Uncoupling Protein 2 Promoter Polymorphism −866G/A Affects Peripheral Nerve Dysfunction in Japanese Type 2 Diabetic Patients

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OBJECTIVE — To determine genetic predispositions for diabetic polyneuropathy, we investigated the relationship between the −866G/A polymorphism of uncoupling protein (UCP) 2 and neurological manifestations in 197 type 2 diabetic patients.

RESEARCH DESIGN AND METHODS — We first examined whether UCP2 mRNA had been expressed in the dorsal root ganglion (DRG) in four Long-Evans Tokushima Otsuka rats using RT-PCR and electrophoresis. Genotyping of UCP2 promoter polymorphism −866G/A was then performed in 197 unrelated Japanese type 2 diabetic patients, who were subjected to nerve conduction, quantitative vibratory perception, head-up tilt, and heart rate variability tests, by PCR restriction fragment–length polymorphism. The relationships between UCP2 genotype and various nerve functions were analyzed by uni- and multivariable analysis.

RESULTS — Expression of UCP2 mRNA was confirmed in rat DRG. Multiple regression analysis clarified the hypothesis that the G/A + A/A genotype was significantly related to decreased motor nerve conduction velocity and impaired blood pressure maintenance on the head-up tilt test. Multiple logistic regression analysis revealed that the G/A + A/A genotypes are a significant risk factor for sensory nerve conduction slowing and orthostatic hypotension.

CONCLUSIONS — UCP2 promoter gene polymorphism −866 G/A was significantly associated with nerve conduction slowing and vasomotor sympathetic functions. These findings suggest that the higher UCP2 activity related to the A allele has an energy-depleting effect on peripheral nerve function in type 2 diabetic patients.

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Diabetic peripheral polyneuropathy (DPN) is a multifactorial disorder arising from hyperglycemia and/or insulin deficiency. Advanced DPN causes serious complications, such as diabetic foot ulcers, gangrene, and Charcot joint, all of which reduce the quality of life of diabetic patients with DPN (1). Severe cardiovascular autonomic neuropathy also increases the risk of mortality (2). Therefore, elucidation of genetic predispositions for DPN is important for preventing DPN and inhibiting its progression. There are, however, few reports on the genetic predispositions for DPN (3–5).

Oxidative damage due to hyperglycemia is reported to be one of the major factors contributing to the development of DPN (6). The main source of reactive oxygen species (ROS) in diabetes is thought to be the mitochondria (7). Uncoupling proteins (UCPs) can provide a controlled leak of protons across the inner membrane of the mitochondria and thus uncouple oxidative phosphorylation from respiration, with a concomitant decrease in inner mitochondrial membrane potential (8) and free radical generation (9,10). The mitochondrial UCP families, particularly UCP2, which is expressed in various human tissues, are thought to contribute to control of body temperature and energy metabolism as well as to regulation of mitochondrial production of ROS. Therefore, the UCP2 gene is considered to be involved in DPN.

In experimental studies, the protective effects of UCP activation against oxidative stress–induced cell death in neuronal cells (11) and cardiomyocytes (12) have been documented. These findings suggest favorable effects of UCP2 activation in DPN. On the other hand, increased UCP2 levels in β-cells are reported to be associated with reduced insulin secretion via decreased ATP production (13,14). We also found that the −866G/A polymorphism in the UCP2 gene, which enhances transcriptional activity, was associated with an increased risk for type 2 diabetes via decreased insulin secretion (15). These findings indicate unfavorable effects of UCP2 activation on DPN via decreased ATP production. In either case, UCP2 promoter gene polymorphisms may be related to the onset and progression of DPN. In this study, we addressed the question of whether the −866G/A polymorphism in the UCP2 gene is associated with DPN progression. However, expression of UCP2 was found to be low in cultured dorsal root ganglion (DRG) neurons on Western blotting and immunocytochem-

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Abbreviations: ΔBP, change in systolic blood pressure after standing for 5 min; CMAP, compound muscle action potential; DPN, diabetic peripheral neuropathy; DRG, dorsal root ganglion; MCV, motor nerve conduction velocity; ROS, reactive oxygen species; SCV, sensory nerve conduction velocity; SNAP, sensory nerve action potential; UCP, uncoupling protein; VPT, vibratory perception threshold; VPT-F, VPT at tip of index finger; VPT-T, VTP at tip of big toe.

