# Association of diabetic neuropathy with Na/K ATPase gene polymorphism

**P. Vague, D. Dufayet, T. Coste, C. Moriscot, M. F. Jannot, D. Raccah**

Department of Diabetes, University Hospital Timone, Marseille, France

**Summary** Diabetes mellitus induces a decrease in Na/K ATpase activity in man and animals, and this decrease plays a role in the development of diabetic neuropathy. Na/K ATPase is encoded by various genes, of which the ATP1 A1 gene is expressed predominantly in peripheral nerves and in erythrocytes. To investigate whether a polymorphism in the Na/K ATPase genes could explain the predisposition of some patients with insulin-dependent diabetes mellitus (IDDM) to develop polyneuropathy, a restriction fragment length polymorphism (RFLP) of the ATP1 A1 gene was studied together with erythrocyte Na/K ATPase activity in 81 Caucasian patients with more than 10 years' duration of IDDM. Associations with diabetic neuropathy, retinopathy and nephropathy were sought. Digestion of the first intron of the ATP1 A1 gene by the Bgl II restriction enzyme revealed a dimorphic allelism. Frequency of the restricted allele was 0.18 in this selected series (however, it was 0.10 in representative samples of IDDM patients and of normal subjects in our area). Mean erythrocyte Na/K ATPase activity was lower in diabetic patients than in 42 control subjects ($292 \pm 10$, vs $402 \pm 13$ nmol Pi $\cdot$ mg protein$^{-1} \cdot$ h$^{-1}$, $p < 0.0001$) and was not related to HbA$_{1c}$ value or to diabetes duration. It was lower in the group of the 28 patients bearing the restricted allele ($241 \pm 10$ vs $319 \pm 11$ nmol Pi $\cdot$ mg protein$^{-1} \cdot$ h$^{-1}$, $p < 0.0001$).

Neuropathy was absent in 50 patients, mild in 15 and severe in 16. When classified accordingly the three groups of patients did not differ with respect to sex, age and duration of diabetes. The respective frequency of the restricted allele among the groups was 10, 73 and 81 %, ($p < 0.0001$) and mean erythrocyte Na/K ATPase activity was respectively: $322 \pm 10.7$ nmol Pi $\cdot$ mg protein$^{-1} \cdot$ h$^{-1}$, $268 \pm 15$ and $229 \pm 17$, ($p < 0.001$). A borderline association between renal status or retinal status and repartition of polymorphism and a borderline correlation between renal status and Na/K ATPase activity were found, but significance disappeared after checking for the presence or absence of neuropathy. IDDM patients bearing the ATP1 A1 variant detected by Bgl II RFLP are much more frequently affected by neuropathy (relative risk 6.5, with 95 % CI 3.3–13). Identification of this risk factor may help to prevent this complication. It is suggested that the restricted allele is in linkage disequilibrium with a genomic mutation allowing diabetes to induce a greater impairment of Na/K ATPase activity which could in turn favour the development of neuropathy. [Diabetologia (1997) 40: 506–511]

**Keywords** Diabetic neuropathy, Na/K ATPase activity, Na/K ATPase gene polymorphism, risk factor, genetic susceptibility.

Neuropathy is a leading cause of disability in diabetes mellitus. Unlike diabetic retinopathy which is an almost constant long-term complication, diabetic neuropathy occurs in only 50 % of cases [1]. This phenomenon is comparable to that observed with diabetic nephropathy [2] which has been associated with genetic susceptibility [3, 4]. A number of facts suggest

that diabetic neuropathy may also involve genetic susceptibility. Although it is widely accepted that duration of diabetes and poor metabolic control [1, 5, 6] are contributory factors, there is no satisfying explanation for interindividual difference with regard to the development of neuropathy. The influence of sex, age, height, and smoking remains controversial [5, 7, 8]. The higher incidence and greater severity of neuropathy in insulin-dependent diabetic (IDDM) patients of North African origin than in European diabetic patients [9] suggests genetic involvement. Similarly, in a study including non-insulin-dependent diabetic (NIDDM) patients, a higher but not statistically significant different prevalence of neuropathy was observed in black and Hispanic Americans than in their white counterparts [7].

