

## **The frequent UCP2 -866G>A polymorphism protects against insulin resistance and is associated with obesity**

A study of obesity and related metabolic traits among 17,636 Danes

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## ORIGINAL ARTICLE

# The frequent *UCP2* –866G>A polymorphism protects against insulin resistance and is associated with obesity: a study of obesity and related metabolic traits among 17 636 Danes

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**CONTEXT:** Uncoupling protein 2 (UCP2) is involved in regulating ATP synthesis, generation of reactive oxygen species and glucose-stimulated insulin secretion in  $\beta$ -cells. Polymorphisms in *UCP2* may be associated with obesity and type 2 diabetes mellitus.

**OBJECTIVE:** To determine the influence of a functional *UCP2* promoter polymorphism (–866G>A, rs659366) on obesity, type 2 diabetes and intermediary metabolic traits. Furthermore, to include these and previously published data in a meta-analysis of this variant with respect to its impact on obesity and type 2 diabetes.

**DESIGN:** We genotyped *UCP2* rs659366 in a total of 17 636 Danish individuals and established case-control studies of obese and non-obese subjects and of type 2 diabetic and glucose-tolerant subjects. Meta-analyses were made in own data set and in publicly available data sets. Quantitative traits relevant for obesity and type 2 diabetes were analysed within separate study populations.

**RESULTS:** We found no consistent associations between the *UCP2* –866G-allele and obesity or type 2 diabetes. Yet, a meta-analysis of data from 12 984 subjects showed an association with obesity (GA vs GG odds ratio (OR) (95% confidence interval (CI)): 0.894(0.826–0.968)  $P=0.00562$ , and AA vs GG OR(95% CI): 0.892(0.800–0.996),  $P=0.0415$ . Moreover, a meta-analysis for type 2 diabetes of 15 107 individuals showed no association. The –866G-allele was associated with elevated fasting serum insulin levels ( $P=0.002$ ) and HOMA insulin resistance index ( $P=0.0007$ ). Insulin sensitivity measured during intravenous glucose tolerance test in young Caucasian subjects ( $n=377$ ) was decreased in carriers of the GG genotype ( $P=0.05$ ).

**CONCLUSIONS:** The *UCP2* –866G-allele is associated with decreased insulin sensitivity in Danish subjects and is associated with obesity in a combined meta-analysis.

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**Keywords:** diabetes; insulin sensitivity; genetics; uncoupling protein 2; –866G/A polymorphism; rs659366

## INTRODUCTION

Uncoupling protein 2 (UCP2) is a mitochondrial transporter that uncouples oxidative phosphorylation via induced proton leak from the inner mitochondrial membrane (reviewed in Dalgaard and Pedersen<sup>1</sup> and Dalgaard<sup>2</sup>). It is ubiquitously expressed<sup>3,4</sup> and overexpression of UCP2 is reported to inhibit glucose-stimulated insulin secretion in pancreatic rat islets<sup>5</sup> and INS-1  $\beta$ -cells.<sup>6</sup> *Ucp2*<sup>(–/–)</sup> mice have higher pancreatic islet ATP levels, increased glucose-stimulated insulin secretion and are protected against glucose toxicity in  $\beta$ -cells in some,<sup>7,8</sup> but not all reports,<sup>9</sup> whereas on a high-fat diet they showed increased insulin secretion and decreased plasma triglyceride concentrations compared with wild-type mice.<sup>10</sup> However, no effect of *Ucp2* gene disruption on obesity was observed, even upon a high-fat diet;<sup>11</sup> whereas,

short-term inhibition of *Ucp2* using antisense oligonucleotides ameliorated both insulin resistance as well as improved insulin secretion in a diet-induced mouse model.<sup>12</sup> In comparison, missense mutations of *UCP2* were identified in two families in which congenital hyperinsulinaemia occurred in young children.<sup>13</sup> Each of the two families carried their own mutations, which segregated with the disease and which changed amino acids conserved between species, and functional studies supported a lack of function of mutated UCP2 proteins. Unfortunately, no phenotype information was given on adult members of these two families. Thus, there is clear evidence that UCP2 ablation modulates insulin secretion in mice, and suggestive evidence in humans. This raises the relevant question whether there is an effect of common genetic variation in the *Ucp2* gene in human subjects.

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A frequent -866G>A polymorphism (rs659366) has been identified in *UCP2*.<sup>14</sup> It was found to be located in the core promoter<sup>15</sup> in a region with putative binding sites for two  $\beta$ -cell transcription factors. The -866A-allele has been reported to associate with both decreased and increased adipose tissue *Ucp2* mRNA levels;<sup>14,16</sup> however, reporter constructs with the -866A-allele show increased activity in adipocytes<sup>14</sup> and in  $\beta$ -cells.<sup>17</sup> It is therefore most likely that the -866A-allele causes increased promoter activity and increases *Ucp2* mRNA levels.