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

istry (11). Therefore, we first confirmed that UCP2 mRNA is expressed in rat DRG by RT-PCR and electrophoresis. The relationship between the −866G/A polymorphism and neurological manifestations was then evaluated by genotyping 197 diabetic subjects.

RESEARCH DESIGN AND METHODS

UCP2 mRNA expression in rat DRG

Four Long-Evans Tokushima Otsuka rats (Otsuka Pharmaceutical Laboratory), aged 5 weeks and weighing ~100 g, were used for experiments. After pentobarbital anesthesia (50 mg/kg i.p.), rats were killed and the DRG, spinal cord, and muscles were quickly removed and dissected free of connective tissues. Specimens were immediately immersed in RNAlater (Takara, Kyoto, Japan) and were extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) (16). Target RNA (4.5 μg) was reverse transcribed into cDNA using MultiScribe reverse transcriptase (Applied Biosystems, Tokyo, Japan) according to the manufacturer's instructions. Primer sequences were as described previously: UCP1 forward, TCCCTCAG GATTGGCCTCTAC; UCP1 reverse, GT CATCAAGCCAGCC GAGAT; UCP2 forward, GTTTCAAGGCCACCGAT GTG, UCP2 reverse, GGGAA AGTGAT GAGATCTGCAAT; UCP3 forward, ACTGGAGGCGAGAGGAAATACA; UCP3 reverse, ATGTTGGGCCAAGTC CCTTT (17). The PCR products obtained were loaded onto 2.0% agarose gels and evaluated by electrophoresis. Because of the small amount of DRG and spinal cord tissue, samples from two rats were combined and used for RNA extraction.

Diabetic subjects and clinical characteristics

A total of 197 unrelated Japanese type 2 diabetic patients, who were subjected to four somatic and autonomic nerve function tests, were recruited after giving written informed consent. The study was approved by the ethics committee of Wakayama Medical University. Diabetes was diagnosed according to the criteria of the World Health Organization (18). Patients with type 1 diabetes, maturity-onset diabetes of the young, and severe liver or renal dysfunction were excluded. Subjects consisted of 116 men and 81 women. Mean age was 54.23 ± 12.34 (mean ± SD) years, mean BMI was 24.21 ± 4.05 kg/m^2, and mean age at diagnosis was 42.61 ± 12.14 years. Of the subjects, 69 were outpatients and 128 were inpatients of the Wakayama Medical University Hospital; 72.5, 23.2, and 4.3% were treated with insulin, oral hypoglycemic agents and diet/exercise therapy, respectively. Diabetic retinopathy was evaluated by an ophthalmologist, and 104 (52.8%) patients who had simple, preproliferative, or proliferative retinopathy were considered retinopathy positive. A previous history of diabetic foot ulcer was observed in 9 (4.6%) patients. Subjects with systolic blood pressure >140 mmHg and/or diastolic blood pressure >90 mmHg or those receiving antihypertensive treatment were defined as hypertensive. Subjects with total cholesterol >5.69 mmol/l (220 mg/dl) and/or triglycerides >1.70 mmol/l (150 mg/dl) or those taking antihyperlipidemic medication were defined as hyperlipidemic. The prevalence of patients with hypertension and hyperlipidemia was 44.7 and 47.7%, respectively.

Nerve function tests

Four objective and quantitative tests (nerve conduction study, quantitative vibratory perception threshold (VPT) test, head-up tilt test, and heart rate variability test) were performed to evaluate the various aspects of somatic and autonomic nerve function. All examinations were conducted in a temperature-controlled room at 25°C.

