Cardiovascular Morbidity and Early Mortality Cluster in Parents of Type 1 Diabetic Patients With Diabetic Nephropathy

LISE TARNOW, MD
PETER ROSSING, MD
FLEMMING S. NIELSEN, MD
JOHAN A. FAGERUDD, MD
ODETTE POIRIER, PHD
HANS-HENRIK PARVING, MD

OBJECTIVE — A familial predisposition was proposed to be a determinant of the increased morbidity and mortality from cardiovascular disease in type 1 diabetic patients with diabetic nephropathy. The insertion allele of an insertion/deletion polymorphism in the ACE (ACE/ID) gene seems to protect against coronary heart disease in nondiabetic and diabetic subjects. The aim of the present study was to evaluate these hypotheses in parents of a large group of type 1 diabetic patients with and without diabetic nephropathy.

RESEARCH DESIGN AND METHODS — We investigated cardiovascular morbidity and mortality of parents of 163 type 1 diabetic patients with nephropathy and parents of 163 sex- and age-matched normoalbuminuric patients with type 1 diabetes.

RESULTS — Kaplan-Meier curves showed that total parental mortality was significantly increased in parents of type 1 diabetic patients with nephropathy (121 of 244 [~50%]) as compared with parents of normoalbuminuric type 1 diabetic patients (119 of 269 [~44%]) (P = 0.008 [log-rank test]) partially due to an increase in cardiovascular deaths (48 of 244 [~ 20%] vs. 42 of 269 [~16%], $P < 0.05$). In addition, more patients with nephropathy, as compared with the normoalbuminuric group, had at least one parent with fatal/nonfatal cardiovascular disease (46% [95% CI 38–54] vs. 36% [28–44], P = 0.05). Fathers of patients homozygous for the I-allele of the ACE/ID polymorphism had significantly less myocardial infarction as compared with other genotypes (P = 0.03), regardless of the nephropathic state of the offspring.

CONCLUSIONS — Cardiovascular morbidity and early mortality clusters in parents of type 1 diabetic patients with diabetic nephropathy. The ACE/ID polymorphism helps explain the increased morbidity from cardiovascular disease.

Diabetes Care 23:30–33, 2000

Several studies have demonstrated familial clustering of diabetic nephropathy, suggesting a genetic component in the pathogenesis of this devastating microvascular complication (1–3). The increased cardiovascular morbidity and mortality observed not only in diabetic patients with kidney disease (4) but in their nondiabetic parents (5,6) has led investigators to suggest a possible analogous process that leads to atherosclerosis and glomerulosclerosis in diabetic patients. A common putative contributor is the insertion/deletion polymorphism (ACE/ID) in the ACE gene because the I-allele has been suggested to protect against diabetic nephropathy (7,8) and against coronary heart disease in studies of nondiabetic (9,10) and diabetic subjects (7,11–13).

In contrast to previously mentioned studies (5,6), Nørgaard et al. (14) found no increase in parental history of cardiovascular morbidity or mortality in Danish middle-aged parents of patients with nephropathy compared with the parents of patients with normoalbuminuria. The purposes of the present study were to evaluate familial predisposition to cardiovascular disease in a large group of parents of type 1 diabetic patients with and without diabetic nephropathy and, furthermore, to investigate the association between the ACE/ID polymorphism and parental myocardial infarction.

RESEARCH DESIGN AND METHODS

From a study of all type 1 diabetic patients with diabetic nephropathy who had their glomerular filtration rate measured during 1993 at the Steno Diabetes Center, 199 nephropathic patients and 192 type 1 diabetic patients with persistent normoalbuminuria were included in a case-control study (11,15). The two groups were matched with regard to sex, age, and duration of diabetes. Diabetic nephropathy was defined as a persistent urinary albumin excretion rate >300 mg/24 h with the presence of diabetic retinopathy and the absence of clinical or laboratory signs of other kidney or urinary tract diseases.

