The UCP1 Gene Polymorphism A-3826G in Relation to DM2 and Body Composition in Czech Population

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Key words
- type 2 diabetes mellitus
- uncoupling protein 1
- A-3826G polymorphism
- insulin sensitivity
- obesity in humans

Abstract

Mitochondrial uncoupling contributes to the control of energy expenditure. The brown fat specific uncoupling protein 1 (UCP1) mRNA was detected in intraperitoneal and extraperitoneal adipose tissue in adult humans. The A-3826G polymorphism in the UCP1 gene promoter region was found to be associated with reduced mRNA expression indicating that the polymorphism is of functional importance.

Objective: To determine allelic frequencies and genotypic distribution of the A-3826G polymorphism and to study its possible association with anthropometric parameters and biochemical markers of glucose and lipid metabolism in type 2 diabetes mellitus (DM2) patients (n = 295), in offsprings of DM2 patients (n = 113), and in healthy adults without family history of DM2 (n = 120).

Results and Discussion: In the whole cohort of 528 subjects, the G allele was observed with a frequency of 0.26. Genotypic distribution did not differ between diabetics and controls. However, in the offsprings of DM2 patients, significantly higher BMI and a trend towards higher waist to hip ratio, waist to height ratio, waist circumference, and subcutaneous fat mass was observed in the AG genotype compared with the wild-type. Similar tendency was evident in the control group. This indicates possible involvement of the A-3826G polymorphism in the regulation of body composition.

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Abbreviations

UCP	uncoupling protein
DM2	type 2 diabetes mellitus
BMI	body mass index
WHR	waist to hip ratio
WHeR	waist to height ratio
DI	disposition index
G_0	fasting serum blood glucose
I_0	fasting serum insulin
oGTT	oral glucose tolerance test
ivITT	intravenous insulin tolerance test
PCR	polymerase chain reaction
RFLP	restriction fragment length polymorphism.

Introduction

The gene for mitochondrial uncoupling protein 1 (UCP1) is expressed in brown fat (Cinti et al., 1989). It belongs to the family of mitochondrial carrier proteins (Cassard et al., 1990). By decreasing the electrochemical gradient over the inner mitochondrial membrane, UCP1 uncouples oxidative phosphorylation, which leads to the production of heat instead of energy storage in the form of ATP (Klingenberg et al., 1990). The thermogenic function of brown fat tissue is under the control of catecholamines acting through adrenoreceptors. As a consequence of norepinephrine binding to the adipocyte plasma membrane, lipolysis is stimulated and non-esterified fatty acids are released. These fatty acids serve as a substrate in the process of brown fat thermogenesis, but they also act as the cytosolic second messengers activating UCP1 (Locke et al., 1982). Although a controversy exists concerning the physiological significance of brown adipose tissue in adults and its possible contribution to body weight control, genetic analysis of various human cohorts suggested a participation of UCP1 in fat tissue content regulation (Fumeron et al., 1996; Heilbronn et al., 2000; Clement et al., 1996; Oppert et al., 1994; Esterbauer et al., 1998). There is a large amount of evidence that brown adi-

pocytes are present and activated in human adults in common nonpathologic and certain pathologic situations (Garruti and Ricquier, 1992; Lean et al., 1986; Ricquier et al., 1982; Zancanaro et al., 1994; Hany et al., 2002; Minotti et al., 2004; van Marken Lichtenbelt et al., 2003; Yang et al, 2003). Hence, disturbed UCP1 function as a result of nucleotide substitution in the regulatory promoter region could represent one of the mechanisms contributing to the complex control of adiposity in humans. A tight functional relationship between central obesity and type 2 diabetes mellitus (DM2) is well established. Therefore, the aim of our study was to determine allelic frequencies and genotypic distribution of the A-3826G polymorphism in a group of Czech adult DM2 patients, offspring of DM2 patients, and in Czech adult healthy non-relative population without family history of DM2. The possible association of the allelic variants with anthropometric parameters and with biochemical markers of glucose tolerance, insulin sensitivity, lipid metabolism and other biochemical features was studied.

