Genetic and Environmental Regulation of Na/K Adenosine Triphosphatase Activity in Diabetic Patients

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Even if the pathogenesis of diabetic neuropathy is incompletely understood, an impaired Na/K adenosine triphosphatase (ATPase) activity has been involved in this pathogenesis. We previously showed that a restriction fragment length polymorphism (RFLP) of the ATP1-A1 gene encoding for the Na/K ATPase's alpha 1 isoform is associated with a low Na/K ATPase activity in the red blood cells (RBCs) of type 1 diabetic patients. We thus suggested that the presence of the variant of the ATP1A1 gene is a predisposing factor for diabetic neuropathy, with a 6.5% relative risk. Furthermore, there is experimental evidence showing that lack of C-peptide impairs Na/K ATPase activity, and that this activity is positively correlated with C-peptide level. The aim of this study was to evaluate the respective influence of genetic (ATP1-A1 polymorphism) and environmental (lack of C-peptide) factors on RBC's Na/K ATPase activity. Healthy and diabetic European and North African subjects were studied. North Africans were studied because there is a high prevalence and severity of neuropathy in this diabetic population, and ethnic differences in RBC's Na/K ATPase activity are described. In Europeans, Na/K ATPase activity was significantly lower in type 1 (285 ± 8 nmol Pi/mg protein/h) than in type 2 diabetic patients (335 ± 13 nmol Pi/mg protein/h) or healthy subjects (395 ± 9 nmol Pi/mg protein/h). Among type 2 diabetic patients, there was a significant correlation between RBC's Na/K ATPase activity and fasting plasma C-peptide level ($r = 0.32$, $P < .05$). In North Africans, we confirm the ethnic RBC's Na/K ATPase activity decrease in healthy subjects (296 ± 26 *v* 395 ± 9 nmol Pi/mg protein/h, $r < 0.05$), as well as in type 1 diabetic patients (246 ± 20 *v* 285 ± 8 nmol Pi/mg protein/h; $P < .05$). However, there is no relationship between the ATP1A1 gene polymorphism and Na/K ATPase activity. ATP1A1 gene polymorphism could not explain the ethnic difference. We previously showed that Na/K ATPase activity is higher in type 1 diabetic patients without the restriction site on ATP1A1 than in those heterozygous for the restriction site. This fact was not observed in healthy subjects. In type 2 diabetic patients, association between ATP1A1 gene polymorphism and decreased enzyme activity was found only in patients with a low C-peptide level. Therefore, the ATP1-A1 gene polymorphism influences Na/K ATPase activity only in case of complete or partial C-peptide deficiency, as observed in type 1 and some type 2 diabetic patients, without any correlation with hemoglobin A_{1c} (HbA_{1c}). Correlation observed between C-peptide levels and RBC's Na/K ATPase suggests that the deleterious effect of C peptide deficiency on Na/K ATPase activity is worse in the presence of the restriction site. This may explain the high relative risk of developing the neuropathy observed in type 1 diabetic patients bearing the variant allele.

Na/K ADENOSINE triphosphatase (ATPase) in its purified form, is composed of 2 subunits, an α subunit, which has 4 isoforms[1,2,3] and a β subunit with 3 isoforms.[4,5] The expression of these isoforms is tissue-specific.

It has been shown that the enzyme is implied in diabetic neuropathy. It seemed interesting to us to study the α 1 isoform, as it is predominantly expressed in peripheral nerves[6] and exclusively in red blood cells (RBCs).[7] The ATP1A1 gene on chromosome1p21[8] encodes this isoform. RBC's Na/K ATPase activity is decreased in human type 1 diabetes[9-11] and in rats with streptozotocin-induced diabetes.[12] This activity is also decreased in several tissues prone to diabetic complications, such as sciatic nerve,[13] retina,[14] and heart.[15] Moreover, we also observed a significant correlation in the decrease in activity in RBCs and sciatic nerve.[12]

The alteration in Na/K ATPase activity is implicated in the pathogenesis of diabetic neuropathy.[16,17] Indeed, the decrease in RBC's Na/K ATPase activity is more pronounced in the presence of diabetic neuropathy in type 1 diabetic patients.[10]