All eligible patients and their parents (37%) were interviewed by a modified and standardized World Health Organization cardiovascular questionnaire (16). Cardiovascular disease was diagnosed if anamnestic evidence of myocardial infarction, angina pectoris, intermittent claudication, or cerebral ischemia was given. Data on deceased or nonattending parents were obtained from the surviving spouses (14%) or their offspring (49%). Fatal myocardial infarction, heart failure, ruptured aortic aneurysm, or cerebrovascular accident were classified as cardiovascular deaths. A parental history of presence/absence of cardiovascular morbid-

From Steno Diabetes Center (L.T., P.R., F.S.N., H.-H.P.), Gentofte, Denmark; the Department of Medicine (J.A.F.), Helsinki University Hospital, Helsinki, Finland; and Institut Nationale de la Santé et de la Recherche Medicale U525 (O.P.), Paris, France.

Address correspondence and reprint requests to Dr. Lise Tarnow, Steno Diabetes Center, Niels Steensens Vej 2, DK-2820 Gentofte, Denmark. E-mail: lise.tarnow@dadlnet.dk.

Received for publication 9 August 1999 and accepted in revised form 29 September 1999.

Abbreviations: ACE/ID, ACE insertion/deletion.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

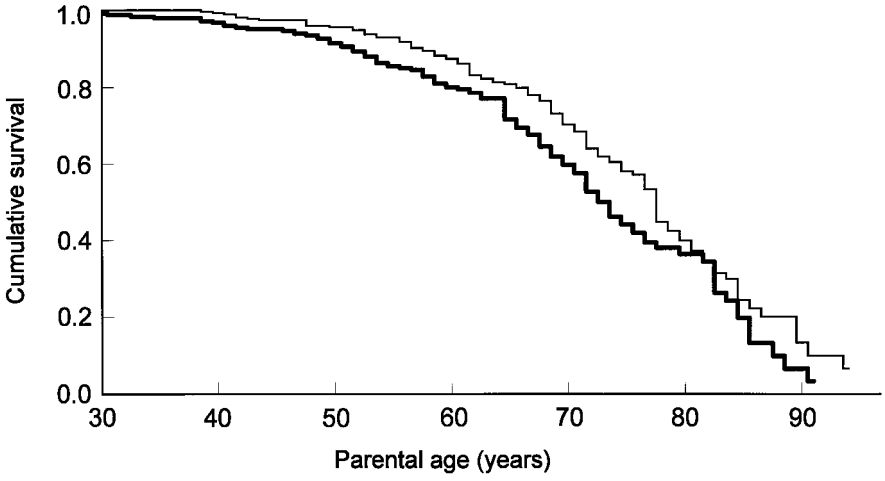

Figure 1—*Kaplan-Meier plot of survival of 244 parents of 163 patients with type 1 diabetes with nephropathy (bold line) and in 269 parents of 163 patients with long-standing type 1 diabetes and persistent normoalbuminuria (thin line). Log-rank test* P = *0.008.*

ity and mortality was available for 163 patients in both groups. In addition, reliable information on age and cause of death was obtained for 244 (75%) of the parents of patients with nephropathy and for 269 (83%) of parents of patients with normoalbuminuria. The nonresponding nephropathic patients did not differ from included patients with nephropathy with respect to sex (25 men), age (43.4 ± 9.8 years), or duration of diabetes (29.2 ± 8.4 years). In the normoalbuminuric group, sex distribution was similar (20 men), whereas mean age (48.1 ± 9.1 years) and duration of diabetes (29.8 ± 9.1 years) were higher in nonresponders as compared with responders (P = 0.001 and 0.03, respectively).

The ACE/ID polymorphism was determined by polymerase chain reaction in all type 1 diabetic patients with and without diabetic nephropathy, as described previously (15). Informed consent was obtained and the study was approved by the local ethical committee.