Study Subjects and Methods

We analysed the frequency of the A-3826G polymorphism in 528 adult (older than 20 years) subjects. This cohort included healthy adult individuals without family history of DM2 (n = 120, M/F = 42/78; age = 32.5 ± 11.0 years, BMI = 23.3 ± 3.8 kg/m^2), DM2 patients (n = 295, M/F = 112/183; age = 58.8 ± 7.0 years, BMI = 30.5 ± 5.5 kg/m^2), and healthy direct offspring of type 2 diabetics (n = 113, M/F = 41/72; age = 38.2 ± 10.4 years, BMI = 25.5 ± 4.2 kg/m^2). These offspring were healthy volunteers who had diabetic one or both parents and were not relatives of DM2 patients participating in our study. DM2 patients were diagnosed by criteria of the World Health Organization in the Institute of Endocrinology, Prague. All of them were well compensated either by diet (41.6%), or by diet and peroral antidiabetic drugs (53.6%), or by insulin (18.6%). The study protocols were in accordance with institutional ethic guidelines and national rules and all the subjects gave their written informed consent to participate in the study.

Anthropometric data were obtained in the fasting state. Body weight, height, waist and hip circumferences were measured in all participants in order to calculate body mass index (BMI) and to evaluate visceral fat accumulation by means of waist circumference, waist to hip ratio (WHR) and waist to height ratio (WHeR). Furthermore, 14 skinfolds were measured in all offspring and controls and in subgroup of the diabetics. Body composition (% of subcutaneous fat mass, % of muscle mass, and % of bone mass from the total body weight) was then calculated using the ANTROPO program (Bláha, 1991).

After an overnight fast, a venous blood samples were obtained in order to determine biochemical parameters. Glucose metabolism was characterized by blood glucose (G_0), glycosylated hemoglobin, glycosylated proteins, proinsulin, C-peptide, and, in probands not on insulin therapy, also by immunoreactive insulin (I_0). Lipid profile was assesed by total cholesterol, high-density lipoprotein cholesterol, triglyceride, and low-density lipoprotein cholesterol concentrations. For more detailed subjects characterisation, the determination of leptin, growth hormone, cortisol, sex hormone binding globulin, dehydroepiandrosterone, TSH, free T3, and free T4 were performed. The 24-hour volume of urine was collected and epinephrine, norepinephrine, dopamine, vanillylmandelic acid, homovanillic acid, and hydroxyindoleacetic acid were extracted from these samples and their concentrations were evaluated. The group of offspring and controls underwent the three-hour oral glucose tolerance test (oGTT) with 75 g of glucose load and intravenous insulin tolerance test (ivITT) according to Young et al. (Young et al., 1996). To assess insulin sensitivity and β-cell function of the subjects, we used the homeostasis models HOMA R and HOMA F assessment (Matthews et al., 1985) (HOMA R = $I_0{}^*G_0/22.5$, HOMA F = $20{}^*I_0/(G_0\text{-}3.5)$). Moreover, disposition index (DI) was calculated as follows (Kahn et al., 1993): DI = HOMA F$^*(G_0/I_0)$.

For detection of the A-3826G polymorphism, DNA was extracted from peripheral leucocytes and genotype for the two restriction variants by PCR-RFLP method. PCR amplification of the segment with the A-3826G polymorphism site was carried out in a volume of 12 μl, containing 20 ng of genomic DNA, 2.9 pmol of each primer, 2.5 mM $MgCl_2$, 2 mM dNTPs (Takara), 10× PCR Buffer received together with Taq DNA polymerase (Gold 5 U/μl, Perkin Elmer); 0.18 units of the enzyme was used.

The PCR conditions were: denaturation at 94 °C for 12 min followed by 35 cycles of denaturation for 20 sec, annealing at 62 °C for 30 sec, extension at 72 °C for 1 min and final extension at 72 °C for 10 min. Following primers were used (Valve et al., 1998): forward primer 5′-CCA GTG GTG GCT AAT GAG AGAA-3′ and reverse primer 5′-GCA CAA AGA AGA AGC AGA GAGG-3′.

