Insulin-dependent diabetic sibling pairs are concordant for sodium-hydrogen antiport activity[1]

ROBERTO TREVISAN, PAOLA FIORETTO, JOSE' BARBOSA, and MICHAEL MAUER

Department of Clinical and Experimental Medicine and Department of Internal Medicine and National Research Center for the Study of Aging, University of Padua, Italy, and Department of Medicine and Department of Pediatrics, University of Minnesota Medical School, Minneapolis, Minnesota, USA

Insulin-dependent diabetic sibling pairs are concordant for sodium-hydrogen antiport activity.

Background. Recent findings of enhanced Na^+/H^+ antiport activity in cultured fibroblasts and immortalized lymphoblasts from type 1 diabetic patients with nephropathy support the view that a phenotypic or genotypic factor(s) underlies nephropathy risk. This study evaluated the kinetic properties of Na^+/H^+ antiporter in cultured fibroblasts from families with two siblings affected by type 1 (insulin-dependent) diabetes.

Methods. Seventeen diabetic sibling pairs were studied. The age was 38 ± 10 years (mean $\pm$ SD) in probands, the first to develop diabetes, and 39 ± 7 in siblings; the duration of diabetes was, by definition, longer in probands (24 ± 12 vs. 17 ± 8 years in siblings). Na^+/H^+ antiport activity was determined using a microfluorometric technique with the pH sensitive dye 2',7'-bis(2-carboxyethyl)-5(6)-carboxyfluorescein in skin fibroblasts cultured for at least six passages.

Results. There were no significant differences between probands and siblings for the following parameters: glycated hemoglobin, $8.3 \pm 0.8\%$ in probands and $8.6 \pm 1.4\%$ in siblings; creatinine clearance, 103 ± 24 ml/min/1.73 m^2 in probands and 103 ± 25 in siblings; albumin excretion rate, 6.8 (1 to 860) μg/min (median and range) in probands and 4.9 (2 to 1334) in siblings. Intracellular pH and buffering capacity were superimposable in the sibling pairs. The V_{max} for the antiport was 39.2 ± 14.7 mmol/liter cell/min in probands and 40.3 ± 17.6 in siblings. The internal pH for half-maximal activation (Km) and Hill coefficient was also similar in probands and siblings. There were correlations between probands and siblings in values for intracellular pH ($r = 0.51$, $P < 0.04$), V_{max} ($r = 0.84$, $P < 0.0001$), and buffering capacity ($r = 0.53$, $P < 0.03$). Glycated hemoglobin values over five years were not significantly correlated in the sibling pairs ($r = 0.3$, $P > 0.1$). V_{max} was related with the albumin excretion rate ($r = +0.49$, $P = 0.005$) and glycated hemoglobin ($r = +0.41$, $P = 0.017$) in the total cohort of sibling pairs. However, multiple regression analysis, using V_{max} as the dependent variable, found no correlations between any of the subjects' clinical and demographic variables.

Conclusions. Familial concordance for Na^+/H^+ antiport activity in long-term cultured skin fibroblasts from type 1 diabetic siblings suggests that at least some of the *in vitro* phenotypical characteristics of these cells are likely to be genetically determined and to be, at least in part, independent of *in vivo* metabolic control.

The epidemiological evidence that only a subset of diabetic patients is susceptible to renal damage [1, 2] and the demonstration of clear familiar clustering of diabetic nephropathy [3, 4] are consistent with the possibility that genetic factors may partially explain the risk of or protection from diabetic renal disease. Predisposition to hypertension and cardiovascular disease may be important determinants of susceptibility to renal complications of diabetes because raised blood pressure (BP) [5] and cardiovascular disease [6] are more prevalent in nondiabetic parents of type I diabetic patients with nephropathy.

