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A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male non-diabetic Pima Indians

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OBJECTIVE: UCP2 and UCP3 are newly discovered uncoupling proteins, which are thought to underlie the variability in energy metabolism in humans. Mutations in the *UCP2* and/or *UCP3* gene have been associated with sleeping metabolic rate. Recently we reported that skeletal muscle UCP3 mRNA expression was positively correlated with sleeping metabolic rate in Pima Indians. To study whether genetic variation in the promoter region of *UCP3* contributed to the variation in expression of UCP3, we screened part of the proximal promoter region for polymorphisms.

METHODS: In the first part of the study, the proximal promoter region of UCP3 was screened by direct sequencing in 24 non-diabetic Pima Indians (range body mass index (BMI): 18–47 kg/m^2) (Schrauwen *et al*. *Diabetes* 1999; 48: 146–149) and skeletal muscle UCP3 mRNA expression was measured by RT-PCR. In the second part of the study, we typed the polymorphism found in the first part of the study in 67 Pima Indians (32 males, 35 females) from the upper and lower extremes of the BMI distribution.

RESULTS: We identified a novel C to T substitution in the UCP3 promoter, 6bp upstream of the putative TATA signal, and 55 bp upstream of the transcription starting site. Among 18 male subjects, skeletal muscle UCP3 mRNA expression was significantly higher in the C/T & T/T group compared to the C/C homozygotes ($P < 0.02$). However, in the group of 67 Pima Indians genotype frequencies were not different in the obese and lean groups.

CONCLUSION: We identified a novel polymorphism in the proximal promoter region of UCP3, which was associated with increased skeletal muscle expression of UCP3 in male non-diabetic Pima Indians. Considering the suggested role of UCP3 in energy metabolism, this polymorphism might be of physiological importance in the regulation of energy balance.

Keywords: UCP3 promoter; obesity; genotype

Introduction

The interest in the study of energy metabolism has recently been renewed by the discovery of two new mitochondrial proteins, UCP2 and UCP3, which exhibit uncoupling activity.[1,2] UCP2 is widely expressed,[1] with abundant expression in adipose tissue and skeletal muscle, while UCP3 is predominantly expressed in skeletal muscle.[3] UCP3 is expressed equally (ratio 1:1) as a long and a short form, the latter lacking exon 7, which results in a truncated protein.[3] These uncoupling proteins are likely candidates to underlie the variability in energy metabolism in humans and may thus be involved in the development of obesity.

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UCP2 maps to chromosome 11q13[1] and *UCP3* is thought to be only 8 kb away from *UCP2*.[4] Urhammer *et al*[5] reported a common polymorphism in the *UCP2* gene. This polymorphism, a single nucleotide substitution, which results in an alanine to a valine amino acid substitution at position 55, was not associated with body mass index (BMI), fat mass or waist-to-hip ratio (WHR) in Danish Caucasians. Furthermore, Elbein *et al*[6] did not find linkage between markers in the 11q13 region and BMI in 42 North European families with type 2 diabetic siblings. However, in the Quebec Family study, resting metabolic rate (RMR) was genetically linked to DNA microsatellite markers in the vicinity of 11q13.[7] Despite the lack of genetic linkage of the UCP2/UCP3 region in Pima Indians,[8] we recently reported an association between polymorphisms in *UCP2* and *UCP3* and sleeping metabolic rate in this population.[4] Furthermore, we recently found that the skeletal muscle expression of UCP3 mRNA was positively correlated to resting metabolic rate, in male Pima Indians.[9]

To study whether genetic variation in the promoter region of *UCP3* contributes to the variation in expression of UCP3 and is associated with BMI, we screened part of the proximal promoter region for polymorphisms in Pima Indians.

Methods

In the first part of this study, the proximal promoter region of *UCP3* was screened in 24 non-diabetic Pima Indians (18 males, 6 females), in whom we had previously measured skeletal muscle UCP3 expression.[9] Subjects had a range in BMI from 18 to 47 kg/m^2 (mean $\pm$ s.e.m.: 34 $\pm$ 1.4). In the second part of the study, 67 non-diabetic, full blooded Pima Indians (32 males, 35 females) were selected from the extremes in BMI distribution (30 lean, 37 obese). All subjects were in good health as determined by physical examination and routine blood and urine tests. All subjects were clinically euthyroid and serum thyroid stimulating hormone concentrations were within the normal range. None took prescribed or over-the-counter medications. Glucose tolerance was assessed by an oral glucose tolerance test according to the WHO criteria,[10] and insulin concentrations were also measured (Concept 4, ICN, Forsham, PA). This study was approved by the ethics committee of the National Institute of Diabetes and Digestive and Kidney Diseases and by the Tribal Council of the Gila River Indian Community, and all subjects gave written informed consent prior to participation.

