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Review Article

Genetic Variance in *Uncoupling Protein 2* in Relation to Obesity, Type 2 Diabetes, and Related Metabolic Traits: Focus on the Functional −866G>A Promoter Variant (rs659366)

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Uncoupling proteins (UCPs) are mitochondrial proteins able to dissipate the proton gradient of the inner mitochondrial membrane when activated. This decreases ATP-generation through oxidation of fuels and may theoretically decrease energy expenditure leading to obesity. Evidence from $Ucp^{(-/-)}$ mice revealed a role of UCP2 in the pancreatic β-cell, because β-cells without UCP2 had increased glucose-stimulated insulin secretion. Thus, from being a candidate gene for obesity UCP2 became a valid candidate gene for type 2 diabetes mellitus. This prompted a series of studies of the human UCP2 and UCP3 genes with respect to obesity and diabetes. Of special interest was a promoter variant of UCP2 situated 866bp upstream of transcription initiation (−866G>A, rs659366). This variant changes promoter activity and has been associated with obesity and/or type 2 diabetes in several, although not all, studies. The aim of the current paper is to summarize current evidence of association of UCP2 genetic variation with obesity and type 2 diabetes, with focus on the −866G>A polymorphism.

1. Introduction

Uncoupling protein 2 (UCP2) and uncoupling protein 3 (UCP3) belong to a large family of mitochondrial transmembrane carriers. UCP2 was identified in 1997 based on its homology to the brown fat uncoupling protein (UCP, then renamed UCP1) [1, 2]. Shortly thereafter, UCP3 was cloned also based on homology to UCP1 and UCP2 [3, 4]. Later, more distantly related proteins were identified and named UCP4 and UCP5 (BMCP1) [5–7]. The physiological role of UCP1 is well established; it is responsible for nonshivering thermogenesis in brown fat, in which it induces proton leak across the inner mitochondrial membrane [8, 9]. Now 14 years later, the physiological functions of UCP2 and UCP3 are still under debate, as is the role of genetic variation in these. The aim of this paper is to recapitulate the currently published literature on human genetic variation in the UCP2 genomic region concerning development of obesity, type 2 diabetes, and related metabolic disorders with focus on the −866G>A promoter polymorphism (rs659366).

2. Physiological Functions of UCP2 and UCP3

UCP2 is ubiquitously expressed [1, 2] whereas UCP3 is found predominantly in skeletal muscle and brown adipose tissue [3, 4, 10], and their expression is both induced by fasting, and peroxisome proliferators as well as hyperglycemia, which indicates a role connected with the availability of fuel substrates [11–14]. However, the upregulation in response to thyroid hormone, cold, β3-adrenergic agonists, and high fat diets also suggests involvement in regulation of energy expenditure [15–17].

Neither UCP2 nor UCP3 affects basal proton conductance of the mitochondrial inner membrane [18–23]. However, they do induce proton leak across the inner mitochondrial membrane when activated by, for example, fatty acids, superoxide, or free radical derived peroxidation products of membrane phospholipids [24, 25]. UCP2 and UCP3 may decrease the formation of superoxide and reactive oxygen species (ROS) by mild uncoupling of the respiratory chain, whose activity is increased under these circumstances [21, 24, 26] (Figure 1). This is concordant with the induction

of UCP2 and UCP3 during cold, fasting and high fat feeding, since these conditions require lipid oxidation and thus high activity of the respiratory chain [18]. On the other hand, it has recently been suggested that UCP2 restricts pyruvate efflux from the mitochondria and hence ensures availability of substrates for the citric acid cycle, which would then explain the increase in glucose oxidation compared with lipid oxidation in *Ucp2*$^{(-/-)}$ mouse embryonic fibroblasts [27, 28]. Whether this proposed function of UCP2 is shared with UCP3 is not known, and this hypothesis requires more investigation as it is less supported by experimental evidence as the theory of mild uncoupling.

