Original Article

Variation in the *UCP2-UCP3* Gene Cluster Predicts the Development of Type 2 Diabetes in Healthy Middle-Aged Men

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The impact of the *UCP2* −866G>A and *UCP3* −55C>T variants on prospective risk of type 2 diabetes was examined over 15 years in 2,936 healthy middle-aged men (mean age 56 years). Conversion to diabetes (n = 169) was associated with higher BMI, blood pressure, cholesterol, triglycerides and C-reactive protein. The hazard ratio (HR) for diabetes of a BMI >30 kg/m^2 was 3.96 (95% CI 2.87–5.47). Homozygosity for the *UCP2*A or *UCP3*T alleles accelerated the onset of diabetes, with significant differences in risk of diabetes at 10 years (HR [95% CI] *UCP2*AA vs. GA+GG 1.94 [1.18–3.19], P = 0.009; *UCP3*TT vs. CC+CT 2.06 [1.06–3.99], P = 0.03) but less so at 15 years (*UCP2*AA 1.42 [0.92–2.19], P = 0.1; *UCP3TT* 1.57 [0.87–2.04], P = 0.13). Men who were homozygous for both *UCP2*AA and *UCP3*TT (1.5% of men) had a risk for diabetes at 10 years of 4.20 (1.70–10.37), P = 0.002. These genotype effects were additive with obesity, and men with a BMI >30 kg/m^2 and this genotype combination had a 10-year risk of diabetes of 19.23 [5.63–63.69], P < 0.0001. Functional promoter variants *UCP2* and *UCP3* increase the prospective risk of diabetes. Although the mechanism of the *UCP2* effect is likely to be caused by increased expression in the pancreas and subsequent reduced insulin secretion, the mechanism of the *UCP3* effect is currently unknown. Both effects are exacerbated by obesity. *Diabetes* 55:1504–1511, 2006

The rate of mitochondrial oxidative metabolism appears to be important in the development of type 2 diabetes with reduced expression of key genes in oxidative metabolism and mitochondrial function, not only in patients with type 2 diabetes (1) but also in subjects with pre-diabetes, even when glucose tolerance is normal (2). Fuel substrate oxidation, via the respiratory chain, leads to development of a proton gradient across the mitochondrial membrane, which can be dissipated to produce ATP. This gradient can also be dissipated without the production of ATP by uncoupled metabolism, with the release of energy as heat. Therefore, the rate of oxidative metabolism depends not only on ATP requirements but also the extent to which uncoupled fuel substrate oxidation is present (3).

Uncoupling protein (UCP)2 and UCP3 are members of a mitochondrial carrier protein superfamily that can facilitate the exchange of substrates across the mitochondrial inner membrane. They show high (~55%) amino acid homology with UCP1 (4), the first in the family to be identified, which occurs in brown adipose tissue. UCP1 plays an important role in nonshivering thermogenesis (5), dissipating the proton gradient by proton transfer across the mitochondrial membrane with the release of heat. The uncoupling of metabolism per se also causes an increase in glycolysis and GLUT4 synthesis and translocation, as well as increased breakdown of fatty acids. This leads to reduced blood glucose and fat mass and improved glucose tolerance (6). The *UCP2* and *UCP3* genes are located within 8 kb of each other on chromosome 11q13 (7), and the reported association of obesity and diabetes traits (8–13) with variation in the *UCP2-UCP3* gene cluster suggests the importance of this locus in determining risk of these disorders.

A substantial role for UCP2 or UCP3 in thermogenesis in humans is unlikely because these proteins are not upregulated by cold, and it has been suggested that the main function of UCP2 is protection from damage caused by reactive oxygen species (ROS) or oxidative stress (14,15). The production of ROS depends, in part, on mitochondrial membrane potential, and reduction of this potential by uncoupling reduces ROS production. In pancreatic β-cells, uncoupling by UCP2 has a further effect on glucose-stimulated insulin secretion. A reduction in insulin secretion is seen when *UCP2* is overexpressed in isolated rat islet cells or human insulinoma cells, and glucose-stimulated insulin secretion is improved in the *UCP2* knockout mouse (15). A common variant in the promoter (−866G>A, rs659366) is associated with higher *UCP2* mRNA levels and was associated with reduced insulin secretion or type 2 diabetes in Austrian (16), Italian (17), and Japanese samples (18), as well as with reduction in insulin secretion in healthy adults and post–myocardial infarction survivors (19). In keeping with a role in the control of ROS production, −866AA homozygotes have also been reported to have higher oxidative stress markers in men with diabetes, greater risk of cardiovascular disease in healthy men (20), and higher carotid atherosclero-

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Received for publication 20 December 2005 and accepted in revised form 30 January 2006.