Nerve conduction study. Nerve conduction studies were performed using an electromyograph (Synax 1200; NEC, Tokyo, Japan). Motor nerve conduction velocity (MCV) between the wrist and elbow, compound muscle action potential (CMAP) of the ulnar nerve, sensory nerve conduction velocity (SCV) between the wrist and elbow, and sensory nerve action potential (SNAP) of the median nerve were measured using standard methods. Electric stimuli were produced at supramaximal intensity (0.1 ms duration square wave with 25% above the current necessary to evoke maximal amplitude) and the CMAP produced by wrist stimulation was evaluated. A median nerve sensory conduction study was performed by antidromic techniques, and recording ring electrodes were placed on the index finger. Electric stimulation was performed with a bipolar electrode at the midposition of the medial elbow and wrist. The average of 10 responses stimulated supramaximally at the wrist was evaluated as SNAP. Examinations were performed bilaterally, and the average value was used for analysis. Skin temperature was measured at the forearms and was maintained at >32°C.

Quantitative vibratory perception threshold test. The VPT at 125 Hz was measured bilaterally at the tips of index fingers (VTP-F) and big toes (VPT-T). VPTs were semiquantitatively assessed using a vibratory sensation meter (AU02A; RION Company, Tokyo, Japan). The average of the two sides was used for analysis.

Autonomic nerve function tests (head-up tilt test and heart rate variability test). Sympathetic vasomotor function was evaluated by a head-up tilt test using a tilt table (Sakai, Tokyo, Japan) and an automatic sphygmomanometer (BP-88; Colin Company, Komaki, Japan). Supine brachial blood pressure was measured after patients rested for 15 min on a bed and was measured again after 5 min of passive standing in the 70° head-up position. Orthostasis-induced decreases in systolic blood pressure after standing for 5 min (ΔBP) were thus examined.

Parasympathetic cardiovagal function was also evaluated by a heart rate variability test. Coefficients of variation of R-R intervals on the electrocardiogram at rest (CV-R) and during deep breathing (CV-DB) were determined with an electrocardiograph (Autocardiner FCP-2201; Fukuda Denshi, Tokyo, Japan). CV-R and CV-DB were calculated in 100 R-R intervals after 15 min of bed rest and during deep breathing (6 breaths/min), respectively.

Genotyping of UCP2

Genomic DNA was isolated from peripheral blood according to standard procedures. UCP2 genotype was analyzed by PCR–restriction fragment–length polymorphism using *Mlu*I, as previously described (19). Primers were 5′-GACGC TGCTTCTGCCAGGAC-3′ (forward) and 5′-AGGCGTCAGGAGATGGACCG-3′ (reverse).

Comparisons between UCP2 genotype and clinical factors

We divided the patients into two groups based on UCP2 genotype, as previously reported (15): G/G genotype and G/A + A/A genotype. Differences in clinical factors, such as age, sex, BMI, HbA_{1c} (A1C), onset age of diabetes, duration of diabetes, prevalence of insulin therapy, diabetic retinopathy, hyperlipidemia, hypertension, numbness in the soles or

Table 1—*Clinical characteristics and neurological data of type 2 diabetic patients divided into two groups based on UCP2 genotype*