In the pancreatic β-cell, UCP2 is important for appropriate glucose-stimulated insulin secretion. Overexpression of UCP2 inhibits glucose-stimulated insulin secretion in pancreatic rat islets and INS-1 β-cells [36–38], which is well explained by the theory of mild uncoupling because the resulting decrease in ATP-levels decreases closure of the ATP/ADP sensitive potassium channels, and therefore decreases insulin secretion (Figure 1). Concordant with this, *Ucp2*$^{(-/-)}$ mice have increased glucose-stimulated insulin secretion and higher pancreatic islet ATP levels and are protected against glucose-toxicity in β-cells [29, 39], and on a high-fat diet they show increased insulin secretion and decreased plasma triglyceride concentrations [40]. This is in line with *in vitro* studies of *Ucp2*$^{(-/-)}$ islets of Langerhans, which are resistant to palmitate-induced β-cell dysfunction [41]. UCP2 mRNA is upregulated in obese ob/ob mice, and ob/ob mice lacking UCP2 showed restored first-phase insulin secretion and reduced level of hyperglycaemia [29]. No effect of *Ucp2* gene disruption on obesity was observed, even upon a high-fat diet or on a background of genetic obesity [29, 32]; however, short-term inhibition of *Ucp2* using antisense oligonucleotides ameliorated insulin resistance and improved insulin secretion in a diet-induced mouse model [42]. Recently, β-cell function of *Ucp2*$^{(-/-)}$ on an in-bred C57Bl background was reported to be opposite to earlier published reports, in that β-cells without UCP2 showed lower glucose-stimulated insulin secretion, but maintained higher levels of reactive oxygen species [43]. The reason for these contradictory results are at the moment unknown but may be explained by yet unidentified modifier genes different from the initial mixed C57Bl and 129 background to congenic back-crossed strains [44]. Moreover, because two different theories exist for explaining the role of UCP2 in β-cells, it is difficult to extrapolate from mouse data to data obtained in humans. The specific contribution between hypersecretion of insulin versus the deleterious effect of ROS on β-cells in humans versus mice is unknown (Figure 1). Similar to UCP2, UCP3 has recently been found to be expressed in pancreatic β-cells, where it also influenced insulin secretion [45], but the physiological function of UCP3 in β-cells is not known.

Disruption of the UCP3 gene in mice does not cause an obese phenotype, but levels of oxidative stress are increased in skeletal muscle of *Ucp3*$^{(-/-)}$ mice [46, 47]. There may be compensatory effects when removing UCP3 or UCP2 from skeletal muscle (or β-cells) in which they are coexpressed; generation of UCP2 and UCP3 double knockout mice may resolve this issue. Overexpression of UCP3 in skeletal muscle or UCP3 together with UCP2 in skeletal muscle have been reported to create a lean phenotype in mice [48, 49]. It is uncertain whether these data are reliable as it has been shown that overexpression of mitochondrial carriers may lead to over-load of the inner mitochondrial membrane and artifactual data [20]. Thus, over-expressing UCP2 or UCP3 in mice may not be a good or reliable strategy for interrogating their physiological function.

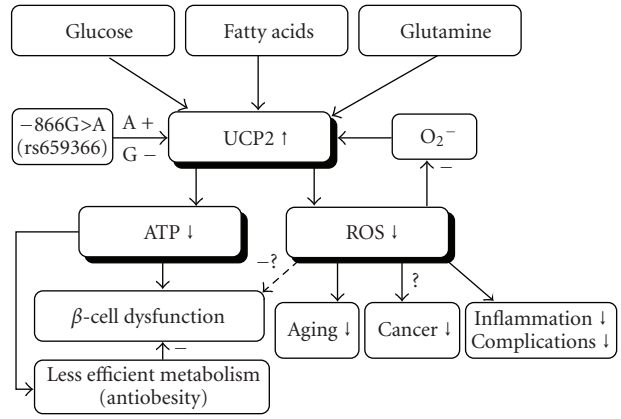

Figure 1: Mechanism by which UCP2 activation may lead to obesity and type diabetes. UCP2 upregulation by nutrients (glucose, lipids, fatty acids, glutamine (protein-rich diet)) increases UCP2 mRNA transcription or translation. UCP2 activity is increased by superoxide radicals. Increased UCP2 amount or activity causes β-cell dysfunction [29] and may contribute to decreased metabolic efficiency by decreasing ATP-generation. UCP2 activation decreases oxidative stress and may therefore decrease aging [30], cancer progression [31], and inflammation [32]. Decreased ROS levels may also protect β-cell [33]. Decreased ATP-generation by UCP2 upregulation may cause less efficient metabolism and protect against obesity, which will decrease demand for insulin secretion by the β-cell.

Interestingly, decreased ROS due to partial uncoupling by UCP2 or UCP3 could represent a link to the "thinness and longevity" phenomenon observed when diet restriction of rodents increases their life span by up to 50% [50] (Figure 1). In fact, *Ucp2*$^{(-/-)}$ mice, having increased oxidative stress in their mitochondria, live significantly shorter than WT litter mates [30], supporting the hypothesis that mitochondrial-derived free radicals are involved in aging [51]. Recently, it was shown that UCP2 mRNA levels were increased in colon cancer samples, also suggesting a link between levels of oxidative stress modulated by UCP2 and development of cancer [52] (Figure 1).