CRP, C-reactive protein; NPHSII, Second Northwick Park Heart Study; ROS, reactive oxygen species; UCP, uncoupling protein.

DOI: 10.2337/db05-1645

TABLE 1
Baseline characteristics and their association with the development of type 2 diabetes

	No diabetes	With diabetes	HR (95% CI)*	*P* value
n	2,767	169	—	—
Age (years)	56.0 (3.5)	56.3 (3.4)	1.16 (0.93–1.46)	0.19
Systolic blood pressure (mmHg)†	136.3 (18.7)	141.3 (19.3)	1.30 (1.11–1.52)	0.001
Diastolic blood pressure (mmHg)	84.4 (11.4)	86.2 (11.3)	1.14 (0.98–1.34)	0.09
BMI (kg/m^2)†	26.0 (3.3)	28.6 (3.7)	1.86 (1.65–2.10)	<0.0001
Obesity	340 (12.3)	59 (35.1)	3.96 (2.87–5.47)	<0.0001
Smoking	28.6 (791)	32.0% (54)	1.29 (0.93–1.79)	0.13
Cholesterol (mmol/l)	5.72 (1.01)	5.90 (0.98)	1.20 (1.03–1.39)	0.03
Triglycerides (mmol/l)†	1.75 (0.92)	2.27 (1.7)	1.55 (1.34–1.80)	<0.0001
Fibrinogen (g/l)†	2.71 (0.52)	2.78 (0.53)	1.17 (1.00–1.36)	0.11
CRP (mg/l)†	2.92 (3.44)	4.05 (4.36)	1.37 (1.16–1.61)	<0.001
CRP excluding >20 mg/l	2.41 (2.47)	3.40 (3.26)	1.46 (1.21–1.75)	<0.0001

CRP measurements made after diabetes was recorded are excluded from the analysis ($n = 2$). *Age- and practice-adjusted HR for 1-SD increase in all variables except smoking (current/non), obesity (>30/<30), and age (5-year increase); †geometric mean (approximate SD).

sis in asymptomatic women (21), although this allele is associated with a lower rate of neuropathy in patients with type 1 diabetes (22). This may, in part, be attributable to different behavior of the variants in different tissues under different environmental conditions. The effect of PAX6 on *UCP2* G and A constructs varies according to cell type (16). The −866A allele was also associated with lower subcutaneous adipose tissue mRNA, but not with type 2 diabetes, in a mixed-race sample from the U.S. (23), although this may be explained by differences in genetic and environmental background.

UCP3 is expressed almost uniquely in skeletal muscle tissue (4), which is consistent with an important role in the regulation of metabolism because skeletal muscle accounts for nearly 50% of resting metabolic rate. UCP3 expression is increased in conditions that increase fatty acid oxidation, and because free fatty acids are obligatory for UCP3 function, a role in fat oxidation is likely (24). Impaired fatty acid metabolism is an important precursor in insulin resistance and is an important determinant of the amount of fat stored as intramyocellular lipid, which is negatively correlated with insulin sensitivity (25). Mice in which *UCP3* is overexpressed are lean and resistant to diet-induced diabetes (26). In humans a rare functional (exon 6 splice donor site) mutation is associated with a 50% reduction in fat oxidation (12), a state associated with obesity and diabetes. Although the results of *UCP3* mRNA levels in patients with type 2 diabetes have been variable, a reported 50% lower UCP3 protein compared with healthy control subjects (27) is consistent with a role for UCP3 function in protection from the development of diabetes. A variant in the *UCP3* gene associated with higher mRNA levels has been identified (*UCP3* −55C>T, rs1800849) (28). This variant has been associated with changes in fat distribution (waist-to-hip ratio) and BMI (29). In one study the −55T allele was associated with reduced risk of type 2 diabetes (30), but this was not replicated in other studies (29,31). This variant has also been associated with lower rates of neuropathy in a small study of type 1 diabetes (22).

The purpose of this research was to clarify these inconsistencies and to further elucidate the role of identified genetic variation in the *UCP2-UCP3* gene cluster in the development of type 2 diabetes. Specifically, we studied the impact of these variants on prospective risk of type 2 diabetes in a large cohort of healthy Caucasian men over a period of 15 years' follow-up. We also examined the interaction of obesity and UCP2/UCP3 genotype in determining risk.