	G/G	G/A + A/A	*P* value
n	58	139	
Age (years)	54.9 ± 12.3	54.0 ± 12.4	0.646
Sex (male/female)	29/29	87/52	0.102
BMI	24.6 ± 4.0	24.1 ± 4.1	0.442
A1C (%)	8.85 ± 1.84	8.82 ± 2.13	0.928
Age at diabetes onset (years)	43.5 ± 13.4	42.2 ± 11.6	0.517
Duration of diabetes (years)	11.7 ± 8.0	12.4 ± 7.8	0.604
Insulin therapy (%)	36/54 (66.7)	98/131 (74.8)	0.260
Retinopathy (%)	27/50 (54.0)	67/128 (52.4)	0.842
Hyperlipidemia (%)	26/53 (49.1)	61/129 (47.3)	0.828
Hypertension (%)	24/53 (45.3)	59/131 (45.0)	0.976
Numbness in soles or toes of both feet (%)	25/57 (43.8)	62/139 (44.6)	0.924
Spontaneous pain in both feet (%)	8/57 (14.0)	15/139 (10.8)	0.522
Orthostatic dizziness (%)	9/57 (15.8)	26/137 (19.0)	0.599
Areflexia in patellar tendon reflex (%)	7/51 (13.7)	32/130 (24.6)	0.109
Areflexia in Achilles tendon reflex (%)	25/51 (49.0)	67/130 (51.5)	0.760
MCV (m/s)	51.76 ± 5.06	49.76 ± 5.47	0.018
Prevalence of impaired MCV (%)	14/58 (24.1)	54/139 (38.8)	0.048
CMAP (mV)	6.98 ± 2.44	6.69 ± 2.10	0.442
Prevalence of impaired CMAP (%)	1/54 (1.9)	13/132 (9.8)	0.061
SCV (m/s)	57.64 ± 5.17	56.45 ± 5.93	0.197
Prevalence of impaired SCV (%)	16/58 (27.6)	63/138 (45.7)	0.019
SNAP (μV)	19.62 ± 16.29	19.23 ± 14.40	0.875
Prevalence of impaired SNAP (%)	20/54 (37.0)	34/129 (26.4)	0.149
VPT-F (dB)	5.77 ± 5.78	7.50 ± 6.85	0.098
Prevalence of impaired VPT-F (%)	24/58 (41.4)	74/139 (53.2)	0.129
VPT-T (dB)	21.14 ± 9.36	22.35 ± 10.33	0.447
Prevalence of impaired VPT-T (%)	25/57 (43.9)	65/139 (46.8)	0.711
CV-R (%)	2.14 ± 1.03	1.91 ± 1.13	0.201
Prevalence of impaired CV-R (%)	25/56 (44.6)	71/135 (52.6)	0.317
CV-DB (%)	4.04 ± 2.85	4.12 ± 3.14	0.862
Prevalence of impaired CV-DB (%)	16/56 (28.6)	57/135 (42.2)	0.077
ΔBP (mmHg)	9.2 ± 13.6	17.0 ± 17.8	0.003
Orthostatic hypotension (%)	1/58 (1.7)	16/139 (11.5)	0.026

Data are means ±SD or *n* (%). *n* = 197.

toes of both feet, spontaneous pain in both feet, diminished Achilles tendon reflex, and a history of diabetic foot ulcer, between the G/G and G/A + A/A genotypes were examined.

Comparisons between UCP2 genotype and neurological function

Actual data from neurological examinations and the prevalence of impaired values were compared between the GG and G/A + A/A genotypes. Because nerve conduction data (MCV, SCV, CMAP, and SNAP) and vibratory thresholds (VPT-F and VPT-T) were distributed normally, values exceeding the range of means ± 2 SD of the healthy subjects in our institute (20) were judged as impaired. CV-R and CV-DB results that were converted into logarithms were distributed normally, and data that were more than the means −2 SD of logarithms of healthy subjects (21) were considered impaired. Because ΔBP values in the head-up tilt test were not distributed normally, decisions regarding impairment were made using the presence of orthostatic hypotension, as defined by the American Autonomic Society criteria (22): a decrease in upright systolic/diastolic blood pressure of at least 20/10 mmHg together with frequent orthostatic symptoms, such as lightheadedness and presyncope.

