Functional Polymorphisms of *UCP2* and *UCP3* Are Associated With a Reduced Prevalence of Diabetic Neuropathy in Patients With Type 1 Diabetes

GOTTFRIED RUDOFSKY, JR., MD
ANTONIA SCHROEDTER
ANDREAS SCHLOTTERER, PHD
OLGA E. VORON'KO, PHD
MARTIN SCHLIMME, PHD
JOERG TAFEL, MD
BEREND H. ISERMANN, MD
PER M. HUMPERT, MD
MICHAEL MORCOS, MD
ANGELIKA BIERHAUS, PHD
PETER P. NAWROTH, MD
ANDREAS HAMANN, MD

OBJECTIVE — We studied the association between polymorphisms in the *UCP* genes and diabetes complications in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS — We analyzed 227 patients with type 1 diabetes using PCR and subsequent cleavage by restriction endonucleases for the promoter variants A-3826G in the *UCP1* gene, G-866A in the *UCP2* gene, and C-55T in the *UCP3* gene.

RESULTS — No effect of the A-3826G polymorphism in the *UCP1* gene on diabetes complications was found. Patients who were heterozygous or homozygous for the G-866A polymorphism in the *UCP2* gene or the C-55T polymorphism in the *UCP3* gene had a significantly reduced prevalence of diabetic neuropathy (*UCP2*: odds ratio 0.44 [95% CI 0.24–0.79], $P = 0.007$; *UCP3*: 0.48 [0.25–0.92], $P = 0.031$), whereas there was no association with other diabetes complications. This effect was stronger when G-866A and C-55T occurred in a cosegregatory manner (*UCP2* and *UCP3*: 0.28 [0.12–0.65], $P = 0.002$). Furthermore, a multiple logistic regression model showed an age- and diabetes duration–independent effect of the cosegregated polymorphisms on the prevalence of diabetic neuropathy ($P = 0.013$).

CONCLUSIONS — Our data indicate that both the G-866A polymorphism in the *UCP2* gene and the C-55T polymorphism in the *UCP3* gene are associated with a reduced risk of diabetic neuropathy in type 1 diabetes. Thus, the results presented here support the hypothesis that higher expression of uncoupling protein might prevent mitochondria-mediated neuronal injury and, ultimately, diabetic neuropathy.

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Diabetic neuropathy is the most common neuropathy in the western world. Hyperglycemia triggers a number of mechanisms thought to underlie diabetic neuropathy (1). Next to others, increased production of reactive oxygen species (ROS) (2,3) and formation of advanced glycation end products (AGEs) (4,5) are of pathophysiological importance. In patients with diabetes, mitochondrial ROS generation, especially at high blood glucose levels, is one of the main sources of oxidative stress (6,7). Increased levels of ROS are known to promote nonenzymatic glycation and AGE formation. AGEs and, in particular, carboxymethyllysine accumulate in peripheral nerves of patients with diabetes (4,8,9). Subsequent enhanced AGE-RAGE (receptor of AGEs) interaction leads to sustained nuclear factor-κB activation (10,11) with consecutively increased interleukin-6 and tumor necrosis factor-α expression, resulting in neuronal dysfunction and, ultimately, diabetic neuropathy (11–13).

To neutralize ROS and prevent harm, several antioxidant defense mechanisms exist. One of these is represented by the uncoupling proteins (UCPs). Cellular energy and mitochondrial membrane function in response to glucose are regulated in part by a group of UCPs. Members of this family of inner mitochondrial membrane proteins function as anion carriers. The originally identified uncoupling protein, now classified as UCP1, is expressed exclusively in brown adipocytes and can disperse the mitochondrial proton gradient, bypassing the production of ATP and resulting in the generation of heat (14,15). Recent studies have demonstrated that UCPs can prevent mitochondrial ROS formation. The different members of the UCP family have distinct tissue distributions (14,16–19). Tissue localization as well as regulation confer to different roles of the family members. The function of UCP1 is believed to be the generation of body heat as response to cold exposure (14,18). The genes encoding for UCP2 and UCP3 are expressed in numerous tissues, with UCP3 expression occurring predominantly in skeletal muscle but also in neurons (20) and UCP2 being ubiquitously expressed in spleen, lung, stomach, white adipose tissue, neuronal tissue, endothelial cells, and others (17,21–23).

Recently, it has been shown that an adenovirus-mediated overexpression of UCP1 and UCP3 in vitro prevents neurons from glucose-induced degeneration by preventing mitochondrial hyperpolarization and formation of ROS (20).