The GG genotype was shown to associate with an increased risk of obesity among 596 and 791 white Europeans.<sup>14</sup> No consensus has been achieved regarding the association between -866G>A and adiposity; contrasting the original observation,<sup>14</sup> a range of smaller or equally sized studies reported no association with levels of body mass index (BMI) or waist-to-hip ratio.<sup>15,18-27</sup> However, among 2695 healthy, British men, the AA genotype was more prevalent among obese participants,<sup>28</sup> and a haplotype containing the -866G-allele showed association with childhood obesity.<sup>29</sup> Also, a modestly increased 'fat BMI' was reported in Danish obese men carrying the A-allele.<sup>30</sup> No association with juvenile-onset obesity was observed.<sup>22,31</sup> Therefore, it is still not clear whether this variant is associated with obesity; in order to determine this, larger studies and collective meta-analyses are needed.

A number of studies have examined the relationship of -866G>A with components of the metabolic syndrome, such as hyperglycaemia, obesity, hypertension and dyslipidaemia (reviewed in Chan and Harper<sup>32</sup> and Fisler and Warden<sup>33</sup>). Assuming that a more subtle intermediary obesity-related phenotype is affected by the -866G>A polymorphism, a number of interesting observations have been made; among 681 French type 2 diabetic patients the *UCP2* G-allele was associated with elevated triglyceride and total cholesterol concentrations and increased risk of dyslipidaemia<sup>34</sup> and decreased HDL (high-density lipoprotein)-cholesterol levels were reported among 658 Korean women carrying the A-allele.<sup>25</sup> Contrary, a lack of association with lipid levels has also been reported.<sup>18-20,23</sup>

For type 2 diabetes the general notion is also unclear, as reports have been made of association of the -866A-allele with increased<sup>20,35,36</sup> and decreased<sup>16,23,37,38</sup> risk of type 2 diabetes as well as no association at all.<sup>24,34,39</sup> An early onset of type 2 diabetes has been related to both the A-allele<sup>17,36</sup> and the G-allele<sup>37</sup> and an early requirement for insulin treatment has been observed among A-allele carriers.<sup>17,34</sup> Lower basal insulin secretion was initially reported among A-allele carriers,<sup>14</sup> but was contrasted by subsequent studies<sup>15,19-21,23</sup> where no association was found. The A-allele was related to reduced glucose-stimulated first-phase insulin secretion among 137 Japanese type 2 diabetic patients<sup>17</sup> and in isolated pancreatic islets from non-diabetic subjects,<sup>19</sup> which is in accordance with the A-allele causing increased promoter activity and presumably also increased *UCP2* protein levels. Also, observations of a lower disposition index have been made,<sup>19,35</sup> although this could be induced by changes in insulin sensitivity rather than insulin secretory capacity. Indeed, increased insulin resistance assessed by a hyperinsulinaemic-euglycaemic clamp or an IVGTT (intravenous glucose tolerance test) among A-allele carriers has been reported in some<sup>20,35</sup> but not all<sup>19,21,29</sup> studies. A recent meta-analysis of the -866G/A polymorphism for association with type 2 diabetes mellitus concluded that this variant does not confer increased risk of diabetes.<sup>40</sup>

Thus, this variant has been extensively characterized in a number of small-scale studies with variable outcomes and it is therefore highly relevant to perform studies in larger, yet still well-characterized, study groups in order to establish the role of the *UCP2* -866G>A polymorphism. Many of these early studies were underpowered compared with their identified effect sizes and some are likely to be false-positive reports. Therefore, using data from a total of 17 636 Danes, we aim in the present study to

clarify the relationship of the *UCP2* -866G>A polymorphism with a range of metabolic traits, type 2 diabetes and obesity. In addition, we carried out meta-analyses of obesity and type 2 diabetes by combining our data with previously published and publicly available data. Our conclusions from this study are that the G-allele of the -866 polymorphism is associated with obesity in one of our study groups and in a combined meta-analysis, whereas this variant is not associated with type 2 diabetes either in our study groups or in the combined meta-analysis. Furthermore, the G-allele of this variant is consistently associated with increased insulin resistance.