These observations have raised interest in the search for intermediate phenotypes, which are significantly associated with diabetic nephropathy. Such intermediate phenotypes could be useful for early definition of risk and might help clarify the molecular mechanisms leading to diabetic nephropathy. Elevated Na^+/H^+ antiporter activity has been associated with diabetic renal disease in both type I and type II diabetic patients [7–13]. The Na^+/H^+ antiporter is a membrane transport system found in all eukaryotic cells. Exchanging intracellular protons for extracellular sodium, the antiporter plays a central role in three major cellular events: (*a*) intracellular pH (pH_i) regulation, (*b*) cell volume control, and (*c*) stimulus-response coupling and cell proliferation [14]. This transport system also plays an important role in renal sodium reabsorption. There are at least five different isoforms of Na^+/H^+ antiport. The first, referred to as NHE-1, is expressed in most cell types, is sensitive to amiloride, and is activated by growth factors [14].

Kinetic analyses of Na^+/H^+ antiporter activity in long-term cultured cells from type I diabetic patients with nephropathy demonstrated that maximal velocity was increased when compared with cells from normoalbuminuric patients and nondiabetic control subjects [7, 8, 12]. The presence of this enhanced activity in cells from diabetic nephropathy patients is consistent with genetic control of this phenotype. However, influences of advanced renal damage or glycemic control on cellular Na^+/H^+ antiporter activity could not be excluded by these studies.

Type I diabetic siblings offer a unique human model for the exploration of the relationship between renal status, metabolic control, and *in vitro* Na^+/H^+ antiporter activity. This study evaluated whether cultured skin fibroblasts from type I diabetic sibling pairs share common phenotypic characteristics of *in vitro* Na^+/H^+ antiporter activity.

METHODS

Patients

Patients were recruited from a registry of type I diabetic patients in the United States Midwest that was maintained by one of the authors (J. Barbosa). Adult type I diabetic patients with one or more type I diabetic siblings were eligible for study. All siblings with a diabetes duration of at least 10 years were asked to participate, with the exception of one family, in which siblings with a diabetes duration of six and five years, respectively, were recruited. The proband was the first patient to develop diabetes; the second was the sibling. All willing sibling pairs meeting these criteria were accepted without any exclusion for the presence or absence of long-term diabetic complications. However, neither sibling could have advanced renal disease (serum creatinine of more than 2.0 mg/dl). Twenty-one eligible sibling pairs provided informed consent for this study, which was approved by the Committee on the Use of Human Subjects in Research at the University of Minnesota. Seventeen of these 21 pairs had successful skin fibroblast cultures established and are the subject of this report. These patients are the subjects of a five-year prospective study.

A group of 12 normal subjects without a family history of hypertension and cardiovascular disease served as controls.

Study protocol

Patients were admitted to the Clinical Research Center (CRC) at the University of Minnesota for three days of testing. Three carefully timed (12 hr) urine samples were collected for the measurement of albumin excretion rate (AER) by fluorescent immunoassay (normal values, less than 15 μg/min). Values of 20 to 200 μg/min were defined as microalbuminuria, and values of more than 200 μg/min were defined as proteinuria. Creatinine clear-

ance (C_{Cr}) using these urine collections was used to estimate the glomerular filtration rate (normal range, 90 to 130 ml/min/1.73 m^2). Plasma and urine creatinine concentrations were measured using a modification of the Jaffé reaction. Arterial BP was measured on multiple occasions by trained CRC nursing staff with a standard mercury sphygmomanometer, and mean values are presented. Hypertension was defined as systolic BP of 140 mm Hg or more or diastolic BP of 90 mm Hg or more or known treatment for hypertension. Hemoglobin A_{1c} (HbA_{1c}) was measured by high-performance liquid chromatography (HPLC) in a single laboratory (normal values, 4.0 to 6.5%). The mean of prospective multiple values for each patient over a five-year longitudinal study period was used (median, six measurements per patient, range 2 to 16).

Cell culture

Skin biopsy was done under local anesthesia with a 3 mm punch at the time of kidney biopsy and at the already-anesthetized percutaneous kidney biopsy site. The biopsy material was cut into 1 mm^3 fragments and transferred to a 25 cm^2 cell culture flask. Fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 5 mM glucose supplemented with 10% fetal calf serum (FCS). After the fourth passage, cells were harvested and stored in liquid nitrogen. Frozen cells were transported on dry ice from the University of Minnesota to the University of Padova for characterization of Na^+/H^+ antiport activity. For each experiment, fibroblasts were then thawed and grown as described earlier here. All experiments were performed between the 6th and 10th passages.