RESEARCH DESIGN AND METHODS

For the prospective Second Northwick Park Heart Study (NPHSII), from April 1989 to April 1994, 3,012 healthy Caucasian men, who were aged 50–64 years and were registered with nine primary care practices in the U.K., were recruited for prospective surveillance. The study was approved by the institutional ethics committees and performed in accordance with the declaration of Helsinki. All subjects gave written informed consent. To be eligible, subjects had to be free of unstable angina, myocardial infarction, or evidence of silent infarction, coronary surgery, aspirin or anticoagulant therapy, cerebrovascular disease, malignancy (except skin cancer other than melanoma), or any condition precluding informed consent. Weight, height, and blood pressure measurements were recorded, and venous blood samples were collected for plasma and DNA analysis. Participants were recalled annually for 5 years for interview and repeat venous blood collection. Self-report by questionnaire was used to identify case subjects at baseline. Exclusion criteria precluded subjects requiring insulin or oral hypoglycemics from entry into NPHSII. New case subjects were identified by practice note search for physician-diagnosed and -treated type 2 diabetes according to current national guidelines.

Genotyping. Genotypes were determined by leukocyte DNA PCR amplification, using published primers and conditions for both *UCP2* (32) and *UCP3* (33). The products where then resolved by microarray diagonal gel electrophoresis as previously described (20) and confirmed by two independent technicians blind to subject outcome, with discrepancies resolved by repeat genotyping.

Statistical analysis. Analysis was performed using Intercooled STATA (Version 8.2; STATA, College Station, TX). Baseline characteristics were transformed to a normal distribution as appropriate. Obesity was defined as BMI >30 kg/m^2. Results are presented as hazard ratios (HRs) obtained from Cox regression models with their corresponding 95% CIs, adjusted for age and practice (recruitment site), and with further adjustment as described in the RESULTS section. Frequencies were compared by χ^2 test. Population-attributable fraction (%) was estimated as: pd × [(HR − 1)/HR], where pd is the proportion of cases exposed to the risk factor.

RESULTS

There were a total of 3,012 eligible Caucasian men, among whom at least one genotype was available for 2,671 (91%). A total of 76 men had type 2 diabetes at baseline and were excluded from prospective analysis. After 15 years of follow-up, a further 169 men had developed type 2 diabetes. Baseline BMI, C-reactive protein (CRP), triglycerides, cholesterol, and blood pressure were all associated with increased risk of development of type 2 diabetes (Table 1), with BMI conferring the highest risk (HR 1.86 [95% CI 1.65–2.10], $P < 0.0001$ per increase of 1 SD). A stepwise model was used to determine which of these variables

TABLE 2
Baseline characteristics with an independent association with the risk of development of type 2 diabetes (stepwise model)

	HR (95% CI)*	*P* value
BMI (kg/m^2)	1.72 (1.49–1.99)	<0.0001
Triglycerides (mmol/l)	1.27 (1.08–1.49)	0.004
CRP (mg/l)	1.21 (1.01–1.46)	0.04

*Age- and practice-adjusted HR for 1-SD increase.

were independently associated with type 2 diabetes (Table 2). Cholesterol and blood pressure were no longer associated with type 2 diabetes, and BMI remained the most significant predictor. Figure 1 shows the 3.96-fold (2.87–5.47) higher rate of development of type 2 diabetes in obese subjects, defined as BMI >30 kg/m^2.

For both the *UCP2* and *UCP3* variants, the genotype frequency was as expected from Hardy-Weinberg proportions, and the rare allele frequency was 0.37 (95% CI 0.35–0.38) and 0.23 (0.21–0.24) for the *UCP2* and the *UCP3* variants, respectively. The two variants showed weak but significant positive linkage disequilibrium (D′ = 0.28, $P < 0.001$). There was no significant difference in baseline BMI, blood pressure, lipid parameters, age, or CRP between the different genotypes for either variant (data not shown).

Risk of development of type 2 diabetes

UCP2 *−866G>A*. The genotype frequency did not differ significantly between those who developed diabetes and those who did not (no diabetes vs. diabetes [GG/GA/AA%]: 40/47/13 vs. 42/40/18, $P = 0.16$), but a trend to a higher frequency of the AA genotype was seen when the baseline subjects with type 2 diabetes were included (40/47/13 vs. 42/40/18, $P = 0.06$). The 15-year risk of type 2 diabetes associated with the AA genotype compared with the GG+GA men, adjusted for age and recruitment site, was 1.47 (95% CI 0.97–2.23), $P = 0.07$, excluding baseline cases and 1.49 (1.03–2.14), $P = 0.03$, including baseline cases. This risk effect was maintained after adjustment for BMI, blood pressure, cholesterol, triglycerides, and CRP, with an increased 15-year risk of type 2 diabetes of 1.59 times (1.03–2.45), $P = 0.04$, in AA homozygotes (1.59 [1.06–2.17], $P = 0.02$, including baseline cases). A Kaplan-Meier plot (Fig. 2) indicates that the *UCP-2* AA genotype appears to be associated with the development of diabetes ~5–10 years earlier, but there is a "catch up" in incidence in the GG+GA men by 15 years. As a consequence, the genotype difference for risk of diabetes at 10 years was highly significant (HR [AA vs. GG/GA]: 1.94 [1.18–3.19], $P = 0.009$). In obese men, the risk was increased even further, with obese *UCP2*AA men having a risk of type 2 diabetes at 15 years of 5.55 (2.95–10.45), $P < 0.001$, compared with nonobese *UCP2*GG+GA men.