Several polymorphisms in the promoter regions of the different *UCP* genes are known (24–27). Of these, G-866A and C-55T polymorphisms in *UCP2* and *UCP3*, respectively, have been shown to

From the Division of Endocrinology and Metabolism, Department of Medicine, University of Heidelberg, Heidelberg, Germany.

Address correspondence and reprint requests to Andreas Hamann, MD, Division of Endocrinology and Metabolism, Department of Medicine, Im Neuenheimer Feld 410, D-69120 Heidelberg, Germany. E-mail: andreas_hamann@med.uni-heidelberg.de.

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Abbreviations: AGE, advanced glycation end product; ROS, reactive oxygen species; UCP, uncoupling protein.

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

Table 1—*Characteristics of patients with the different genotypes for the* UCP1, UCP2, *and* UCP3 *gene*

	UCP1				
	AA	AG	GG	AG and GG	*P* value
n	130	85	12	97	
Diabetes duration (years)	19.5 ± 2.1	19.3 ± 2.6	14.4 ± 4.7	18.7 ± 2.4	0.65*
Female rate (%)	49.2 ± 8.6	50.6 ± 10.6	33.3 ± 26.7	48.5 ± 9.9	1.00†
Age (years)	43.0 ± 2.0	44.4 ± 2.8	40.2 ± 5.4	43.8 ± 2.5	0.70*
Nicotine use (%)	28.9 ± 7.9	20.0 ± 8.5	16.7 ± 21.4	19.7 ± 7.9	0.26†
Hypertension (%)	32.3 ± 8.0	29.4 ± 9.7	33.3 ± 26.7	29.9 ± 9.1	0.77†
A1C (%)	7.0 ± 0.3	7.1 ± 0.3	6.7 ± 0.6	7.0 ± 0.3	0.97*
Cholesterol (mg/dl)	194 ± 6	193 ± 9	183 ± 17	192 ± 8	0.49*
Triglycerides (mg/dl)	87 ± 9	93 ± 15	115 ± 30	96 ± 16	0.70*
BMI (kg/m^2)	25.3 ± 0.6	25.1 ± 0.9	23.9 ± 1.2	25.0 ± 0.8	0.15*
Neuropathy (%)	30.8 ± 7.9	22.4 ± 8.9	33.3 ± 26.7	23.7 ± 8.5	0.29†
Nephropathy (%)	22.3 ± 7.2	21.2 ± 8.7	25.0 ± 24.5	21.6 ± 8.2	1.00†
Retinopathy (%)	30.0 ± 7.9	27.1 ± 9.4	16.7 ± 21.4	25.8 ± 8.7	0.55†

Data are % or means ± asymptotic 95% CIs. *P* values were calculated for the comparison of wild-type carriers and the carriers of the hetero- and homozygous variants as described under RESEARCH DESIGN AND METHODS. *Mann-Whitney *U* test; †Fisher's exact test.

be associated with increased mRNA levels (24–26). To confirm the UCP hypothesis and to verify its importance in vivo, we studied a possible association of variants in the genes encoding UCP1, UCP2, and UCP3 with nephropathy, retinopathy, and neuropathy in patients with type 1 diabetes.

RESEARCH DESIGN AND METHODS

— The study group consisted of 227 Caucasian subjects with type 1 diabetes. Patients were consecutively enrolled between January 1998 and October 2002 from the outpatient clinic of the Department of Endocrinology (University of Heidelberg, Heidelberg, Germany). Because the study was performed as a monocentric study, care was taken to avoid any carriers of the polymorphisms studied being obviously related to each other. Therefore, the patients taking part were questioned whether they had family members with diabetes treated in the recruiting center. These patients were excluded from the study.

Diagnostic criteria

Diabetic nephropathy was defined as microalbuminuria of >20 mg/l in two or three samples of morning urine obtained within 12 months (24–26). Patients with urinary infections were excluded. The absence or presence of neuropathy was defined according to Diabetes Control and Complication Trial criteria (28). Neuropathy was diagnosed by a decreased or missing ankle reflex and symptoms, by reduced vibration sensitivity with symptoms, or by neuropathic foot ulcer. Symptoms were defined as numbness, dysesthesia or paresthesia, hypersensitivity to touch, burning pain, aching, or stabbing pain in the feet. Diabetic retinopathy was defined by ophthalmoscopic examination performed by ophthalmologists with special interest in diabetic retinal disease. Patients were not stratified according to the severity of diabetes complications.