## MATERIALS AND METHODS

### Participants

The *UCP2* rs659366 polymorphism was genotyped in 17 636 Danes comprising (1) the population-based Inter99 sample of middle-aged Danes sampled at Research Centre for Prevention and Health ( $n=6162$ , clinical trial reg. no. NCT00289237),<sup>41</sup> (2) type 2 diabetic patients sampled through the out-patient clinic at Steno Diabetes Center ( $n=1720$ ), (3) a population-based group of middle-aged glucose-tolerant subjects recruited from Steno Diabetes Center ( $n=733$ ), (4) the ADDITION study group sampled through the Department of General Practice at University of Aarhus ( $n=8644$ , clinical trial reg. no. NCT00237548), which is a population-based, high-risk screening and intervention study for type 2 diabetes in general practice<sup>42</sup> and (5) a population-based sample of young, healthy Danish Caucasians recruited from Research Centre for Prevention and Health ( $n=377$ ). Clinical characteristics have been described.<sup>43,44</sup> A part of study sample 3 (32%) was also investigated in relation to rs659366 in a previous publication.<sup>15</sup>

Study groups 1 and 3 underwent a standard 75-g oral glucose tolerance test and study group 5 was examined using a frequently sampled tolbutamide-modified IVGTT.<sup>45</sup> Informed written consent was obtained from all subjects before participation. The study was approved by the Ethical Committee of Copenhagen County and was in accordance with the principles of the Helsinki Declaration. Type 2 diabetes and intermediary stages were defined according to the WHO criteria.<sup>46</sup>

For case-control studies of obesity, cases were defined as having a BMI  $\geq 30 \text{ kg m}^{-2}$  and control subjects as having a BMI  $< 25 \text{ kg m}^{-2}$ . Dyslipidaemia was defined as fasting serum triglycerides  $\geq 1.7 \text{ mmol l}^{-1}$  or HDL-cholesterol  $< 0.9 \text{ mmol l}^{-1}$  for men or  $< 1.0 \text{ mmol l}^{-1}$  for women and/or current or previous treatment with lipid-lowering drugs. Hypertension was defined as mean systolic blood pressure  $\geq 140 \text{ mm Hg}$  and/or mean diastolic blood pressure  $\geq 90 \text{ mm Hg}$ , and/or current or previous treatment with antihypertensive drugs.

### Biochemical and anthropometrical measurements

Height and body weight were measured in light indoor clothes and without shoes, and BMI was calculated as weight (kg)/(height (m))<sup>2</sup>. Waist circumference was measured in the standing position midway between the iliac crest and the lower costal margin and hip circumference at its maximum. Blood samples were drawn after a 12-h overnight fast. Plasma glucose was analysed by a glucose oxidase method (Granatest, Merck, Darmstadt, Germany). HbA<sub>1c</sub> was measured by ion-exchange high-performance liquid chromatography (normal reference range: 4.1-6.4%) and serum insulin (excluding des<sup>31,32</sup> and intact proinsulin) was measured using the AutoDELFIA insulin kit (Perkin-Elmer Wallac, Turku, Finland). Serum triglyceride and total- and HDL-cholesterol were analysed using enzymatic colorimetric methods (GPO-PAP and CHOD-PAP, Roche Molecular Biochemicals, Mannheim, Germany). HOMA-IR was calculated as fasting plasma glucose ( $\text{mmol l}^{-1}$ ) multiplied by fasting serum insulin ( $\text{pmol l}^{-1}$ ) and divided by 22.5. BIGTT-S<sub>i</sub> was calculated as described.<sup>47</sup>

### Genotyping

The *UCP2* rs659366 polymorphism was genotyped using KASPar (KBioscience, Hoddesdon, UK). Discordance between 965 random duplicate samples included in the genotyping was 0% and the genotyping

success rate was 97%. All genotype groups obeyed Hardy-Weinberg equilibrium.

### Statistical analyses

Fisher's exact test was applied to examine differences in allele frequencies, and logistic regression with adjustment for age and sex was applied to examine differences in genotype distributions between affected and unaffected subjects. A general linear model was used to test quantitative variables for differences between genotype groups among non-diabetic and untreated subjects. Quantitative traits were analysed with age, sex and BMI as covariates, and BMI was corrected for age and sex. An additive genetic model was used to compare the effects of carrying 0, 1 or 2 G-alleles. Meta-analyses were performed as described<sup>48</sup> and only studies providing complete information on numbers of subjects of each genotype were eligible for inclusion. Population attributable risk (in %) was calculated as by Northridge.<sup>49</sup> Several of the investigated traits are correlated (obesity, type 2 diabetes, insulin resistance) and comparisons for these by genotype are therefore not completely independent. Significance values were not corrected for multiple testing, because independent study groups were investigated for replication. A *P*-value of less than 0.05 was considered significant. All analyses were performed using SPSS version 14.0 (IBM, New York, NY, USA) and RGui version 2.7.0 (<http://www.r-project.org/>, Institute for Statistics and Mathematics, Vienna, Austria).