The substitution of G for A abolishes a Bcl I restriction site. RFLPs were detected after 5 h digestion in 55 °C with 3 U of enzyme. The A allele gives two fragments (157 bp and 122 bp) whereas the G allele gives one 279 bp fragment.

Statistical data treatment: The χ^2-test was used to assess differences in genotypic distribution between the group of DM2 patients and controls and between the group of offspring and controls (**Table 1**). As the number of GG homozygotes was not high enough for separate statistical analysis, they were added to heterozygotes and G-allele carriers and non-carriers were compared. Odds ratio and the 95% confidence intervals were calculated to evaluate the risk of DM2 for the G-allele carriers. For evaluation of the relations between G-allele presence and anthropometric as well as biochemical characteristics, the non parametric Mann-Whitney robust test was used in individual subgroups of subjects with different diabetes status. The differences were considered statistically significant if p-level < 0.05. Two-tailed p-level values are reported (**Table 2**). In addition, the offspring and control group were distributed into subgroups based on quartiles of BMI, WHR, waist circumference, WHeR, and also sc. fat mass content, separately males and females. Then, with respect to the quartile distribution, both genders were analysed together according to genotypic frequencies in the particular quartile subgroups (**Table 3**).

The statistical analyses were performed using NCSS 2001 software.

Table 1 Allelic Distribution and Frequency of the UCP1 gene A-3826G Polymorphism in Controls, Offspring, and DM2 Patients

Group	A-3826A	A-3826G	G-3826G	G allele freq.
Controls	61 (50.83%)	49 (40.83%)	10 (8.33%)	0.29
Offspring	60 (53.10%)	51 (45.13%)	2 (1.77%)	0.24
Diabetics	157 (53.22%)	124 (42.03%)	14 (4.75%)	0.26
Total	278 (52.65%)	224 (42.42%)	26 (4.92%)	0.26

Table 2 Relationships between Anthropometric Characteristics and G-allele Presence

	women							men						
	AA			AG or GG			p	AA			AG or GG			p
	n	mean ± SD	median	n	mean ± SD	median		n	mean ± SD	median	n	mean ± SD	median	
controls														
BMI(kg/m^2)	40	22.9 ± 3.8	22.3	36	24.1 ± 4.6	22.9	0.202	21	23.1 ± 2.6	22.6	20	23.0 ± 3.4	23.3	0.825
WHR	40	0.73 ± 0.07	0.72	36	0.76 ± 0.07	0.74	0.073	21	0.82 ± 0.05	0.82	20	0.83 ± 0.05	0.82	0.473
WheR	40	0.43 ± 0.06	0.41	36	0.45 ± 0.07	0.44	0.085	21	0.44 ± 0.04	0.43	20	0.45 ± 0.05	0.46	0.315
Waist(cm)	40	72.1 ± 9.5	69.8	36	75.8 ± 10.2	72.7	0.092	21	80.1 ± 8.0	79.0	20	81.9 ± 8.7	83.5	0.382
Sc.Fat(%)	40	24.2 ± 7.3	23.8	36	25.3 ± 8.3	24.0	0.578	21	13.9 ± 4.9	13.4	20	17.8 ± 7.5	17.6	0.076
offspring														
BMI(kg/m^2)	41	23.9 ± 3.7	22.8	31	26.0 ± 4.2	26.2	**0.022**	19	24.9 ± 3.3	25.9	20	28.5 ± 4.3	28.1	0.014
WHR	41	0.76 ± 0.06	0.75	31	0.77 ± 0.07	0.76	0.474	18	0.87 ± 0.07	0.86	20	0.93 ± 0.08	0.95	0.027
WheR	41	0.46 ± 0.06	0.45	31	0.48 ± 0.07	0.47	0.086	18	0.48 ± 0.06	0.48	20	0.54 ± 0.08	0.55	0.015
Waist(cm)	41	76.3 ± 9.9	74.1	31	80.3 ± 11.5	79.6	0.109	18	87.8 ± 11.0	86.8	20	95.9 ± 13.3	96.8	0.045
Sc.Fat(%)	40	25.1 ± 7.5	24.4	30	26.6 ± 6.6	26.6	0.238	17	16.2 ± 6.7	13.9	18	21.7 ± 7.8	19.7	0.018
diabetics														
BMI(kg/m^2)	97	31.4 ± 5.0	31.0	80	31.3 ± 7.2	30.3	0.392	53	28.7 ± 3.8	28.4	56	29.7 ± 4.4	29.5	0.169
WHR	99	0.91 ± 0.08	0.89	80	0.85 ± 0.08	0.87	**0.000**	53	0.96 ± 0.08	0.96	55	0.97 ± 0.06	0.98	0.450
WHeR	97	0.65 ± 0.08	0.63	80	0.62 ± 0.10	0.61	**0.049**	53	0.60 ± 0.06	0.59	55	0.61 ± 0.08	0.61	0.193
Waist(cm)	99	104.5 ± 12.6	105.0	80	100.8 ± 14.5	100.0	0.055	53	104.1 ± 10.5	103.0	55	106.7 ± 13.0	106.0	0.155
Sc.Fat(%)	6	26.5 ± 4.2	26.1	6	33.4 ± 5.6	33.9	0.066	8	17.4 ± 5.8	17.6	10	19.3 ± 6.7	19.1	0.564