A genetic predisposition for diabetic neuropathy was suspected in North African people suffering from earlier and more severe neuropathy.[18] In this ethnic group, as well as in Afro Americans[19] and Sepharadic Jews,[20] Na/K ATPase activity is lower than in Caucasians.[10] This fact could explain the susceptibility of North African diabetic patients to develop neuropathy. The gene encoding the Na/K ATPase's alpha 1 isoform could be a candidate gene for the predisposition to diabetic neuropathy. The relative risk to develop diabetic neuropathy is 6.5% in type 1 diabetic patients bearing the variant allele[21] on the first intron of ATP1A1.[1]

In type 2 diabetes, the pathophysiology of polyneuropathy is more complex that in type 1 diabetes,[22] and a genetic predisposition is more difficult to demonstrate. We have observed that RBC's Na/K ATPase activity in type 2 diabetic patients was not related to blood glucose control, but to the level of blood C-peptide.[23] Indeed, in vitro, C-peptide has a direct stimulatory effect on Na/K ATPase activity in some cell models.[24] In vivo, preliminary data showed that infusion of C-peptide in type 1 diabetic patients increases RBC's Na/K ATPase activity.[25] Furthermore, low pancreatic β cells secretion capacity seems to be a risk factor for diabetic neuropathy in type 2 diabetic patients.[26] These data suggest that C-peptide level is correlated with RBC's Na/K ATPase activity, and that C-peptide is implicated in diabetic neuropathy pathogenesis.

For these reasons, we asked whether the ATP1-A1 polymorphism is associated with RBC's Na/K ATPase activity modifications in healthy subjects and in type 2 diabetic patients, as it does in type 1 insulinopenic patients.[21] The influence of the

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ethnic origin, European versus North African, was also evaluated. We also tested whether C-peptide level could influence the relationship between ATP1A1 polymorphism and RBC's Na/K ATPase activity.

MATERIALS AND METHODS

Subjects

Their characteristics are summarized in Table 1.

European subjects. Different groups of subjects of European ancestry were studied. Seventy-five healthy individuals (17 women) with a mean age of 36.1 ± 1.2 years (range, 18 to 57) were recruited among hospital staff. Eighty-one patients with established type 1 diabetes under regular outpatient follow-up in our department were consecutively selected. The following selection criteria were necessary: diagnosis of diabetes before the age of 30, initiation of insulin treatment less than 1 year after diagnosis, more than 5 years duration of diabetes, and absence of C-peptide in the serum. To assess the prevalence of the ATP1-A1 polymorphism in a representative type 1 diabetic population, restriction fragment length polymorphism (RLFP) analysis was also performed in 50 type 1 diabetic subjects diagnosed between November 1991 and October 1993. One hundred and seventeen patients (52 women) with established type 2 diabetes were enrolled. Selection criteria were: diagnosis of diabetes after the age of 30 and more than 3 years after diagnosis without insulin treatment. Among these patients, 44 were actually treated with insulin. Indication for insulin was either failure of oral agents or severe degenerative complications.

North African subjects. Sixteen healthy subjects of North African ancestry (10 women) were enrolled among hospital staff. Thirty type 1 diabetic patients (17 women) were selected using the same criteria as those for European type 1 diabetic subjects.

Among the diabetic patients, absence or presence of diabetic peripheral neuropathy was evaluated according to the Diabetes Control and Complications Trial (DCCT) criteria.[27] These criteria consisted of signs and symptoms including numbness, dysesthesias, and/or paraesthesias, hypersensitivity to touch, burning pain, and/or aching, stabbing pain in hands and/or feet, neuropathic foot ulcer, and decreased or absent deep tendon reflexes. Patients were considered as suffering from neuropathy if 1 or more criteria were found. Neuropathy was present in 31 (38%) type 1 European diabetic patients, 10 (33%) North Africans, and 61 (52%) type 2 diabetic patients. However, this study was not intended to analyze Na/K ATPase genotype or activity according to presence or absence of neuropathy. Indeed, the risks for diabetic neuropathy are numerous: age, diabetes duration, glycemic control, hypertension, tobacco, and so on. In the absence of a careful selection of patients, it would be critical to try to correlate presence of neuropathy with Na/K ATPase genotype or phenotype as we did in type 1 diabetic patients in a previous study.[21] This clinical parameter has not been evaluated in these results.

