Letters to the editor

Increased blood pressure and erythrocyte sodium/lithium countertransport activity are not inherited in diabetic nephropathy

Dear Sir,

We would like to comment on the data recently published by Jensen et al. [1].

In the Discussion, the authors state that 55% of the group of diabetic patients with nephropathy had at least one parent with hypertension whereas only 34% of the group with normoalbuminuria had a hypertensive parent. These proportions yield an odds ratio of 2.5 which is not significantly different from our odds ratio of 3.7 [2]. Also, note that the difference in these proportions (55% – 34% = 21%) is similar to that reported by us (77% – 50% = 27%) [2]. The lower proportion of parents with hypertension, overall, in the study by Jensen et al. reflects their choice of using a more restrictive definition of hypertension than we did.

Consistent with the difference in hypertension between the parents of patients with nephropathy and control patients without nephropathy, Figure 3 shows a striking difference between the parent groups with regard to the distribution of sodium/lithium countertransport (Na/Li CTT). Among parents of patients with nephropathy, the maximum velocity ($V_{\max}$) of Na/Li CTT had a bimodal distribution, while the distribution of Na/Li CTT among the parents of the normoalbuminuric group was unimodal. Different shapes for the distribution of $V_{\max}$ of Na/Li CTT in parents of patients with nephropathy and control patients without nephropathy would be expected only if there is an association between parental CTT and nephropathy in diabetic offspring. Jensen et al. based their conclusion that the distributions were not significantly different on a statistical test that ignored the evidence implicit in their different shapes. Significance tests for bimodality are available, but if any were applied to this data, the authors do not report it.

Table 1. Comparison of $V_{\max}$ of sodium/lithium countertransport (Na/Li CTT) in patients with nephropathy and without (no nephropathy) nephropathy according to level of glycaemic control during the first 10 years of Type 1 (insulin-dependent) diabetes. [2]

$V_{\max}$ of Na/Li CTT	Good and fair control		Poor control	
	No nephropathy	Nephropathy	No nephropathy	Nephropathy
	<i>n</i>	<i>n</i>	<i>n</i>	<i>n</i>
< 0.35 mmol · l erythrocytes ⁻¹ · h ⁻¹	12	4	11	3
> 0.35 mmol · l erythrocytes ⁻¹ · h ⁻¹	21	5	10	21
Odds ratioTTT	0.7		7.7	
95% C.I.	(0.2–3.2)		(1.8–33.8)	

Furthermore, the relatively small mean difference between patients with nephropathy and control patients without nephropathy with regard to the $V_{\max}$ of Na/Li CTT is a consequence of the decision by Jensen et al. to use a study design that has little power to reject the null hypothesis. The authors identified 54 Type 1 (insulin-dependent) patients aged under 31 years with clinical nephropathy from among *all those ever attending* two specialized diabetes clinics. Yet, they sampled 58 control patients (out of thousands) by *consecutive selection* among attendees in the same clinics. This latter strategy samples patients with a probability inversely proportional to the interval between visits. Thus, their control subjects were likely to be frequent clinic attenders who usually have better glycaemic control and a lower risk of nephropathy; a finding reported by the Steno group and by us [3, 4]. Yet, in our study [2], predisposition to essential hypertension appeared to be a risk factor for diabetic nephropathy *only* among patients with poor glycaemic control. As can be seen in Table 1, when our study group was divided into two subgroups according to the level of glycaemic control during the first 10 years of Type 1 diabetes, a significant difference in the $V_{\max}$ of Na/Li CTT between patients with nephropathy and control patients without nephropathy was seen only in the subgroup with poor control. Interestingly, if we compare all the patients with nephropathy in Table 1, regardless of glycaemic control, with only those control patients without nephropathy who had good or fair glycaemic control (i.e. a group possibly similar to the control patients without nephropathy selected by Jensen et al.), the difference in the means of the $V_{\max}$ of the Na/Li CTT is not statistically significant.