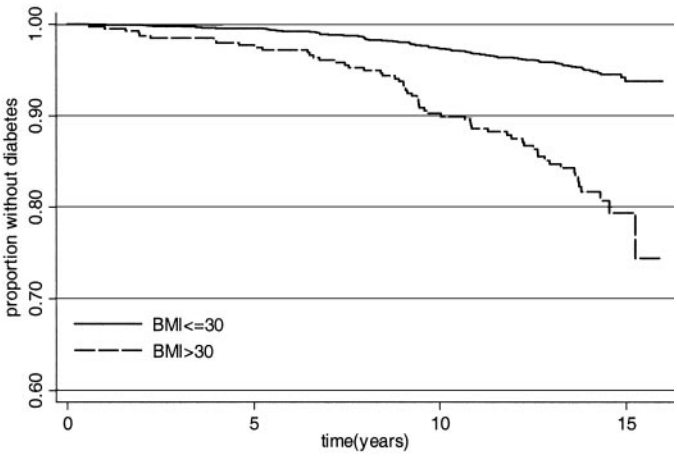

FIG. 1. Kaplan-Meier plot for the development of type 2 diabetes by the presence or absence of obesity.

UCP3 *−55C>T*. A Kaplan-Meier plot for risk of type 2 diabetes associated with *UCP3* is also shown in Fig. 2. There was a significant risk of type 2 diabetes associated with the TT genotype at 10 years (TT vs. CT+CC 2.06 [95% CI 1.06–3.99], $P = 0.03$) but not at 15 years (1.50 [0.85–2.66], $P = 0.16$). Risk of type 2 diabetes was again exacerbated by obesity, with the risk associated with *UCP3*TT genotype increasing to 5.65 (2.07–15.46), $P < 0.001$, at 15 years in the obese men.

***Combined* UCP2-UCP3 *genotypes*.** The percentage of men developing type 2 diabetes by combined *UCP2-UCP3* genotype is shown in Tables 3 and 4. After adjustment for age and place of recruitment, men homozygous for both rare alleles (the AA-TT genotype) were the only group showing a significant increased risk of type 2 diabetes at 10 years (HR 4.20 [95% CI 1.70–10.37], $P = 0.002$) or at 15 years (2.37 [1.07–5.2], $P = 0.03$) (Fig. 3). To obtain a more robust estimate of the effect of the combined genotypes, the subjects were combined into four groups based on homozygosity for the variant alleles (*UCP2-UCP3*: 1 = GG+CC, 2 = GG+GA-TT, 3 = AA-CC+CT, 4 = AA-TT). Risk of type 2 diabetes increased across the groups with increasing numbers of rare alleles, progressing from no variant homozygotes to two variant homozygotes (1.5% of men) at 10 years ($P = 0.002$) and 15 years ($P = 0.04$) (Fig. 3). The unadjusted population-attributable fraction at 10 years for the groups was 0.1% (−3.5 to 5.3), 5.4% (−3.4 to 13.3), and 5.2% (0.2–10), respectively, for groups 2, 3, and 4. Total population-attributable fraction for all variation in the *UCP2-UCP3* genotype from homozygous wild-type was 11.6% (0.4–21.8), corresponding to ~10 cases of type 2 diabetes in the sample at 10 years.

***Combined genotype and obesity*.** When the effect of the *UCP2-UCP3* genotype was combined with obesity (above and below 30 kg/m^2), nonobese AA-TT men showed an increased risk of type 2 diabetes of 2.50 (95% CI 0.91–6.86), $P = 0.08$ (Fig. 4), whereas obese AA-TT men had an increased risk of 19.23 (5.83–63.39), $P < 0.001$. The effects of the individual genotypes were additive, with no evidence of an interaction between the effect of *UCP2* and *UCP3* genotype ($P = 0.15$). There was also no evidence of interaction between the effect of combined genotype group and obesity ($P = 0.83$). When the population-attributable fraction model was adjusted for obesity, the population-attributable fraction at 10 years of the combined genotype was 11.0% (0–21.1), which compares to a population-attributable fraction of 25.6 (13.6–36.0) for obesity. This means that in the absence of variation in *UCP2-UCP3*, there would be 9 fewer cases of type 2 diabetes over 10 years, whereas in the absence of obesity, there would be 23 fewer cases.