Assay Procedures

Na/K ATPase activity measurement. Venous blood samples were collected from fasting subjects on sodium citrate (0.11 mmol/L) for Na/K ATPase activity measurement and on EDTA (0.34 mmol/L) tubes for the other assays. Samples were taken at around 8:00 A.M before the morning insulin injection.

Immediately after collection, leukocytes and platelets were removed by filtering through a cellulose microcrystalline column as described by Beutler et al.[28] Na/K ATPase activity was estimated with a spectrophotometric determination of inorganic phosphate released from ATP, in the presence and absence of ouabain, a specific enzyme inhibitor, using a method adapted from Rahmani-Jourdheuil et al,[29] as previously published.[10] The sensibility threshold of the method was 2.10^{-7} mol/L. The intra- and interassay coefficients of variation were 2.8% and 3.9%. Results are given in nmol Pi/mg protein/h.

C-peptide and hemoglobin A_{1c} measurement. Plasma C-peptide was measured by conventional radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX) with a sensitivity of 0.05 nmol/L. The intra-and interassay coefficients of variation were 2.8% and 3.9% (fasting normal C-peptide level was 0.4 to 0.8 nmol/L). Hemoglobin A_{1c} HbA_{1c} was measured by high-performance liquid chromatography (BioRad, Hercules, CA, normal range, 4% to 6.5%).

RFLP analysis. DNA was obtained from leukocytes by standard technique, which was previously published and is summarized as follows.[21] Intron 1 of the ATP1 A1 gene was amplified using a forward primer 5′ ACC-GCC-ACC-ATG-GGG-AAG-GGG3′ and a reverse primer 5′ CTC-ATA-CTT-ATC-ACG-TCC-AAC3′. The primers surrounding the first intron were determined from sequence data for the ATP1 A1 gene[30] and obtained from Eurogentec (Angers, France). The polymerase chain reaction (PCR) was performed in a volume of 100 μL containing 1 μg of genomic DNA, 1 μmol of each of the 2 primers, 500 μmol each of the 4 desoxynucleic acids, 10 μL G Buffer, 4 mmol/L MgCl2 and 3 U of a mixture of Taq and Pwo polymerases (Expand long template PCR system, Boehringer Mannheim, Meylan, France) allowing amplification of long fragments at 68°C with a low error rate. The PCR reaction (Perkin Elmer 2400, Norwalk, CT) began with denaturation at 92°C for 2 minutes followed by 30 cycles of

Table 1. Characteristics of Subjects

	Europeans					North Africans	
			Type 2 Diabetes				
	Controls (n = 75)	Type 1 Diabetes (n = 81)	Whole Group (n = 117)	Oral Treatment (n = 73)	Insulin (n = 44)	Controls (n = 16)	Type 1 Diabetes (n = 30)
Female/male ratio	17/58	39/42	52/65	31/42	21/23	10/6	17/13
Age (yr)	361 ± 1.2	39.5 ± 1.5	60.7 ± 1.0	58.8 ± 1.0	64.4 ± 1.6	33.6 ± 2.8	36.7 ± 3.2
Diabetes duration (yr)		23.8 ± 1.0	14.2 ± 0.9	12.7 ± 0.9	17.8 ± 1.1		12.2 ± 1.3
BMI (kg/m^2)	22.8 ± 0.9	23.1 ± 1.5	29.2 ± 0.6	29.9 ± 0.6	27.1 ± 0.8	21.8 ± 0.4	21.3 ± 0.3
HbA_{1c}(%)		8.7 ± 0.3	8.8 ± 0.2	8.4 ± 0.2	9.9 ± 0.3		9.7 ± 0.4
Fasting blood glucose (mg/dL)		170 ± 10	150 ± 10	130 ± 20	180 ± 30		190 ± 20
Na/K ATPase (nmolPi/mg protein/h)	395 ± 9	285 ± 8*	335 ± 13	364 ± 16	264 ± 12*†	296 ± 26‡	246 ± 20‡

NOTE. Results as means ± SEM.

*Different from controls of the same ethnic origin ($P < .05$).

†Different from type 2 diabetic patients "whole group" or "oral treatment" ($P < .05$).