Genotyping

Genomic DNA was prepared from peripheral blood using the QIAmp Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The regions encompassing the three different polymorphic sites were amplified by PCR as previously described (27,28,30). PCR products were digested using the restriction enzymes *Bcl*I, *Mlu*I, and *Bsa*JI for the polymorphisms *UCP1* A-3826G, *UCP2* G-866A, and *UCP3* C-55T, respectively.

Statistical analysis

Because of the small numbers of subjects with homozygous genotypes of each *UCP* variant, they were combined with the heterozygous genotype for statistical analysis. Group characteristics are presented by *UCP* genes with sample size, means, and 95% CIs for continuous data. For binary data, sample size, rate, and 95% CIs are given. This description was for the investigation of homogeneity of groups. All *P* values in Table 1 were for exploratory use only. Pairwise linkage disequilibrium was examined with Haploview software (http://www.broad.mit.edu/personal/jcbarret/haploview/). Haplotype analyses combining the G-866A and the C-55T polymorphisms of the *UCP2* and *UCP3* genes were performed with the partition ligation-expectation maximization algorithm (31). Haplotype distributions were compared between groups by a likelihood-ratio test. The three primary outcomes (neuropathy, retinopathy, and nephropathy) were analyzed with multiple regression analysis. Covariates were included for adjustment of potential confounding (age [years], sex, diabetes duration [years], BMI [weight in kilograms divided by the square of height in meters], HbA_{1c} [A1C] [percent]). Because of the three primary outcomes, the α-level was divided; 1.67% for each test was used to guarantee a global 5% error rate. SPSS release 11.0 was used.

RESULTS

— The study was performed as a monocentric cross-sectional pilot study, in which 227 nonrelated Caucasian patients with type 1 diabetes were analyzed (Table 1).

First, the A-3826G polymorphism in the promoter of *UCP1* was analyzed: 130 patients (57.3%) were homozygous for the wild-type allele (AA), 85 patients (37.4%) were heterozygous for the polymorphism (AG), and 12 (5.3%) were homozygous for the polymorphism (GG). According to Hardy-Weinberg equilibrium, an allele frequency of 0.76 for the wild-type A allele and 0.24 for the G allele was calculated. Neither allele nor genotype frequencies were significantly different in men or women. Further, the genotype groups we compared did not differ with respect to the baseline clinical

Table 1—*Continued*

UCP2					*UCP3*				
GG	GA	AA	GA and AA	*P* value	CC	CT	TT	CT and TT	*P* value
93	99	35	134		144	74	9	83	
19.7 ± 2.7	19.0 ± 2.2	18.4 ± 4.2	18.8 ± 1.9	0.72*	20.8 ± 2.0	12.2 ± 2.8	19.1 ± 7.7	17.7 ± 2.6	0.06*
45.2 ± 10.1	51.5 ± 9.8	51.4 ± 16.5	51.5 ± 8.5	0.42†	52.1 ± 8.2	44.3 ± 11.3	44.4 ± 32.5	44.6 ± 10.7	0.33†
44.6 ± 2.4	43.4 ± 2.4	40.1 ± 4.1	42.5 ± 2.1	0.20*	44.6 ± 2.0	41.6 ± 2.9	42.2 ± 6.4	41.3 ± 2.6	0.06*
24.2 ± 8.7	23.2 ± 8.3	28.6 ± 15.0	25.6 ± 7.4	1.00†	27.6 ± 7.4	21.4 ± 9.3	44.4 ± 32.5	24.1 ± 8.7	0.43†
35.5 ± 9.7	28.3 ± 9.5	33.3 ± 15.6	28.4 ± 7.6	0.31†	33.8 ± 7.8	25.7 ± 10.0	33.3 ± 30.8	27.7 ± 9.6	0.38†
7.0 ± 0.3	6.9 ± 0.3	7.3 ± 0.6	7.0 ± 0.3	0.93*	6.8 ± 0.2	7.3 ± 0.4	7.0 ± 0.9	7.3 ± 0.4	0.08*
196 ± 8	191 ± 7	192 ± 11	191 ± 6	0.51*	192 ± 6	194 ± 9	217 ± 16	197 ± 8	0.38*
93 ± 14	85 ± 13	99 ± 20	89 ± 11	0.65*	84 ± 9	102 ± 20	120 ± 63	103 ± 18	0.19*
25.5 ± 0.8	25.0 ± 0.6	24.9 ± 1.1	25.0 ± 0.5	0.42*	25.1 ± 0.5	25.3 ± 1.0	25.2 ± 2.2	25.4 ± 0.9	0.96*
37.6 ± 9.8	19.2 ± 7.8	25.7 ± 14.5	20.9 ± 6.9	0.007†	33.1 ± 7.7	20.3 ± 9.2	22.2 ± 27.1	20.5 ± 8.5	0.031†
25.8 ± 8.9	16.2 ± 7.3	28.6 ± 15.0	19.4 ± 6.7	0.26†	18.3 ± 6.4	25.7 ± 10.0	44.4 ± 32.5	28.9 ± 9.8	0.07†
26.9 ± 9.0	29.3 ± 9.0	28.6 ± 15.0	29.1 ± 7.7	0.77†	27.5 ± 7.3	30.0 ± 10.4	33.3 ± 30.8	30.1 ± 9.9	0.76†