Statistics

Data are expressed as means ± SD or medians (range) as appropriate. Comparisons between groups of normally or log-normally distributed variables were performed with an unpaired Student's t test. Prevalences are given by actual values with 95% CI and compared by χ^2 test. Survival was compared using Kaplan-Meier plots with the log-rank test. A P value <0.05 (two-sided) was considered statistically significant. Analyses were performed with a commercial software package (SPSS, Chicago).

RESULTS — The groups of type 1 diabetic patients with and without nephropathy were comparable with regard to sex distribution (97 vs. 98 men), age (40 ± 10 vs. 42 ± 10 years), and duration of diabetes (27 ± 8 vs. 26 ± 8 years). Urinary albumin excretion rate, serum creatinine, and cholesterol were elevated: 781 mg/24 h (16–14, 543), 98 µmol/l (54–575), and 5.7 ± 1.2 mmol/l in patients with nephropathy vs. 7 mg/24 h (1–30), 77 µmol/l (40–116), and 4.7 ± 1.0 mmol/l in normoalbuminuric patients ($P <$ 0.001). More patients with nephropathy received antihypertensive medication than did patients with normoalbuminuria (75% [68–82] vs. 11% [6–16], respectively) ($P <$ 0.001). Because of ongoing antihypertensive treatment, some patients with previously persistent macroalbuminuria had a urinary albumin excretion rate <300 mg/24 h at the time of investigation.

A Kaplan-Meier survival curve showed that the parents of patients with nephropathy had reduced survival rates when compared with the parents of patients with normoalbuminuria (log-rank test P = 0.008) (Fig. 1). In the parents of those with nephropathy, 28 had died from myocardial infarction (22%), 14 from stroke (11%), 6 from other cardiovascular diseases (5%), 39 from cancer (31%), and 39 from other causes (31%). In the parents of patients with normoalbuminuria, 28 had died from myocardial infarction (23%), 11 from stroke (9%), 4 from other cardiovascular diseases (3%), 41 from cancer (34%), and 38 from other causes (31%). Kaplan-Meier curves for cardiovascular mortality were plotted, and they indicated that the parents of those with nephropathy were more likely to suffer a cardiovascular death (log-rank test $P < 0.05$) (Fig. 2). Similar analyses for death from myocardial infarction or from stroke revealed no significant differences in survival (log-rank test P = 0.22 and 0.15, data not shown).

The prevalence of cardiovascular morbidity and mortality in parents did not differ between patients with and without nephropathy (Table 1), whereas more patients with nephropathy had at least one parent with fatal/nonfatal cardiovascular disease (46% [38–54]) as compared with the normoalbuminuric group (36% [28–44]) (P = 0.05). Based on data from the subset of subjects in

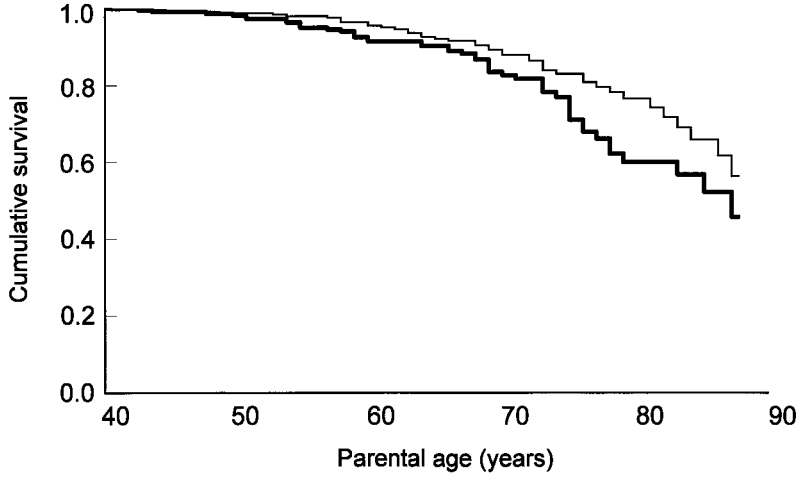

Figure 2—*Kaplan-Meier plot of survival free of cardiovascular death in 244 parents of 163 patients with type 1 diabetes with nephropathy (bold line) and in 269 parents of 163 patients with long-standing type 1 diabetes and persistent normoalbuminuria (thin line). Log-rank test* P < *0.05.*