Results

The allelic frequency of the A-3826G polymorphism was assessed in the whole cohort of participants. The Hardy-Weinberg expectations were fulfilled in each group. The genotypic distribution was not significantly different between the diabetic and the control group ($\chi^2 = 2.02$; $p = 0.36$) nor between the offspring and the control group ($\chi^2 = 5.17$; $p = 0.07$), see **Table 1**. The occurrence of the G allele was not associated with increased risk of DM2.

Relationships between phenotypic features and the G-allele carriership in the particular groups and genders shows the **Table 2**. In the control group, slightly higher medians in almost all tested anthropometric parameters (BMI, WHR, WHeR, waist circumference, and subcutaneous fat mass) are apparent in the G-allele carriers, both in males and females. Analyses of biochemical markers of glucose and lipid metabolism revealed no association with the polymorphism in this group.

In a group of offspring of DM2 patients, significantly higher BMI was observed in the G-allele carriers compared with the wild-type, both in males and females. Furthermore, higher WHR, WHeR, waist circumference and subcutaneous fat mass was evident in the G-allele carriers in both genders. In men these differences were significant. No biochemical markers showed an association with the polymorphism in this group.

In diabetic patients, similarly to controls and offspring, no genotype-related association with any screened biochemical parameter was observed. However, in diabetic women, lower WHR and WHeR were found in the G-allele carriers compared with diabetic women with the AA genotype. The differences in waist circumference and subcutaneous fat mass were also evident. No such a tendency was observed in diabetic men.

To widen the analysis of the G-allele association with body composition, controls and offspring were subdivided into subgroups based on quartiles of BMI, on quartiles of WHR, WHeR, and also on quartiles of waist circumference and sc. fat mass content. Then, with respect to the different quartile distribution between genders, men and women were analysed together. Percentage of the G-allele carriers were examined and compared in these quartile subgroups, see **Table 3**. BMI higher than the 3rd quartile was the most frequent in the G-allele carriers, whereas BMI lower than the 1st quartile was abundant in the AA genotype. Similar genotypic distribution was observed when WHR, waist circumference, WHeR, and sc. fat content was analysed, all χ^2-statistics were significant or, in case of sc. fat content, of borderline significance.

Discussion

Recently, new discoveries about the significance of brown fat have sparked interest in this organ as a potential tool in the fight against obesity in adult humans (Avram et al., 2005). UCP1 protein, exclusively expressed in brown adipocytes, is the mediator of thermogenesis in response to adrenergic stimulation.

