether a polymorphism near the promoter region of the IGF-I gene was related to the development of MA.

Subjects and methods

Study population

All subjects included in the present study were participants of the Rotterdam Study. The Rotterdam Study is a single-center prospective follow-up study in which all residents aged 55 years and over of the Rotterdam suburb Ommoord were invited to participate. The Medical Ethics Committee of Erasmus Medical Center Rotterdam approved the study and written informed consent was obtained from all participants. The aim of the Rotterdam study and the design of the study has been described previously (20). The baseline examination of the Rotterdam Study was conducted between 1990 and 1993. A total of 7983 participants (response rate 78 percent) were examined.

Because of practical and financial reasons, only a proportion of the Rotterdam Study ($n = 1069$) underwent a fasting glucose tolerance test. From this group we selected our cases and controls for a case-control study in which we assessed the relation between both IGF-I genotypes, glucose tolerance and albuminuria (21). Criteria for the present study were age younger than 75 years, no use of anti-epileptics, corticosteroids, hormonal replacement therapy, cytostatics and people with dementia. After blood was drawn from the participants after an overnight fast, participants underwent an oral glucose tolerance test with 75 g glucose. Diabetic patients who were treated with anti-diabetic medication did not undergo a glucose tolerance test. Therefore, post load glucose measurements were only available for persons not using anti-diabetic medication. For the oral glucose tolerance test blood was drawn after 2 h. Normal glucose tolerance (NGT) was defined as fasting glucose below 6.1 mmol/l and 2-h post load below 7.8 mmol/l. Individuals with high glucose levels not meeting criteria for diabetes, but were too high to be considered normal were diagnosed as having prediabetes. Prediabetes includes persons with impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT). IFG was defined as fasting glucose between 6.1 and 7.0 mmol/l and IGT as a 2-h post load glucose between 7.8 and 11.1 mmol/l. A diagnosis of diabetes mellitus was made if subjects were treated for diabetes or had a fasting glucose level of 7.0 mmol/l or above and/or a 2-h post load glucose of 11.1 mmol/l or above (22). All individuals meeting criteria for diabetes or prediabetes were to be considered as subjects with an abnormal glucose tolerance (AGT). To determine insulin sensitivity we used the homeostasis model assessment (HOMA)(23).

Measurements

A questionnaire was used to collect data on glucose status, smoking status and use of anti-hypertensive medication. Blood pressure was measured in a sitting position at the right upper arm with a random-zero sphygmomanometer and the average obtained of two measurements at one occasion was used.

Blood sampling and storage have been described elsewhere (24). Serum was separated by centrifugation and quickly frozen in liquid nitrogen. For the present study, we used blood measurements performed on fasting blood samples. Glucose levels were measured by the glucose hexokinase method (Medgenix Diagnostics, Brussels, Belgium) with an intra-assay variation of less than 2.5 and 6.0% respectively. Fasting insulin was measured by a commercially available assay (IRMA, Medgenix Diagnostics) with an intra- and inter-assay variation of 3–6 and 5–12 percent, respectively. Serum creatinine levels were assessed using an automated enzymatic procedure (Roche, Mannheim, Germany). Creatinine clearance was determined by the formula of Cockcroft and Gault (25). Serum cholesterol and triglycerides were determined by a standard laboratory method.

Participants collected timed overnight urine one day before the examination, albumin and creatinine were measured. Albumin and creatinine in urine were determined by a turbidimetric method and measured by a Hitachi 917 analyzer (Roche/Hitachi Diagnostics, Mannheim, Germany). Detection limit ranged from 2 to 400 mg/l and therefore all measurements below 2 mg/l were set at 1 mg/l. MA was defined as an albumin-to-creatinine ratio (ACR) of >2.5 mg/mmol in males and 3.5 mg/mmol in females (26). From 34 subjects, no data of MA were available. Fourteen subjects (NGT: four subjects, AGT: ten subjects) met the criteria for macroalbuminuria, they were included in the analysis.

