Heritability of albumin excretion rate in families of patients with Type II diabetes

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Abstract

Aims/hypothesis. To study whether albumin excretion rate is an inherited trait in families of patients with Type II (non-insulin-dependent) diabetes mellitus.
Methods. We used three different approaches. Heritability of albumin excretion rate was studied in 267 nuclear families from the Botnia Study in Western Finland using parent-offspring regression. Albumin excretion rate was also measured in 206 non-diabetic offspring of 119 Type II diabetic parents with or without albuminuria (albumin excretion rate > 20 μg/min). Finally, albumin excretion rate was measured in altogether 652 siblings of 74 microalbuminuric and 320 normoalbuminuric probands. To study the potential confounding effect of blood pressure, the heritability of blood pressure was estimated in 718 nuclear families.
Results. Using parent-offspring regression, the heritability of albumin excretion rate was about 30 %, being the strongest from mothers to sons (35–39 % resemblance). The heritability for systolic blood pressure ranged from 10 to 20 % and for diastolic blood pressure from 10 to 27 %. Offspring of albuminuric Type II diabetic parents had higher albumin excretion rates (median 5.4 [range 1.0–195] vs 4.0 [1.0–23] μg/min, $p = 0.0001$) and a higher frequency of microalbuminuria (11 vs 2 %, $p = 0.012$) than offspring of normoalbuminuric parents. Further, siblings of microalbuminuric probands had higher albumin excretion rates than siblings of normoalbuminuric probands (4.1 [0.6–14.5] vs 3.6 [0.2–14.4] μg/min, $p < 0.01$).
Conclusion/interpretation. The data suggest that albumin excretion rate is an inherited trait in families of patients with Type II diabetes. [Diabetologia (1999) 42: 1359–1366]

Keywords Heritability, albumin excretion rate, Type II (non-insulin-dependent) diabetes mellitus, microalbuminuria, diabetic nephropathy.

Diabetic nephropathy develops in up to 30 % of patients with Type I (insulin-dependent) diabetes mellitus after 30 years duration, whereas the cumulative risk in Type II (non-insulin-dependent) diabetes mellitus varies between 25 and 50 % depending upon ethnic background and age at onset [1–3]. Although hyperglycaemia in itself represents the most important single risk factor for the development of diabetic nephropathy, genetic predisposition is also considered to have an important role. A familial clustering of diabetic nephropathy has been shown in both Type I and Type II diabetes [4–6]. Therefore an intensive search for genes which increase susceptibility to diabetic nephropathy has been initiated.

For genetic studies it is essential to correctly define the phenotype as early as possible. Microalbuminuria has served this purpose as it predicts diabetic nephropathy in both Type I and Type II diabetes [7–10]. Microalbuminuria is, however, also associated with cardiovascular disease [11] and it is not entirely clear whether the same genetic mechanisms confer suscep-

Table 1. Clinical characteristics of parents and offspring from 267 families participating in the parent-offspring estimation of heritability of AER

	Fathers	Mothers	Sons	Daughters
n	156	178	225	253
Age (years)	65.0 ± 0.9	65.8 ± 0.8	39.2 ± 0.8	39.9 ± 0.7
BMI (kg/m^2)	26.6 ± 0.3	27.8 ± 0.4	25.7 ± 0.3	24.6 ± 0.3
WHR	0.970 ± 0.004	0.857 ± 0.006	0.938 ± 0.005	0.833 ± 0.006
SBP (mmHg)	144 ± 2	144 ± 2	126 ± 1	122 ± 1
DBP (mmHg)	82 ± 1	81 ± 1	78 ± 1	76 ± 1
AER (μg/min)	5.0 (0.3-155.5)	3.9 (0.2-173.3)	4.3 (0.3-105.0)	4.3 (0.2-162.1)
FB-glucose (mmol/l)	6.6 ± 0.2	6.7 ± 0.2	5.1 ± 0.1	4.9 ± 0.1
FS-insulin (pmol/l)	72 ± 5	74 ± 4	49 ± 2	44 ± 1
Insulin AUC	29 510 ± 1870	35 300 ± 1990	29 330 ± 1320	29 390 ± 1110
Type II diabetes (%)	48	53	3	3
Hypertension (%)	30	48	9	12