Decreased Na/K ATPase activity in nervous tissue has been implicated in the development of diabetic neuropathy [10]. Na/K ATPase (EC3.6.1.37) is a membrane ubiquitous enzyme that ensures transport of three sodium ions out of the cell and two potassium ions into the cell in association with hydrolysis of a molecule of ATP. This transport not only maintains sodium and potassium concentration gradients between the intracellular and extracellular milieus, but is also critical to a number of important processes including membrane potential generation and nerve conduction [11]. A decrease in Na/K ATPase activity has been reported in the erythrocytes of IDDM patients and diabetic rats [12–17]. Moreover, the impairment of this enzyme activity is proportional in sciatic nerve and erythrocytes of diabetic rats [17]. Na/K ATPase activity in erythrocytes varies according to ethnic origin. It is lower in blacks and North Africans, who are predisposed to neuropathy in the case of diabetes [9], than in Caucasians and Scandinavians [18, 19].

On the other hand, IDDM patients with neuropathy have lower erythrocyte Na/K ATPase activity than patients without neuropathy when matched for diabetes control and duration [14–16]. These findings raise the possibility that a constitutional decrease in Na/K ATPase activity could be a predisposing factor to diabetic neuropathy.

Na/K ATPase is composed of two subunits, a catalytic alpha subunit and a beta subunit whose function probably involves membrane binding. The alpha subunit occurs in three isoforms: alpha 1, alpha 2, and alpha 3, which are coded by different genes and are differently expressed depending on tissue type and developmental stage. In adult animals, alpha 1 is present in all tissues, being predominant in nervous tissue and exclusive in erythrocytes [20, 21]. Having noted a decreased Na/K ATPase activity in erythrocytes of IDDM patients, we chose the ATP1 A1 gene coding for the alpha 1 isoform as the candidate gene for predisposition to diabetic neuropathy. The ATP1 A1 gene is located on the short arm of chromosome 1 and is composed of 23 exons and 22 introns. A restriction polymorphism with Bgl II enzyme has been described in the first intron [22]. The purpose of the present study was to correlate erythrocyte Na/K ATPase activity and restriction polymorphism of the first intron of the ATP1 A1 gene to the presence of neuropathy, nephropathy, and retinopathy in a consecutive series of 81 IDDM patients with long-term diabetes.

## Materials and methods

*Study subjects.* Unrelated patients with IDDM under regular outpatient treatment in our department were consecutively selected if they were C-peptive negative, had diabetes for more than 10 years, and were not taking any medication known to influence Na/K ATPase (e. g. calcium blockers, thyroxine, glucocorticoid, mineral corticoid or digitalis-like drugs).

A total of 81 patients (39 female) ranging in age from 20 to 66 years (mean age: $39.5 \pm 1.5$ years) were enrolled, after they had given their informed consent for the study. As this kind of study requires ethnic homogeneity, all the patients were of Caucasian origin in order to avoid artefactual associations generated by population stratification. Mean duration of diabetes was $23.8 \pm 1$ years (range 11–43). Mean body mass index was $23.1 \pm 1.5$ kg/m$^2$. Seventeen patients were being successfully treated for hypertension. All patients had normal thyroid status as determined by normal thyrotropin levels.

On the basis of fundus ophthalmoscopy and angiofluorography the severity of retinopathy was classified into three categories: no retinopathy, moderate retinopathy defined by the presence of microaneurysms and other moderate to severe non-proliferative abnormalities, and severe retinopathy consisting of fibrous proliferation, new vessels, vitreous or peritinal haemorrhage, or scars of photocoagulation either in scatter or confluent patches, presumably directed at new vessels.

Diabetic nephropathy was considered to be absent if urinary albumin excretion (nephelometric assay) was lower that 30 mg per day on two occasions, incipient if it was between 30 and 300 mg per day and overt when macro-albuminuria was present.