3. UCPs: Candidate Genes for Obesity and Type 2 Diabetes

Because UCP2 and UCP3 decrease mitochondrial membrane potential and mediate proton leak [53], they are candidate genes for obesity and type 2 diabetes. UCP2 and UCP3 are coexpressed in skeletal muscle, which contributes the most to the basal metabolic rate [54]. Mutations reducing the activity

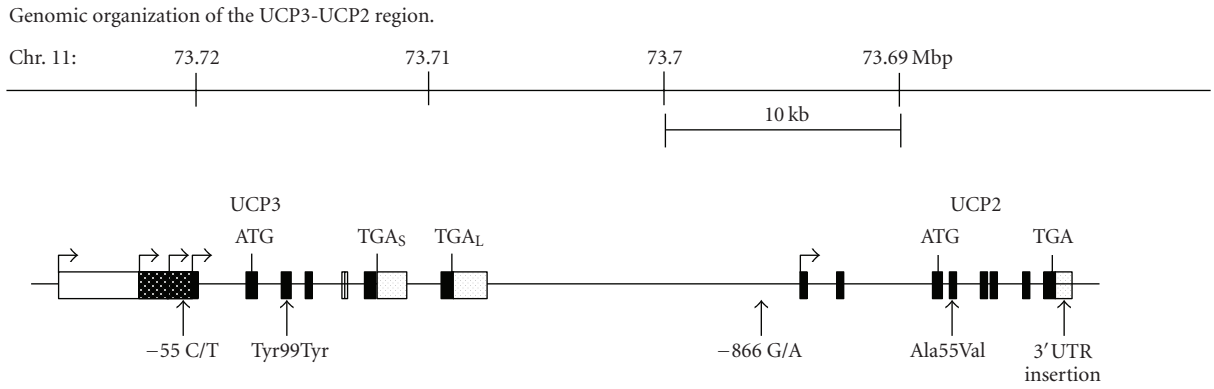

Figure 2: Diagram of the UCP2-UCP3 genomic region with indications of common genetic variation. Genomic organization of the UCP2-UCP3 region on chromosome 11. ATG: start codon, TGA: stop codon. Bent arrows indicate reported transcription start sites (from [34]). UCP3 protein exists in a short and a long form due to alternative polyadenylation sites, indicated by TGA_S and TGA_L [35].

or expression of either protein could theoretically diminish energy expenditure by an increase in coupling of oxidative phosphorylation, and thereby contribute to development of obesity. Mutations in UCP2 regulatory regions causing increased levels could cause or worsen decreased glucose-stimulated insulin secretion directly through a decreased ATP/ADP ratio in the pancreatic β-cell and promote development of type 2 diabetes.

The most consistent trait found in *Ucp2*$^{(-/-)}$ and *Ucp3*$^{(-/-)}$ mice has been the increased levels of superoxide radicals and oxidative stress. Insulin resistance may be caused by increased intracellular ROS levels [55], which are influenced by the expression or activity of UCPs [56]. UCP2 may also modulate the severity of low-grade inflammation present in obesity and obesity-associated type 2 diabetes, because ROS levels generated by macrophages and other immune cells are increased in *Ucp2*$^{(-/-)}$ mice [32]. This also points to an important role of UCP2 in atherosclerosis, since *Ucp2*$^{(-/-)}$ mice fed an atherogenic diet developed more atherosclerosis [57]. Similarly, oxidative stress may be causative for late diabetic complications [58], and as modulators of mitochondrial ROS levels, UCP2 and UCP3 may affect the severity of diabetic complications.

4. Human UCP2 and UCP3 Genetic Variation

UCP2 and UCP3 are the likely result of an ancestral gene-duplication, because they are situated close to each other on chromosome 11q13 [64] (Figure 2). Because UCP2 and UCP3 are considered candidate genes for development of obesity and type 2 diabetes, they have been studied extensively. There is a low number of frequent genetic variants, which have been investigated in a large number of studies (Table 1 and Figure 2), and most identified variants have been of low frequency and have therefore not been so intensively studied. There are 3 common polymorphisms in UCP2, which are well studied: a promoter variant, −866G>A (rs659366), a missense polymorphism in codon 55 changing an alanine to a valine (Codon 55 Ala/Val, rs660339), and a 45 bp insertion-deletion polymorphism in the 3′untranslated region (UTR) of the UCP2 gene (3′UTR ins/del). In UCP3, there is one common and well-studied polymorphism: a promoter variant, −55 C/T (rs1800849) (Table 1) [63, 65–69].