Statistical analysis

Data are shown as means ± SD, values, or percentages. Differences in continuous clinical factors (age, onset age of diabetes, duration of diabetes, and BMI), nerve conduction data, and VPT between the two UCP2 genotypes were analyzed by unpaired *t* test. Comparisons of autonomic function data between the two genotypes were performed by Mann-Whitney *U* test. Proportions of categorical clinical factors (sex, insulin therapy, retinopathy, hypertension, orthostatic hypotension, numbness in soles or toes of both feet, spontaneous pain in both feet, diminished Achilles tendon reflex, and a history of diabetic foot ulcer) were compared by χ^2 test between the two UCP2 genotypes. Prevalence of impairment in neurological examination in the two UCP2 genotypes was also analyzed by χ^2 test.

Multiple regression analysis was performed to examine the relationships between the actual results of nerve function tests and clinical background factors, including the UCP2 genotype. Results of nerve function tests were set as dependent variables for the analyses. Six clinical background factors (age, sex, duration of diabetes, A1C levels, retinopathy, and UCP2 genotype: G/G = 0, G/A + A/A = 1) were selected as independent variables. Multiple logistic regression analysis was also used to determine independent associations between UCP2 genotype and the presence of impairment in neurological examinations and two subjective symptoms (numbness in soles or toes of both feet, spontaneous pain in both feet) using the same independent variables. All analyses were performed with the StatView program for Windows (version 5.01; SAS Institute, Cary, NC).

RESULTS

UCP2 mRNA expression in rat DRG

The elution patterns for the PCR products are shown in Fig. 1. UCP1 mRNA was observed in one of the spinal cords and in both DRG samples. UCP2 and UCP3 mRNA were noted in all samples of muscle, spinal cord, and DRG.

UCP2 genotype

The G/G, G/A, and A/A genotypes of UCP2 were observed in 58 (29.5%), 98 (49.7%), and 41 (20.8%) of the 197 patients, respectively. G and A allele frequencies were 54.3 and 45.7%, respectively.

Relationships between UCP2 genotype, clinical factors and neurological data

There were no significant differences with regard to age, sex, BMI, A1C levels, onset

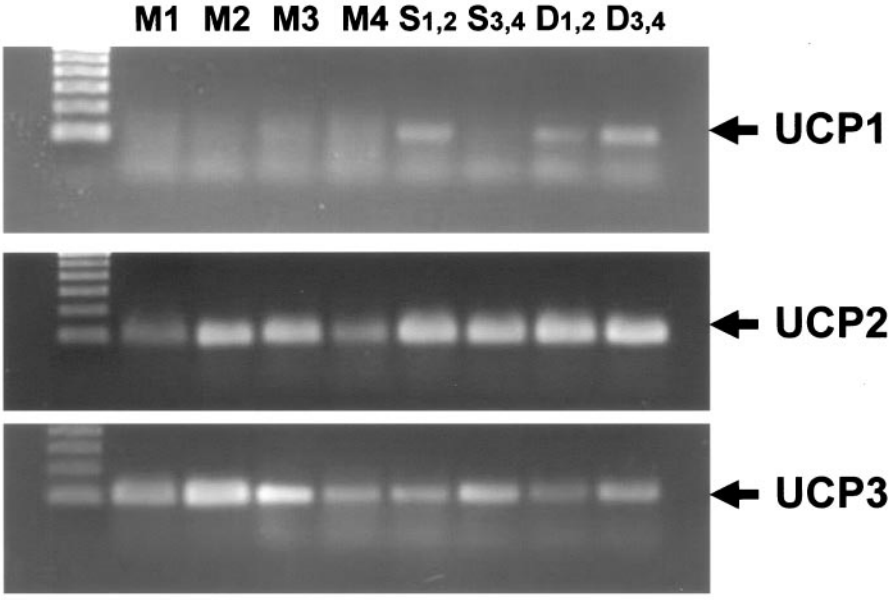

Figure 1—*Elution patterns of UCP1, UCP2, and UCP3 mRNA amplified by RT-PCR. Four male Long-Evans Tokushima Otsuka rats were used. M1–M4 indicate muscle samples from four animals. $S_{1,2}$, $S_{3,4}$ and $D_{1,2}$, $D_{3,4}$ indicate the spinal cord and DRG samples, respectively; because of the small sizes of these organs, tissue samples from two animals were combined and analyzed. UCP1 mRNA was weakly detected in one spinal cord and two DRG samples. UCP2 mRNA was clearly detected in both spinal cord and DRG samples, as well as in two muscle samples. UCP3 mRNA was detected in muscle, spinal cord, and DRG.*