## RESULTS

### Obesity case-control studies

The UCP2 -866G>A polymorphism was genotyped in 17 636 Danes, and we carried out case-control studies of obesity (BMI <25 kg m<sup>-2</sup> vs BMI ≥30 kg m<sup>-2</sup>) and WHO-defined type 2 diabetes. The case-control study of obesity was performed separately for the Steno/Inter99 and ADDITION study samples due to differences in ascertainment protocols, and in the Steno/Inter99 sample (*n* = 4700) we observed a nominal association with -866G>A (Table 1). The genotype distributions showed that the GG and GA genotypes were more prevalent in obese subjects of the Steno/Inter99 groups, indicating that the G-allele predisposes to obesity. However, the results from the ADDITION study (*n* = 4022) showed no evidence for association with obesity (Table 1).

### Type 2 diabetes mellitus case-control study

For type 2 diabetes, there were no differences in minor allele frequencies or genotype distributions between cases (*n* = 3338)

and controls (*n* = 4904) (Supplementary Table A). Moreover, when analysing the ADDITION cohort prospectively, genotype distributions and minor allele frequencies were similar among non-diabetic people who converted to type 2 diabetes after a 5-year follow-up (GG 67, GA 82, AA 18) and those who did not convert (GG 1330, GA 1660, AA 586). There was no difference in the age of onset of diabetes in the different genotype groups and additional exploratory association studies of dyslipidaemia and hypertension were all negative (data not shown).

### Meta-analysis for impact of UCP2 -866G>A on obesity and type 2 diabetes

We carried out a meta-analysis of the UCP2 -866G>A polymorphism in relation to obesity and type 2 diabetes using the data obtained from the present study along with available data from previously published reports (Figure 1). For type 2 diabetes, the included studies were highly heterogeneous (*P* = 1.4 × 10<sup>-5</sup>)—probably due to large ethnic differences<sup>20,21,23,35,38</sup>—and no overall association was observed (*P* = 0.3). Among studies of obesity, homogeneity was nearly obtained (*P* = 0.02) when excluding studies of Asian subjects,<sup>50,51</sup> which amounted to 4% of subjects. There was an overall association of the -866G>A polymorphism with obesity (*P* = 0.003) (Figure 1). The odds ratio in the meta-analysis was 0.89 for the GA or AA genotypes vs the GG risk genotype in a total of 12 984 subjects of European descent (GA vs GG odds ratio (OR) (95% confidence interval (CI)): 0.894(0.826–0.968), *P* = 0.00562, and AA vs GG OR(95% CI): 0.892(0.800–0.996), *P* = 0.0415). The population attributable risk for obesity related to these odds ratios was 0.8% for the GG genotype in the combined meta-analysis and 1.8% in the Steno/Inter99 study group. Thus, in line with our data from the Steno/Inter99 study group this meta-analysis confirms that the common G-allele is associated with obesity.

### Impact of -866G>A on quantitative traits

We analysed a range of quantitative metabolic traits in relation to -866G>A in the Inter99 study population (Table 2). Fasting plasma glucose and serum insulin were significantly higher among the GG genotype (*P* = 0.02 and *P* = 0.002). G-allele carriers were more insulin resistant as estimated by the homoeostasis model assessment (HOMA-IR) (*P* = 0.0007), which was confirmed by a decreased insulin sensitivity index estimate (BIGTT-S<sub>i</sub>, *P* = 0.03). In the sample of 377 young, healthy Caucasians who underwent a tolbutamide-modified IVGTT carriers of the G-allele had decreased insulin sensitivity (*P* = 0.05), although most prominently when using a recessive model (Table 3).

Although the GG genotype was significantly associated with obesity in the Inter99/Steno study group and in the meta-analysis, BMI according to genotype was not affected in the Inter99 or ADDITION study groups (Table 2 and Supplementary Table B). In the sample of 377 young, healthy Caucasians, fat percentage was lower in GG-carriers (mean ± s.d.: GG 22 ± 8%, GA 24 ± 7%, AA 23 ± 9%, *P* = 0.02), but BMI was unchanged (Table 3). For waist-to-hip ratio, waist circumference, fasting serum lipids and blood pressure we found no relationship with the -866G>A polymorphism (Tables 2 and 3 and Supplementary Table B). Analyses were made with adjustment for age and sex and both with and without adjustment for BMI, but this did not essentially change the results.