Measurement of intracellular pH and Na^+/H^+ antiport activity

After thawing, cells were seeded onto cover slips using approximately 2 to 3 × 10^5 cells per cover slip. Measurements were made after a growth period of at least 36 hours in DMEM and after achievement of morphologic confluence. That the cells were in the plateau phase of growth, in which there is a steady-state condition with an almost completely ceased cell proliferation, was confirmed by evaluation of [^{3}H]-thymidine incorporation in fibroblasts grown in the same conditions. At confluence, [^{3}H]-thymidine incorporation in fibroblasts was 10 to 15 times lower than that observed in cells during the log phase of growth and similar to those observed in fibroblasts cultured in serum-free medium (data not shown).

All experiments were in duplicate and were all performed by an operator masked to the identity of the subjects studied. pH_i was measured using the pH-sensitive dye 2′,7′-bis(2-carboxyethyl)-5(6)-carboxyfluorescein (BCECF)/acetoxymethylester (AM) as previously described [13, 15]. Briefly, fibroblasts on cover slips loaded

with BCECF-AM (10 μmol/liter in DMEM without serum) at 37°C for 20 minutes were inserted into a specially constructed thermostatic cuvette in a Perkin-Elmer LS-50 Luminescence Spectrometer (Perkin-Elmer Corp., Analytical Instruments, Norwalk, CT, USA). The two excitation wavelengths were set at 490 and 435 nm (slit widths 5 nm) with emission at 530 nm (slit widths 5 nm). Autofluorescence was subtracted from fluorescence readings before the calculation of 490/435 ratios.

Intracellular pH in fibroblasts was determined in the HEPES-buffered saline (140 mM NaCl, 5 KCl, 2 mM CaCl$_2$, 1 mM MgSO$_4$, 5 mM glucose, 20 mM HEPES/Tris, 1 g/liter bovine serum albumin, pH 7.4). The 490/435 ratios were subsequently converted to pH values at the end of each experiment by construction of a calibration curve to prevent bias from potential differences in the position of the cover slip. This calibration of the fluorescent signal was achieved, as previously described, using high-concentration K$^+$ buffers of various pH values containing 7 μmol/liter of nigericin [16].

After the determination of intracellular pH in fibroblasts, cells were clamped at different intracellular pH (6.0, 6.2, 6.4, 6.6, 6.8, and 7.0; a single cover slip per each different acid loading) using a KCl buffer with nigericin (7 μmol/liter) [9]. When this acid-loading procedure was completed, nigericin was removed by incubation for five minutes in a Na$^+$-free buffer (135 mM choline chloride, 2 mM CaCl$_2$, 1 mM MgCl$_2$, 5 mM glucose, 20 mM HEPES/Tris, pH 7.4) containing 1 g/liter defatted albumin. The absence of any intracellular pH recovery over a period of five minutes demonstrated that the cells cannot regulate their pH in the absence of external Na$^+$.

The cells were then exposed again to the HEPES-buffered saline (Na$^+$ = 140 mmol/liter, pH 7.4) to activate the Na$^+$/H$^+$ exchange. Upon addition of this sodium-rich medium, intracellular pH increased linearly with time for at least 20 seconds. The same experiment of acid loading to different pH and studying external Na$^+$-dependent H$^+$ efflux was repeated in the presence of 1 mM amiloride, an inhibitor of Na$^+$/H$^+$ antiport activity in order to determine the exchange caused by the antiport. The rate of change in intracellular pH caused by Na$^+$/H$^+$ antiport was considered the difference between the slope of the initial rate of alkalization in HEPES-buffered saline and that measured in the presence of amiloride. All slopes had correlation coefficients exceeding 0.96 and were obtained from 40 readings at 0.5-second intervals taken over 20 seconds from the addition of the solutions.