age of diabetes, duration of diabetes, proportion of insulin therapy, prevalence of diabetic retinopathy, hyperlipidemia, or hypertension, numbness in soles or toes of both feet, spontaneous pain in both feet, orthostatic dizziness, or diminished patellar and Achilles tendon reflex between the two diabetic groups (G/G and G/A + A/A). Data for neurological examinations and prevalence of impaired values in G/G and G/A + A/A genotypes are shown in Table 1. Although there were no significant differences in CMAP, SCV, SNAP, VPT-F, VPT-T, CV-R, and CV-DB between the G/G and G/A + A/A groups, MCV in the G/G group was significantly higher than that in the G/A + A/A group. ΔBP values in the G/G group were also significantly smaller than those in the G/A + A/A group. The prevalences of MCV or SCV impairment and orthostatic hypotension were significantly lower in the G/G group compared with the G/A + A/A group.

The results of multiple regression analyses are summarized in Table 2. UCP2 gene polymorphism was significantly related to MCV and ΔBP, independently of age, sex, duration of diabetes, hemoglobin A1C levels and retinopathy. As independent deteriorating factors for MCV, the following were identified: UCP2 genotype (G/A + A/A, $\beta = -0.149$, $P = 0.0323$), A1C ($\beta = -0.286$, $P < 0.0001$), and retinopathy ($\beta = -0.353$, $P < 0.0001$). As independent deteriorating factors for ΔBP, UCP2 genotype (G/A + A/A, $\beta = 0.186$, $P = 0.012$) and retinopathy ($\beta = 0.268$, $P = 0.0008$) were identified. Multiple logistic regression analysis revealed that UCP2 genotype (G/A + A/A) was a significant risk factor for impairment of MCV (odds ratio 2.589, $P = 0.0324$) or SCV (2.275, $P = 0.0347$) independent of age, sex, A1C, duration of diabetes, and retinopathy. Retinopathy and high A1C were significant risk factors for MCV or SCV impairment. Age was negatively correlated with MCV impairment. There was no significant relationship between subjective symptoms and UCP2 genotype.

CONCLUSIONS — UCP2 is expressed in various human tissues and is thought to play a range of physiological roles, such as nonshivering thermogenesis, energy production, and redox balance. Expression of UCP2 in the peripheral nervous system had not previously been confirmed; this is the first report to demonstrate UCP2 mRNA expression in the rat DRG. One of the major functions of UCP2 is its action as a sensor and negative regulator of ROS production. Macrophages from UCP2-deficient mice were found to generate higher levels of ROS (10). Overexpression of UCPs has been reported to inhibit oxidative stress–induced programmed cell death in cultured neurons (11) and cardiomyocytes (12). Although these in vitro studies suggest cytoprotective effects for UCP2 activation, there have been no clinical studies indicating that UCP2 activation is able to prevent oxidative injury. UCP2 is also thought to negatively regulate energy production by increasing proton leak without oxidative phosphorylation across the inner mitochondrial membrane. UCP2-knockout mice were reported to have higher ATP content in their islets and showed higher insulin secretion (14,23). Adenovirus-induced overexpression of UCP2 in pancreatic β-cells caused impaired insulin secretion (13). Therefore, UCP2 activation affects the progress of DPN in either a promotive or an inhibitory manner, as both oxidative stress and energy deficiency elicited by nerve ischemia are thought to cause DPN.