Data are mean ± SEM, except AER which is given as median (range). AUC, incremental area under the curve; FB, fasting blood; FS, fasting serum

Table 2. Clinical characteristics of parents and offspring from 718 families in which the heritability of blood pressure was estimated

	Fathers	Mothers	Sons	Daughters
n	577	794	899	968
Age (years)	64.0 ± 0.5	65.1 ± 0.4	40.0 ± 0.4	39.9 ± 0.4
BMI (kg/m^2)	26.8 ± 0.1	27.6 ± 0.2	26.1 ± 0.1	25.0 ± 0.1
WHR	0.967 ± 0.003	0.866 ± 0.003	0.941 ± 0.003	0.830 ± 0.003
SBP (mmHg)	142 ± 1	143 ± 1	128 ± 1	124 ± 1
DBP (mmHg)	83 ± 1	81 ± 1	79 ± 1	77 ± 1
FB-glucose (mmol/l)	6.7 ± 0.1	7.0 ± 0.1	5.5 ± 0.1	5.1 ± 0.1
FS-insulin (pmol/l)	77 ± 3	83 ± 5	58 ± 2	50 ± 4
Insulin AUC	29 560 ± 1110	32 070 ± 1110	26 800 ± 730	28 430 ± 660
Type II diabetes (%)	44	47	12	9
Hypertension (%)	30	43	10	12

Data are mean ± SEM or percentages. AUC, incremental area under the curve; FB, fasting blood; FS, fasting serum

tibility to both diabetic nephropathy and cardiovascular disease. Little information is available on the heritability of albumin excretion rate (AER) as a continuous variable, which is a prerequisite for the use of AER as a quantitative trait in genetic studies. We tested this hypothesis by using three different approaches in the same study cohort.

Firstly, we studied heritability of AER in nuclear families using parent-offspring regression [12]. Secondly, we quantified AER in offspring of albuminuric and normoalbuminuric Type II diabetic parents assuming that, if genetically determined, AER should be higher in the offspring of the albuminuric than in the offspring of normoalbuminuric parents. Thirdly, a similar approach was used to compare AER in siblings of microalbuminuric and normoalbuminuric probands. Because AER is strongly related to blood pressure [13], we also assessed the heritability of blood pressure.

Subjects and methods

Subjects. All study subjects were recruited from the Botnia Study, which was initiated in 1990 in the western part of Finland with the aim to identifying genetic and early metabolic

defects in people at increased risk of developing Type II diabetes [14]. Details of the study cohort and sampling strategy have been presented earlier [14]. In short, all available Type II diabetic patients from four primary health care units plus their first-degree relatives were invited to participate in the study. Of the diabetic patients 95 % and of their non-diabetic first-degree relatives 76 % participated in the study. Non-diabetic subjects and diabetic patients with fasting blood glucose concentrations less than 10 mmol/l underwent an oral glucose tolerance test (OGTT) during which a timed urine sample was collected for the determination of the albumin concentration. Patients with Type I diabetes were excluded from the study.

Protocol 1: parent-offspring regression. To estimate parent-offspring regression of AER in families, we studied offspring of non-diabetic and diabetic patients from 267 families with an average of 1.8 children per family (Table 1). Of the fathers 48 %, of the mothers 53 % and of the offspring 3 % had Type II diabetes. The number of families available for the above analyses were restricted by the urine collection strategy. Therefore, a greater number of families, that is 718 families with an average of 2.5 children per family, were available for the estimation of the heritability of blood pressure (Table 2). Of the mothers 43 %, of the fathers 30 % and of the offspring 10 % had hypertension.