Cells were reclamped at the same intracellular pH and exposed to 10 mM ammonium chloride as previously described to measure intracellular buffering capacity [17]. Buffering capacity at each intracellular pH was calculated from (*a*) the equilibrium between NH$_4^+$, NH$_3$, and pH in the extracellular medium, as determined by

the Henderson-Hasselbach equation using a pKa for NH$_3$ of 8.89, and (*b*) the formula $\Delta[NH_4]_i/\Delta pH_i$, as described [17]. Intracellular buffering capacity (mmol/liter/pH unit) was multiplied by change in intracellular pH in order to convert rates of intracellular alkalization (in pH units/min) to H$^+$ equivalent efflux rates (in mmol/liter/min).

All chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA), except for BCECF-AM, which was purchased from Calbiochem Co. (La Jolla, CA, USA).

Statistical analyses

Statistical calculations were performed with Statistical Package for the Social Sciences (SPSS-PC). AER values, not normally distributed, are presented as median and range and were logarithmically transformed prior to statistical analysis.

The Hill equation was used to model the Na$^+$/H$^+$ antiport kinetics [18], to obtain values for estimated maximum rate from pH$_i$ activation curve (V$_{max}$), the pH$_i$ for half-maximal activation (pH$_{0.5}$), and the apparent Hill coefficient for the internal H$^+$ binding sites of the antiport. A Hill coefficient of more than one indicates positive cooperativity for H$^+$ binding. The computer program used to derived these kinetic parameters was a recursive nonlinear least squares algorithm (P-fit; Biosoft Corporation, Cambridge, UK). Calculated SEM was in the range of 5 to 10% for V$_{max}$ and less than 4.0 for pH$_{0.5}$ and Hill coefficient.

Data are presented as mean ± SD unless otherwise stated. Comparisons between type I diabetic siblings were done by paired *t*-test. Correlations of values among sibling pairs were done by linear regression analysis. One-way analysis of variance was performed to examine the influence of sibship on renal functional parameters and kinetic parameters of Na$^+$/H$^+$ exchange. This was done by comparing the variance between siblings to the variance among all patients for each variable. Simple linear regression and multiple regression analyses were performed to determine if age, duration of diabetes, gender, AER, or HbA$_{1c}$ were related to the measured kinetic parameters of Na$^+$/H$^+$ antiport activity. A two-tailed *P* value of less than 0.05 was considered significant.

RESULTS

Clinical features and renal function

Probands and siblings were similar in age, gender, and body mass index (BMI; Table 1). The 12 controls (7 males) were similar in age (37 ± 6 years) and BMI (24.5 ± 2.1 kg/m^2) to those of diabetic sibling pairs (Table 1). Type I diabetes duration was, by definition, longer in the probands than in their respective siblings (Table 1). HbA$_{1c}$ over five years and daily insulin dose

Table 1. Demographic, clinical and biochemical features for the 17 type I diabetic sibling pairs

|	Probands	Siblings	Regression r values P values	ANOVA F values P values
N (male/female)	17 (9/8)	17 (8/9)		
Age *years*	38 ± 10	39 ± 7	0.75	6.06
			0.001	0.0003
Duration of diabetes *years*	24 ± 12	17 ± 8	0.88	4.19
			0.001	0.003
BMI *kg/m²*	25.1 ± 2.9	25.2 ± 2.8	0.29	2.24
			0.27	0.054
Hemoglobin A_{1c} %	8.3 ± 0.8	8.6 ± 1.4	0.026	1.09
			0.92	0.43
Insulin dose *U/day*	42 ± 19	43 ± 16	0.155	1.44
			0.55	0.23
Mean blood pressure *mm Hg*	90.1 ± 9.4	84.3 ± 7.9	0.154	1.12
			0.56	0.41
Albumin excretion rate *μg/min*	6.8 (1–860)	4.9 (1.8–1334)	0.654 0.006	4.350 0.003
Creatinine clearance *ml/min/1.73 m²*	103 ± 24	103 ± 25	0.321	2.07
			0.21	0.074

Data are given as mean ± SD, except the albumin excretion rate which is given as median and range.