UCP2-UCP3 *haplotypes*. As shown in Table 5, all four haplotypes were present in this sample. As would be predicted by the single nucleotide polymorphism analysis, only the AT combination was associated with increased risk of diabetes at 10 years (AT vs. GC 1.63 [95% CI 1.12–2.36], $P = 0.01$), and this effect was no longer statistically significant at 15 years (1.27 [0.93–1.73], $P = 0.13$).

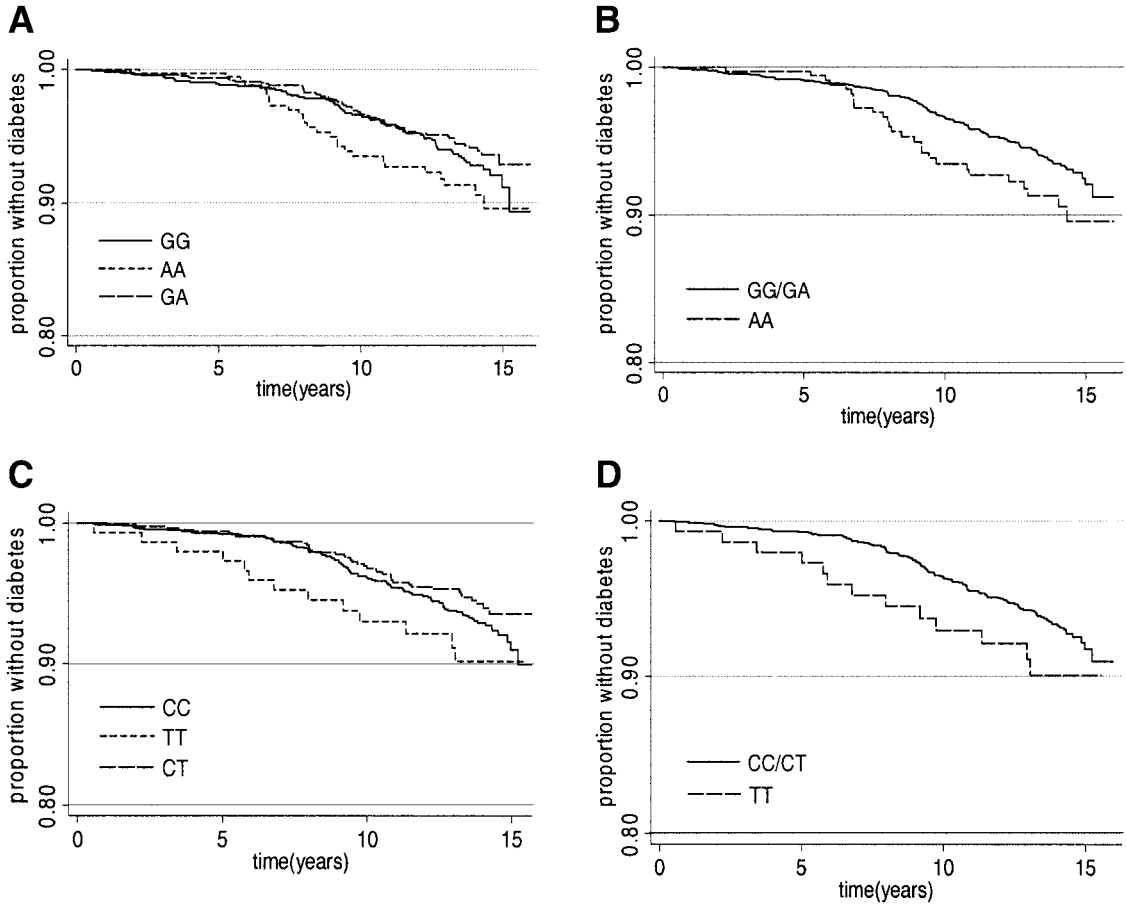

FIG. 2. Kaplan-Meier plot for the development of type 2 diabetes by UCP2G>A genotype (*A*), AA genotype based on a recessive model (*B*), UCP3C>T genotype (*C*), and TT genotype based on a recessive model (*D*).