In other words, the small differences between the patients with nephropathy and control patients without nephropathy found by Jensen et al. resulted from under-representation in their control group of patients who did not develop diabetic nephropathy in the presence of poor glycaemic control and who probably attended the clinic infrequently.

In addition to selecting a study design that is not very powerful against the null hypothesis, we believe that the assay method used to determine the $V_{\max}$ of Na/Li CTT by Jensen and co-workers further diminished the power of their study to reject the null hypothesis. Their method has less precision than the method used by us and others [2]. The greater variability of the assay blurs the differences between patients with nephropathy and control patients without nephropathy and increases the sample size required to achieve a given power. We are, furthermore, puzzled by the authors' citation of non-diabetic control values from other laboratories rather than their own past work in which control values varied from 0.31 to 0.42 mmol · l erythrocytes⁻¹ · h⁻¹ [5, 6].

Given all the points mentioned above we think that the data reported by Jensen et al. actually support the hypothesis put forward by us and others that genetic predisposition to hypertension plays a significant role in the development of renal disease in Type 1 diabetes [2, 7].

Yours sincerely,
L. Laffel, J. H. Warram and A. S. Krolewski

References

1. Jensen JS, Mathiesen ER, Norgaard K, Hommel E, Borch-Johnsen K, Funder J, Brahm J, Parving HH, Deckert T (1990) Increased blood pressure and erythrocyte sodium/lithium countertransport activity are not inherited in diabetic nephropathy. *Diabetologia* 33: 619–624
2. Krolewski AS, Canessa M, Warram JH, Laffel LMB, Christlieb AR, Knowler WC, Rand LI (1988) Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med* 318: 140–145
3. Borch-Johnsen K, Kreiner S, Deckert T (1985) Diabetic nephropathy – Susceptibility to care? A cohort-study of 641 patients with type I (insulin-dependent) diabetes. *Diab Res* 3: 397–400
4. Krolewski AS, Warram JH, Christlieb AR, Busick EJ, Kahn CR (1985) The changing natural history of nephropathy in type I diabetes. *Am J Med* 78: 785–794
5. Ibsen KK, Jensen HA, Wieth JO, Funder J (1982) Essential hypertension: sodium-lithium countertransport in erythrocytes from patients and from children having one hypertensive parent. *Hypertension* 4: 703–709
6. Gall MA, Rossing P, Jensen JS, Funder J, Parving HH (1991) Red cell Na⁺/Li⁺ countertransport in non-insulin-dependent diabetics with diabetic nephropathy. *Kidney Int* 39: 135–140
7. Mangili R, Bending JJ, Scott G, Li LK, Gupta A, Viberti GC (1988) Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 318: 146–150

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Response from the Authors

Dear Sir,

Genetic predisposition to essential hypertension, represented by increased maximal erythrocyte sodium/lithium countertransport activity, has been suggested as a marker for the risk of developing diabetic nephropathy in Type 1 (insulin-dependent) diabetes mellitus [1]. Originally Viberti et al. reported a significantly higher blood pressure level in the parents of 17 albuminuric and 17 non-albuminuric Type 1 diabetic patients [2]. They found a significantly higher blood pressure level in the parents of the albuminuric than in the parents of the non-albuminuric patients, but only 42% of the possible parents were investigated. In our study we investigated 80 parents of patients with nephropathy and 78 parents of patients with urinary albumin excretion below 20mg/24 h (i.e. normoalbuminuria) [3]. Blood pressure in the two groups was 137/83 ± 21/10 vs 133/81 ± 20/10 mm Hg, and the prevalence of hypertension was 25 vs 19%, respectively. The prevalence of parents receiving antihypertensive medication did not differ. Our results have recently been confirmed by Viberti's group [4]. They found a mean blood pressure of 99 (77–121) vs 98 (89–118) mm Hg in parents of albuminuric and non-albuminuric Type 1 diabetic patients. Furthermore, there was no significant difference in the prevalence of antihypertensive treatment between the two groups of parents.