Amplification of the UCP3 promoter upstream ($\sim$1 kb) and part of exon 1 was performed by PCR in a 25 µl volume containing 1.5 mmol/l $MgCl_2$, 0.25 units of Amplitaq DNA polymerase (Perkin Elmer, Foster, CA), and 5 pmol of the oligonucleotide primers, sense 5′-CATGACTTCCCTGAGATTCC and antisense 5′-CTCCCTAGGGCTCCATCC. After 35 cycles, PCR products were purified by the PCR purification kit (Qiagen, Valencia, CA). Product concentration was estimated on a 1% agarose gel. $\sim$200 ng DNA template was used for cycle sequencing following the instruction (Applied Biosystems, Foster City, CA) with the sense primer 5′-CCTATCGTGGGAGGCCTTG, the above mentioned antisense primer and dichlororhodamine dye mix. After removal of excess dye terminators with the Microspin G-50 column (Pharmacia Biotech, Piscataway, New Jersey), samples were electrophoresed and analysed on an ABI Prism 377 automated sequencer (PE Applied Biosystems, Foster, CA). The expression of UCP3 mRNA was determined as previously described.[9]

Results

We identified a previously undescribed C to T substitution in the UCP3 promoter, 6 bp upstream of the putative TATA signal, and 55 bp upstream of the

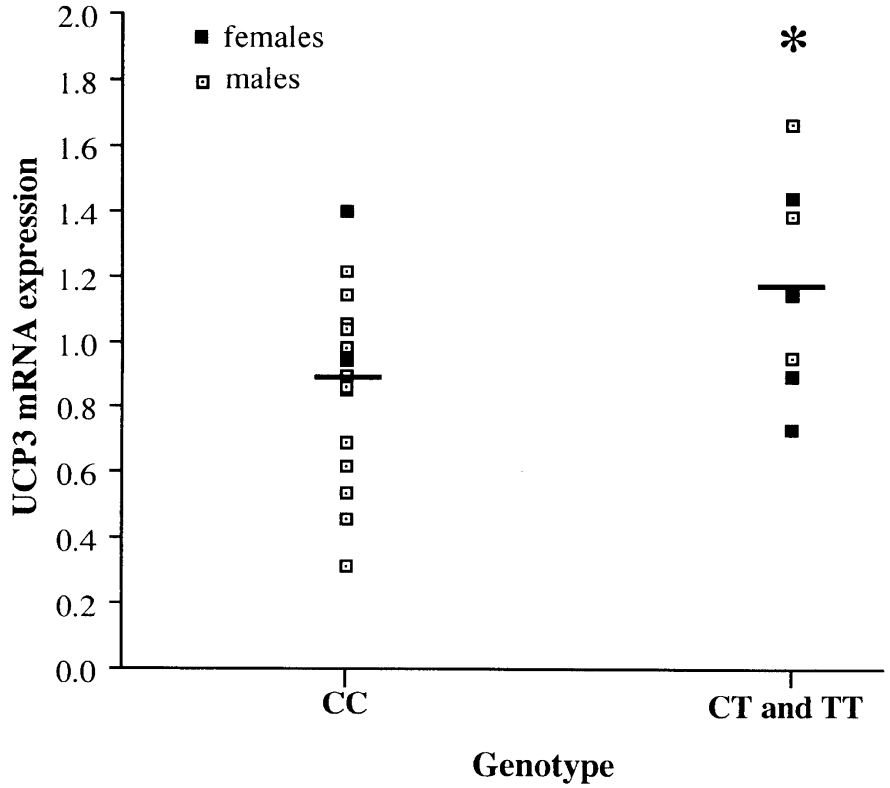

Figure 1 Skeletal muscle UCP3 mRNA expression in different genotypes of a UCP3 promoter variant.
* = $P < 0.05$ compared to CC.

transcription starting site, among the 24 subjects. One was homozygous for the polymorphism (T/T), 6 heterozygous and 17 homozygous for C/C. Because there was only one T/T homozygote, we compared UCP3 mRNA expression between the C/C group and the C/T & T/T group.

In the 18 male subjects, UCP3 mRNA expression was significantly higher in the C/T and T/T group compared to the C/C homozygotes (Figure 1), 1.33 ± 0.21 *vs* 0.84 ± 0.07, $P = 0.012$). When the six female subjects were included UCP3 mRNA expression was still significantly higher in the C/T & T/T group compared to the C/C homozygotes but the *P*-value dropped (Figure 1, 1.17 ± 0.13 *vs* 0.88 ± 0.07, $P < 0.04$), possibly implying a gender effect. However, the expression of UCP3 mRNA was not significantly different between males and females and the small number of female subjects does not allow conclusions on the effect of the polymorphism on UCP3 mRNA expression in female subjects.