Absence or presence of neuropathy was defined according to Diabetes Control and Complications Trial (DCCT) criteria [23] consisting of signs, symptoms including numbness, dysaesthesias and/or paraesthesias, hypersensitivity to touch, burning pain and/or aching, stabbing pain in hands and/or feet, and neuropathic foot ulcer, and decreased or absent deep tendon reflexes. Neuropathy was classified as severe if two or three of the criteria (signs, symptoms and reflexes) were affected, as mild neuropathy if one of the three criteria was met and absent if any one of these criteria was found.

*Measurement of Na/K ATPase activity.* Venous blood samples were collected on sodium citrate (0.11 mmol/l) from fasting subjects at around 08.00 hours before the morning insulin injection. Immediately after collection leucocytes and platelets were removed by filtering through cellulose micro-crystalline column as described by Beutler et al. [24]. Na/K ATPase activity was assayed in isolated erythrocyte membranes as the difference between inorganic phosphate released from vanadate-free ATP during separate assays with and without 1 mmol/l ouabain, a specific inhibitor of Na/K ATPase as previously reported [14, 16] with slight modifications in the storage at $-80\,°C$ of the erythrocyte membranes.

Control blood samples were obtained from 42 healthy subjects matched with regard to sex, age and ethnic origin.

*Analysis of restriction fragment length polymorphism (RFLP).*
DNA was obtained from leucocytes by standard technique. Intron 1 of the ATP1 A1 gene was amplified using a forward primer 5′ ACC/GCC/ACC/ATG/GGG/AAG/GGG 3′ and a reverse primer 5′ CTC/ATA/CTT/ATC/ACG/TCC/ACC 3′. The primers surrounding the first intron were determined from sequence data for the ATP1 A1 gene [25] and obtained from Eurogentec (Angers, France). The polymerase chain reaction (PCR) was carried in a volume of 100 μl containing 1 μg of genomic DNA, 1 μmol of each of the two primers, 500 μmol each of the four deoxynucleic acids, 3 units of a mixure of Taq and Pwo polymerases (Expand long template PCR system; Boehringer-Mannheim Meylan, France) allowing amplification of long fragments at 68 °C with very low error rate [26].

The PCR reaction (Perkin Elmer 480, Norwalk, Conn. USA) began with denaturation at 92 °C for 2 min followed by 30 cycles of denaturation at 92 °C for 10 s, annealing at 60 °C for 30 s, extension at 68 °C for 10 min. After the tenth cycle, the duration of the extension step was increased by 20 s at each cycle.

The last extension step was prolonged for 7 min at 68 °C. Amplified fragments were digested with the Bgl II enzyme at 37 °C for 1 h and analysed by electrophoresis at 100 mA on 0.7 % agarose gel for 3 h.

As this series of study subjects was not representative of the population of IDDM subjects, RFLP analysis was also performed on the DNA samples from 58 IDDM subjects diagnosed consecutively between November 1991 and October 1993. It was also performed on the DNA of the 42 control subjects in whom erythrocyte Na/K ATPase activity was determined and of 50 blood donors from our city.

Enzyme activity assays, RFLP analysis and clinical classification were performed blindly by three different investigators (T. C., D. D. and M. F. J. respectively).

*Statistical analysis.* Results are presented as mean ± SEM. Differences between groups were tested by analysis of variance, by Student's *t*-test for means and the Mann-Whitney U-test for non-normal variables. Correlation between variables and genotype was determined using the chi-squared test.

**Table 1.** Characteristics of patients with and without neuropathy

| | Neuropathy | | | |
| --- | --- | --- | --- | --- |
| | Absent | Mild | Severe | |
| Number | 50 | 15 | 16 | |
| Sex (female, male) | 23/27 | 8/7 | 8/8 | NS |
| Age (years) | $37.5 \pm 1.5$ | $42.8 \pm 3.5$ | $42.7 \pm 3.5$ | $p = 0.05$ |
| Diabetes duration (years) | $22.3 \pm 1.2$ | $26.2 \pm 2.7$ | $26.5 \pm 2.3$ | $p = 0.05$ |
| Presence of retinopathy (%) | 42 | 67 | 87.5 | $p < 0.05$ |
| Presence of nephropathy (%) | 14 | 40 | 62.5 | $p < 0.05$ |
| HbA$_{1C}$ (%) | $8.4 \pm 0.2$ | $9.7 \pm 0.4$ | $8.7 \pm 0.4$ | $p < 0.05$ |
| Na/K ATPase activity (nmol Pi · mg protein$^{-1}$ · h$^{-1}$) | $322 \pm 10$ | $268 \pm 15$ | $229 \pm 17$ | $p < 0.0001$ |

