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 UCP2 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.
UCPs in diabetic neuropathy

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

<table>
<thead>
<tr>
<th>UCP1</th>
<th></th>
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<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td>AG and GG</td>
<td>p value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>130</td>
<td>85</td>
<td>12</td>
<td>97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female rate (%)</td>
<td>19.5 ± 2.1</td>
<td>19.3 ± 2.6</td>
<td>14.4 ± 4.7</td>
<td>18.7 ± 2.4</td>
<td>0.65*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>43.0 ± 2.0</td>
<td>44.4 ± 2.8</td>
<td>40.2 ± 5.4</td>
<td>43.8 ± 2.5</td>
<td>0.70*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nicotine use (%)</td>
<td>28.9 ± 7.9</td>
<td>20.0 ± 8.5</td>
<td>16.7 ± 21.4</td>
<td>19.7 ± 7.9</td>
<td>0.26†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>32.3 ± 8.0</td>
<td>29.4 ± 9.7</td>
<td>33.3 ± 26.7</td>
<td>29.9 ± 9.1</td>
<td>0.77†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1C (%)</td>
<td>7.0 ± 0.3</td>
<td>7.1 ± 0.3</td>
<td>6.7 ± 0.6</td>
<td>7.0 ± 0.3</td>
<td>0.97*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>194 ± 6</td>
<td>193 ± 9</td>
<td>183 ± 17</td>
<td>192 ± 8</td>
<td>0.49*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>87 ± 9</td>
<td>93 ± 15</td>
<td>115 ± 30</td>
<td>96 ± 16</td>
<td>0.70*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.3 ± 0.6</td>
<td>25.1 ± 0.9</td>
<td>23.9 ± 12</td>
<td>25.0 ± 0.8</td>
<td>0.15*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neurropathy (%)</td>
<td>30.8 ± 7.9</td>
<td>22.4 ± 8.9</td>
<td>33.3 ± 26.7</td>
<td>23.7 ± 8.5</td>
<td>0.29†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nephropathy (%)</td>
<td>22.3 ± 7.2</td>
<td>21.2 ± 8.7</td>
<td>25.0 ± 24.5</td>
<td>21.6 ± 8.2</td>
<td>1.00†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retinopathy (%)</td>
<td>30.0 ± 7.9</td>
<td>27.1 ± 9.4</td>
<td>16.7 ± 21.4</td>
<td>25.8 ± 8.7</td>
<td>0.55†</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 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). Neurorathy 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 ophtalmoscopic 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 Bcll, MluI, and BsaJI 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 were 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 Haplovieview software (http://www.broad.mit.edu/personal/jcbarret/haplovieview/). 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], HbA₁c [%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 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
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 wild-type allele (CC), and 35 patients (15.4%) were homozygous for the wild-type allele (AA). 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 characteristics shown in Table 1. In addition, no difference in genotype frequencies was found with respect to diabetic retinopathy (P = 0.06), or had a slightly different prevalence of diabetic nephropathy (P = 0.07). 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 43.1 ± 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, r2 = 0.058, χ2 = 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 (χ2 = 3.48, P = 0.35), diabetic neuropathy (χ2 = 5.86, P = 0.18), or diabetic retinopathy (χ2 = 0.99, P = 1.00). Because GG and CC genotypes of the G-866A and the C-55T polymorphisms 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

<table>
<thead>
<tr>
<th>UCP2</th>
<th>UCP3</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GA</td>
<td>CT</td>
<td>37 (61.7)</td>
</tr>
<tr>
<td>AA</td>
<td>CT</td>
<td>16 (26.7)</td>
</tr>
<tr>
<td>AA</td>
<td>TT</td>
<td>4 (6.7)</td>
</tr>
<tr>
<td>GA</td>
<td>TT</td>
<td>3 (5.0)</td>
</tr>
</tbody>
</table>

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.
UCPs in diabetic neuropathy

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

<table>
<thead>
<tr>
<th>Combined genotype</th>
<th>n</th>
<th>Diabetes duration (years)</th>
<th>Female rate (%)</th>
<th>Age (years)</th>
<th>Nicotine use (%)</th>
<th>Hypertension (%)</th>
<th>A1C (%)</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>BMI (kg/m²)</th>
<th>Hypertension (%)</th>
<th>Retinopathy (%)</th>
<th>Nephropathy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG and CC</td>
<td>70</td>
<td>20.4 ± 3.1</td>
<td>50.0 ± 11.7</td>
<td>45.4 ± 2.9</td>
<td>23.1 ± 9.9</td>
<td>35.7 ± 11.2</td>
<td>6.7 ± 0.3</td>
<td>195.1 ± 8.3</td>
<td>83.6 ± 12.5</td>
<td>25.3 ± 0.7</td>
<td>41.4 ± 11.5</td>
<td>20.0 ± 9.4</td>
<td>25.7 ± 10.2</td>
</tr>
<tr>
<td>GA/AA and CT/TT</td>
<td>69</td>
<td>18.1 ± 2.8</td>
<td>50.0 ± 12.7</td>
<td>41.3 ± 3.2</td>
<td>17.1 ± 9.5</td>
<td>25.0 ± 11.0</td>
<td>7.1 ± 0.4</td>
<td>196.1 ± 8.9</td>
<td>95.1 ± 20.5</td>
<td>25.2 ± 0.8</td>
<td>16.7 ± 9.4</td>
<td>23.3 ± 10.7</td>
<td>30.0 ± 11.6</td>
</tr>
</tbody>
</table>

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 (O₂⁻) producing electron transport systems. The importance of O₂⁻ has been demonstrated in cultured endothelial cells transfected to overexpress the enzyme manganese superoxide dismutase. As superoxide dismutase reduces mitochondrial O₂⁻ to H₂O₂, overexpression of manganese superoxide dismutase blocked the hyperglycemia-dependent O₂⁻ 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-
neuropathy in type 1 diabetic patients. However, the data presented here indicated a reduced risk for diabetic neuropathy. A polymorphism in a gene close to the localization of both genes on chromosome 11q13. UCP3 is reported to be important contributors to diabetic neuropathy. The G-866A polymorphism in the promoter of the UCP3 gene, several reports described a significant lower BMI of 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. 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. 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.

References

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