To establish whether this polymorphism was associated with BMI, we typed this polymorphism in 67 Pima Indians (32 males, 35 females) from the upper (BMI: $48.1 \pm 5.3\,kg/m^2$) and lower (BMI: $24.2 \pm 1.5\,kg/m^2$) extremes of the BMI distribution. The polymorphism did not alter any restriction site and therefore genotyping was performed by direct sequencing in all subjects. In the lean subjects, the allele frequency was 88.3% for C and 11.7% for T, whereas in the obese subjects the allele frequency was 78.4% for C and 21.6% for T (NS). The overall allele frequency in this population was 83% for C and 17% for the T allele. Genotype frequencies were 76.7% for CC, 23.3% for CT and 0% for TT in the lean subjects and 64.9% for CC, 27.0% for CT and 8.1% for TT in the obese subjects (Table 1). To investigate whether the allele frequency of this population was different from Caucasians, we determined allele frequency of the promoter polymorphism in 56 CEPH Caucasians. Allele frequency was 75% for the C allele and 25% for the T allele (NS).

Discussion

The recent discovery of UCP2 and UCP3 in various tissues has renewed the interest in human energy metabolism. Uncoupling proteins uncouple oxygen consumption from ATP production and therefore stimulate the dissipation of energy as heat. In the present study we examined whether genetic variation in the proximal promoter region of *UCP3* contributed to the variability in the expression of UCP3 in Pima Indians. Mutation screening revealed one novel polymorphism, a C to T conversion, located 6 bp upstream of the TATA signal, and 55 bp upstream of the transcription initiation site. Although no known transcription factor binding sites exist in this location, the location of this polymorphism in the proximal promoter region and its proximity to the putative TATA signal is interesting and warrants further study.

The main goal of the present study was to examine whether genetic variation in the *UCP3* promoter region contributed to variation in UCP3 expression. We therefore screened the promoter region of the *UCP3* gene for this novel polymorphism in 24 nondiabetic Pima Indians, in whom we had previously measured UCP3 mRNA expression. On average, UCP3 mRNA expression was 0.96 ± 0.07 (ratio with β-actin) with a wide range from $0.31 - 1.66$. The C to T mutation in the proximal promoter region of *UCP3* was significantly associated with UCP3 mRNA expression, at least in the male subjects. In the male subjects, those with the T allele had, on average, 58% higher expression of UCP3 in skeletal muscle compared to those subjects without the T allele. The number of female subjects was too limited to make conclusion whether this effect was gender specific. These results, together with the location of the mutation, are promising and suggest a role for this polymorphism in the regulation of *UCP3* gene expression in humans.

Recently, we showed that the mRNA expression of UCP3 was positively correlated with sleeping metabolic rate in Pima Indians,[9] suggesting that a low UCP3 mRNA expression may contribute to a low sleeping metabolic rate. Since a low relative resting metabolic rate is a predisposing factor for weight gain,[11] it was expected that individuals with low *UCP3* gene expression would eventually have higher body weight. Indeed, we also found a negative correlation between BMI and *UCP3* gene expression.[9] To further investigate the role of the UCP3 promoter polymorphism, we selected 67 Pima Indians with extremes in BMI and examined, by direct sequencing, whether the allele frequency of the C to T mutation was different between the lean and obese groups. No significant differences in allele frequency between the two groups were found. This might indicate that the effect of the C to T mutation on UCP3 mRNA expression is minor and not translated in an effect on BMI. However, since obesity is a highly heterogeneous trait even strong effects of polymorphisms on UCP3 mRNA expression would not necessarily result in extreme differences in BMI. The selection of Pima Indians with extremes in BMI may have resulted in a selection of subjects where major genes are respon-

Table 1 Geneotype frequency of a UCP3 promoter variant in obese (BMI: $48.1 \pm 5.3\,kg/m^2$) and lean subjects ($24.2 \pm 1.5\,kg/m^2$)

	Genotype	*n*	*Lean*	*Obese*
Males	CC	18	9	9
	CT	12	6	6
	TT	2	0	2
Females	CC	29	14	15
	CT	5	1	4
	TT	1	0	1

BMI = body mass index.

sible for the lean or obese phenotype. Therefore, further studies are required to test whether this polymorphism is associated with BMI in other populations or with other traits, like sleeping metabolic rate, which are more likely to be directly influenced by UCP3 mRNA expression. Finally, it should be kept in mind that future studies are needed to reveal whether differences in UCP3 mRNA are translated into differences in UCP3 protein content.

Conclusion

In conclusion, we identified a novel polymorphism in the proximal promoter region of UCP3, near the putative TATA signal. The C to T mutation was associated with increased expression of UCP3 in skeletal muscle of male non-diabetic Pima Indians. Considering the potential role of UCP3 in energy metabolism, this polymorphism might be of physiological importance in the regulation of energy balance.

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