Results are given as mean ± SEM

**Table 2.** Characteristics of patients according to retinal status

| | Retinopathy | | | |
| --- | --- | --- | --- | --- |
| | Absent | Mild | Severe | |
| Number | 34 | 16 | 31 | |
| Age (years) | $38.3 \pm 2$ | $34.3 \pm 2$ | $43.7 \pm 2$ | $p < 0.05$ |
| Diabetes duration (years) | $22.1 \pm 1.8$ | $21.7 \pm 1.4$ | $27 \pm 1.5$ | $p = 0.05$ |
| HbA$_{1C}$ (%) | $8.9 \pm 0.3$ | $8.6 \pm 0.3$ | $8.7 \pm 0.3$ | NS |
| Na/K ATPase activity (nmol Pi · mg protein$^{-1}$ · h$^{-1}$) | $300 \pm 12.5$ | $315 \pm 25$ | $271 \pm 14$ | NS |

Results are given as mean ± SEM

## Results

### Na/K ATPase activity

Mean Na/K ATPase activity was lower in the 81 diabetic patients than in the 42 control subjects ($292 \pm 9$ vs $402 \pm 13$ nmol · Pi · mg protein$^{-1}$ · h$^{-1}$, $p < 0.0001$). This finding is consistent with our previous results [14, 16].

Enzyme activity in the patient group was not related to sex ($p = 0.48$), duration of diabetes ($r = 0.1$, $p = 0.34$), or HbA$_{1c}$ ($r = 0.05$, $p = 0.63$). A negative relationship with age was of borderline significance ($r = -0.23$, $p = 0.04$).

As shown in Table 1, 31 of 81 patients presented with neuropathy, which was severe in 16. Patients with diabetic neuropathy did not differ from those without neuropathy with respect to sex, but they were slightly older and had had diabetes for a slightly longer period of time. Diabetes was slightly less well controlled in patients with neuropathy based on HbA$_{1c}$ values. Nephropathy and retinopathy were more frequent in patients with neuropathy. Na/K ATPase activity was significantly lower in patients with neuropathy. Patients with severe neuropathy did not differ from those with a mild form except for an even lower Na/K ATPase activity.

Twenty-three patients presented with nephropathy, which was overt in 12 cases. No differences in the various parameters studied were observed between patients with and without nephropathy. However, Na/K ATPase activity was $304 \pm 11$ nmol Pi · mg protein$^{-1}$ · h$^{-1}$ in patients without nephropathy as compared to $263 \pm 15$ in patients with nephropathy. This borderline significance ($p = 0.05$) disappeared after separate analysis of two subgroups with or without neuropathy.

Forty-seven patients presented with retinopathy, which was severe in 31 cases (Table 2). They did not differ from those without retinopathy, except for age and duration of diabetes. No difference in Na/K ATPase was observed between groups.

## Restriction fragment length polymorphism

*Frequency of the polymorphism.* Following the action of the Bgl II enzyme on the PCR product, agarose gel electrophoresis revealed three different fragments of 3, 8 and 11 kilobases (kb). The 11 kb fragment corresponds to the undigested allele. The 3 and 8 kb fragments were always found together and corresponded to the products of the digested allele.

Of the 81 diabetic patients in this study, 53 were homozygous for the unrestricted allele (one 11 kb fragment only), 28 were heterozygous (11, 8 and 3 kb fragments) and none of the patients had two restricted alleles. However, one North-African patient presented homozygous restricted alleles (8 and 3 kb fragments). This patient was a 32-year-old female who had been suffering from diabetes for 14 years and presented severe neuropathy. She was not included in the study because of her different ethnic origin.