characteristics shown in Table 1. In addition, no difference in genotype frequencies was found with respect to diabetes complications (Table 1).

Next the G-866A polymorphism in the promoter of *UCP2* was analyzed: 93 patients (41.0%) were homozygous for the wild-type allele (GG); 99 patients (43.6%) were heterozygous (GA) for the A allele, and 35 patients (15.4%) were homozygous for the A allele (AA) (allele frequency of 0.63 for the G allele and 0.37 for the A allele). Neither allele nor genotype frequencies were significantly different in men or women. Further, the genotype groups we compared did not differ with respect to the baseline clinical characteristics shown in Table 1. In addition, no difference in genotype frequencies was found with respect to diabetic nephropathy ($P = 0.26$) and diabetic retinopathy ($P = 0.77$). In contrast, a highly significant lower prevalence of diabetic neuropathy was seen for carriers of the GA and AA genotypes (odds ratio [OR] 0.44 [95% CI 0.24–0.79], $P = 0.007$) (Table 1).

When the C-55T polymorphism in the promoter of *UCP3* was analyzed, 144 patients (63.4%) were homozygous for the wild-type allele (CC), 74 patients (32.6%) were heterozygous for the T allele (CT), and 9 patients (4.0%) were homozygous for the T allele (TT) (allele frequency of 0.80 for the wild-type C allele and 0.20 for the T allele). Neither allele nor genotype frequencies were significantly different in men or women. Carriers of the CC genotype were slightly older (CC 44.6 ± 2.0 years, CT and TT 41.3 ± 2.6 years; $P = 0.06$) and had a longer diabetes duration (CC 20.8 ± 2.0 years, CT and TT 17.7 ± 2.6 years; $P = 0.06$), whereas CT and TT carriers had slightly worse diabetes control (CC 6.8 ± 0.2%, CT and TT 7.3 ± 0.4%; $P = 0.08$). As for the *UCP1* and *UCP3* polymorphisms, the genotype groups we compared did not differ with respect to the other baseline clinical characteristics shown in Table 1. In addition, no difference in genotype frequencies was found with respect to diabetic retinopathy ($P = 0.76$), whereas a weak trend for an increased prevalence of diabetic nephropathy in the CT and TT genotype carriers was found that, however, did not reveal statistical significance (CC 18.3 ± 6.4%, CT and TT 28.9 ± 9.8%; $P = 0.07$). In contrast, a significant lower prevalence for diabetic neuropathy was seen for the carriers of the CT and TT genotypes (OR 0.48 [95% CI 0.25–0.92], $P = 0.031$) (Table 1).

To analyze whether specific haplotypes were associated with diabetes complications in patients with type 1 diabetes, linkage disequilibrium and haplotype structure of G-866A and C-55T polymorphisms in *UCP2* and *UCP3* were studied. Calculations of the linkage disequilibrium showed a weak positive linkage disequilibrium between these polymorphisms ($D' = 0.36$, $r^2 = 0.058$, $\chi^2 = 12.81$, $P < 0.001$). The haplotype frequencies were estimated, and association analyses were performed with respect to each diabetes complication. These revealed no significant differences in the haplotype distribution among patients with or without diabetic nephropathy ($\chi^2 = 3.48$, $P = 0.35$), diabetic neuropathy ($\chi^2 = 5.86$, $P = 0.18$), or diabetic retinopathy ($\chi^2 = 0.99$, $P = 1.00$). Because GG and CC genotypes of the G-866A and the C-55T polymorphism were associated significantly with diabetic neuropathy, characteristics and diabetes complications for the G-C haplotype were compared with those for the other haplotypes. No significant differences were found among the groups we compared (data not shown).