Table 1—*Prevalences of cardiovascular disease and risk factors in parents of 163 type 1 diabetic patients with diabetic nephropathy and in parents of 163 patients with long-standing type 1 diabetes and persistent normoalbuminuria*

	Parents of type 1 diabetic patients		
	With nephropathy	With normoalbuminuria	*P* value
Age at investigation (years)	66.9 ± 8.5	69.4 ± 9.1	0.02
Age at time of death (years)	63.6 ± 13.9	67.4 ± 12.0	0.03
Prevalence of:			
Cardiovascular disease (fatal and nonfatal)	27% (22–32)	23% (18–27)	0.20
Myocardial infarction (fatal and nonfatal)	13% (9–16)	13% (9–17)	NS
Stroke (fatal and nonfatal)	11% (7–14)	7% (5–10)	0.17
Antihypertensive medication	27% (22–31)	24% (20–29)	NS
Antidiabetic treatment	14% (10–18)	13% (9–17)	NS

Data are means ± SD or prevalences (95% CI).

which information on cardiovascular disease was obtained both from the parents and the diabetic patients, we found that 68% of parents with cardiovascular disease were classified correctly by their diabetic offspring with nephropathy as compared with a sensitivity of 63% in parents of patients with normoalbuminuria (NS). The corresponding specificity was 93 vs. 96% in the nephropathic versus the normoalbuminuric group, respectively (NS).

Of patients with known ACE/ID genotype (n = 323), 62 had a paternal history of myocardial infarction and 21 had a maternal history of myocardial infarction, whereas 245 had no affected parents. Fathers of patients homozygous for the I-allele had significantly less myocardial infarction (11%, n = 8) as compared with fathers of patients with ID or DD genotypes (22%, n = 54) (P = 0.03).

CONCLUSIONS — We found increased early mortality rates in parents of type 1 diabetic patients with nephropathy as compared with parents of patients with long-standing type 1 diabetes and persistent normoalbuminuria. Excess mortality was related to premature death due to cardiovascular disease. Type 1 diabetic patients with nephropathy were more likely to have a parental history of cardiovascular disease. The fathers of patients with the II genotype had experienced a myocardial infarction less frequently. Thus, the present study, which is so far the largest of its kind, confirms and extends the finding that early cardiovascular mortality clusters in parents of patients with nephropathy (5,6,17).

One limitation to our study is the possibility that patients with nephropathy, who are significantly more ill, may be sensitized to identifying a family history of cardiovascular disease. However, this limitation is unlikely to bias the data on mortality. In addition, the prevalences of cardiovascular morbidity reported by parents and by patients with and without diabetic nephropathy are in agreement.

Originally, Nørgaard et al. (14) reported no difference in mortality between parents of 58 type 1 diabetic patients with diabetic nephropathy and 54 patients without diabetic nephropathy. Moreover, no increase in prevalence of cardiovascular disease was found among parents of nephropathic patients (14). On the contrary, Earle et al. (5), in a similar study of 61 patients with micro- and macroalbuminuria and 61 normoalbuminuric patients, found that more of the parents of patients with elevated urinary albumin excretion rate had died, largely from cardiovascular disease. Recently, no difference between groups was found by comparison of the absolute death status in a Finnish study (17). But by taking the time course into consideration in the Kaplan-Meier survival analysis, a higher overall mortality rate among parents of patients with overt nephropathy (n = 137) was demonstrated as compared with parents of patients without nephropathy (n = 54) (17). A similar finding was reported from a study of 118 patients with severe kidney disease and a normoalbuminuric control group (n = 118), suggesting that the parents of patients with nephropathy are at an increased risk of premature vascular death (6).