It is very difficult to compare our study [3] with the study of Krolewski et al. [1]. They obtained their information about blood pressure by mailed questionnaires to the parents, so they did not actually measure the blood pressure. Furthermore, we defined hypertension according to the criteria of The World Health Organisation. In

families where both parents were investigated we found that 55 vs 34% of the patients with nephropathy and normoalbuminuria, respectively, had at least one hypertensive parent. The proportions reported by Krolewski et al. (77 vs 50%) also included families with only one living parent, and information about blood pressure of the dead parent was given from the surviving spouse.

Bimodal distribution of sodium/lithium countertransport (Na/Li CTT) has been found in major populations [5–7]. In our study [3] both groups of parents showed indications of bimodality, but testing for this reached significance only in the parents of patients with nephropathy. Our use of non-parametric statistics which is independent of the type of distribution would have unveiled a significant difference in Na/Li CTT in the two groups of parents if present.

Our diabetic patients with normoalbuminuria were identified from the files of the two hospitals including all patients attending the clinics. Thus, the likelihood of being included in the study was independent of the frequency of attendance to the clinics. The reasons for restricting the study to patients developing diabetic nephropathy before the age of 31 years were that we wanted to avoid too many dead parents, and that we wanted to study a group of patients demonstrating the capacity to develop diabetic nephropathy rather early, as these two factors would increase the probability of finding a difference if present. Furthermore, with a diabetes duration of 17 ± 4 years and a urinary albumin excretion rate of 10 (6–14) mg/24 h our normoalbuminuric diabetic patients were at very low risk of developing nephropathy with time [8]. The pre-study calculations of the required sample sizes are carefully reported in the paper [3].

Krolewski et al. have massaged their results by arbitrarily dividing their patient groups in subgroups with respect to values of Na/Li CTT and quality of glycaemic control (Index of Frequency of Hyperglycaemia during the First Decade of Diabetes) without giving any arguments for their choices of dividing levels. But since the diabetic state in itself seems to correlate to elevated Na/Li CTT [3, 9–11], it is a little confusing why the control subjects of Krolewski et al. with good and fair glycaemic control have higher Na/Li CTT than the control subjects with poor glycaemic control [1].

We do not know on what basis Laffel et al. claim that their Na/Li CTT assay method [1] has more precision than ours [3, 9, 12]. They give no information about coefficients of variation of the method as given by us. It should be noted that we calculated Na/Li CTT by linear regression analysis based on measurement of lithium concentrations in the cell media at four different times (0, 30, 60 and 90 min) whereas Krolewski et al. used only 0 and 60 min. Furthermore, our laboratory was blinded with respect to the blood sample group.

Concerning the values of non-diabetic control subjects ours are in accordance with many other studies [9, 13–19]. (Krolewski et al. had no non-diabetic control group.)

Recent studies have demonstrated that the mechanism of raised Na/Li-CTT in hypertension and in Type 1 diabetes are different [11, 19]. The increased activity in essential hypertension results from increased sodium affinity at the outside of the Na/Li countertransporter. The increased activity in diabetes is caused by increased maximum velocity. Thus, familial predisposition to essential hypertension cannot explain the raised Na/Li CTT in Type 1 diabetes. Furthermore, when blood pressure and prevalence of hypertension in parents of patients with nephropathy and normoalbuminuria are the same, familial predisposition to essential hypertension plays no role in the development of diabetic nephropathy.