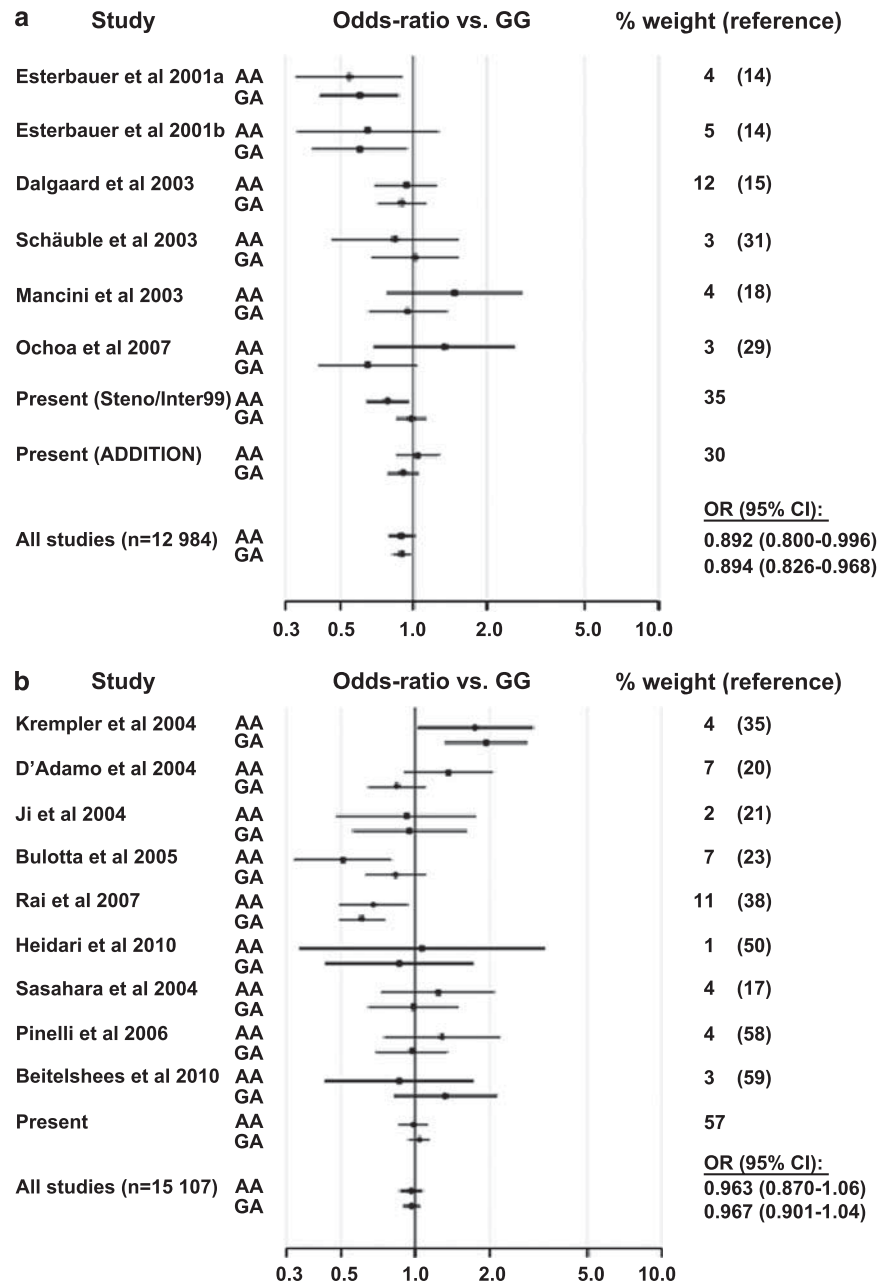
## DISCUSSION

Our main positive finding was the association of the UCP2 -866G>A polymorphism with insulin resistance among 5781 middle-aged Danes and in 377 young, healthy Danes, where subjects with the G-allele consistently had lower insulin sensitivity. The second positive finding was a decreased OR for obesity

**Table 1.** Genotype distribution and minor allele frequencies of the UCP2 -866G>A polymorphism for the participants stratified according to obesity

	BMI <25 kg m <sup>-2</sup>	BMI ≥30 kg m <sup>-2</sup>	P <sub>GD</sub>	P <sub>MAF</sub>
<i>Steno/Inter99</i>	<i>n</i> = 3153	<i>n</i> = 1547		
GG	1133 (36)	583 (38)	0.03	0.03
GA	1499 (48)	754 (49)		
AA	521 (17)	210 (14)		
MAF (95% CI)	40.3 (39.1–41.5)	37.9 (36.2–39.7)		
<i>ADDITION</i>	<i>n</i> = 1567	<i>n</i> = 2455		
GG	534 (34)	874 (36)	0.2	0.9
GA	799 (51)	1183 (48)		
AA	234 (15)	398 (16)		
MAF (95% CI)	40.4 (38.7–42.2)	40.3 (38.9–41.7)		