5. Effects of the −866G>A Variant on Transcriptional Activity of the *UCP2* Promoter

The −866G>A polymorphism is situated in the proximal promoter of *UCP2* and putatively changes one or more transcription factor binding sites [60, 70]. Several studies determined whether the activity of the promoter changes with genotype. In insulin producing cells, the β-cell transcription factor PAX6 binds preferentially to the A-allele, which increases reporter-gene activity of constructs containing the A-allele [70, 71]. Sesti et al. (2003) showed decreased glucose-stimulated insulin secretion from isolated human islets having the GA-genotype vs. the GG-genotype [72], suggesting that increased *UCP2* mRNA from the A-allele translates into increased UCP2 protein, induced proton leak, decreased ATP/ADP ratio, and decreased glucose-stimulated insulin secretion in accordance with the phenotype of the *Ucp2*$^{(-/-)}$ mice. In adipocytes, the −866 A-allele was associated with both decreased [73] or increased [74] levels of adipose tissue *UCP2* mRNA. However, reporter-gene constructs with the −866 A-allele showed increased activity in adipocytes [70], similar to findings in insulin-producing cells. Thus, the minor A-allele directs higher rates of transcription from the *UCP2* promoter compared with the G-allele.

6. UCP2 Genetic Variation in Relation to Obesity

The frequent −866G>A polymorphism (rs659366) has been extensively investigated for association with obesity and

Table 1: Studied high frequency variants of the UCP2 and UCP3 genes.

Gene	Variant	Acc. number	Approximate frequency (ref)
UCP2	Promoter −1957G>A	rs649446	29.0% (A-allele) [59]
UCP2	Promoter −866G>A	rs659366	37.0% (A-allele) [60]
UCP2	Codon 55 Ala/Val	rs660339	39.6% (Val) [61]
UCP2	3′UTR ins>del	—	29.6% (ins-allele) [62]
UCP3	Promoter −55C>T	rs1800849	26.9% (T-allele) [63]
UCP3	Exon 3 Tyr99Tyr	rs1800006	30.0% (T-allele) [59]
UCP3	Exon 5 Tyr210Tyr	rs2075577	16.0% (T-allele) [59]

related subphenotypes. The AA genotype was initially shown to associate with a reduced risk of obesity among 596 and 791 white Europeans [74]—an observation that has been replicated [75], but more studies report either increased prevalence of the A-allele in obesity [76–78] or no association at all [59, 60, 72, 79–88] (Table 2). The total number of subjects in the studies reporting no association with obesity for the A-allele is above 14000 and by far outnumbers the initial observation, and the number of participants in the three studies reporting association of the A-allele with obesity or increasing indices of adiposity is approximately 4000. Therefore, it is most likely that the −866 A-allele has a very modest effect if any on development of obesity, but in order to evaluate, this a proper meta-analysis is necessary.

Assuming that a more subtle intermediary obesity-related phenotype is affected by the −866G>A polymorphism, a number of observations have been made; among 681 French type 2 diabetic patients, the variant was associated with elevated triglyceride and total cholesterol concentrations and increased risk of dyslipidaemia [90], and in line with this, decreased HDL-cholesterol levels were reported among 658 Korean women [59]. Lack of association with lipid levels has also been reported [72, 79, 80, 82]. Carriers of the G-allele of the −866G>A polymorphism lost more weight than A-homozygotes in a study of diet-induced body fat reduction in 301 Korean women undergoing a very-low-calorie programme [92]. Finally, in 296 obese children, homozygosity of the A-allele was related to increased resting-energy expenditure, increased glucose oxidation rate, and lower lipid oxidation rate [89], and among 185 Pima Indians, the −866G>A polymorphism was associated with increased 24-hour energy expenditure [83].