The apparent discrepancies among these studies can be explained by the varying degrees of kidney disease of the probands included in each study: whereas some studies focus on patients who are micro- and macroalbuminuric (5,18) or who have overt nephropathy with predominantly well-preserved kidney function (14, present study), other studies include larger cohorts of patients ($n > 50$) who have renal failure (6,17). In addition, the methods of analysis differ, as the survival curve analyses consider the age of parents at the time of events they are more sensitive (6,17, present study) as compared with cross-sectional analyses (14,18). Furthermore, although the parents of patients with nephropathy were reported to suffer from premature death as compared with parents of normoalbuminuric patients in the present study as in several other studies (5,6,17), the mean age at death was 64 years in this parental group in the present study and a previous study (5). The relatively young age (58 years) of the parents in the study by Nørgaard et al. (14) might therefore partly explain the lack of familial predisposition reported, as suggested by Earle et al. (5).

By application of similar criteria for the definition of cardiovascular disease, our data confirm and extend the findings of Earle et al. (5) that the premature mortality in parents of patients with nephropathy is predominantly cardiovascular. In accordance, Lindsay et al. (6) found parents of those with nephropathy more likely to suffer a vascular death in general and a death from stroke in particular. Neither the previous studies (5,14,17,18) nor the present study have reported an excess number of deaths related to stroke in parents of nephropathic patients.

One plausible genetic risk factor contributing to the familial predisposition to cardiovascular disease is the ACE/ID polymorphism, of which the DD genotype is associated with almost twice the plasma concentration of ACE than individuals without the deletion (15,19) and possibly with increased risk of diabetic nephropathy (7,8). If the frequency of the DD genotype is indeed increased in patients with myocardial infarction (9,10), those affected should transmit the D-allele to their offspring more often than unaffected parents do, and both DD and ID genotypes would therefore be expected to be more frequent in those with a parental history of myocardial infarction. Our finding of fewer myocardial infarctions among fathers of

patients homozygous for the I-allele is in accordance with this hypothesis. In agreement, a previous study reported an excess of DD genotype among nondiabetic Caucasian subjects with a parental history of fatal myocardial infarction (20). Furthermore, our data confirm previous studies in type 1 (7,11) and type 2 (12,13) diabetic patients, all of which report an association between the ACE/ID polymorphism and coronary heart disease.

In conclusion, our data support the hypothesis that cardiovascular morbidity and early mortality clusters in parents of type 1 diabetic patients with diabetic nephropathy. The ACE/ID polymorphism helps explain the increased morbidity from coronary heart disease. Further studies are required to confirm this association and to reveal other genetic factors that contribute to the genetic susceptibility of cardiovascular disease in type 1 diabetic patients with diabetic nephropathy.