Next, we asked whether the effect of the polymorphisms in the *UCP2* and *UCP3* genes on diabetic neuropathy is stronger when expressed in a cosegregatory manner. Therefore, two groups were formed. In the first group, all patients ($n = 70$) carrying only the wild-type allele of the G-866A polymorphism of *UCP2* and the C-55T polymorphism of *UCP3* were combined and compared with a second group of patients ($n = 60$) carrying heterozygous or homozygous alleles of both polymorphisms. The formation of the second group is shown in Table 2.

According to the baseline characteristics, patients carrying the wild-type alleles of both genes were significantly older

Table 2—*Formation of the* UCP2 *and* UCP3 *groups*

UCP2	*UCP3*	*n* (%)
GA	CT	37 (61.7)
AA	CT	16 (26.7)
AA	TT	4 (6.7)
GA	TT	3 (5.0)

Patients ($n = 60$) carrying the hetero- or homozygous alleles of the G-866A polymorphism of *UCP2* and the C-55T polymorphism of *UCP3* forming the combined UCP group (*UCP2* and *UCP3*) are shown. GA, heterozygous *UCP2*; AA, homozygous *UCP2*; CT, heterozygous *UCP3*; TT, homozygous *UCP3*.

Table 3—*Characteristics of patients in the combined genotype* UCP2 *and* UCP3 *group*

	GG and CC	GA/AA and CT/TT	*P* value
n	70	69	
Diabetes duration (years)	20.4 ± 3.1	18.1 ± 2.8	0.34*
Female rate (%)	50.0 ± 11.7	50.0 ± 12.7	1.00†
Age (years)	45.4 ± 2.9	41.3 ± 3.2	0.04*
Nicotine use (%)	23.1 ± 9.9	17.1 ± 9.5	0.60†
Hypertension (%)	35.7 ± 11.2	25.0 ± 11.0	0.25†
A1C (%)	6.7 ± 0.3	7.1 ± 0.4	0.25*
Cholesterol (mg/dl)	195.1 ± 8.3	196.1 ± 8.9	0.80*
Triglycerides (mg/dl)	83.6 ± 12.5	95.1 ± 20.5	0.52*
BMI (kg/m^2)	25.3 ± 0.7	25.2 ± 0.8	0.65*
Neuropathy (%)	41.4 ± 11.5	16.7 ± 9.4	0.002†
Nephropathy (%)	20.0 ± 9.4	23.3 ± 10.7	0.67†
Retinopathy (%)	25.7 ± 10.2	30.0 ± 11.6	0.70†

Data are % or means ± asymptotic 95% CIs. *Mann-Whitney *U*-test; †Fisher's exact test.

than patients with polymorphisms (−866GG and −55CC 45.4 ± 2.9 years, −866GA/AA and −55CT/TT 41.3 ± 3.2 years; $P = 0.04$) (Table 3). The other baseline clinical characteristics did not differ among the genotype groups we compared. In addition, no difference in genotype frequencies was found with respect to diabetic nephropathy ($P = 0.67$) and diabetic retinopathy ($P = 0.70$). However, the occurrence of both polymorphisms in *UCP2* and *UCP3* together was associated with a highly significant lower prevalence of diabetic neuropathy compared with wild-type carriers for *UCP2* and *UCP3* (OR 0.28, [95% CI 0.12–0.65], $P = 0.002$) (Table 3).

Because the combined groups differed significantly with respect to patient's age, a multiple logistic regression model was performed to exclude any influence of age and diabetes duration. There was no association either with nephropathy or with retinopathy (data not shown). The multiple logistic regression model demonstrated an independent association of the presence of the cosegregated alleles with diabetic neuropathy in patients with type 1 diabetes ($P = 0.013$) (Table 4).

CONCLUSIONS — Our results show a significantly reduced risk for diabetic neuropathy in carriers who were heterozygous or homozygous for the A allele of the G-866A polymorphism in the *UCP2* gene and for the T allele of the C-55T polymorphism in the *UCP3* gene. The calculated linkage disequilibrium points only to a weak association between the G-866A polymorphism of the *UCP2* gene and the C-55T polymorphism of the *UCP3* gene. Further, because of the rather low linkage disequilibrium, the haplotype analysis showed no association of any tested haplotype with diabetes complications. This suggests that the two polymorphisms are inherited almost independently of each other. When we studied an additional effect of a combined genotype, the effect was even stronger when the polymorphisms for the *UCP2* and *UCP3* genes were cosegregated. This finding argues for an additive effect, which is independent of age, diabetes duration, sex, and A1C. In contrast, no association of the A-3826G polymorphism in the *UCP1* gene with diabetic neuropathy was observed, most probably due to the fact that UCP1 expression is restricted to brown adipose tissue and absent in neuronal tissue (14,18).