Numerous studies do not support a functional impact of the 3′UTR insertion or the Ala55Val polymorphism in causing obesity or type II diabetes. Few association studies have found differences in allele or genotype frequencies of the Ala55Val polymorphism between obese and/or type 2 diabetic subjects and control subjects [61, 93, 94] and this variant is generally not considered to predispose to obesity or type 2 diabetes. The 3′UTR insertion polymorphism has been related to measures of energy expenditure or increased BMI [83, 95, 96]. In heterozygous state, the 3′UTR insertion has been associated with increased sleeping metabolic rate and 24-h energy expenditure and lower BMI in Pima Indians, in agreement with a role of UCP2 in controlling energy expenditure [97]. Moreover, the insertion homozygous genotype was associated with increased BMI in South Indian females and increased serum leptin levels in British women [95]. However, in Danish subjects there was no association with obesity or weight gain over a 26-year followup [62]. The 3′UTR 45 bp insertion could exert its effect through altered mRNA stability; however, there was no difference in UCP2 mRNA levels between genotypes in skeletal muscle from Pima Indians [97], but *in vitro* mRNA stability assays showed that the insertion allele had less stable mRNA [74].

7. Type 2 Diabetes and the Metabolic Syndrome with Regard to UCP2 Genetic Variation

Mar Gonzalez-Barroso et al. (2008) reported on two families in which congenital hyperinsulinemia occurred and who carried heterozygous mutations in UCP2 [98]. The two families each carried their own mutations, which segregated with the disease and which changed amino acids conserved between species. Functional studies of recombinant yeast showed lower proton leak of the mutant UCP2s, and the mutants were not able to suppress insulin secretion in β-cells when over-expressed as opposed to wild-type UCP2. Thus, the phenotype of carriers of heterozygous null-alleles of UCP2 were in fact very similar to the phenotype of $Ucp2^{(-/-)}$ mice on mixed-strain genetic background [29], but opposite the phenotype of $Ucp2^{(-/-)}$ mice in congenic lines [43]. However, it is not known how the hyperinsulinism associated with UCP2 null-mutations affects β-cells later in life; oxidative stress is increased in $Ucp2^{(-/-)}$ mice, and over time this is associated with declining β-cell function. On the other hand, $Ucp2^{(-/-)}$ mice do not become diabetic [43]. Thus, studying adult and aging carriers of the identified UCP2 mutations is likely to be very rewarding for elucidating the contribution of UCP2 towards maintenance of glucose tolerance in humans.

Given that UCP2 null mutations cause hyperinsulinemia, the −866 A-allele, having increased transcriptional activity, would be expected to show association with decreased β-cell function and ultimately with type 2 diabetes. When examining measures of insulin secretion, the −866 A-allele was associated with decreased glucose-stimulated insulin secretion among 137 Japanese type 2 diabetic patients undergoing frequently sampled IVGTT [71] and also in isolated pancreatic islets from nondiabetic subjects [72].

Table 2: Summary of association studies of the UCP2 promoter −866G>A (rs659366) polymorphism in relation to obesity and related metabolic traits.

Ethnic population	n_{obese} (Frequency of A-allele in %)	$n_{control}$ (Frequency of A-allele in %)	Phenotypes	Reference
Caucasian	340 (46.5) 109 (31.2)	256 (52.2) 589 (38.2)	Common G-allele predisposed to obesity	Esterbauer et al. 2001 [74]
Caucasian	749 (39.6)	816 (40.7)	Not associated with obesity or BMI within groups	Dalgaard et al. 2003 [60]
Caucasian	122 (28.2) 76 (34.9)	374 (29.0)	Not associated with obesity or BMI within groups	Mancini et al. 2003 [79]
Caucasian	—	302 (32.1)	Not associated with BMI within group	Sesti et al. 2003 [72]
Caucasian	— —	565 (32.4) 483 (33.6)	Not associated with BMI in control or diabetic patients	D'Adamo et al. 2004 [80]
Japanese	—	134 342	Not associated with BMI, but with hypertension	Ji et al. 2004 [81]
Caucasian	296 (37.0)	—	A-allele associated with decreased lipid oxidation	Le Fur et al. 2004 [89]
Caucasian	—	327 (34.6) 746 (28.6)	Not associated with BMI within group	Bulotta et al. 2005 [82]
Pima Indians	864 (54.0) 263 (55.5)	— —	Not associated with BMI within group. AA genotype increased 24 hr EE	Kovacs et al. 2005 [83]
Korean	—	658	Not associated with BMI within group. Associated with decreased HDL-levels	Cha et al. 2007 [59]
Caucasian	—	598 653	Not associated with BMI within group. A-allele associated with decreased W/H-ratio and lower fasting p-insulin	Gable et al. 2007 [84]^P
Filipino	—	1755 (29.7)	Not associated with BMI within group	Marvelle et al. 2008 [85]
Caucasian	375 (41.3)	2316 (35.8)	A-allele associated with obesity and associated with increased risk of CHD and systolic BP. AA genotype associated with increased oxidative stress	Dhamrait et al. 2004 [77]
Caucasian	192 (38.3)	170 (38.2)	AA genotype significantly associated with obesity and insulin resistance in children	Ochoa et al. 2007 [76]
Caucasian	225 (39.6)	294 (38.9)	AA genotype associated with various indices of obesity	Kring et al. 2008 [78]
Caucasian	277	188	Not associated with early-onset obesity	Schäuble et al. 2003 [86]
Caucasian	—	681 (36.9)	Not associated with BMI in type 2 diabetic patients, but AA genotype associated with increased triglyceride and cholesterol levels	Reis et al. 2004 [90]
Various	—	3784 (35.4–46.7)	Not associated with obesity, allele-frequencies not given for obese subjects	Hsu et al. 2008 [87]
Korean	—	1469 (~48)	GG genotype associated with obesity in children but protective in adults	Jun et al. 2009 [75]
Caucasian	—	507	AA genotype decreased total cholesterol and decreased LDL-cholesterol. Not associated with BMI within group	Salopuro et al. 2009 [88]^P
Indian	200 (42.0)	240 (32.2)	A-allele associated with obesity and hyperinsulinemia (in obese subjects)	Srivastava et al. 2010 [91]