Yours sincerely,

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References

1. Krolewski AS et al. (1988) Predisposition to hypertension and susceptibility to renal disease in insulin-dependent diabetes mellitus. *N Engl J Med* 318: 140–145

2. Viberti GC et al. (1987) Raised arterial pressure in parents of proteinuric insulin-dependent diabetics. *Br Med J* 295: 515–517
3. Jensen JS et al. (1990) Increased blood pressure and erythrocyte sodium/lithium countertransport activity are not inherited in diabetic nephropathy. *Diabetologia* 33: 619–624
4. Walker JD et al. (1990) Sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy and their parents. *Br Med J* 301: 635–638
5. Boerwinkle E et al. (1986) Analysis of the distribution of erythrocyte sodium-lithium countertransport in a sample representative of the general population. *Genet Epidemiol* 3: 365–378
6. Dadone MM et al. (1984) Genetic analysis of sodium-lithium countertransport in 10 hypertension prone kindreds. *Am J Med Genet* 17: 565–577
7. Turner ST et al. (1987) Sodium-lithium countertransport in erythrocytes of ambulatory hypertensive and normotensive patients. *Hypertension* 9: 24–34
8. Mathiesen E et al. (1990) The relationship between blood pressure and urinary albumin excretion in the development of microalbuminuria. *Diabetes* 39: 245–249
9. Gall MA et al. (1991) Red cell Na^+/Li^+ countertransport in non-insulin-dependent diabetics with diabetic nephropathy. *Kidney Int* 135–140
10. Trevisan M et al. (1990) Sodium-lithium countertransport and glucose metabolism. *J Hyperten* 8 [Suppl 3]: S124
11. Rutherford PA et al. (1990) The mechanism of raised sodium-lithium countertransport in Type 1 diabetes mellitus is different from that in essential hypertension. *Diabetic Med* 7 [Suppl 2]: 1A
12. Ibsen KK et al. (1982) Sodium-lithium countertransport in erythrocytes from patients and from children having one hypertensive parent. *Hypertension* 4: 703–709
13. Mangili R et al. (1988) Increased sodium-lithium countertransport activity in red cells of patients with insulin-dependent diabetes and nephropathy. *N Engl J Med* 318: 146–150
14. Trevisan M et al. (1988) Hypertension, non-insulin-dependent diabetes and intracellular sodium metabolism. *Hypertension* 11: 264–268
15. Turner ST et al. (1985) Sodium-lithium countertransport and blood pressure in healthy blood donors. *Hypertension* 7: 955–962
16. McDonald A et al. (1987) Epidemiological studies of sodium transport and hypertension. *Hypertension* 10 [Suppl 1]: 42–47
17. Weder AB et al. (1986) Red-cell lithium-sodium countertransport and renal lithium clearance in hypertension. *N Engl J Med* 314: 198–201
18. Adragna NC et al. (1985) Effect on exercise on cation transport in human red cells. *Hypertension* 7: 132–139
19. Rutherford PA et al. (1990) Increased erythrocyte sodium-lithium countertransport activity in essential hypertension is due

to an increased affinity for extracellular sodium. *Clin Science* 79: 365–369

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Family cell lines available for diabetes research

Dear Sir,

We have read with great interest the letter by Lernmark et al. and commend the National Disease Research Interchange and Juvenile Diabetes Foundation International for their initiative in establishing a bank of human cell lines for the study of molecular genetics of Type 1 (insulin-dependent) diabetes mellitus.

Your readers may be interested to know that the British Diabetic Association has undertaken a parallel and complementary initiative independently to establish a similar repository (The BDA Alec & Beryl Warren Repository). We have collected cells from 100 multiplex families of whom at least two siblings have Type 1 diabetes. Our intention is to make this resource available to molecular geneticists worldwide. Before replication of these cell lines and/or extracting DNA, it would be helpful to have expressions of interest from scientists who would wish to have access to such a resource.

Further information including a preliminary catalogue of the first 45 families, HLA typed and ready for replication, is available from Dr. M. Murphy, British Diabetic Association, 10 Queen Anne Street, London W1M 0BD, UK: Telephone 071-323 1531, FAX No. 071-637 3644.

Yours sincerely,
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References

1. Lernmark Å, Ducat L, Eisenbarth G, Ott J, Dermott MA, Rubinstein P, Spielman R (1991) Human cell lines from families available for diabetes research. *Diabetologia* 34: 61 (Letter)

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