Genotyping of the IGF-I promoter polymorphism

The human IGF-I gene contains a polymorphic CA repeat 1 Kb upstream of the promoter region (27). IGF-I genotypes were determined as described previously (17). The most common allele contains 19 CA-repeats, which equals a length of 192-bp (28). In a previous study, we examined the role of the various lengths of the alleles of the IGF-I promoter polymorphism and observed an optimum for IGF-I levels and body height (29). Mean IGF-I levels and body height for homozygous carriers of the 192-bp allele, and

homozygous carriers of the 194-bp-allele of this IGF-I gene promoter polymorphism were equal and significantly higher than the values measured in subjects homozygous for alleles smaller than 192-bp and longer than 194-bp, respectively, suggesting an optimum for IGF-I expression by these two variants. Based on carrier ship of the 192-bp and 194-bp alleles, we therefore distinguished two different IGF-I genotypes in the present study: As wild type genotype we considered carriers homozygous for the 192-bp or for the 194-bp allele, and carriers heterozygote for these two alleles, participants with this genotype are further in the text denoted as carriers of the wild type. Participants with all other combinations of alleles were considered carriers of the variant genotype, which is further in the text denoted as variant carriers. Seven hundred and eighty seven (73.6%) were carriers of the wild type and 282 subjects were variant carriers (26.4%).

Carotid ultrasonography

Carotid atherosclerosis was assessed by duplex scan ultrasonography of the carotid arteries, using a 7.5 MHz linear array transducer (ATL, Ultramark IV). Measurements of intima media thickness (IMT) were performed offline from the still images recorded on videotape. Details about this measurement have been published previously (30). Briefly, the interfaces of the far and near walls of the distal common carotid artery are marked over a length of 10 mm. We used the average of the measurements of three still images of both the left and right arteries. Common carotid IMT was determined as the mean IMT of near and far wall measurements of both the left and right arteries. Results from a reproducibility study of IMT measurements have been published elsewhere(31). The mean differences±S.D. in common carotid IMT between paired measurements of sonographers, readers, and visits were -0.004 ± 0.10, 0.066 ± 0.07, and -0.013 ± 0.13 mm, respectively.

Data analyses

Except when otherwise mentioned, data are presented as mean±S.D.Diabetic and non-diabetic groups were compared with the Mann–Whitney test. Means of parameters were compared between genotypes after stratification for glucose tolerance using analyses of variance. Insulin, albuminuria and ACR did not met the criteria for normality and were logarithmic transformed before analysis in order to obtain approximate normal distribution. Therefore results of these parameters are presented as geometric means with 95% confidence intervals (CI). Distribution of sex, use of antihypertensive drugs, lipid lowering drugs and frequency of the metabolic syndrome between genotypes was compared using a chi-square test.

Risk for MA was calculated by a binary logistic regression analysis and all results are presented unadjusted, and when explicitly mentioned, after adjustment for age, sex, mean blood pressure, fasting glucose and insulin levels and smoking. All analyses were performed using the SPSS for Windows software package, version 10.0.5 (SPSS Inc., Chicago, IL, USA).

Results

Table 1 presents clinical characteristics of 1069 subjects (595 subjects with NGT and 474 subjects with an AGT (254 subjects with pre-diabetes and 220 subjects with diabetes) stratified by IGF-genotype. Subjects with AGT were older and most of them were male in comparison to subjects with NGT (Table 1). Serum creatinine, blood glucose and cholesterol levels, blood pressure and body mass index (BMI) were higher in subjects with AGT than in subjects with NGT (Table 1). Subjects with AGT frequently used more antihypertensive drugs and had larger intima media ratios and more insulin resistance than subjects with NGT. There were no differences in general characteristics between carriers of the wild type and variant carriers of this IGF-I gene promoter polymorphism in the group of NGT or AGT (Table 1).

Table 2 shows the relation between the two IGF-I genotypes and parameters of renal function, comparing subjects with AGT to subjects with NGT. Subjects with AGT had significantly higher urinary albumin concentrations and ACRs than subjects with NGT. In addition, mean serum creatinine levels, creatinuria and clearance were higher in subjects with AGT than in subjects with NGT. In variant carriers with AGT, mean albuminuria and ACR were higher than in carriers of the wild type, but these differences did not reach statistical significance.