^{P}Denotes prospective study. Abbreviations: CHD: coronary heart disease; BP: blood pressure; EE: energy expenditure; BMI: body mass index; HDL: high density lipoprotein; W/H: waist to hip.

These observations are in accordance with the A-allele directing increased UCP2 expression and causing decreased insulin secretion (but also lower ROS-levels). Decreased basal insulin secretion was initially reported among A-allele carriers [74] but was contrasted by subsequent studies [60, 72, 80–82], which showed no association. Also, early onset of type 2 diabetes has been correlated with the A-allele [71, 99], but also with the G-allele [100], whereas early requirement for insulin treatment was observed in A-allele carriers [71, 90] (Table 3).

Observations of a lower disposition index in −866A carriers have been made [70, 72], although this could also be induced by changes in insulin sensitivity rather than insulin secretory capacity. It is possible that the −866 A

Table 3: Summary of association or prospective studies of the UCP2 promoter −866G>A (rs659366) polymorphism in relation to type 2 diabetes and intermediary phenotype.

Ethnic population	$n_{diabetes}$ (Allele frequency in %)	$n_{control}$ (Allele frequency in %)	Phenotypes	Reference
Caucasian	201 (41.2)	391 (32.5)	A-allele associated with type 2 diabetes increased disposition index	Krempler et al. 2002 [70]
Caucasian	565 (32.4)	483 (33.6)	AA genotype decreased insulin sensitivity and was associated with type 2 diabetes	D'Adamo et al. 2004 [80]
Caucasian	—	2595 (37.0)	AA genotype increased risk of type 2 diabetes, especially combined with obesity	Gable et al. 2006 [99]^P
Caucasian	—	302 (28.8)	A-allele associated with decreased insulin secretion. Isolated islets of A-allele carriers had decreased *in vitro* insulin secretion	Sesti et al. 2003 [72]
Caucasian	131 (33.0)	118 (48.0)	G-allele associated with type 2 diabetes and increased adipose tissue mRNA	Wang et al. 2004 [73]
Caucasian	746 (28.6)	327 (34.5)	G-allele associated with type 2 diabetes	Bulotta et al. 2005 [82]
Caucasian	—	2216 (38.1)	GG genotype increased risk of type 2 diabetes	Lyssenko et al. 2005 [100]^P
Indian	762 (35.0)	924 (41.0)	G-allele associated with type 2 diabetes	Rai et al. 2007 [101]
Caucasian	—	3122 (36.7)	GG genotype increased risk of MI in men	Cheurfa et al. 2008 [102]^P
Caucasian	—	589 (38.2)	AA genotype borderline associated with increased fasting insulin levels	Esterbauer et al. 2001 [74]
Pima Indian	864 (54.0) 263 (55.5)	— —	Not associated with type 2 diabetes within group. AA genotype borderline associated with decreased insulin sensitivity	Kovacs et al. 2005 [83]
Various	1584	2198 (35.4–46.7)	Not associated with type 2 diabetes	Hsu et al. 2008 [87]
Japanese	413 (47.2)	172 (43.1)	Not associated with type 2 diabetes, but A-allele showed higher transcriptional activity and carriers had decreased AIR	Sasahara et al. 2004 [71]
Caucasian	—	235 (43.2) 410 (34.5)	No association with changes fasting p-glucose or s-insulin in glucose-tolerant subjects	Dalgaard et al. 2003 [60]
Caucasian	—	507	AA genotype decreased total cholesterol and decreased LDL-cholesterol.	Salopuro et al. 2009 [88]^P
Caucasian	—	296 (37.0)	No influence on insulin sensitivity	Le Fur et al. 2004 [89]
Caucasian	375 (41.3)	2316 (35.8)	A-allele associated with increased type 2 diabetes risk, increased risk of CAD and systolic BP, and increased oxidative stress	Dhamrait et al. 2004 [77]
Various	—	901 (39.4)	Diabetic A-allele carriers poor survival after MI	Palmer et al. 2009 [103]^P
Caucasian		453 (33.0–36.0)	AA genotype associated with increased oxidative stress and CAD	Stephens et al. 2008 [104]
Caucasian	—	227 (39.3)	Diab. neuropathy lower in AA genotype	Rudofsky et al. [105]
Caucasian	—	280 (39.3)	GG genotype associated with low-grade inflammation, but not insulin levels	Labayen et al. 2009 [106]
Caucasian	—	383 (31.9)	GG genotype associated with increased CRP	Lapice et al. 2010 [107]