This study provided data on the relationship between the UCP1 gene A-3826G promoter region polymorphism and body composition in Czech population. We determined the allelic frequencies and genotypic distributions in 528 Czech probands, 295 of whom were DM2 patients, 113 were direct offspring of DM2 patients, and 120 were healthy controls without family history of DM2. The allelic frequency of the polymorphism in the

Table 3 G-carriership in Relation to Quartile Subgroups of BMI, WHR, Waist Circumference, Waist to Height Ratio, and Sc. Fat Mass in Nondiabetic Subjects

	G-carriership	<1st quartile	1st-2nd quartile	2nd-3rd quartile	>3rd quartile	
BMI (kg/m^2)	AA	17.5%	13.6%	11.4%	10.5%	χ^2 = 10.32
	AG or GG	7.5%	11.8%	13.6%	14.0%	p = **0.016**
WHR	AA	15.9%	15.0%	13.2%	8.9%	χ^2 = 10.62
	AG or GG	8.8%	10.1%	12.3%	15.8%	p = **0.014**
Waist (cm)	AA	15.0%	15.9%	13.2%	8.7%	χ^2 = 9.97
	AG or GG	9.7%	9.7%	11.9%	16.0%	p = **0.019**
WHeR	AA	16.7%	16.3%	11.0%	8.8%	χ^2 = 16.30
	AG or GG	7.9%	9.3%	14.1%	15.9%	p = **0.001**
Sc. Fat (%)	AA	16.2%	14.0%	13.5%	9.5%	χ^2 = 7.79
	AG or GG	8.6%	11.7%	11.7%	14.9%	p = **0.050**

whole cohort of participants was similar to other Caucasian populations (Oppert et al., 1994; Schaffler et al., 1999). We investigated possible association of the polymorphism with markers of glucose and lipid metabolism and with susceptibility to DM2. In literature, the G variant has been associated with reduced UCP1 mRNA expression indicating that the polymorphism is of functional importance (Esterbauer et al., 1998). In agreement with this finding, several trials showed that obese women with the G variant were more likely to gain weight over time (Clement et al., 1996; Oppert et al., 1994) and less likely to lose weight (Fumeron et al., 1996). In one study conducted on Australian overweight women, the G variant was reported to increase the susceptibility to obesity (Heilbronn et al., 2000). Furthermore, a greater frequency of the G variant was found in subjects with DM2 and an association was observed between fasting glucose level and the G variant in women with DM2, independently of increased BMI (Heilbronn et al., 2000). On the other hand, some studies did not confirm the association with insulin resistance and DM2 (Schaffler et al., 1999). Thus, the functional impact of this substitution and its association with DM2 and obesity remains an open question deserving further investigation.

Genotypic distribution in our groups of probands demonstrated that the substitution is probably not associated with increased DM2 risk in the Czech population. However, in nondiabetic subjects, G-allele carriers occurred more frequently in the quartile groups with highest levels of BMI, WHR, waist circumference, and WHeR. In support of this finding, in offspring of diabetics higher BMI was observed both in males and females carrying the G-allele compared to the wild-type. Also other anthropometric parameters (WHR, WHeR, waist circumference, subcutaneous fat mass) tended to be higher in offspring carrying the G-allele in comparison with the wild-type. When the G-allele carriers were compared with the wild-type in the group of controls, slightly higher values in anthropometric data were also evident. On the other hand, the G-allele carriers in the group of diabetics do not show higher values in the measured anthropometric parameters. This might be, at least in part, a consequence of drug treatment. A majority of drug-treated diabetics in our group was treated by derivatives of sulfonylurea (66%). It is well known that this treatment can influence body composition (UKPDS 33, 1998). Thus, it is likely that pharmacological intervention interfere with the eventual small effect of the G allele on body mass regulation.

The present findings should be interpreted within the context of their limitations. Our study population was anthropometrically and biochemically well characterized but, for genetic studies, it was relatively small when organised into three subgroups analysed separately: diabetics, offspring of diabetics, and controls. Despite this known limitation, we have demonstrated that the G-allele carriers of the UCP1 gene promoter polymorphism A-3826G are the most frequent in quartile groups with the highest BMI, WHR, waist circumference, and WHeR in the offspring and control group. Furthermore, the G-allele carriers have significantly higher body mass index than the wild-type in offspring group. This could indicate the possible impact of the polymorphism on body mass regulation. This effect is more apparent in individuals exposed to genetic load predisposing to DM2.

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