The common UCP2 promoter polymorphism −866G/A in humans has been reported to be associated with various pathological conditions. The A allele was associated with increased transcriptional activity and increased mRNA levels in human fat cells (24). The A allele was also found to reduce the risk of obesity in Caucasians (17), but not in Japanese (15). On the other hand, our group (15) and Krempler et al. (24) previously reported that the A allele of the −866 G/A polymorphism decreased insulin secretion and increased the risk of type 2 diabetes. There are, however, no reports on the association between UCP2 gene polymorphism and DPN.

Our study revealed that the −866 G/A and A/A genotypes of UCP2 are significantly associated with nerve conduction slowing and impaired blood pressure regulation on a head-up tilt test. This suggests that higher UCP2 activity related to the A allele of −866 G/A polymorphism causes deterioration of peripheral nerve function by energy depletion rather than

Table 2—*Relationships between neurological function and clinical background factors evaluated by multiple regression analysis (A) and multiple logistic regression analysis (B)*

		Independent variables					
		A: β (*P* value)					
Dependent variables	Regression analysis R^2 (*P* value)	Age	Sex	A1C	Duration	Retinopathy	UCP2 genotype (−866G/A) (G/G = 0, G/A + A/A = 1)
MCV	0.197 (<0.0001)	0.032 (0.6597)	−0.091 (0.1967)	−0.286 (<0.0001)	−0.001 (0.9944)	−0.353 (<0.0001)	−0.149 (0.0323)
SCV	0.195 (<0.0001)	−0.113 (0.1269)	−0.059 (0.4156)	−0.286 (<0.0001)	−0.031 (0.6999)	−0.364 (<0.0001)	−0.061 (0.3914)
ΔBP	0.139 (0.0003)	0.094 (0.2167)	0.065 (0.3816)	0.093 (0.2075)	0.048 (0.5524)	0.268 (0.0008)	0.186 (0.0112)

		B: Adjusted OR (95% CI), *P* value					
Dependent variables	R^2	Age	Sex	A1C	Duration	Retinopathy	UCP2 genotype (−866G/A) (G/G = 0, G/A + A/A = 1)
Impairment of MCV	0.197	0.949 (0.918–0.981), 0.0023	1.501 (0.692–3.259), 0.3040	1.273 (1.056–1.533), 0.0112	0.971 (0.920–1.025), 0.2894	6.518 (2.628–16.166), <0.0001	2.589 (1.083–6.191), 0.0324
Impairment of SCV	0.146	0.978 (0.948–1.008), 0.1482	1.090 (0.542–2.194), 0.8082	1.264 (1.058–1.510), 0.0099	1.002 (0.954–1.054), 0.9256	4.977 (2.306–10.742), <0.0001	2.275 (1.061–4.880), 0.0347
Orthostatic hypotension	0.107	1.037 (0.976–1.102), 0.2422	3.038 (0.782–11.801), 0.1085	1.078 (0.819–1.418), 0.5933	0.992 (0.916–1.075), 0.8467	1.873 (0.546–6.428), 0.3185	6.408 (0.801–51.266), 0.0800
Numbness in soles ortoes of both feet	0.048	1.002 (0.973–1.031), 0.9156	0.886 (0.461–1.701), 0.7152	1.167 (0.993–1.371), 0.0602	1.051 (1.002–1.101), 0.0408	1.420 (0.720–2.801), 0.3111	0.865 (0.4351–1.718), 0.6783
Spontaneous pain in both feet	0.047	1.018 (0.973–1.066), 0.4360	1.127 (0.425–2.988), 0.8106	1.054 (0.829–1.339), 0.6692	1.020 (0.955–1.089), 0.5585	2.283 (0.781–6.672), 0.1314	0.610 (0.229–1.620), 0.3211

β, standard regression coefficient.

neuroprotective effects against oxidative stress in type 2 diabetic patients.