The frequencies of the genotypes were not significantly different from that predicted by the Hardy Weinberg equilibrium ($\chi^2 = 2.92$, NS). The frequency of the unrestricted allele was 0.82 and that of the restricted allele was 0.18.

In the control series and in the series of consecutively diagnosed IDDM subjects the frequency of the restricted allele was lower, being 0.10 and 0.11, respectively.

*Correlation between restriction site polymorphism and Na/K ATPase activity.* Na/K ATPase activity in homozygous diabetic patients without the restriction site was higher than in heterozygous diabetic patients with restriction site ($319 \pm 11$ vs $241 \pm 10.5$ nmol Pi $\cdot$ mg protein$^{-1} \cdot$ h$^{-1}$, $p < 0.0001$). In contrast, among healthy subjects no relationship was observed between the gene polymorphism and Na/K ATPase activity with an average 400 nmol Pi $\cdot$ mg protein$^{-1} \cdot$ h$^{-1}$ in subjects homozygous for the unrestricted allele and 393 among heterozygous subjects.

*Correlations between restriction site polymorphism, diabetes duration, HbA$_{IC}$ and diabetic complications.* No correlation was found between restriction site polymorphism and sex ($p = 0.8$) duration of diabetes ($p = 0.33$), or HbA$_{1C}$ ($p = 0.43$). Replication in a second set of IDDM patients with variable diabetes duration confirmed these results.

Table 3 shows the association between the RFLP and diabetic complications. There was a close association between the presence of the restriction site and neuropathy. Neuropathy was present in almost all the patients bearing the restriction site. The relative risk conferred by its presence was 6.5 (95% confidence interval: 3.3–13). A borderline correlation was found between restriction site polymorphism and nephropathy and retinopathy, respectively $\chi^2 = 6.8$, $p = 0.03$ and $\chi^2 = 6.9$, $p = 0.03$. However, in this series

**Table 3.** Frequency of subjects with restricted allele according to complications status

|  | Absent | Mild | Severe |
|---|---|---|---|
| **Neuropathy** | | | |
| No restricted allele | 46 | 4 | 3 |
| Presence of restricted allele | 4 (8%) | 11 (73%) | 13 (81%) |
| $\chi^2 = 35.6$ $p < 0.0001$ | | | |
| **Nephropathy** | | | |
| No restricted allele | 43 | 5 | 5 |
| Presence of restricted allele | 15 (25%) | 6 (55%) | 7 (58%) |
| $\chi^2 = 6.8$ $p = 0.03$ | | | |
| **Retinopathy** | | | |
| No restricted allele | 27 | 11 | 15 |
| Presence of restricted allele | 7 (20%) | 5 (31%) | 16 (51%) |
| $\chi^2 = 6.9$ $p = 0.03$ | | | |

of patients, microvascular complications were frequently found together. When separate analysis of the subgroups with or without neuropathy was performed, no associations between the presence of the restriction site polymorphism and nephropathy or retinopathy were observed.

## Discussion

Using the candidate gene approach to ascertain a genetic susceptibility for diabetic neuropathy in a series of long-term IDDM patients, we observed an association between peripheral neuropathy, a decrease in erythrocyte Na/K ATPase activity and a restriction polymorphism in a Na/K ATPase gene, ATP1 A1 with a high level of statistical significance. Our results showed a weak association with diabetic nephropathy or diabetic retinopathy, which disappeared after controlling for the neurologic status of the patients.

A number of findings have implicated a decrease in Na/K ATPase activity in nervous tissue in the development of diabetic neuropathy [10]. A decrease in this enzymatic activity enhances intra-axonal sodium concentration and blocks nerve membrane depolarization. In the sciatic nerve of diabetic rats decreased Na/K ATPase activity is associated with a slowing of nerve conduction [27–29].