were similar (P values of 0.580 and 0.869, respectively) in probands and siblings (Table 1). There was no effect of sibship on these parameters by regression analysis or analysis of variance. Five probands and two siblings had AER values exceeding 20 μg/min. Two probands and two siblings were proteinuric (AER more than 200 μg/min), whereas three probands had microalbuminuria (AER between 20 and 200 μg/min). HbA_{1c} values over five years were significantly higher in diabetic patients with abnormal (9.30 ± 1.03%) versus normal AER (8.11 ± 0.9%, P = 0.005). AER was similar in probands and siblings, and there was a correlation (r = +0.65) between values in probands and siblings. Analysis of variance confirmed a significant concordance in AER values (P = 0.003) between sibling pairs (Table 1). Mean blood pressure was slightly but not significantly higher in probands than in siblings. Three probands were hypertensive, and two were on antihypertensive treatment. There was no effect of sibship on blood pressure or C_{Cr}.

Sodium-hydrogen antiporter activity

Cell pH activation for the Na^+/H^+ exchange, that is, the initial rates of amiloride-sensitive hydrogen efflux in cultured fibroblasts in the recovery from intracellular acid loading, across a wide pH range was similar at all intracellular pH tested between probands and siblings and was similar to those of normal control subjects (Fig. 1).

Resting intracellular pH and intracellular buffering capacity in fibroblasts from probands was superimposable on that of cells from their respective siblings (Table 2). The kinetic parameters obtained from cell pH activation curves for the Na^+/H^+ exchange, V_{max}, $pH_{0.5}$, and Hill coefficient were similar in probands and siblings (Table 2). Figure 2 shows the individual values of V_{max} in probands and their respective siblings.
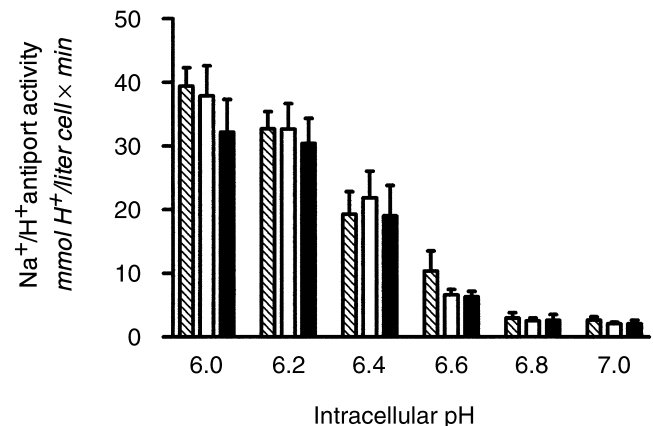
Fig. 1. Initial rate of amiloride-sensitive H^+ efflux in the recovery from an intracellular acid loading across a wide intracellular pH range in cultured fibroblasts from type I diabetic sibling pairs and normal control subjects. Cells were acid loaded as described in the **Methods** section. Symbols are: (▨) probands; (□) siblings; (■) normal subjects. Each bar represents the mean ± SD.

Resting intracellular pH (7.3 ± 0.04), intracellular buffering (26 ± 2.81 mmol/liter per pH unit) and the kinetic parameters of Na^+/H^+ exchange (V_{max}, 32.5 ± 12.5; $pH_{0.5}$, 6.4 ± 0.2; and Hill coefficient, 2.2 ± 0.4) were not significantly different in fibroblasts derived from control as compared with diabetic subjects.

V_{max}, intracellular pH, and buffering capacity were significantly correlated in the sibling pairs, as confirmed by analysis of variance (Table 2). $pH_{0.5}$ and Hill coefficient, on the contrary, were not concordant between probands and siblings (Table 2).