DISCUSSION

This 15-year study of the prospective risk of type 2 diabetes in healthy middle-aged Caucasian men confirmed the significance of obesity as a major risk factor for diabetes, and it also found a significant impact on risk of developing type 2 diabetes associated with variants in the genes for *UCP2*(−866A) and *UCP3*(−55T). Subjects who developed type 2 diabetes also had higher blood pressure, triglycerides, cholesterol, and CRP at baseline, although only BMI, triglycerides, and CRP were associated independently with increased risk. The association with CRP and triglycerides may have arisen because subjects going on to develop type 2 diabetes were already more insulin resistant at baseline. The risk associated with BMI was over a third higher than any other metabolic parameter, and the size of the risk associated with obesity, defined here as BMI >30 kg/m^2, was approximately four times that associated with the genetic variants. In effect, after 10 years of follow-up, 1 of 4 cases of type 2 diabetes would have been prevented if there were no obesity present in the sample, whereas 1 of 10 cases would be prevented if there were no genetic variation at *UCP2* −866 or *UCP3* −55. The haplotype analysis showed only a significant risk of type 2 diabetes in those carrying the *UCP2*A/*UCP3*T haplotype, suggesting that both variants are required to produce a biologically important impact on disease development. It may also be that these single nucleotide polymorphisms

TABLE 3
Combined *UCP2-UCP3* genotypes and risk of type 2 diabetes at 10 years

UCP2	*UCP3*	% with diabetes (*n*)	HR (95% CI)*
GG	CC	3.2 (24/741)	1.00
	CT	2.3 (6/265)	0.59 (0.24–1.44)
	TT	9.4 (3/32)	3.00 (0.89–10.04)
GA	CC	3.8 (25/663)	1.09 (0.62–1.91)
	CT	2.2 (10/461)	0.63 (0.30–1.31)
	TT	1.4 (1/71)	0.38 (0.05–2.81)
AA	CC	3.7 (6/161)	1.14 (0.46–2.79)
	CT	5.7 (8/141)	1.64 (0.73–3.67)
	TT	13.6 (6/44)	4.20 (1.70–10.37)

*Age- and practice-adjusted HR.

TABLE 4
Combined *UCP2-UCP3* genotypes and risk of type 2 diabetes at 15 years

UCP2	*UCP3*	% with diabetes (*n*)	HR (95% CI)*
GG	CC	6.8 (50/741)	1.00
	CT	4.5 (12/265)	0.54 (0.28–1.03)
	TT	9.4 (3/32)	1.36 (0.42–4.37)
GA	CC	5.4 (36/663)	0.77 (0.50–1.18)
	CT	5.0 (23/461)	0.69 (0.42–1.14)
	TT	4.2 (3/71)	0.56 (0.17–1.81)
AA	CC	6.8 (11/161)	1.05 (0.54–2.02)
	CT	6.4 (9/141)	0.93 (0.45–1.89)
	TT	15.9 (7/44)	2.37 (1.07–5.25)

*Age- and practice-adjusted HR.

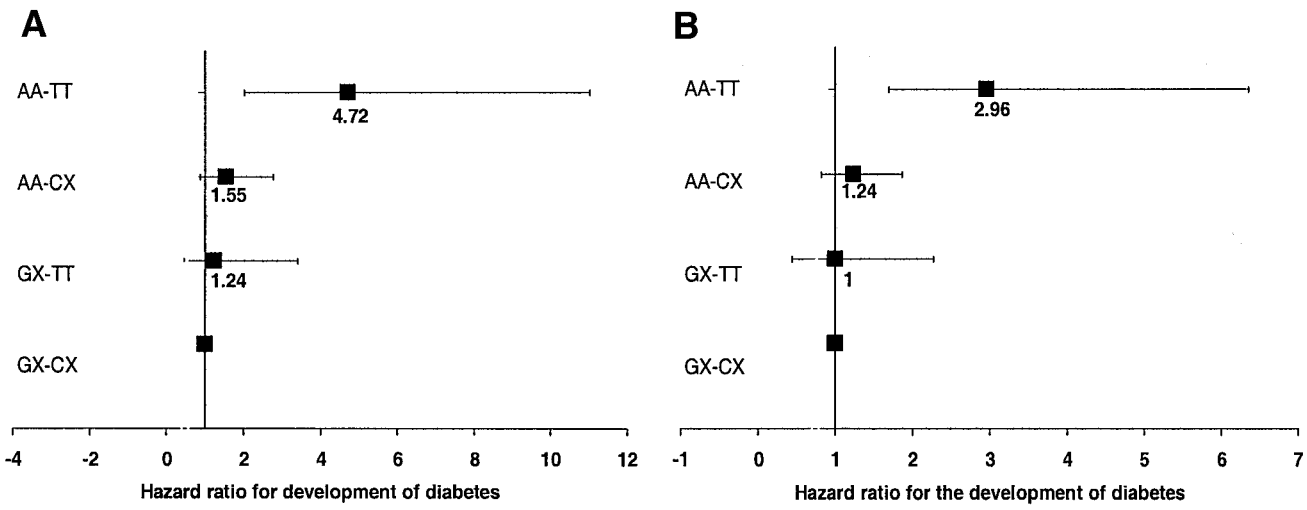

FIG. 3. HRs for the development of type 2 diabetes by *UCP2-UCP3* genotype at 10 (*A*) and 15 (*B*) years of follow-up.