Protocol 2: AER in offspring of albuminuric and normoalbuminuric Type II diabetic probands. The effect of parental Type II diabetes plus albuminuria on AER in the offspring was stud-

Table 3. Clinical characteristics of albuminuric and normo-albuminuric Type II diabetic probands with p values adjusted for BMI

	Albuminuric probands	Normo-albuminuric probands	p value
n (men/women)	67 (37/30)	52 (24/28)	
Age (years)	70.0 ± 1.2	70.3 ± 0.9	
Duration of diabetes (years)	11.1 ± 1.0	10.3 ± 0.6	
Treatment:			0.004
– diet only (%)	29	44	
– OHA (%)	56	52	
– insulin (%)	36	8	
BMI (kg/m^2)	29.6 ± 0.7	27.3 ± 0.5	(0.011)
WHR – male subjects	0.989 ± 0.014	0.984 ± 0.010	
– female subjects	0.917 ± 0.018	0.859 ± 0.010	
FB-glucose (mmol/l)	9.1 ± 0.4	8.7 ± 0.3	
FS-insulin (pmol/l)	159 ± 21	88 ± 11	0.019
SBP (mmHg)	154 ± 3	147 ± 2	
DBP (mmHg)	83 ± 2	80 ± 1	
Hypertension (%)	52	58	
History of CHD (%)	43	37	
Triglycerides (mmol/l)	2.09 ± 0.12	1.96 ± 0.19	
Cholesterol (mmol/l)	5.61 ± 0.15	5.95 ± 0.17	
HDL-cholesterol (mmol/l)	1.13 ± 0.03	1.29 ± 0.04	0.003
AER (µg/min)	–	4.6 (0.6-14.3)	
Retinopathy (%)	31	15	

All data are means ± SEM or percentages except AER which is given as median (range). OHA, oral hypoglycaemic agents; CHD, coronary heart disease; FB, fasting blood; FS, fasting serum

Table 4. Clinical characteristics of microalbuminuric and normoalbuminuric probands

	Micro-albuminuric probands	Normo-albuminuric probands	p value
n (men/women)	74 (46/28)	320 (163/158)	
Age (years)	54.5 ± 2.0	53.5 ± 0.9	
BMI (kg/m^2)	26.9 ± 0.5	26.2 ± 0.2	
WHR – men	0.974 ± 0.009	0.958 ± 0.004	
– women	0.857 ± 0.020	0.836 ± 0.005	
SBP (mmHg)	147 ± 4	134 ± 1	< 0.0001
DBP (mmHg)	83 ± 2	80 ± 1	
FB-glucose (mmol/l)	6.3 ± 0.3	5.5 ± 0.1	0.001
FS-insulin (pmol/l)	77 ± 7	57 ± 2	0.002
Insulin AUC (pmol/l · min)	37030 ± 3120	34880 ± 1470	
AER (µg/min)	25.3 (14.6-193.5)	3.6 (0.3-14.4)	
Type II diabetes (%)	30	17	0.009

All data are means ± SEM or percentages except AER which is given as median (range). AUC, incremental area under the curve; FB, fasting blood; FS, fasting serum

ied in 108 (49 men/59 women) non-diabetic offspring of 67 (37 men/30 women) Type II diabetic patients with abnormal AER and in 98 (40 men/58 women) non-diabetic offspring of 52 (24 men/28 women) Type II diabetic patients with normal AER. To improve the specificity of the normoalbuminuric status, a diabetes duration of at least 5 years was required in the latter parents. Data on the Type II diabetic probands are shown in Table 3.

Protocol 3: AER in siblings of albuminuric and normoalbuminuric probands. We also studied 141 siblings of 74 microalbuminuric probands and 511 siblings of 320 normoalbuminuric probands. Of the probands 30% and 17% respectively had Type II diabetes (Table 4). If there were several probands with microalbuminuria in a sibship, the proband was randomly chosen and the other microalbuminuric siblings were excluded.