Abbreviations: BMI, body mass index; CI, confidence interval; GD, genotype distribution; MAF, minor allele frequency. Data are number of subjects with each genotype (% of each group) and MAF in percentages. All *P*-values were calculated using Fisher's exact test and comparing genotype distributions (*P*<sub>GD</sub>) and MAFs (*P*<sub>MAF</sub>). Also, logistic regression was applied with adjustment for age and sex; however, this did not change the results. All genotype groups obeyed Hardy-Weinberg equilibrium.



**Figure 1.** Meta-analysis of obesity (a) and type 2 diabetes (b). The meta-analysis of obesity uses data from references<sup>14,15,18,29,31</sup> in addition to data obtained in the present study. The meta-analysis of type 2 diabetes uses data from references<sup>17,20,21,23,35,38,50,58,59</sup> in addition to data from the present study.

of the UCP2 -866A-allele in a meta-analysis comprising 12 984 subjects. The population attributable risk for obesity for the combined meta-analysis was 0.8%, which is low but in the range previously found for obesity- or diabetes-associated gene variants.<sup>52</sup> However, when examining quantitative traits, BMI was not increased by this variant. Thus, although the variant was associated with obesity, its action may primarily be due to lower peripheral insulin sensitivity as this quantitative trait was consistently lower in GG genotype carriers.

Insulin resistance has been reported to be causally linked with oxidative stress,<sup>53</sup> and absence of UCP2 increases superoxide production<sup>8</sup> and causes oxidative stress.<sup>9</sup> Overexpression of UCP2 in cells decreases their nutrient-induced generation of reactive

oxygen species.<sup>54</sup> The widespread expression pattern of UCP2 makes possible a dual function in obesity (energy metabolism) and type 2 diabetes (glucose metabolism),<sup>55</sup> which is consistent with our findings that this variant is associated with decreased insulin sensitivity and obesity among G-allele carriers. The G-allele of the -866G>A polymorphism, based on its lower *cis*-acting capability, is predicted to be associated with increased reactive oxygen species levels and insulin resistance. Obesity increases UCP2 expression in various tissues,<sup>1</sup> and a possible mechanism for the observed association between the -866G-allele and both insulin resistance and obesity is that the obesity-induced increase of UCP2 mRNA could be lower for the -866G-allele compared with the A-allele, resulting in increased reactive



**Table 2.** Anthropometric and metabolic characteristics of 5781 middle-aged untreated Danes (Inter99) stratified according to *UCP2* -866G>A genotypes

	GG	GA	AA	$\beta$ (95% CI)	P
<i>n</i> (men/women)	2164 (1087/1077)	2694 (1331/1363)	923 (451/472)		
Age (years)	46 $\pm$ 8	46 $\pm$ 8	46 $\pm$ 8		
BMI ( $\text{kg m}^{-2}$ )	26.2 $\pm$ 4.4	26.2 $\pm$ 4.6	26.1 $\pm$ 4.6	-0.02 (-0.18; 0.15)	0.9
Waist circumference (cm)	86 $\pm$ 13	87 $\pm$ 13	86 $\pm$ 14	0.17 (-0.03; 0.36)	0.09
Waist-to-hip ratio	0.86 $\pm$ 0.08	0.86 $\pm$ 0.09	0.86 $\pm$ 0.09	0.001 (-0.001; 0.003)	0.2
<i>Plasma glucose (mmol l<sup>-1</sup>)</i>					
Fasting	5.6 $\pm$ 0.9	5.5 $\pm$ 0.8	5.5 $\pm$ 0.7	-0.03 (-0.06; -0.01)	0.02
30-min	8.8 $\pm$ 2.0	8.6 $\pm$ 1.8	8.7 $\pm$ 1.8	-0.05 (-0.11; 0.02)	0.2
120-min	6.3 $\pm$ 2.2	6.2 $\pm$ 2.1	6.2 $\pm$ 2.1	-0.04 (-0.11; 0.04)	0.3
AUC	223 $\pm$ 136	218 $\pm$ 135	220 $\pm$ 132	-1.67 (-6.46; 3.11)	0.5
<i>Serum insulin (pmol l<sup>-1</sup>)</i>					
Fasting	35 (25-53)	34 (23-50)	32 (23-49)	-3.0% (-4.9; -1.1)	0.002
30-min	247 (176-355)	246 (176-351)	236 (170-348)	-1.1% (-3.1; 0.8)	0.3
120-min	158 (101-259)	156 (95-254)	152 (90-251)	-4.0% (-6.7; -1.2)	0.005
AUC	18 870 (13 120-27 740)	18 680 (13 100-27 100)	18 270 (12 740-26 670)	-1.8% (-3.9; 0.4)	0.1
<i>Insulin resistance (mmol l<sup>-1</sup>-pmol l<sup>-1</sup>)</i>					
HOMA-IR	8.7 (5.8-13.4)	8.1 (5.5-12.7)	7.9 (5.4-12.2)	-3.5% (-5.5; -1.5)	0.0007
BIGTT-S <sub>i</sub>	9.1 $\pm$ 4.1	9.3 $\pm$ 4.1	9.5 $\pm$ 4.1	0.17 (0.02; 0.33)	0.03
<i>Fasting serum lipids (mmol l<sup>-1</sup>)</i>					
Triglyceride	1.1 (0.8-1.6)	1.1 (0.8-1.5)	1.0 (0.8-1.5)	-1.4% (-3.2; 0.4)	0.1
Total cholesterol	5.5 $\pm$ 1.1	5.5 $\pm$ 1.1	5.5 $\pm$ 1.1	-0.002 (-0.040; 0.035)	0.9
HDL-cholesterol	1.4 $\pm$ 0.4	1.4 $\pm$ 0.4	1.4 $\pm$ 0.4	-0.003 (-0.016; 0.010)	0.7