‡Different from European control subjects.

denaturation at 92°C for 10 seconds, annealing at 60°C for 30 seconds, and extension at 68°C for 10 minutes. After the tenth cycle, the duration of the extension step was increased by 20 seconds at each cycle. The last extension step was prolonged for 7 minutes at 68°C. Amplified fragments were digested with the BgI II enzyme at 37°C for 1 hour and analyzed by electrophoresis at 100 mA on 1% agarose gel for 2 hours.

Statistical Analysis

Student's *t* test for means and the Mann-Whitney U-test for non-normal variables give data as means ± SEM. Differences between groups were tested by analysis of variance (ANOVA). Correlation between variables and genotype was determined using the χ^2 test. *P* values less than .05 were considered statistically significant.

RESULTS

Frequency of the Restriction Site Polymorphism

The results obtained in the various groups are presented in Table 2. The frequencies of the genotypes were not significantly different from those predicted by the Hardy-Weinberg equilibrium.

Among the Caucasian control subjects, the variant allele frequency was 10%. In the series of 50 consecutively diagnosed type 1 diabetic patients, the variant allele frequency was 10%. This series may be considered representative of the type 1 diabetic population in our area. In the clinic-based series of type 1 and type 2 diabetic patients, this allele frequency was, respectively, 17.3% and 20.1%. Among North African individuals, the prevalence of the polymorphism did not differ between healthy and type 1 diabetic subjects. It also did not differ from that of the European subjects.

RBC's Na/K ATPase Activity in Diabetes

These results are presented in Table 1.

European individuals. In type 1 diabetic patients, Na/K ATPase activity was lower than in healthy controls. In type 2 diabetic patients, this activity was not significantly different from that of healthy controls. However, the group of type 2 diabetic patients treated by insulin had lower mean enzyme activity than those treated by oral treatment or healthy controls. In the same way, type 2 diabetic patients with low residual insulin secretion (C-peptide lower than 0.4 nmol/L) had diminished Na/K ATPase activity compared with the patients with more preserved insulin secretion (181 ± 21 *v* 334 ± 617 nmol Pi/mg protein/h; $P < .0001$). There was a significant correlation, shown by linear regression analysis, between enzymatic activity and C-peptide level ($r = .32$; $P < .01$). This relationship was much stronger ($r = .64$, $P < .001$) among insulin-treated type 2 diabetic patients, in whom C-peptide levels were variable, ranging from low to normal values.

Sex, body mass index, age, and fasting blood glucose did not influence Na/K ATPase activity in any group. The relationship observed between Na/K ATPase activity and HbA_{1c} ($r = .29$, $P = .009$) and diabetes duration ($r = .31$, $P = .002$) disappeared after adjustment for C-peptide.

North African individuals. RBC's Na/K ATPase activity was lower in type 1 diabetic patients than in healthy controls, although the level of statistical significance was not reached. Healthy subjects, as well as type 1 diabetic patients of North African ancestry, had lower Na/K ATPase activity than each European control group.

Relationship Between Restriction Site Polymorphism and Na/K ATPase Activity in the Different Subgroups

RBC's Na/K ATPase activity was not influenced by the presence of the ATP1 A1 polymorphism in control European subjects (397 ± 24 *v* 401 ± 12 nmol Pi/mg protein/h), as well as in the North African subjects (291 ± 10 *v* 303 ± 21 nmol Pi/mg protein/h). In contrast, in type 1 diabetes, Na/K ATPase was significantly lower in patients bearing the variant allele in European patients (241 ± 10 *v* 319 ± 11 nmol Pi/mg protein/h, $P = .0001$), as well as in North African patients (207 ± 25 *v* 282 ± 20 nmolPi/mg protein/h, $P = .05$).

In type 2 diabetes, Na/K ATPase activity was also lower in patients with the variant allele than in patients without (280 ± 17 nmol Pi/mg protein/h *v* 339 ± 13, $P = .008$). This is also true for the subgroup treated with insulin (215 ± 12 nmol Pi/mg protein/h *v* 267 ± 13, $P = .03$), but not in a subgroup on oral treatment (328 ± 22 nmol Pi/mg protein/h *v* 377 ± 17, $P = .07$). These results are shown in Fig 1.