Albumin excretion rate. We measured AER from a timed urine collection during an OGTT except in the Type II diabetic parents of protocol 2, in whom AER was measured from overnight or 24 h-urine collections. In a subset of subjects (n = 442) AER measured during the OGTT and AER measured during the previous night were compared, showing a strong positive correlation (r = 0.605, p < 0.0001). The methods were also compared by plotting the difference between the methods against their mean [15]. In the AER range below the limit of 15 µg/min the mean AER difference between the methods was 0.44 µg/min (95% C.I. –8.00 to 9.66) and with AER above the limit 11.5 µg/min (95% C.I. –22.02 to 59.52).

In urine collections during the OGTT, microalbuminuria was defined as an AER between 15 and 200 µg/min, which is lower than the consensus threshold of 20 µg/min but similar and even lower values have earlier been used to predict the outcome of microalbuminuria [16]. Non-diabetic subjects with manifest albuminuria (AER > 200 µg/min) were excluded from the analyses to avoid inclusion of subjects with non-diabetic kidney disease.

The diagnosis of diabetic nephropathy in the Type II diabetic probands in protocol 2 was based on medical records or multiple overnight or 24 h-urine collections. Patients with any evidence of non-diabetic renal disease were excluded from the study. Type II diabetic parents were considered to have abnormal AER if their AER was 20 µg/min or more in overnight or 30 mg/24 h or more in 24 h-urine samples.

Oral glucose tolerance test. In all studies, subjects with a fasting blood glucose less than 10 mmol/l underwent a standard 120 min OGTT (75-g glucose) after 12 h of overnight fasting. During the OGTT venous blood samples were taken at –5, 0, 30, 60 and 120 min for determination of blood glucose and serum insulin concentrations. The fasting values represent the mean of the –5 and the 0 min samples. World Health Organisation criteria of 1985 were used to classify the subjects [17]. Incremental insulin area was calculated by the trapezoidal rule. Triglycerides, total and HDL-cholesterol were determined from fasting serum samples.

Waist circumference was measured on standing subjects with a soft tape in the midaxillary line, midway between the lowest rib margin and the iliac crest. Hip circumference was measured over the widest part of the gluteal region [18]. The waist-to-hip ratio (WHR) was then calculated and used as a measure of abdominal obesity. The body mass index (BMI) was calculated as weight (kg) divided by height (m^2). Blood

Table 5. Heritability ($h^2 \pm$ SEM) of albumin excretion rate, systolic and diastolic blood pressure estimated from parent-offspring regression and subdivided by sex. All data are adjusted for age and glucose

	Fathers	Mothers
All offspring		
AER	0.29 ± 0.15	0.31 ± 0.12
AER adjusted for SBP	0.27 ± 0.15	0.34 ± 0.13
SBP	0.05 ± 0.06	0.16 ± 0.04
DBP	0.15 ± 0.07	0.21 ± 0.06
Sons		
AER	0.15 ± 0.17	0.35 ± 0.15
AER adjusted for SBP	0.12 ± 0.18	0.39 ± 0.16
SBP	0.11 ± 0.07	0.20 ± 0.05
DBP	0.19 ± 0.09	0.27 ± 0.07
Daughters		
AER	0.34 ± 0.19	0.29 ± 0.16
AER adjusted for SBP	0.31 ± 0.20	0.35 ± 0.16
SBP	0.10 ± 0.07	0.10 ± 0.06
DBP	0.20 ± 0.08	0.10 ± 0.07

Table 6. Clinical characteristics of non-diabetic offspring of Type II diabetic patients with abnormal (OffALB) and normal AER (OffNORMO)