Subjects with an AGT had an increased risk of developing MA (Odds Ratio (OR): 2.5; 95% CI: 1.5–4.0; $P < 0.001$) in comparison to subjects with NGT. Compared to subjects with NGT, the risk for MA was higher (OR: 3.1; 95% CI: 1.2–7.7; $P = 0.02$) in variant carriers with AGT, than in carriers of the wild type of this IGF-I gene polymorphism (OR: 2.2; 95% CI: 1.2–4.0; $P = 0.009$; Fig. 1).

Table 3 shows the prevalence and relative risk of MA in subjects with AGT and subjects with NGT stratified for IGF-I genotype. Variant carriers with AGT had a higher prevalence of MA than carriers of the wild type of this IGF-I gene polymorphism, subsequently the risk of MA for variant carriers was investigated. In subjects with AGT, the relative risk for MA was non-significantly increased in variant carriers when compared with carriers of the wild type: (Relative Risks (RR)): 1.5; 95% CI: 0.8–2.9; $P = 0.19$). When this analysis was repeated after adjustment for age, gender, fasting insulin, fasting glucose, mean blood pressure and smoking, the risk of MA in variant

Table 1 General characteristics of the study population, comparing subjects with normal glucose tolerance (NGT) and subjects with abnormal glucose tolerance (AGT) after stratification for IGF-I genotype.

	NGT			AGT			
	Wild type	Variant type	P2	Wild type	Variant type	P2	**P1**
Number of subjects	444	151		343	131		
Age	66.2±5.6	66.0±4.8	0.67	67.7±5.8	66.8±5.8	0.11	<0.001
Sex (M/F)	202/242	71/80	0.75	184/159	70/61	0.97	0.01
Creatinine (μmol/l)	89.3±15.0	89.4±13.9	0.93	92.2±18.0	91.0±17.9	0.61	0.27
Fasting glucose (mmol/l)	5.5±0.4	5.4±0.3	0.85	7.2±2.0	7.3±2.2	0.74	<0.001
2-h glucose post-load (mmol/l)	5.3±1.2	5.3±1.2	0.76	9.4±3.9	9.2±4.0	0.67	<0.001
Mean systolic bp (mmHg)	136±21	135±19	0.56	143±20	142±22	0.87	<0.001
Mean diastolic bp (mmHg)	76±11	75±11	0.52	78±11	78±12	0.67	0.002
Total Cholesterol (mmol/l)	6.63±1.06	6.54±0.98	0.37	6.53±1.13	6.46±1.09	0.47	0.35
HDL-Cholesterol (mmol/l)	1.33±0.32	1.33±0.32	0.88	1.22±0.30	1.20±0.30	0.48	<0.001
Triglycerides (mmol/l)	1.50±0.66	1.44±0.50	0.31	1.95±1.10	1.96±1.00	0.96	<0.001
BMI (kg/m^2)	25.8±3.1	26.0±3.3	0.69	27.5±3.6	27.4±3.7	0.73	<0.001
Current smoking (%)	10.0	14.1	0.21	14.6	8.9	0.39	0.53
Antihypertensive drugs (%)	22.7	18.5	0.30	34.1	38.9	0.18	<0.001
Lipid lowering drugs (%)	5.0	2.6	0.24	7.3	6.1	0.67	0.08
IMT (mm)	0.75±0.13	0.75±0.12	0.69	0.78±0.15	0.79±0.11	0.51	<0.01
HOMA-IR	1.92±0.06	2.05±0.10	0.36	1.78±0.07	1.78±0.11	0.98	0.04

P1, *P*-value comparing Subjects with NGT and subjects with AGT after adjustment for age and sex; P2, *P*-value comparing wild type with variant type adjusted for age and sex; HOMA-IR, homeostasis model assessment of insulin resistance.

carriers with AGT became significant: (RR: 2.1; 95% CI: 1.1–4.4; $P = 0.04$). Compared with carriers of the wild type genotype, the relative risk for MA also increased in variant carriers with NGT after these adjustments, but it still remained not significant (RR: 1.6; 95% CI: 0.6–4.0; $P = 0.35$).

Discussion

Compared to subjects with NGT, variant carriers with AGT had a higher risk to develop MA than carriers of the wild type genotype. In addition, variant carriers with AGT had a borderline and significantly higher prevalence of MA than carriers of the wild type genotype. This findings became significant after adjustment for a number of possible confounding factors, which have been previously found to be involved in the development of MA. Our findings suggest that this IGF-I gene polymorphism modulates the susceptibility and/or progression of MA as soon as a person develops AGT.