are markers for an additional functional sequence change (or changes) at this locus that has yet to be discovered. The relatively low frequency of this haplotype (9%) and the recessive nature of their effect precluded a robust estimate of the additive effect with obesity. The additive effect of the combination of obesity and the presence of the gene variants increased the risk to extremely high levels, although such subjects were rare in the sample. In obese subjects with one homozygous variant, the risk of type 2 diabetes increased to over five times that of nonobese subjects nonhomozygous for either variant. Homozygotes for both variant alleles had a risk of diabetes from 2.5 times higher in the nonobese to nearly 20 times higher in obese subjects.

The *UCP2* and *UCP3* genes are located close together in a gene cluster, but the pattern of their expression is very different. UCP2 expression is widespread, including monocytes, endothelial cells, and, crucially, the β-cells in the pancreas (8), whereas UCP3 is almost exclusively expressed in skeletal muscle, with a much smaller amount in heart, bone marrow, and thyroid. Therefore, the variants' mechanism of action for type 2 diabetes risk is likely to be different. Both variants have been associated with obesity or waist-to-hip ratio in cross-sectional studies (16,29,32,33), but BMI and waist-to-hip ratio did not differ at baseline by genotype, and there was no significant difference in weight gain by genotype (data not shown). Therefore, the effect on type 2 diabetes development is likely to be a primary effect based on changes in physiological functioning of the two proteins.

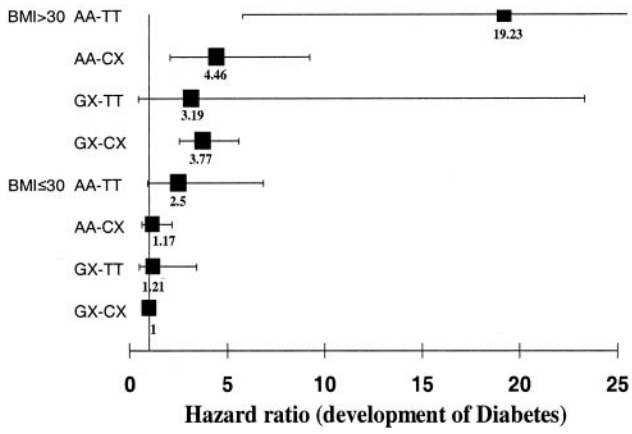

FIG. 4. The HR for the development of type 2 diabetes at 15 years of follow-up by *UCP2-UCP3* genotype in subjects with and without obesity.

The *UCP2* −866A allele has consistently been associated with risk of type 2 diabetes in cross-sectional studies in European subjects, and the mechanism for this appears likely to be caused by increased UCP2 expression. The location of the −866G>A variant, within a multifunctional *cis* regulatory site involving putative binding sites for pancreatic and hypoxia-induced transcription factors, suggests that it is likely to be functional (32). Consistent with this, a promoter construct of the −866A allele was associated with 1.2-fold higher expression versus −866G in INSE-1 cells derived from rat β-cells (16). UCP2 expression uncouples ATP production from glucose metabolism, reducing ATP production, and because insulin secretion depends on the ATP-to-ADP ratio as a marker of glucose metabolism in the pancreatic β-cell (34), secretion is reduced as a result. The higher risk of developing type 2 diabetes associated with variation in the *UCP2* gene is thus likely to be caused by a pancreatic effect on insulin secretion (Fig. 5*A*). Risk is exacerbated by obesity because obese subjects are already likely to be insulin resistant and will require higher insulin secretion to maintain normal glucose homeostasis.

UCP3 expression has been associated with higher uncoupling in vitro (35). However, *UCP3* expression is increased both by fasting and by an isocaloric high-fat diet, neither of which increases metabolic rate or uncoupling. Also, an increase in expression does not change mitochondrial membrane potential. These findings suggest that *UCP3* does not have a conventional uncoupling function in humans (36). There is evidence that *UCP3* expression is increased in situations where fatty acid metabolism is increased, such as fasting (37), lipid infusion (38), or acute exercise (39), and it is decreased when fatty acid metabolism is decreased, such as during chronic exercise (40) and with weight reduction (41), suggesting a role for UCP3 in regulating muscle substrate metabolism. Overexpression of *UCP3* in mice protects them from diabetes, with a 68% increase in mitochondrial palmitate oxidation. This effect is specific to UCP3 because there is no increase in