References

1. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease: evidence of genetic susceptibility to diabetic nephropathy. *N Engl J Med* 320: 1161–1165, 1989
2. Borch-Johnsen K, Nørgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving H-H: Is diabetic nephropathy an inherited complication? *Kidney Int* 41:719–722, 1992
3. Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39: 940–945, 1996
4. Borch-Johnsen K, Kreiner S: Proteinuria: value as predictor of cardiovascular mortality in insulin dependent diabetes mellitus. *Br Med J* 294:1651–1654, 1987
5. Earle K, Walker J, Hill C, Viberti GC: Familial clustering of cardiovascular disease in patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 326: 673–677, 1992
6. Lindsay RS, Little J, Jaap AJ, Padfield PL, Walker JD, Hardy KJ: Diabetic nephropathy is associated with an increased familial risk of stroke. *Diabetes Care* 22:422–425, 1999
7. Marre M, Jeunemaitre X, Gallois Y, Rodier M, Chatellier G, Sert C, Dusselier L, Kahal Z, Chaillous L, Halimi S, Muller A, Sackmann H, Bauduceau B, Bled F, Passa P, Alhenc-Gelas F: Contribution of genetic polymorphism in the renin-angiotensin system to the development of renal complications in insulin-dependent diabetes. *J Clin Invest* 99:1585–1595, 1997
8. Fujisawa T, Ikegami H, Kawaguchi Y, Hamada Y, Ueda H, Shintani M, Fukuda M, Ogihara T: Meta-analysis of association of insertion/deletion polymorphism of angiotensin I-converting enzyme gene with diabetic nephropathy and retinopathy. *Diabetologia* 41:47–53, 1998
9. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Bard JM, Bara L, Ricard S, Tiret L, Amouyel P, Alhenc-Gelas F, Soubrier F: Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. *Nature* 359: 641–644, 1992
10. Samani NJ, Thompson JR, O'Toole L, Channer K, Woods KL: A meta-analysis of the association of the deletion allele of the angiotensin-converting enzyme gene with myocardial infarction. *Circulation* 94:708–712, 1996
11. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Boelskifte S, Borch-Johnsen K, Parving H-H: Insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene is associated with coronary heart disease in IDDM patients with diabetic nephropathy. *Diabetologia* 38:798–803, 1995
12. Ruiz J, Blanché H, Cohen N, Velho G, Cambien F, Cohen D, Passa P, Froguel P: Insertion/deletion polymorphism of the angiotensin-converting enzyme gene is strongly associated with coronary heart disease in non-insulin-dependent diabetes mellitus. *Proc Natl Acad Sci U S A* 91:3662–3665, 1994
13. Keavney BD, Dudley CRK, Stratton IM, Holman RR, Matthews DR, Ratcliffe PJ, Turner RC: UK Prospective Diabetes Study (UKPDS) 14: association of angiotensin-converting enzyme insertion/deletion polymorphism with myocardial infarction in NIDDM. *Diabetologia* 38:948–952, 1995
14. Nørgaard K, Mathiesen ER, Hommel E, Jensen JS, Parving H-H: Lack of familial predisposition to cardiovascular disease in type 1 (insulin-dependent) diabetic patients with nephropathy. *Diabetologia* 34: 370–372, 1991
15. Tarnow L, Cambien F, Rossing P, Nielsen FS, Hansen BV, Lecerf L, Poirier O, Danilov S, Parving H-H: Lack of relationship between an insertion/deletion polymorphism in the angiotensin-I-converting enzyme gene and diabetic nephropathy and proliferative retinopathy in IDDM patients. *Diabetes* 44: 489–494, 1995
16. Rose GA, Blackburn H: *Cardiovascular Survey Methods*. Geneva, World Health Org., 1968
17. Fagerudd JA, Petterson-Fernholm KJ, Grönhagen-Riska C, Groop P-H: The impact of a family history of type 2 (non-insulin-dependent) diabetes mellitus on the risk of diabetic nephropathy in patients with type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 42:519–526, 1999
18. de Cosmo S, Bacci S, Piras GP, Cignarelli M, Placentino G, Margaglione M, Colaizzo D, Di Minno G, Giorgino R, Liuzzi A, Viberti GC: High prevalence of risk factors for cardiovascular disease in parents of IDDM patients with albuminuria. *Diabetologia* 40: 1191–1196, 1997
19. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F: An insertion/deletion polymorphism in angiotensin I converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 86:1343–1346, 1990
20. Tiret L, Kee F, Poirier O, Nicaud V, Lecerf L, Evans A, Cambou JP, Arveiler D, Luc G, Amouyel P, Cambien F: Deletion polymorphism in the angiotensin-converting-enzyme is associated with a parenteral history of myocardial infarction. *Lancet* 341:991–992, 1993