Hyperglycemia-induced superoxide overproduction by the mitochondrial electron transport chain is regarded as an integrator of the various metabolic changes contributing to the development of diabetic neuropathy. A high mitochondrial membrane potential results in increased half-life of the superoxide (O_2^-)-producing electron transport systems. The importance of O_2^- has been demonstrated in cultured endothelial cells transfected to overexpress the enzyme manganese superoxide dismutase. As superoxide dismutase reduces mitochondrial O_2^- to H_2O_2, overexpression of manganese superoxide dismutase blocked the hyperglycemia-dependent O_2^- release (6,32). A similar inhibition was observed by overexpression of UCP1 and UCP3, causing uncoupling of the mitochondrial respiratory chain and subsequent collapse of the voltage gradient (6,20,32). The results presented here support the concept that hyperglycemia-mediated mitochondrial dysfunction, leading to increased radical generation, might play an important role in the development of diabetic neuropathy and provide evidence for the first time that increased expression of UCP might protect against neuronal destruction not only in vitro but also in vivo (20).

Our findings are supported by functional promoter studies for polymorphisms in *UCP2* and *UCP3* genes, which demonstrated their functional significance by showing enhanced transcriptional activity and increased mRNA levels (24–26). Enhanced transcription might provide a higher expression of UCP2 and UCP3 in the inner mitochondrial membrane and thereby prevent or reduce the hyperglycemia-induced depolarization of the inner mitochondrial membrane of neurons, normally seen in states of chronic diabetes (33).

Recent data suggest that *UCP2* and *UCP3* polymorphisms might contribute to obesity (24). In our study, none of the investigated polymorphisms were associated with significant differences in the body weight of the different genotype car-

Table 4—*Multiple regression analysis assessing the independent association of the combined polymorphism of the* UCP2 *and* UCP3 *genes and the presence of diabetic neuropathy in patients with type 1 diabetes*

	Standard coefficient	SE	*P* value
Sex	−1.402	0.071	0.164
A1C	0.219	0.033	0.827
Age	3.418	0.003	0.001
BMI	2.691	0.012	0.008
Diabetes duration	2.791	0.003	0.006
Combined genotype	−2.536	0.036	0.013

Negative values of the standard coefficient mean an inverse association with diabetic neuropathy.

riers. Although there exists strong evidence that some of the known *UCP2* polymorphisms are associated with obesity (34), data for the G-866A polymorphism are contradictory. In the first reports of this polymorphism, an influence on body weight was described (24,25), whereas in several following studies, this finding was not confirmed (35–38). For the C-55T polymorphism in the promoter of the *UCP3* gene, several reports described a significant lower BMI for carriers of the TT genotype (29,39). However, other studies did not find an association, suggesting that the influence on BMI might depend on the ethnic background of the study population (26,40). Up to now, association of polymorphisms in the *UCP* genes were not studied in patients with type 1 diabetes. In the study performed here, we did not see an association with body weight. However, larger studies with different ethnic groups are needed to finally answer this question. Remarkably, knockout models for *UCP2* or *UCP3* could not confirm a strong influence on body weight regulation (41–44).

One might suggest that the interpretation of our study is limited because the patients carrying a variant allele in *UCP2* and *UCP3* in the combined *UCP2* and *UCP3* group were slightly younger than the wild-type carriers (GG and CC 42.5 to 48.3 years; GA/AA and CT/TT 38.1 to 44.5 years). However, next to age, glucose control and duration of disease are known to be important contributors to diabetic neuropathy (27). These influencing variables are distributed equally in the groups of the combined genotype analysis that we compared. Multiple regression analysis identified the polymorphisms as an independent risk factor for diabetic neuropathy. Finally, analysis of each polymorphism alone showed an equal distribution of these risk factors, suggesting that the age distribution in the combined genotype group is unlikely to confound the results.

We cannot fully exclude the possibility that the G-866A polymorphism in the *UCP2* gene and the C-55T polymorphism in the *UCP3* gene are in linkage disequilibrium with an unidentified causative variant in a gene close to the localization of both genes on chromosome 11q13. However, the data presented here indicate that variants in *UCP2* and *UCP3* are associated with a reduced risk for diabetic neuropathy in type 1 diabetic patients.

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