In summary, the presence of the variant allele was associated with decreased Na/K ATPase activity in European or North African patients suffering from type 1 or type 2 diabetes and with poor residual insulin secretion. The correlation observed between C-peptide level and Na/K ATPase activity in type 2 diabetic patients persisted when the subjects, with or without the variant allele, were analyzed separately. The correlation coefficient was 0.33 for the group of 46 patients bearing the

Table 2. ATP1A1 Restriction Polymorphism Repartition

	Europeans				North Africans	
	Controls (%)	Consecutively Diagnosed Type 1 Diabetes (%)	Type 1 Diabetes (%)	Type 2 Diabetes (%)	Controls (%)	Type 1 Diabetes (%)
(NN)	81.3 (n = 62)		65.4 (n = 53)	62.0 (n = 71)	81.3 (n = 13)	73.4 (n = 22)
(NR)	17.3 (n = 12)		34.6 (n = 28)	35.0 (n = 42)	18.7 (n = 3)	23.3 (n = 7)
(RR)	1.4 (n = 1)		0	3.0 (n = 4)	0	3.3 (n = 1)
"Wild type" allele frequency	90.0	90.0	82.7	79.9	90.6	85.0
"Variant" allele frequency	10.0	10.0	17.3*	20.1*	9.4	15.0

Abbreviations: NN = subjects homozygous for the "wild type" allele; NR = subjects heterozygous with restriction site; RR = subjects homozygous for the "variant" allele.

*Statistically different from controls restriction polymorphism repartition in each ethnic group.

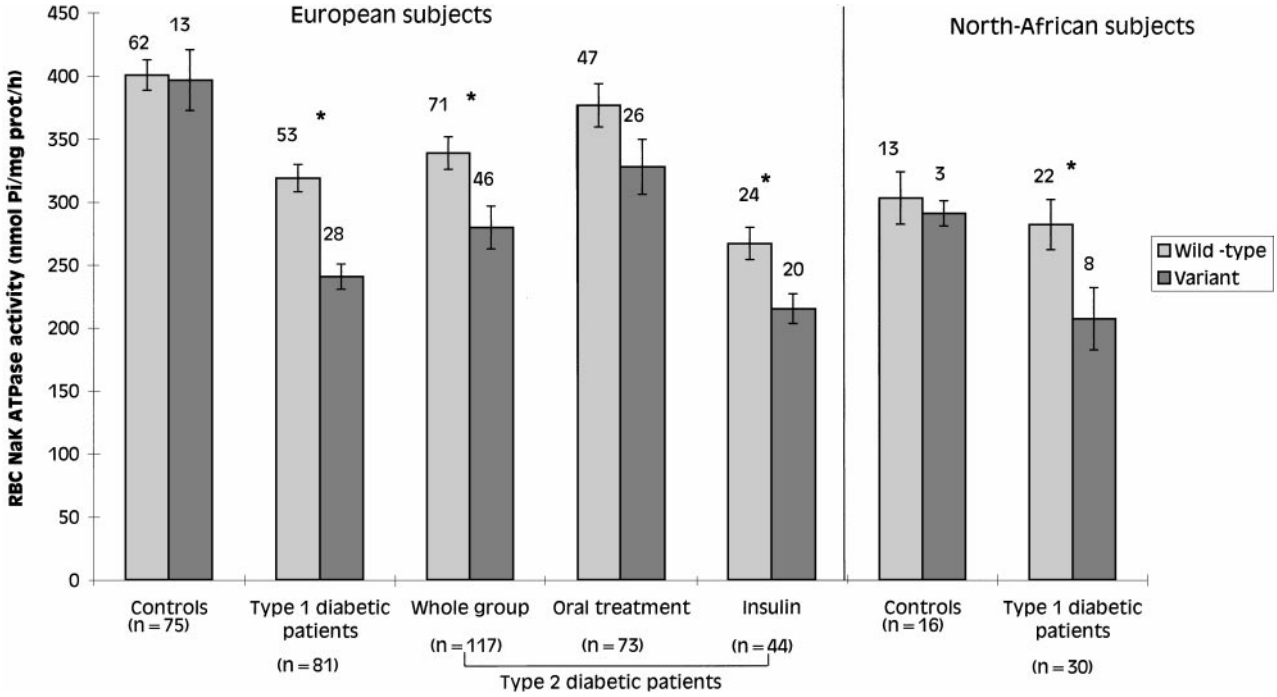

Fig 1. RBC Na/K ATPase activity according to ATP1A1 polymorphism in the various groups of subjects. Light columns, subjects bearing 2 "wild-type" alleles; dark columns, subjects bearing at least 1 "variant" allele. *Statistically significant difference ($P < .05$) between RBC Na/K ATPase measured in patients bearing "wild-type" alleles and "variant" alleles.

variant allele ($P = .03$) and 0.28 for the 71 patients without the variant allele ($P = .02$) (Fig 2).