In patients with IDDM, we and others [12–16] have reported depressed Na/K ATPase activity in erythrocyte membranes; this decrease probably reflects a decrease in nerve tissue. In this regard we showed a parallel decrease of Na/K ATPase activity in both erythrocytes and sciatic nerve of diabetic rats [17].

The mechanism by which diabetes impairs Na/K ATPase activity is probably complex and not linked solely to hyperglycaemia. Indeed, we found no correlation between Na/K ATPase activity and either $HbA_{1c}$ or blood glucose in this series of patients in stable glycaemic control, and in a previously published study [16]. Similarly, in patients with NIDDM Na/K ATPase is not related to glycated haemoglobin [30]. Acceleration of the polyol pathway probably plays a role since treatment with aldose reductase inhibitor normalizes Na/K ATPase activity in the nervous tissue of diabetic rats [28, 29]. Relative insulinopenia in hyperglycaemic patients with IDDM could be another factor. In this regard it has been shown that intensive insulin therapy by means of an artificial pancreas for 24 h restores erythrocyte Na/K ATPase activity [13]. Partial restoration of Na/K ATPase activity despite persistent hyperglycaemia has also been noted in muscle cell membranes of diabetic animals undergoing insulin treatment [31–33]. Insulin acts directly on the sodium pump by increasing its sodium affinity, but the number of pumps present on the membrane is unaltered [34]. Moreover, mean erythrocyte Na/K ATPase activity was related to the ATP1 A1 gene polymorphism only in diabetic patients whereas the Na/K ATPase activity was unchanged in control subjects with or without gene polymorphism. This fact suggests that a genomic mutation linked to the polymorphism may affect a binding site of an intracellular insulin messenger. However, the failure to show that mean erythrocyte Na/K ATPase activity is related to the Na/K ATPase gene polymorphism in normal control subjects does not support a primary genetic control of Na/K ATPase activity.

As in two previous studies [14, 16] we again observed that mean erythrocyte Na/K ATPase activity was lower in diabetic patients with than in those without neuropathy. One may suggest that, in some subjects diabetes-induced Na/K ATPase impairment could be more severe, favouring the development of neuropathy.

Constitutional variations in erythrocyte Na/K ATPase activity have been associated with sex and ethnic origin, with lower activity having been reported in men than women, in blacks than whites, in Asians than Scandinavians [18, 19], and in people of Semitic origin than Caucasians [14, 15, 20]. Semitic peoples are predisposed to severe and early diabetic neuropathy [9]. A genetic susceptibility to diabetic neuropathy through the mechanisms controlling Na/K ATPase activity is therefore plausible.

Since we detected a decrease in Na/K ATPase activity in erythrocytes in which the alpha 1 is the only isoform, and since the alpha 1 isoform is predominant in nervous tissue, the gene of the alpha 1 isoform called ATP1 A1 and coded on the short arm of chromosome 1 would be a likely candidate. The primary structure of this gene has been deduced by cDNA sequencing [25], its promoting region has been identified and a restriction polymorphism detected in intron 1 using Bgl II [22]. After PCR amplification of intron 1, we obtained an 11 kb fragment identical to the one previously described and in some samples digestion by Bgl II generated two fragments of 3 and 8 kb identical to those described [22]. This finding, the fit with Hardy Weinberg equilibrium and the high performance of the DNA polymerase mixture [26] allow us to rule out copy errors in the generation of these long PCR fragments.

In this series of 81 patients with IDDM, we observed a frequency of 0.82 for the unrestricted allele and 0.18 for the restricted allele. This distribution is different from that previously reported, i.e. 0.95 and 0.05 respectively in normal subjects from North America [22, 33]. However, the prevalence of the restricted allele was 0.11 in representative samples of healthy subjects and of IDDM patients originating from the Marseilles area. Ethnic differences could be involved. The higher frequency observed in the study subjects is probably related to the fact that patients affected by chronic complications tend to attend the clinic more often than those without. And in fact, the prevalence of neuropathy was 38% in our series after an average duration of diabetes of 20 years, while it was 27% in a large cross-sectional multicentre study for the same diabetes duration [1].