^{P}Denotes prospective study. Disposition index: the product of Si and AIR. Abbreviations: Si: insulin sensitivity; AIR: acute insulin response; MI: myocardial infarct; LDL: low density lipoprotein; CRP: C-reactive protein; CAD: coronary artery disease.

allele is involved in mediating decreased β-cell function as well as decreased insulin sensitivity of adipose tissue, which would be expected to translate into an increased risk of type 2 diabetes. As the −866 A-allele was reported to increase UCP2 mRNA expression [70, 71], it is expected that ROS-levels would be lower in A-carriers. However, since insulin resistance is associated with increases in oxidative stress [55], it is more likely that changes in disposition index are due to differences in insulin secretion rather than insulin resistance. In line with this, insulin resistance

(HOMA-IR) has been reported to be positively correlated with visceral adipose tissue *UCP2* mRNA expression [80]. Following the "mild uncoupling theory" it would be expected that increased UCP2 expression—as a possible consequence of carrying the −866A-allele—would be associated with increased insulin sensitivity. However, experimental studies do not agree on the effect of −866G>A on insulin sensitivity. Using either hyperinsulinaemic-euglycaemic clamp or an intravenous glucose tolerance test in 39, 263, and 181 subjects, respectively, AA genotype carriers were less insulin sensitive [70, 80, 83], whereas in a number of other studies insulin resistance estimated using the HOMA index in 632 Japanese subjects [81], 363 French adolescents [76], and 302 Italian subjects [72] was not affected by the UCP2 −866G>A variant (Table 3). Clearly, more information is needed on the physiological effects of UCP2 on whole body insulin sensitivity.

Association studies of type 2 diabetes have reported association of the −866A-allele with increased risk of type 2 diabetes in studies representing up to 1640 subjects [70, 77, 80, 99], whereas other studies report association of the G-allele with type 2 diabetes backed by studies of more than 2700 subjects [73, 82, 100, 101], and a number of large studies report no association of this variant with type 2 diabetes [83, 87, 90] (Table 3). Prospective studies have shown that subjects carrying the AA genotype were more likely to become type 2 diabetic, or had poor survival following myocardial infarction [77, 99, 103], but the G-allele has also been associated with increased risk of type 2 diabetes [100]. Thus, it is necessary to perform more studies as well as a proper meta-analysis to investigate the impact of this variant on type 2 diabetes.