The relationship between C-peptide and Na/K ATPase activity being stronger ($r = .64$; $P < .0001$) in type 2 diabetic patients on insulin treatment, this subgroup was analyzed separately. This correlation was also observed in patients with the restriction site (n = 20, $r = .74$, $P = .0007$) and in those without the restriction site (n = 24, $r = .57$, $P = .004$).

DISCUSSION

The results show that RBC's Na/K ATPase activity depends on genetic (polymorphism) and environmental (diabetes) factors. This fact is important, as we have already shown that Na/K ATPase activity is decreased in type 1 diabetic patients suffering from diabetic peripheral neuropathy, and more so if they are bearing ATP1A1 polymorphism, or are North Africans.[10] This polymorphism could thus represent 1 of the genetic predisposition to diabetic neuropathy.

The main objective of this study was to analyze the variation of Na/K ATPase activity according to genetic (ethnic origin, ATP1A1 genotype) and environmental factors (hyperglycemia, C-peptide insufficiency) regardless of neuropathy. Confounding factors are numerous, and the number of patients in different groups was too low to analyze the correlation between clinical neuropathy and ATP1A1 polymorphism. Therefore, we focus our discussion on the hypotheses that could explain Na/K ATPase activity variations.

The decrease in Na/K ATPase activity observed in diabetes could be related to hyperglycemia or to insulinopenia. Some years ago, it was shown that the decrease in Na/K ATPase observed in a small series of uncontrolled type 1 diabetic patients was corrected in 24 hours after glycemic normalization,[29] suggesting that hyperglycemia and/or insulinopenia could be involved in the regulation of erythrocyte Na/K ATPase. Hyperglycemia could decrease Na/K ATPase activity via an increased glycosylation of its β subunit. This fact could lead to a modification of the enzyme's role. In fact, the β subunit is involved in the maturation of the enzyme, localization of the ATPase in the plasma membrane, and stabilization of potassium bound intermediate form of the protein.[31-33] Another hypothesis is based on a reversal hyperglycemic-induced defect in myoinositol metabolism leading to altered lipid metabolism and decreased Na/K ATPase activity.[34,35] However, it must be pointed out that a direct action of hyperglycemia on Na/K ATPase activity has never been confirmed.

In our series, diabetic control was similar in patients suffering from type 1 or type 2 diabetes in terms of fasting blood glucose or HbA_{1c}. Nevertheless, type 2 diabetic patients' Na/K ATPase activity was not different, on the average, from that of the control group. Na/K ATPase activity was not correlated with HbA_{1c} in type 2 diabetes.[23,36] Also, Garner[37] reported that hyperglycemia does not modify Na/K ATPase activity.

If hyperglycemia alone cannot explain Na/K ATPase activity variations in diabetes, insulinopenia may be involved. Insulin regulates Na/K ATPase activity.[38-40] However, the mechanism by which insulin activates the sodium pump is complex. Several investigators have documented the stimulating action of insulin.[39,40,41] Santini et al[42] showed that RBC insulin receptors are able to modify transport properties by increasing the conductivity and permeability of the membrane. Lytton et al[43,44]