	OffALB	OffNORMO	*p* value
n (men/women)	108 (49/59)	98 (40/58)	
Age (years)	43.0 ± 0.9	41.9 ± 0.7	
BMI (kg/m^2)	26.5 ± 0.4	24.6 ± 0.4	0.001
WHR – men	0.969 ± 0.010	0.954 ± 0.008	
– women	0.867 ± 0.015	0.836 ± 0.008	
FB-glucose (mmol/l)	5.0 ± 0.1	5.0 ± 0.1	
FS-insulin (pmol/l)	51 ± 3	41 ± 2	0.012
Insulin AUC (pmol/l · min)	$31\,680 \pm 2040$	$26\,810 \pm 1580$	
SBP (mmHg)	126 ± 1	124 ± 1	
DBP (mmHg)	78 ± 1	79 ± 1	
Hypertension (%)	14	10	
Triglycerides (mmol/l)	1.27 ± 0.06	1.25 ± 0.07	
Cholesterol (mmol/l)	5.49 ± 0.09	5.35 ± 0.10	
HDL-cholesterol (mmol/l)	1.39 ± 0.04	1.49 ± 0.04	
AER (µg/min)	5.4 (1–195)	4.0 (1–23)	< 0.0001

All data are means ± SEM or percentages except AER which is given as median (range). AUC, incremental area under the curve; FB, fasting blood; FS, fasting serum

pressure was measured three times, with 5 min intervals, in the sitting position, after an initial 10-min rest. The mean of the three recordings was then calculated and used in the analysis. Hypertension was defined as a sitting systolic blood pressure (SBP) of 160 mmHg or more, a diastolic blood pressure (DBP) of 95 mmHg or more, or current treatment with antihypertensive drugs. Informed consent was obtained from all subjects participating in the study, and the study protocol was approved by the local ethics committees.

Analytical methods. Blood glucose during the OGTT was measured in duplicate by a hexokinase method in the family studies (Boehringer Mannheim, Mannheim, Germany). Serum insulin concentrations were determined by a double-antibody radioimmunoassay (Pharmacia, Uppsala, Sweden). The sensitivity of the method is 15 pmol/l and the inter assay coefficient of variation 5%. Serum total cholesterol, HDL-cholesterol, and triglyceride concentrations were measured on a Cobas Mira analyser (Hoffman LaRoche, Basel, Switzerland). Urine albumin concentration was determined with radioimmunoassay with a detection limit of 2 mg/l and an inter assay coefficient of variation of 5%.

Statistical analysis. All data are expressed as means ± SEM unless otherwise stated. Differences between group means were tested with analysis of variance (ANOVA) or covariance (ANCOVA) and Pearson's chi-squared test was used to test the significance of frequency differences. Due to skew distribution, AER, triglycerides, fasting insulin and area under the insulin curve were log$_e$ transformed. The association between AER and other variables was tested by multiple linear regression analysis. The heritability was estimated by parent-offspring regression, the data being presented individually for each sex. Therefore, sex-specific values for the heritability were calculated as two times the regression coefficients between parents and offspring. To test the hypothesis that heritability of AER is influenced by blood pressure we further adjusted the heritability estimates for blood pressure.

All statistical analysis was done using a BMDP statistical package (BMDP Statistical Software, Los Angeles, Calif., USA). A *p*-value less than 0.05 was considered statistically significant.

Results

Variables associated with AER in a multiple regression analysis. To exclude the effect of other confounding variables on AER, a multiple linear regression analysis with AER as a dependent variable was carried out. The model included sex, age, BMI, systolic and diastolic blood pressure, fasting serum insulin and fasting blood glucose concentrations. Of these variables, age ($p < 0.0001$), fasting blood glucose ($p < 0.0001$) and systolic blood pressure ($p < 0.0001$) contributed significantly to the variance of AER. Therefore, all heritability estimates derived from the parent-offspring regressions were adjusted for age and fasting blood glucose. The effect of systolic blood pressure was assessed separately by adjusting the heritability estimates of AER for systolic blood pressure. The variance of AER was similar in male and female subjects; therefore adjustment for unequal variance between sex was not applied.