Using the candidate gene approach, we observed in this series of patients with IDDM that an allelic variant of the Na/K ATPase gene was strongly associated with neuropathy and with a decrease in erythrocyte Na/K ATPase activity. Association between intron polymorphism and decreased activity of the product is probably due to a linkage disequilibrium with a DNA mutation affecting ATP1 A1 gene expression. This mutation could affect sites of recognition of factors regulating gene expression or the coding sequences altering the function of the enzyme. This would cause decreased enzymatic activity as a result of either a reduction in the number of enzyme molecules or structural changes of the molecules.

In conclusion we suggest considering the presence of the ATP1 A1 gene variant as a predisposing factor for diabetic neuropathy. This could help to elaborate specific preventive treatment trials in affected patients.

# References

1. Young MJ, Boulton AJM, Macleod AE, Williams DRR, Sonksen PH (1993) A multicentre study of the prevalence of diabetic peripheral neuropathy in the United Kingdom hospital clinic population. Diabetologia 36: 150–154

2. Krolewski AS, Warram JH, Rand LI, Kahn CR (1987) Epidemiologic approach to the etiology of type I diabetes mellitus and its complications. N Engl J Med 26: 1390–1398

3. Marre M, Bernadet P, Gallois F et al. (1994) Relationships between angiotensin I converting enzyme gene polymorphism, plasma levels, and diabetic retinal and renal complications. Diabetes 43: 384–388

4. Doria A, Warram JA, Krolewski AJ (1994) Genetic predisposition to diabetic nephropathy. Evidence for a role of the angiotensin-I-converting enzyme gene. Diabetes 43: 690–695

5. Maser RE, Steenkiste AR, Dorman JS et al. (1989) Epidemiological correlates of diabetic neuropathy. Report from Pittsburgh epidemiology of diabetes complications study. Diabetes 38: 1456–1459

6. Pirart J (1977) Diabète et complications dégénératives. Présentation d'une étude prospective portant sur 4400 cas observés entre 1947 et 1973. Diabète Métab 3: 97–107, 173–182, 242–256

7. Harris M, Cowie C, Eastman R (1993) Symptoms of sensory neuropathy in adults with NIDDM in the US population. Diabetes Care 16: 1446–1452

8. Ziegler D, Gries FA, Spüler M, Lessmann F, and the DiaCAN Multicenter Study Group (1993) The epidemiology of diabetic neuropathy. Diabet Med 10: 825–855

9. Vague P, Brunetti O, Valet AM, Attali I, Lassmann-Vague V, Vialettes B (1988) Increased prevalence of neurologic complications among insulin dependent diabetic patients of Algerian origin. Diabète Métab 14: 706–711

10. Greene DA, Lattmer SA, Sima AAF (1987) Sorbitol, phophoinositides, and sodium-potassium-ATPase in the pathogenesis of diabetic complications. N Engl J Med 316: 599–605

11. Lingrel JB, Kuntzzweiler T (1966) Na/K ATPase. J Biol Chem 269: 19 659–21 994

12. Finotti P, Palatini P (1987) Reduction of erythrocyte Na + K + ATPase activity in type 1 (insulin-dependent) diabetic subjects and its activation by homologous plasma. Diabetologia 29: 623–628

13. Rahmani-Jourdheuil C, Mourayre Y, Vague P, Boyer J, Juhan-Vague I (1987) In vivo insulin effect on ATPase activities in erythrocyte membrane from insulin-dependent diabetics. Diabetes 36: 991–995

14. Raccah D, Gallice P, Pouget J, Vague P (1992) Hypothesis low Na/K ATPase activity of the red cell membrane, a potential marker of the predisposition to diabetic neuropathy. Diabète Métab 18: 236–241

15. Issautier T, Kowacic H, Gallice P, Raccah D, Vague P, Crevat A (1994) Modulation defect of sodium pump evidenced in diabetic patients by a microcalorimetric study. Clinica Chimica Acta 228: 161–170

16. Raccah D, Fabreguettes C, Azulay JP, Vague P (1996) Erythrocyte Na/K ATPase activity, metabolic control and neuropathy in insulin dependent diabetic patients. Diabetes Care 19: 564–568