Abbreviations: AUC, area under the curve; BIGTT-S<sub>i</sub>, BIGTT-insulin sensitivity index; BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; HOMA-IR, homoeostasis model assessment of insulin resistance. Data are unadjusted mean  $\pm$  s.d. or medians (interquartile range). Values of serum insulin, values derived from insulin variables and values of serum triglyceride were logarithmically transformed before statistical analysis and their effect sizes ( $\beta$ ) are presented as the increase/decrease in percent. All analyses were made using an additive genetic model, adjusted for age, sex and BMI (BMI was adjusted for age and sex). HOMA-IR was calculated as fasting plasma glucose ( $\text{mmol l}^{-1}$ ) multiplied by fasting serum insulin ( $\text{pmol l}^{-1}$ ) and divided by 22.5. BIGTT-S<sub>i</sub> was calculated as described.<sup>47</sup>

**Table 3.** Anthropometric and metabolic characteristics of 317 young, healthy Danes stratified according to *UCP2* -866G>A genotypes

	GG	GA	AA	$\beta$ (95% CI)	P <sub>rec</sub>
<i>n</i> (men/women)	129 (72/57)	146 (71/75)	42 (23/19)		
Age (years)	25 $\pm$ 3	26 $\pm$ 3	25 $\pm$ 4		
BMI ( $\text{kg m}^{-2}$ )	23.4 $\pm$ 3.6	23.7 $\pm$ 3.4	24.5 $\pm$ 5.2	0.49 (-0.10; 1.09)	0.08
Waist circumference (cm)	78 $\pm$ 10	78 $\pm$ 10	80 $\pm$ 14	-0.10 (-0.86; 0.65)	0.6
Waist-to-hip ratio	0.82 $\pm$ 0.07	0.82 $\pm$ 0.06	0.82 $\pm$ 0.08	0.001 (-0.006; 0.009)	0.5
Fat mass (kg)	17 $\pm$ 8	17 $\pm$ 7	18 $\pm$ 11	-0.23 (-0.59; 0.13)	0.2
Fat percentage (%)	22 $\pm$ 8	24 $\pm$ 7	23 $\pm$ 9	-0.40 (-0.85; 0.04)	0.02
<i>Plasma glucose (mmol l<sup>-1</sup>)</i>					
Fasting	5.0 $\pm$ 0.4	5.0 $\pm$ 0.5	5.0 $\pm$ 0.6	0.01 (-0.06; 0.08)	0.8
<i>Serum insulin (pmol l<sup>-1</sup>)</i>					
Fasting	29 (23-41)	31 (24-48)	32 (25-52)	1.5% (-5.5; 8.5)	0.9
<i>Insulin action</i>					
Acute insulin response ( $\text{min pmol l}^{-1}$ )	1929 (1147-2601)	1959 (1278-2858)	1760 (1006-2630)	-5.5% (-16.7; 5.7)	0.1
Insulin sensitivity ( $10^{-5} \times (\text{min pmol l}^{-1})^{-1}$ )	13 (9-20)	13 (8-18)	14 (11-23)	2.4% (-6.6; 11.4)	0.05
Disposition index	28 040 (16 750-37 090)	24 540 (15 930-38 500)	29 400 (17 190-43 110)	-3.1% (-16.1; 10.0)	0.5
<i>Fasting serum lipids (mmol l<sup>-1</sup>)</i>					
Triglyceride	0.9 (0.7-1.2)	0.9 (0.7-1.2)	0.9 (0.7-1.3)	5.4% (-1.4; 12.2)	0.2
Total cholesterol	4.5 $\pm$ 0.9	4.5 $\pm$ 0.8	4.6 $\pm$ 1.1	0.01 (-0.13; 0.14)	0.7
HDL-cholesterol	1.2 $\pm$ 0.3	1.2 $\pm$ 0.3	1.1 $\pm$ 0.3	-0.01 (-0.05; 0.03)	0.7

Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; HOMA-IR, homoeostasis model assessment of insulin resistance. Data are unadjusted mean  $\pm$  s.d. or medians (interquartile range). Values of serum insulin, values derived from insulin variables and values of serum triglyceride were logarithmically transformed before statistical analysis and their effect sizes ( $\beta$ ) are presented as the increase/decrease in percent. All analyses were made using a recessive genetic model, adjusted for age, sex and BMI (BMI was adjusted for age and sex). HOMA-IR was calculated as fasting plasma glucose ( $\text{mmol l}^{-1}$ ) multiplied by fasting serum insulin ( $\text{pmol l}^{-1}$ ) and divided by 22.5. BIGTT-S<sub>i</sub> was calculated as described.<sup>47</sup> Acute insulin response was calculated as the area under the insulin curve from 0 to 8 min.

6 oxygen species generation and insulin resistance in G-allele carriers.

This promoter variant of *UCP2* has been extensively studied with respect to many different traits associated with obesity or type 2 diabetes; however, the current study is by far the largest performed to date. Although several studies have shown that this promoter variant changes reporter gene activity,<sup>14,17</sup> it may not be the (only) functional variant: The *UCP3-UCP2* genomic region was investigated for 14 SNPs (single-nucleotide polymorphisms) (including -866G>A) spanning the *UCP2* and *UCP3* loci among 3782 women of different ethnicities.<sup>56</sup> Although no single SNP was associated with type 2 diabetes after correction for multiple testing, haplotype analysis indicated an increased type 2 diabetes risk among 968 Caucasian women, and this effect was further accentuated by overweight although no association with BMI was observed. The four-SNP haplotype in question was in high linkage disequilibrium with the -866A-allele, suggesting that yet unidentified variation covered by the haplotype-spanned area may be responsible for the observed relationships of -866G>A with metabolic variables. The *UCP2* -866G>A polymorphism was also genotyped in the genome-wide association study performed by the Wellcome Trust Case Control Consortium,<sup>57</sup> but was not associated with type 2 diabetes. In the Diabetes Genetics Initiative study, the polymorphism was not genotyped. In a recent meta-analysis of this and other variants of the *UCP3-UCP2* gene locus, the -866G>A polymorphism was not associated with increased risk of type 2 diabetes.<sup>40</sup> The present study confirms and extends these conclusions.

In Austrian subjects, the G-allele of the -866G>A polymorphism was also found to associate with obesity, whereas fasting insulin was lower in Austrians carrying the GG genotype. In these subjects, *UCP2* mRNA levels in visceral fat were decreased in subjects of the GG genotype.<sup>14</sup> Decreased insulin sensitivity has been detected in Italians carrying the AA genotype,<sup>20</sup> whereas we observed the opposite. Although the current SNP has different *cis*-acting capability of its alleles (the A-allele giving higher promoter activity), these observations in line with Hsu et al.<sup>56</sup> point to the possibility that yet unidentified variants may account for the effect on insulin resistance.

Insulin resistance (HOMA-IR) has been reported to be positively correlated with visceral adipose tissue *UCP2* mRNA expression in 100 obese Italians undergoing laparoscopic gastric banding,<sup>20</sup> whereas fasting plasma insulin levels alone are negatively correlated with *UCP2* mRNA expression in subcutaneous abdominal fat.<sup>60</sup> However, a difference between expression levels and regulation of expression between different fat depots cannot be excluded.

In conclusion, using a study of a total of 17 636 Danes we show that the *UCP2* -866G>A polymorphism is associated with decreased insulin sensitivity and obesity. In addition, we confirmed the association with obesity by carrying out meta-analyses with obesity and type 2 diabetes of our data combined with publicly available data.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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