Protocol 1: parent-offspring regression of AER. The heritability of AER from fathers and mothers to offspring of both sex was 29% and 30%, respectively. When the data was calculated separately for sons and daughters, the heritability was strongest from mothers to sons (35% resemblance) and weakest from fathers to sons (15%). Adjustment of AER for systolic blood pressure (SBP) slightly increased the
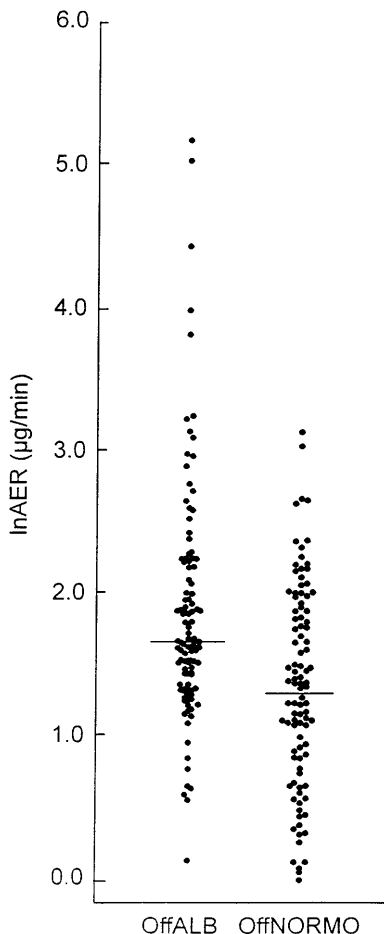
Fig. 1. The distribution of AER in non-diabetic offspring of Type II diabetic parents with abnormal (OffALB) and with normal (OffNORMO) AER. The median AER values shown in the figure correspond to 5.4 and 4.0 µg/min, with $p = 0.0001$ between the groups. lnAER, logarithm of AER

heritability estimates for mothers to offspring by 4% and 6% for sons and daughters, respectively. The same adjustment decreased the heritability estimates for AER slightly from fathers to both sons and daughters by 3%. (Table 5).

Parent-offspring regression of blood pressure. The heritability estimates for blood pressure were generally lower than those for AER. Both SBP and DBP showed higher heritability values from mothers than from fathers (16 and 21% vs 5 and 15% for SBP and DBP, respectively). The heritability estimates for SBP analysed separately for sons and daughters ranged from 10 to 20%, being the highest from mothers to sons. Similarly, the heritability estimates of DBP ranged from 10 to 27%, again being the highest from mothers to sons. (Table 5).

Protocol 2: AER in offspring of albuminuric and normoalbuminuric Type II diabetic probands. The albuminuric Type II diabetic parents had similar age and

duration of diabetes but were more obese ($p = 0.011$) and had higher fasting insulin ($p = 0.019$) and lower HDL-cholesterol ($p = 0.003$) concentrations than the normoalbuminuric Type II diabetic parents (Table 3). Offspring of albuminuric parents had higher BMI ($p = 0.001$), higher fasting insulin concentrations ($p = 0.012$) and AER ($p < 0.0001$) than offspring of normoalbuminuric parents (Table 6). After adjustment of all data for BMI, AER was the only variable that differed significantly between offspring of parents with albuminuria and offspring of parents with normal AER ($p = 0.0001$) (Fig. 1). Of offspring to albuminuric parents 11% and of offspring to normoalbuminuric parents 2% had microalbuminuria ($p = 0.012$).

Protocol 3: AER in siblings of microalbuminuric and normoalbuminuric probands. Siblings of microalbuminuric probands had higher AER ($p < 0.01$) than siblings of normoalbuminuric probands (Table 7). Otherwise, there was no difference in blood pressure or the other variables that were measured between the sibling groups. Of siblings to microalbuminuric probands 14% and of siblings to normoalbuminuric probands 16% had Type II diabetes. Microalbuminuric probands had a higher prevalence of Type II diabetes than normoalbuminuric probands ($p = 0.009$). As diabetes in itself can increase AER, the data were reanalysed using the non-diabetic siblings only. Even in this case AER was higher in siblings of microalbuminuric probands compared with siblings of normoalbuminuric probands ($p < 0.01$) (Table 7). As hypertension can increase AER we also analysed the data separately in normotensive siblings only. Even then AER was higher in siblings of microalbuminuric probands compared with siblings of normoalbuminuric probands (median 4.4 vs 3.4 µg/min, $p < 0.01$).