V_{max} was significantly related with only AER (r = +0.49, P = 0.005) and HbA_{1c} (r = +0.41, P = 0.017) in the total cohort of type 1 diabetic sibling pairs, but not

Table 2. Intercellular pH, buffering capacity and kinetic parameters of sodium-hydrogen exchange of cultured fibroblasts from type I diabetic sibling pairs

	Probands	Siblings	Regression r values P values	ANOVA F values P values
Intracellular pH	7.4 ± 0.03	7.3 ± 0.03	0.51 0.04	3.26 0.01
Buffering capacity at resting pH *mmol/liter per pH unit*	26.0 ± 2.10	24.6 ± 2.2	0.53 0.03	3.36 0.009
V_{max} *mmol/min*	39.2 ± 14.7	40.3 ± 17.6	0.84 0.0001	11.62 0.0001
$pH_{0.5}$	6.4 ± 0.14	6.4 ± 0.22	0.35 0.17	1.94 0.09
Hill coefficient	2.3 ± 0.3	2.3 ± 0.4	0.08 0.75	0.86 0.62

Data are given as mean $\pm$ SD.
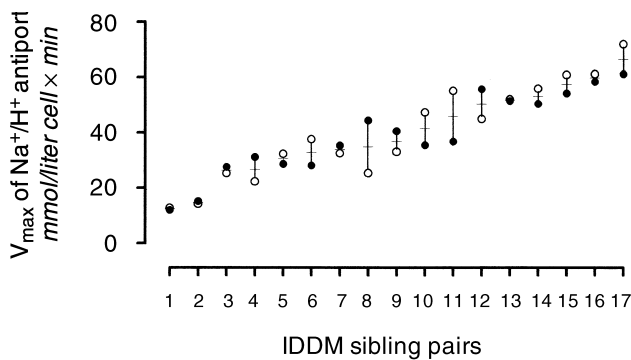
Fig. 2. Individuals values of V_{max} of the sodium-hydrogen antiport in type I diabetic sibling pairs. The 17 pairs were ranked by the mean value of the two siblings in the pair (indicated by an horizontal line) of the V_{max} of the sodium-hydrogen antiport. Symbols are: (●) probands; (○) siblings.

with other clinical variables. However, by multiple regression analysis, using V_{max} as the dependent variable, there were no significant correlations between subjects' age, duration of diabetes, body mass index (BMI), mean blood pressure, insulin dose, C_{Cr}, AER, and mean HbA_{1c}.

The seven type I diabetic patients with abnormal AER (5 probands and 2 siblings) had higher V_{max} (54.8 ± 3.8 mmol/min) than the 27 patients with normal AER (35.7 ± 15.5 mmol/min, $P < 0.005$). The normal AER patients' V_{max} values were similar to those of controls (32.5 ± 12.5 mmol/min, $P = 0.533$). V_{max} in the five probands with abnormal AER was similar to that of their respective siblings (55.17 ± 4.57 vs. 60.45 ± 7.53 mmol/min), although only two of these siblings had AER exceeding 20 µg/min. Therefore, V_{max} in these five sibling pairs, with at least one member of the pair with abnormal AER, was higher (57.81 ± 6.49 mmol/min) than that in the remaining 12 pairs (32.25 ± 12.16 mmol/min, $P < 0.0001$).

DISCUSSION

This study demonstrates for the first time, to our knowledge, that there is a strong familial concordance

in V_{max}, intracellular pH, and buffering capacity of the Na^+/H^+ exchanger in long-term cultured skin fibroblasts from type I diabetic sibling pairs. This observation complements previous reports of concordance of sodium-lithium countertransport activity in identical twins discordant for type 1 diabetes [19] and suggests that these *in vitro* phenotypic characteristics are likely to be, at least in part, genetically determined. Because diabetic nephropathy risk has a strong familial component [3, 4] and because the siblings reported here are also concordant for diabetic nephropathy lesions [20], it is reasonable to hypothesize that cellular processes are genetically regulated. Although the number of patients in this study was small, it is of note that the Na^+/H^+ exchanger activity of patients with increased AER was greater than that of the normoalbuminuric type I diabetic patients, this confirming the association of this cellular phenotype with the presence of renal disease in diabetes [7–12]. Interestingly, Na^+/H^+ exchanger activity also tended to be increased in the normoalbuminuric siblings of patients with increased AER. This argues that higher levels of Na^+/H^+ exchanger activity are probably independent of early renal functional abnormalities.