17. Raccah D, Jannot MF, Issautier D, Vague P (1994) Effect of experimental diabetes on Na/K ATPase activity in red blood cells, peripheral nerve and kidney. Diabète Métab 20: 271–274

18. Beutler E, Kuhl W, Sacks P (1983) Sodium-potassium ATPase activity is influenced by ethnic origin and not by obesity. N Engl J Med 309: 756–760

19. Lasker N, Hopp L, Grossmann S, Bamforth R, Aviv A (1985) Race and sex differences in erythrocytes Na + /K + and (Na + , K + )-adenosine triphosphate. J Clin Invest 75: 1813–1820

20. Lingrel JB, Orlowski J, Shull MM, Price EM (1990) Molecular genetics of Na/K ATPase. Prog Nucl Ac Res Mol Biol 38: 37–89

21. Sweadner KJ (1989) Isozymes of the Na/K ATPase. Biochim Biophys Acta 988: 185–220

22. Shull MM, Pugh DG, Lingrel JB (1990) The human Na/K ATPase α1 gene: characterization of the 5′-flanking region and identification of a restriction fragment length polymorphism. Genomics 6: 451–460

23. DCCT Research Group (1988) Factors in the development of diabetic neuropathy. Baseline analysis of neuropathy in feasibility phase of diabetic control and complications trials. Diabetes 37: 471–481

24. Beutler E, West C, Blume KG (1976) The removal of leucocytes and platelets from whole blood. J Lab Clin Med 88: 328–333

25. Kawakami K, Ohta T, Nojima H, Nagano K (1986) Primary structure of α subunit of human Na, K-ATPase deduced from cDNA sequence. J Biochem 100: 389–397

26. Barnes WM (1994) PCR amplification of up to 35-kb DNA with high fidelity and high yield from bacteriophages templates. Proc Natl Acad Sci 91: 2216–2230

27. Greene DA, Yagihashi S, Lattimer SA, Sima AAF (1984) Nerve Na + K + ATPase, conduction and myoinositol in the insulin-deficient BB rat. Am J Physiol 247: E534–E639

28. Carrington L, Ettlinger CB, Calcutt NA, Tomlinson DR (1991) Aldolase reductase inhibition with Imirestat: effects on impulse conduction and insulin stimulation of Na + / K + -adenosine triphosphatase activity in sciatic nerves of streptozotocin-diabetic rat. Diabetologia 34: 397–401

29. Hirati Y, Okada K (1990) Relation of Na + /K + ATPase to delayed motor nerve conduction velocity effect of aldose reductase inhibitor, ADN-138 on Na + /K + -ATPase activity. Metabolism 39: 563–756

30. Mimura M, Makino H, Kanatsuka A, Asai T, Yoshida S (1984) Na/K ATPase activity in type 2 (non-insulin-dependent) diabetic patients with microalbuminuria. Horm Metab Res 26: 33–38

31. Nishida K, Ohara T, Johnson JW (1992) Na + K + ATPase activity and its αII subunit gene expression in rat skeletal muscle: influence of diabetes, fasting and refeeding. Metabolism 41: 56–63

32. Yuk-Chow NG, Tolerico PH, Book CBS (1993) Alterations in levels of Na + K + ATPase isoforms in heart, skeletal muscle, and kidney of diabetic rats. Am J Physiol 265: E243–E251

33. Dériaz O, Dionne F, Pérusse L, Tremblay A et al. (1994) DNA variation in the genes of the Na, K-adenosinetriphosphase and its relation with resting metabolic rate, respiratory quotient, and body fat. J Clin Invest 93: 838–843

34. Lytton J, Lin J, Di Antonio L, Brodsky J, McGeoch J, McGill D, Guidotti G (1994) Regulation of the Na + K + -pump by insulin. In: Bamberg E, Schoner W (eds) The sodium pump structure, mechanism, hormonal control and its role in disease. Steinkopff, Darmstadt, Springer, New York, pp 670–681