Compared with carriers of the wild type genotype, we observed that the increased risk for MA in variant carriers was relatively small. At first glance, this suggests that the role of this IGF-I gene polymorphism in the development of MA is not very important. However, the susceptibility to MA results probably from an interaction of multiple genetic and environmental factors. Consequently, the relationship between the prevalence of MA (phenotype) and any individual causal locus (genetic variant) will in general be fairly weak, even for major genes. MA will only develop when certain other risk factors are also present, such as hyperglycemia, hypertension and/or smoking. This may have obvious complications for both the number of subjects in the study and the power of analyses that aim to detect individual genetic signals among other genetic and environmental background. This may explain why we observed only a relative small and significant increase in the risk of MA in variant carriers with AGT, compared with carriers of the wild type genotype

Table 2 Relation between IGF-I genotype and parameters of renal function comparing subjects with NGT and subjects with AGT. Data are mean (95% CI) unless otherwise stated.

	NGT			AGT			
	Wild type	Variant carriers	P2	Wild type	Variant carriers	P2	**P1**
Number of subjects	441	151		343	131		
Albuminuria (mg/l)*	2.1 (1.9–2.4)	2.1 (1.8–2.5)	0.97	2.9 (2.5–3.3)	3.6 (2.9–4.7)	0.09	<0.001
Creatininuria (mmol//l)	7.7 (7.4–8.1)	7.5 (6.8–8.1)	0.45	8.1 (7.7–8.6)	8.2 (7.5–8.9)	0.86	0.20
Albumin-to-creatinine ratio (mg/mmol)*	0.32 (0.29–0.37)	0.34 (0.28–0.40)	0.70	0.41 (0.35–0.48)	0.52 (0.40–0.66)	0.12	0.002
Creatinine (μmol/l)	89 (88–91)	89 (87–91)	0.93	92 (90–94)	91 (89–94)	0.61	0.27
Clearance (ml/min)	68 (66–69)	67 (65–69)	0.71	69 (68–71)	70 (67–72)	0.75	<0.001

*Geometric Mean (95% CI); P1, *P*-value comparing subjects with NGT and subjects with AGT after adjustment for age and sex; P2, *P*-value comparing wild type with variant carriers after adjustment for age and sex.

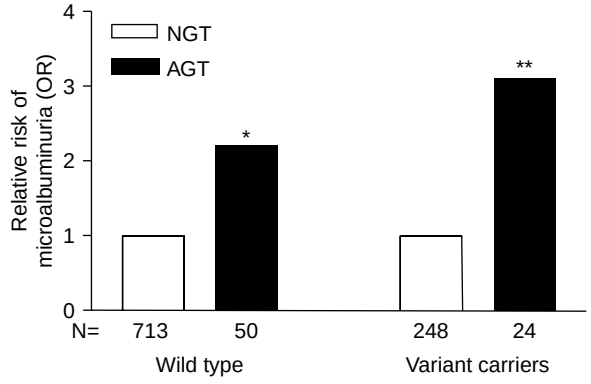

Figure 1 The relative risk of microalbuminuria comparing subjects with NGT and AGT per IGF-I genotype (wild type, left; variant carriers, right). Individuals with NGT were used as the reference group. $*P = 0.009$, $**P = 0.02$ vs the reference group.

of this IGF-I gene polymorphism after adjustment for some possible confounding factors.

The number of subjects with MA was low in our study which might have further attributed to a reduced statistical power of our study. Although the prevalence of MA in our study was low, this prevalence does not differ from previous findings in another big Dutch population-based study (32). In this latter study the prevalence of MA was 6.6% in non-diabetic subjects and 16.1% among diabetic patients. However, in this latter study, the diagnosis of MA was exclusively based on urinary albumin concentrations, while in our study this diagnosis was based on ACRs. A limitation of our study is that MA was assessed on one overnight sample. It is well known that there is considerable intra-individual variability in urinary albumin excretion in time (33). As a consequence, the prevalence of MA in our study may have been significantly both underestimated as well as overestimated.