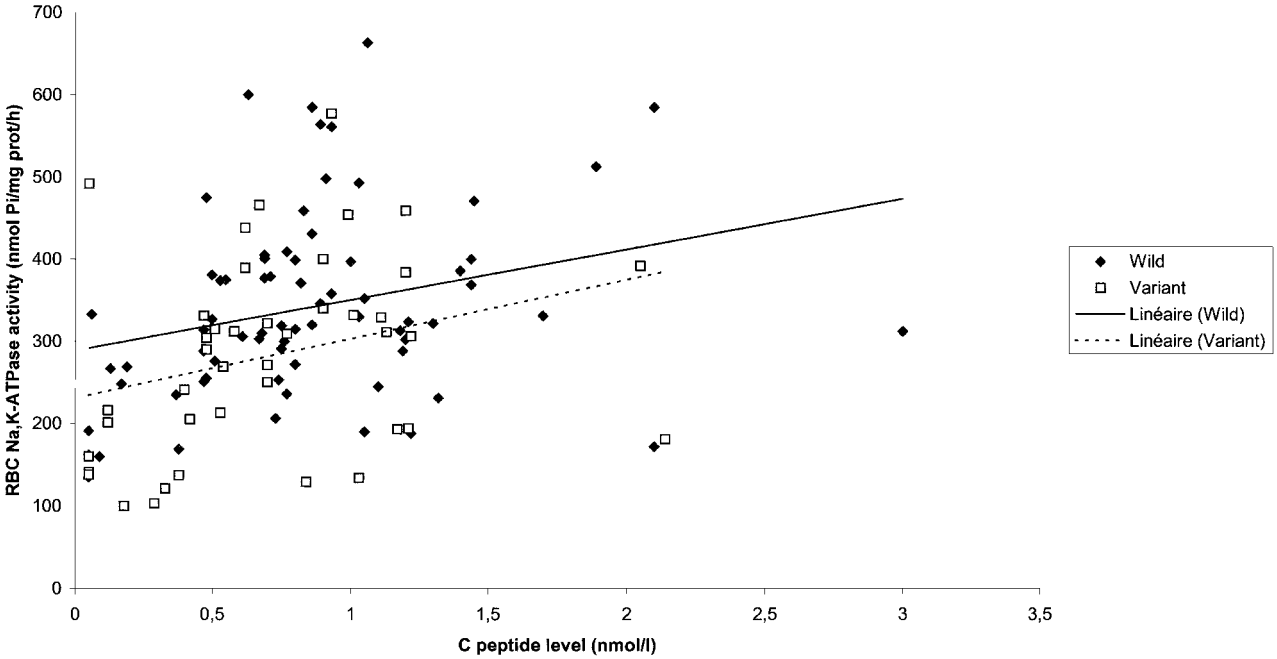

Fig 2. Relationship between RBC Na/K ATPase activity, C-peptide level in type 2 diabetic patients. (□), "variant" allele, (◆), "wild-type" allele, (—) positive correlation for patients bearing "wild" alleles ($r = .28$; $P = .02$); (— - — - —) positive correlation for patients bearing the "variant" allele ($r = .33$; $P = .03$).

reported that insulin acts quickly and at different sites. Insulin increases intracellular sodium concentration and enhances sodium affinity of the pump.[45] It could also induce a redistribution of different subunits of Na/K ATPase from intracellular compartments to the plasma membrane as observed in mammalian skeletal muscle.[46-48] All of these mechanisms are still unclear, but a protein kinase C activation[49] and a phosphatidylinositol 3 kinase stimulation, as well as the ζ isoform of the protein kinase C, seem necessary.[50]

As insulin and C-peptide are cosecreted into the portal circulation in response to intestinal glucose absorption, insulin deficiency in diabetes is associated with a low C-peptide level. In insulin-treated patients with type 1 or type 2 diabetes, an imbalance exists between C-peptide and insulin plasma levels. We have reported that fasting C-peptide level was the only factor independently correlated with Na/K ATPase in type 2 diabetic patients, explaining 23% of its variance.[23] Several arguments suggest that physiologic C-peptide and insulin levels are essential for normal Na/K ATPase activity. It was recently shown that C-peptide stimulates directly Na/K ATPase activity in renal tubule cells.[24] C-peptide may act with a specific receptor, activating G-protein and calcium-dependent signaling pathways, as it is proposed by Rigler et al,[51] Forst et al,[52] and Wahren et al.[53] Several reports underlined C-peptide's biological effects.[54] Giving C-peptide improves parameters altered in diabetic complications. C-peptide reduces glomerular hyperfiltration[24,53-56] and urinary albumin excretion,[56,57] increases blood flow and oxygen uptake,[25,53,54,58,59] stimulates glucose transport in skeletal muscle,[60] decreases blood retinal barrier leakage,[53] attenuates vascular dysfunction,[53,61] and improves autonomic[57,62] or sensory nerve dysfunction.[57]