8. A Possible Role of −866G>A Variant and Oxidative Stress in Cardiovascular Disease and Late Diabetic Complications

Both increased risk of hypertension [81] as well as decreased risk of dying following myocardial infarction [102, 103] has been reported to be associated with the −866A allele, whereas plasma total antioxidant status, which is low when oxidative stress is increased, has been shown to be decreased in AA genotype carriers. Among 2,695 healthy Caucasian men, the risk of coronary heart disease and elevated diastolic blood pressure was increased in men homozygous for the −866A-allele while among 465 diabetic men, the A-allele was associated with increased oxidative stress [77]—an observation that was significantly accentuated by cigarette smoking [104]. Thus, the functional A-allele, which mediates increased UCP2 mRNA levels, is associated with increased oxidative stress. This may be linked with the poor insulin secretion associated with the AA-genotype, leading to increased levels of plasma glucose and HbA1c [71], and perhaps oxidative stress; however, this mechanism is speculative and needs experimental validation. Also, low-grade inflammation has been investigated in the context of the −866G>A polymorphism, where increased C-reactive protein (CRP) was associated with the GG-genotype in a study of 283 diabetic patients. In another study of 280 children and adolescents CRP was unaltered, but fibrinogen, complement C3 and C4 were lower in AA-carriers [106]. Finally, Rudofsky et al. (2006, 2007) showed increased prevalence of the G-allele in type 1 diabetic patients, whereas there was no association with microvascular complications [105, 108].

9. Possible Influence of Other SNPs in the UCP2-UCP3 Genomic Region

The genomic region containing the *UCP2* and *UCP3* genes were investigated for a total of 14 SNPs (including −866G>A) spanning the *UCP2* and *UCP3* loci among 3,782 women of different ethnicities [87]. No single-SNP association with type 2 diabetes was observed following correction for multiple testing; yet, haplotype analysis indicated an association with increased type 2 diabetes risk among 968 Caucasian women, and this effect was further accentuated by overweight although no direct association with BMI was observed. The four-SNP haplotype in question was in high LD with the −866 A-allele, suggesting that as yet unidentified variation covered by the haplotype-spanned area may be responsible for the observed relationships of −866G>A with metabolic variables. The presence of other functional variants may also account for the difference in diabetes or obesity risk-allele reported by a number of studies (Tables 2 and 3).

10. Conclusions and Perspectives

In acute studies using antisense oligonucleotides, UCP2 was involved in both insulin secretion and insulin action [42], whereas $Ucp2^{(-/-)}$ mice have not been reported to have altered insulin sensitivity [29]. Studies of $Ucp2^{(-/-)}$ mouse embryonic fibroblasts have shown that loss of *Ucp2* results in increased glycolysis and decreased fatty acid oxidation—suggesting that UCP2 regulates mitochondrial substrate usage to a greater extent than its original role as an uncoupler of respiratory chain activity from ATP synthesis [27, 28]. Absence of UCP2 causes oxidative stress and superoxide production [32, 39], which is associated with insulin resistance [55]. However, a number of studies report association of the high-expressing allele of the −866G/A variant with oxidative stress, which is at odds with phenotype data from $Ucp2^{(-/-)}$ mice. However, the widespread expression pattern makes possible a dual function in obesity (energy metabolism) and type 2 diabetes (glucose metabolism).

With so many contrasting studies there is, a genuine need for a thorough meta-analysis of the impact of the −866G>A polymorphism in order to conclude whether it predisposes to obesity and/or type 2 diabetes. It is important to note that genome-wide association studies (GWAS) have not identified SNPs in the UCP2-UCP3 locus as being associated with obesity or type 2 diabetes [109, 110]. However, if the mechanism of action of the −866G>A SNP, as some studies indicate, occurs predominantly in already obese and type 2 diabetic subjects to increase late-diabetic complications, such as cardiovascular disease via changes in oxidative stress

levels [77, 103–105], then this polymorphism is unlikely to be identified through a GWAS strategy looking primarily at obesity or type 2 diabetes. Furthermore, early disease onset and a more frequent requirement for insulin may be related to a reduced capacity of insulin secretion. It may well be that the major contribution of genetic variability in UCP2 lies in mediating susceptibility towards complications.

A main conclusion is that variation in the uncoupling protein 2 gene is not associated with major alterations of body weight or risk of type 2 diabetes. It is naturally more difficult to estimate the contribution of UCP genes towards polygenic obesity and type 2 diabetes; however, although many studies do indicate association of the −866G>A variant with obesity and/or type 2 diabetes, the impact of this single variant is low, as is the case with most predisposing variants on polygenic traits. Therefore, large numbers of well-characterised study subjects must be investigated to detect the true effect of a given variant.

Sources

Google Scholar and PubMed were searched for publications in English containing the words "uncoupling protein 2", "uncoupling protein 3", "−866G/A", "−55C/T", "rs659366", "rs660339" "polymorphism", "UCP2", "UCP3", "GWAS", "SNP", and "proton leak", alone or in combinations with "obesity" and "diabetes".

Abbreviations

UCP2: Uncoupling protein 2
UCP3: Uncoupling protein 3
SNP: Single-nucleotide polymorphism
GWAS: Genome wide association study
ROS: Reactive oxygen species.

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