Diabetic patients with the A allele showed a greater decrease in MCV of the ulnar nerve and a higher prevalence of impaired SCV of the median nerve. DPN is electrophysiologically marked by mildly impaired nerve conduction velocity and decreased SNAP. Although nerve conduction slowing is generally considered to reflect demyelination of the nerve fiber, several investigators have suggested that NCV slowing in DPN can be caused by hypofunction or decreased density of Na^+-K^+-ATPase at the nodes of Ranvier (25,26). The function of Na^+-K^+-ATPase is highly dependent on ATP production and insulin secretion in pancreatic β-cells. Thus, decreased Na^+-K^+-ATPase activity elicited by lower ATP production associated with the G/A + A/A genotype might cause the slowing in nerve conduction velocity. Poly(ADP-ribose) polymerase is a nuclear enzyme whose overactivation by DNA strand breaks depletes its substrate NAD^+ and ATP (27). Obrosova et al. reported that poly(ADP-ribose) polymerase activation and energy failure are obligatory steps in functional changes (nerve conduction deficits) in the diabetic nerve, and they speculated that the decrease in the free cytosolic ATP-to-ADP ratio accounted for chronic depolarization and Na^+ channel inactivation with resulting reduced excitability and nerve conduction deficits (28). Therefore, we think that a close relationship between nerve conduction slowing and the −866 G/A and A/A genotypes is plausible. On the other hand, the difference in SNAP and CMAP between patients with the A allele and those without the A allele was not significant. Reduced SNAP is considered to reflect fiber loss in the peripheral nerves (29). Because nerve fiber loss in DPN is thought to occur by endoneurial microangiopathy (30), the relationship between SNAP reduction and energy deficiency abnormalities should be less obvious than nerve conduction slowing. In this study, age was unexpectedly identified as a protective factor against MCV impairment. Although the reason for this is uncertain, the impairment criteria (average +2 SD of age-matched control subjects) may be related; the SD in older individuals tends to be larger than that in younger individuals and thus in older patients this criterion is less strict.

We also found that diabetic patients with the A allele exhibited larger decreases in systolic blood pressure than

those without the A allele on the head-up tilt test. In addition, these individuals showed a higher prevalence of orthostatic hypotension. It is therefore suspected that the reduced energy supply observed in the A allele of the UCP2 gene is related to the progression of orthostatic hypotension. Orthostatic hypotension in DPN is caused mainly by peripheral autonomic dysfunction, particularly efferent sympathetic nerve function (31). On the other hand, we could not find any significant differences in heart rate variability tests, which mainly reflected parasympathetic cardiovagal function, between the patients with and without A allele of the UCP2 gene. Although we do not know the exact reason why only sympathetic nerve function is impaired in the patients with the G/A + A/A genotype, two possible mechanisms are considered. One possible mechanism is based on the difference of nerve fiber size between sympathetic and parasympathetic nerves. Postganglionic sympathetic nerves consist of long small unmyelinated fibers, which are more vulnerable to ischemia and energy deficiency than large myelinated fibers. In contrast, heart rate variability tests (parasympathetic cardiovagal function) reflect the large myelinated fiber function. VPT, which reflected somatosensory myelinated large fiber function, also showed no significant relationship to the −866 G/A polymorphism of the UCP2 gene. Another possibility is that the genetic predisposition to the dysfunction of sympathetic and parasympathetic nerve may differ. Freccero et al. (32) reported that sympathetic and parasympathetic nerve functions correlated in type 1 but not in type 2 diabetic patients. Spontaneous nonobese diabetic mice, which exhibited the rapidly developing sympathetic neuritic dystrophy, have been reported (33).

The subjects analyzed in this study consisted mainly of hospitalized patients with hyperglycemia, advanced microangiopathy, and symptomatic DPN. Therefore, the proportion of insulin therapy in the diabetic patients with the A/A and A/G genotypes (74.8%) was not significantly higher than that in patients with the G/G genotype (67.9%).

In summary, our results suggest that the UCP2 gene may indeed be involved in the pathogenesis of DPN, particularly in nerve conduction dysfunction and vasomotor sympathetic function in Japanese type 2 diabetic patients. Further studies are necessary to identify the effects of UCP2 on neurological function in diabetic patients.

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