Discussion

The study provides convincing evidence that AER is an inherited trait. The parent-offspring regression indicated that about 30% of the variance in AER is genetically determined. In support of a familial clustering of increased AER, offspring of albuminuric parents and siblings of microalbuminuric probands had increased AER.

This study has addressed the heritability of AER using parent-offspring regression in a cohort including both non-diabetic and diabetic subjects. In the DCCT study, clustering of AER was studied in the Type I diabetic probands and their diabetic relatives. Parent-offspring correlations for AER were in line with ours, ranging from 0.10 to 0.17 when both Type I and Type II diabetic patients were included but increased to 0.41 when only Type I diabetic patients were included in the analysis.

Table 7. Clinical characteristics of siblings to microalbuminuric (MICRO) and normoalbuminuric (NORMO) probands

	All siblings		Non-diabetic siblings	
	MICRO	NORMO	MICRO	NORMO
n (men/women)	141 (70/71)	511 (233/274)	121 (62/59)	427 (195/228)
Age (years)	54.8 ± 1.3	54.0 ± 0.6	52.8 ± 1.4	51.8 ± 0.7
BMI (kg/m^2)	26.2 ± 0.3	26.3 ± 0.2	25.6 ± 0.3	25.8 ± 0.2
WHR	0.896 ± 0.007	0.894 ± 0.004	0.892 ± 0.008	0.889 ± 0.004
SBP (mmHg)	138 ± 1	135 ± 1	135 ± 2	132 ± 1
DBP (mmHg)	81 ± 1	82 ± 1	81 ± 1	80 ± 1
FB-glucose (mmol/l)	5.6 ± 0.1	5.5 ± 0.1	5.1 ± 0.1	5.0 ± 0.1
FS-insulin (pmol/l)	53 ± 3	53 ± 2	51 ± 3	46 ± 1
Insulin AUC (pmol/l · min)	34410 ± 2100	32870 ± 980	35260 ± 2160	33150 ± 1020
AER (μg/min)	4.1 (0.6-14.5)	3.6 (0.2-14.4)[a]	4.0 (0.6-14.5)	3.3 (0.2-14.0)[a]
Type II diabetes (%)	14	16		

Data are means ± SEM and percentages, except AER which is given as median (range). AUC, incremental area under the curve; FB, fasting blood; FS, fasting serum. [a] $p < 0.01$ between the microalbuminuric and normoalbuminuric siblings

Earlier cohort studies have presented heritability estimates for blood pressure ranging from 15 to 40 % [19]. Higher estimates, up to 70 %, have been reported in twin studies [20]. Our heritability estimates for blood pressure were 10–27 % when analysed separately for each sex. Hypertension and thereby antihypertensive therapy was three times more common in the parents than in the offspring in our study and no correction was made for the treatment effect. The effect of antihypertensive treatment is usually restricted to patients in the high range of blood pressure. Such effects are not likely to substantially bias the genetic estimates but could result in underestimation of the heritability of blood pressure.

How confident are the heritability estimates? A shared environment within a family might contribute to the observed covariance. In simulation studies it has been shown, however, that environmental factors cannot entirely account for the familial aggregation of a trait [21]. The offspring in the parent-offspring study did not always have both parents alive or available. Given the high mortality rate associated with microalbuminuria, we are likely to miss parents with microalbuminuria and could thus underestimate the heritability of AER. Moreover, the OGTT, during which the urine samples were collected, was done only in subjects with a fasting blood glucose below 10.0 mmol/l. Poorly controlled diabetic subjects, likely to have an increased AER, were therefore excluded from the study. In an attempt to enlarge the study cohort, both diabetic and non-diabetic offspring were studied. Hyperglycaemia in itself could increase AER [22]. The confounding effect of hyperglycaemia seems minor as adjusting the AER data for fasting blood glucose did not change the heritability estimates. Given these shortcomings we consider the presented heritability estimates as minimum estimates of AER heritability.