Hyperglycemia *per se* is unlikely to explain the concordance in Na^+/H^+ exchanger activity between diabetic sibling pairs. Although univariate analysis showed a significant relationship between V_{max} and HbA_{1c} in the group of diabetic patients as a whole, there was no significant concordance in HbA_{1c} between sibling pairs, who, on the other hand, were highly concordant for V_{max} of the antiport. Furthermore, there was virtually identical transport activity in patients with normal AER and matched controls, suggesting that hyperglycemia *per se* does not affect these antiport activities. Because Na^+/H^+ exchanger activity in the siblings was better predicted by the activity in the respective probands than by any other variable, these findings strengthen the hypothesis that Na^+/H^+ exchanger activity is mainly genetically regulated.

Although increased Na^+/H^+ exchanger activity may underlie a predisposition to essential hypertension [21],

it is unlikely that high blood pressure levels could account for our findings, in that no correlation was found between mean blood pressure in their sibling pairs. The cause for the concordance in Na^+/H^+ exchanger activity was not investigated in this study. Most of the sodium-dependent H^+ efflux in fibroblasts is probably attributable to NHE-1 [22], the isoform ubiquitously expressed in all mammalian cells. The concordance observed in type I diabetic sibling pairs could be due to the common presence of different alleles of the NHE-1 gene in the sibling pairs or to similar levels of NHE-1 gene expression [23]. Regarding the latter hypothesis, preliminary studies indicate increased mRNA levels for the NHE-1 gene in cultured skin fibroblasts of type I diabetic patients with rapid versus slow development of diabetic glomerulopathy lesions (abstract; Vats et al, *J Am Soc Nephrol* 52:648A, 1997).

The body of evidence presented supports the proposition that the ion transport abnormality present in some type I diabetic patients is not an epiphenomenon of diabetes or diabetic nephropathy, but may be related to the pathogenesis of this complication. The precise role of this cellular abnormality is far from being clearly understood. However, studies of other cellular features associated with increased Na^+/H^+ antiport activity in cells from type I diabetic patients with nephropathy may help to identify the potential implications of this transport system in the complex processes leading to end-stage renal failure in diabetes. This future work may be greatly facilitated by investigations that identify precise genetic associations with diabetic nephropathy risk.

Enhanced DNA synthesis and abnormalities in the cell cycle and in the cell life span have been reported in fibroblasts of type I diabetic patients with nephropathy [9, 11, 24, 25]. Similar results were also found in immortalized lymphoblasts from such patients, and this was further magnified by the incubation of these lymphoblasts in high glucose concentration [26]. Moreover, quiescent fibroblasts derived from type I diabetic patients with nephropathy exhibit an increased collagen synthesis compared with that of patients without nephropathy and normal controls [27]. Further mRNA for the α1 chain of type I collagen and the α3 integrin subunit is correlated with the rate of development of mesangial expansion in type I diabetic patients [25]. Thus, linkage may exist between increased Na^+/H^+ antiport activity and increased extracellular matrix, the central renal structural abnormality in diabetic nephropathy [28].

Whether the enhanced activity of Na^+/H^+ antiport reflects the appropriate response to an abnormal growth tendency or constitutes a primary permissive factor leading to cell function disturbances is not clear. However, recent data suggest that raised Na^+/H^+ antiport activity is not primary to disturbances in cell growth in that the inhibition of Na^+/H^+ antiporter by ethyl-isopropylamilor-

ide did not prevent the increased incorporation of ^{3}H-thymidine into DNA in cells from type I diabetic patients with nephropathy [11].

Although the connection between enhanced sodium-hydrogen antiport activity and diabetic nephropathy remains unknown, these data support the view that the increased susceptibility to diabetic nephropathy resides in the host cell response to diabetes-induced metabolic derangement and is genetically determined. Whether determination of Na^+/H^+ antiport activity will become a useful marker to identify patients at risk of developing diabetic renal disease will require longitudinal studies.

Reprint requests to Roberto Trevisan, M.D., Ph.D., Divisione di Malattie del Ricambio, Dipartimento di Medicina Clinica e Sperimentale via Giustiniani 2, 35128 Padova, Italy.
E-mail: saurob@ux1.unipd.it

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