Another limitation of our study may be that there is not only an age-related penetrance of AGT, but also an age-related penetrance of MA. Subjects with a NGT at the time of the study may still develop both an altered glucose tolerance and MA in the near future. We previously observed that variant carriers of this IGF-I gene polymorphism have an increased risk of developing diabetes, in comparison to carriers of the wild type genotype. Thus, this suggests that especially in variant carriers with AGT, the risk for MA may have been underestimated. Moreover, the diagnostic umbrella MA probably gathers individuals who have developed MA through a variety of pathological mechanisms (see below). This has obvious implications for the interpretation of our findings.

We did not find relationships between this IGF-I gene promoter polymorphism and renal function parameters, this was not unexpected. In subjects with type 2 diabetes MA is generally considered a stronger predictor of cardiovascular disease than it is of the risk of end-stage renal failure (34). Type 2 diabetes patients with MA are at an increased risk of cardiovascular death compared with patients with normal albuminuria (35, 36). In addition, in non-diabetic subjects, MA is even considered an independent risk factor to developing cardiovascular disease (3, 32). The observed relationship between IGF-I genotype and MA in our study thus probably points mainly to an increased risk of variant carriers for cardiovascular disease. This is in accordance with our previous findings: we observed that variant carriers had a higher risk of developing myocardial infarction (17). In addition, carotid IMT and aortic pulse wave velocity were significantly increased in variant carriers with hypertension (37).

MA may be the first sign that the vascular vessel wall, particularly the endothelium, is injured. It has been found that MA can be the specific consequence of a reduction in the fixed negative charges of the glomerular wall (38, 39).Whether an IGF-I gene genotype of a subject may modulate the susceptibility and/or progression of MA by circulating IGF-I or rather via its effects at the tissue level is at present unknown. However, it is likely that a link should be found in alterations in the composition of the basal membranes of the capillaries and of the extracellular matrices, which has also has been hypothesized for type 1 diabetes.

When our findings will have been replicated by others, these observations may suggest the existence of new etiological pathways for the development of MA, and together, provide some prediction of who will or will not get MA. In addition, it has been suggested that even if the risk is imparted by a certain genotype is low, or that only a subset of subjects carry the predisposing genotype, modulation of that gene by pharmacological intervention may be beneficial (40).

In conclusion, we observed in patients with AGT had a higher risk for MA in variant carriers than in carriers of the wild type genotype of this IGF-I gene promoter polymorphism. Since MA is primarily associated with cardiovascular disease in subjects with type 2 diabetes,

Table 3 The relative risk of microalbuminuria of subjects with AGT and subjects with NGT stratified by IGF-I genotype. Number of subjects for each category are given (% of cases or controls). Risks are given with a 95% confidence interval between brackets.

	Wild type	Variant type
Subjects with AGT		
Microalbuminuria	31 (9.3%)	17 (13.5%)
No microalbuminuria	303 (90.7%)	109 (86.5%)
Relative risk	Reference	1.5 (0.8–2.9)*
Relative risk	Reference	2.1 (1.0–4.4)**
Subject with NGT		
Microalbuminuria	19 (4.4%)	7 (4.8%)
No microalbuminuria	410 (95.6%)	139 (95.2%)
Relative risk	Reference	1.1 (0.45–2.64)*
Relative risk	Reference	1.6 (0.61–4.03)**

*Unadjusted; **After further adjustment for age, gender, mean blood pressure, fasting glucose, fasting insulin and smoking.

our study suggests that variant carriers with an AGT have an increased risk for cardiovascular disease.

Funding

I Rietveld is supported by a grant of the Dutch Diabetes Foundation. The Rotterdam Study is supported by the Erasmus Medical Center and Erasmus University Rotterdam, the Netherlands Organization for Scientific Research (NWO), the Netherlands Organization for Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. The contributions of the general practitioners and pharmacists of the Ommoord district to the Rotterdam Study are greatly acknowledged.

Acknowledgements

We would like to thank L Testers and J Vergeer, R. Oskamp, B de Graaf, F de Rooij and T Rademaker for their help in genotyping and data management. Also I Van Gorp and C van Leeuwen are greatly acknowledged for the measurements of albuminuria and creatinuria.

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Received 3 November 2005
Accepted 14 February 2006