The genetic nature of AER was supported by the findings that non-diabetic offspring of Type II diabetic parents with microalbuminuria and macroalbuminuria had higher AER than offspring of normoalbuminuric parents regardless of whether offspring of microalbuminuric and offspring of macroalbuminuric parents were analysed separately. Similar data have been reported in two other studies involving normotensive offspring with normal glucose tolerance [23, 24]. The observation that offspring of Type II diabetic patients with albuminuria had higher BMI deserves some attention. This could reflect coinheritance of obesity and predisposition to microalbuminuria. Obesity in itself, especially abdominal obesity, has also been associated with increased AER [25, 26]. Further, it has been found that higher BMI predicted higher AER in diabetic siblings of Type II diabetic probands with and without albuminuria [27]. In Pima Indians, in whom Type II diabetes is strongly associated with insulin resistance and obesity [28], renal disease in a Type II diabetic parent increased the risk of diabetes in the offspring 2.5-fold compared with the presence of diabetes alone [29]. Therefore, it is possible that the increased AER and obesity in the non-diabetic offspring in our study could both be associated with insulin resistance [30–33].

In support of the inherited nature of AER, siblings of probands with abnormal AER had increased AER. Diabetic siblings of Type I diabetic patients with diabetic nephropathy are 2.5 to five times more likely to develop nephropathy than siblings of patients without nephropathy [4, 34]. In keeping with these findings, non-diabetic siblings of Type II diabetic patients with microalbuminuria or macroalbuminuria had two times higher AER than siblings of normoalbuminuric Type II diabetic patients in a previous study from Italy [27]. In the present study, only microalbuminuric probands were included and the majority of the probands were non-diabetic. To control for the possible effect of diabetes in itself we analysed the data both including and excluding diabetic siblings of the probands. Both analyses yielded the same results, i.e. higher AER in sibships of microalbuminuric than of normoalbuminuric probands.

What could be the inherited defect resulting in microalbuminuria? Several studies have indicated that increased AER reflects vascular damage and that patients with increased AER often have higher concentrations of von Willebrand factor as a sign of endothelial dysfunction [35–37]. It has been shown that microalbuminuria in Type II diabetes is associated with new cardiovascular events only in the presence of increased von Willebrand factor [36]. A recent study showed that not all Type II diabetic patients with microalbuminuria had renal structural alterations; only those with structural changes appeared to have increased von Willebrand plasma concentrations [38].

Although microalbuminuria has been shown to increase the risk for development of Type II diabetes [39], only a small proportion of the non-diabetic subjects with increased AER in this study will subsequently develop diabetes and thereby be exposed to the risk of developing diabetic nephropathy. In addition, diabetic nephropathy is a relatively rare complication in Scandinavian Type II diabetic patients, probably as a consequence of the late onset of the disease [40]. These patients are at a much higher risk of developing coronary artery disease than of developing diabetic nephropathy. In our 9-year prospective study of 131 Type II diabetic patients, microalbuminuria was associated with high cardiovascular mortality but only one patient developed end-stage renal disease [41]. A high mortality in the microalbuminuria group could thus result in a selection bias with over-representation of survivors with little or no cardiovascular disease.

As discussed, microalbuminuria could also be related to hypertension. Given that adjusting for blood pressure did not statistically significantly change the heritability estimates for AER, it is likely that AER is inherited at least partially independently from blood pressure.

Despite an intensive search for candidate genes for diabetic nephropathy only a few genes can be considered as serious candidates (*ACE*, angiotensinogen, angiotensin II receptor, aldose reductase, heparan sulphate proteoglycan) [42–46]. Notably, most of them are involved in blood pressure regulation. The *ACE* gene insertion/deletion polymorphism has gained most of the attention. A recent meta-analysis of 18 studies firmly established its role in the pathogenesis of diabetic nephropathy or microalbuminuria [47].

In conclusion, heritability estimates indicate that AER is an inherited trait and clusters in families of patients with Type II diabetes. Albumin excretion rate could thus be used as a quantitative phenotype in genetic studies.

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