Na/K ATPase has been implicated in several diabetic complications,[16] and many processes stimulated or modulated by C-peptide are related to membrane permeability or transport.[24,58,61] Restoration of erythrocyte deformability could also facilitate the insulin-stimulating effect.[63] Lastly, Hâring et al[64] reported in 1994 that C-peptide could take the insulin-signaling pathway. Given the difference in RBC's Na/K ATPase activity according to the ethnic origin,[19,20] it may be asked if the genetic background modifies the response to the environmental disturbance. Previous reports have shown that ethnic variations in Na/K ATPase existed. This activity is decreased in RBCs of North African individuals, both in controls and in type 1 diabetic patients.[18,20] Na/K ATPase activity is constitutionally decreased in North Africans who are predisposed to neuropathy in case of diabetes.[10] This drop in activity is even worsened by hyperglycemia of type 1 diabetes.[11] This ethnic variation suggests a genetic control of Na/K ATPase activity.

Two restriction polymorphisms of the ATP1-A1 gene have already been described.[8,65] As the ATP1-A1 gene encodes for the α 1 catalytic isoform, which is exclusively expressed in RBCs and is preponderant in nerve, we studied a RFLP to investigate whether this polymorphism could explain Na/K ATPase variations. This polymorphism was not more frequent in North African controls (9.4% of restricted allele *v* 10% in Europeans) and thus cannot explain the difference in RBC's activity between European and North African people. If Na/K ATPase is genetically well determined, it may appear that another polymorphism is involved.

We observed that Na/K ATPase level is similar in controls bearing or not the restricted allele, whatever the ethnic origin, but it is decreased in diabetic patients, and more so because of

polymorphism. Na/K ATPase is statistically decreased when the restricted allele is present in type 1 diabetic patients, both in Europeans and North Africans, and in type 2 European diabetic patients. This suggests that the particularity conferred by the mutation associated with ATP1-A1 polymorphism renders the enzyme's function more susceptible to the deleterious effect of diabetes. More than hyperglycemia, insulinopenia seems to be determinant on the effect of ATP1-A1 polymorphism. Indeed, Na/K ATPase activity did not differ in the subgroup of type 2 diabetic patients with or without the restricted allele on oral treatment, whereas those treated with insulin differed significantly.

The mechanism by which this polymorphism alters Na/K ATPase is still unclear. It might induce qualitative or quantitative Na/K ATPase abnormalities. In fact, the ATP1A1 gene restriction polymorphism, associated with lower ATPase activity in the case of C peptide insufficiency, is located on the first intron of the gene. It is unlikely that it could affect the function of the enzyme by itself, unless it affects the splicing. Most probably, it is in linked desequilibrium with other polymorphisms either within the gene, within its promoter, or in a nearby gene. Qualitative abnormality could result in a modification of a binding site (ATP, ouabain, or phosphorylation site). Localization in the plasma membrane could also be altered.

Quantitative abnormality could result in transcriptional or translational dysfunction, but a posttranslational degradation is also possible. This restriction polymorphism could affect the sites of recognition of factors regulating gene expression, or the coding sequences, and thus alter the function of the enzyme. This would cause decreased enzymatic activity as a result of either structural changes in the enzyme or a reduction in the number of enzyme molecules. Thus, ATP1-A1 polymorphism could induce an abnormality, which is compensated when C-peptide level is sufficient, but C-peptide deficiency alters Na/K ATPase, and ATP1-A1 polymorphism worsens the situation.

CONCLUSION

NaK ATPase activity is decreased in diabetic patients with defective endogenous insulin secretion, and the genotype (ATP1-A1 polymorphism) influences the phenotype (RBC Na/K ATPase activity), only in case of C-peptide deficiency. This may explain the fact that the polymorphism confers a high risk of diabetic neuropathy in type 1 diabetic patients. This risk remains to be evaluated in a large type 2 diabetes population. On the other hand, the ethnic genetic variations of RBC's NaK ATPase activity between Europeans and North Africans are not explained by the ATP1A1 polymorphism, and supplementary studies will be necessary to characterize this genetic abnormality.

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