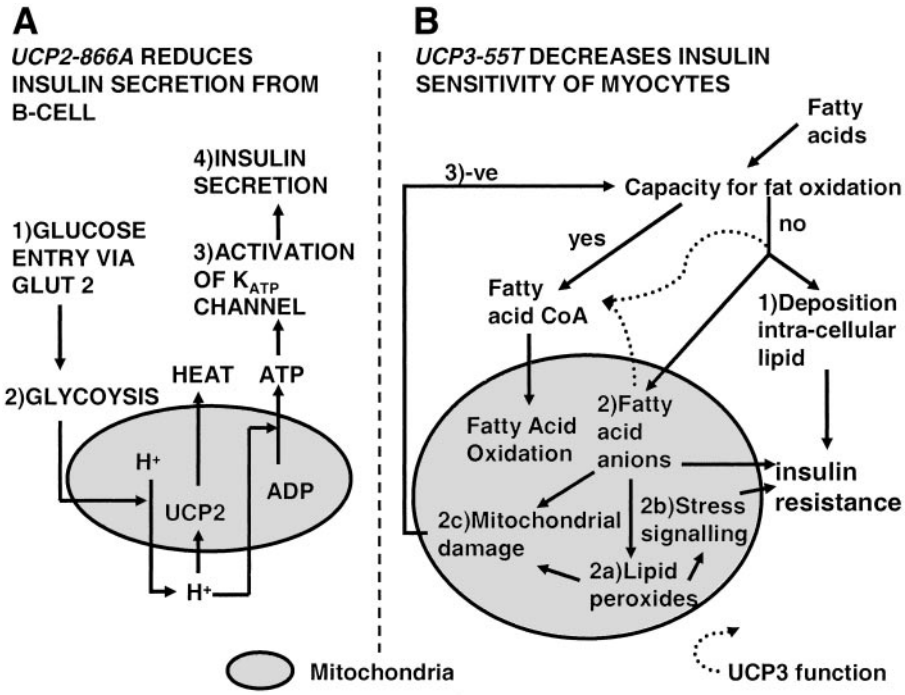

FIG. 5. Mechanism of action of *UCP2-UCP3* gene cluster variants. *A*: *UCP2-866A* increases transcription in the β-cell, reducing the production of ATP from glucose metabolism (1 and 2), reducing potassium influx (3) and insulin secretion (4). *B*: *UCP3-55T* acts through fatty acid metabolism. UCP3 function protects the myocyte from the adverse consequences of fatty acid oversupply (1 and 2) by removing fatty acid anions from mitochondria and maintaining fatty acid oxidative capacity. Impaired function exposes the myocyte to deleterious effects of prolonged exposure of mitochondria to fatty acid anions (2a–2c) with further impairment of fatty acid oxidation (3) and worsening of fatty acid oversupply.

glucose oxidation or change in oxygen consumption or mitochondrial membrane potential, which is seen with increased uncoupling per se (42). This mouse model also had a significant reduction in mitochondrial ROS generation, and, conversely, increased mitochondrial ROS damage was seen in the *UCP3* knockout mouse (43). These data suggest a role for fatty acid availability in the development of type 2 diabetes; when oversupply occurs either because of high delivery or low levels of oxidation, or both, fatty acids can accumulate within the cell. Neutral fatty acids can cross into the mitochondrial membrane, where they will become anionic because of the proton gradient. These fatty acid anions are not able to be metabolized and are trapped in the mitochondria, where they are susceptible to oxidation from the ROS production of the electron transport chain. Lipid peroxides are cytotoxic and highly reactive, and the mitochondrial DNA, RNA, and important enzyme systems, such as the citric acid cycle, are vulnerable to oxidative damage (24,42). Patients with type 2 diabetes have lower capacity for fat oxidation and have increased intramyocellular lipid, which shows increased peroxidation (44). They are also more susceptible to mitochondrial DNA damage and have smaller and more damaged mitochondria (45). *UCP3* expression will thus protect the mitochondria by exporting the fatty acid anions and peroxides from the mitochondrial matrix, protecting mitochondrial function and preventing the development of type 2 diabetes (46) (Fig. 5B).

Because the *UCP3-55T* variant is associated with risk of diabetes in the men studied here, a decrease in function would be predicted. The variant was originally mapped near a TATA box (6 bp) (28), where it may affect transcription, but a recent report (47) places the variant in the 5′ untranslated region near a peroxisome proliferator–activated receptor–responsive element (4 bp), which also suggests it could modify regulation of *UCP3* expression. A reduction in *UCP3* function in −55T carriers has been seen in some but not all previous cross-sectional studies. *UCP3* mRNA expression was higher in CT/TT male nondiabetic Pima Indians, but this study included only 24 subjects and was based on only seven copies of the T allele (28). The T allele was associated with higher BMI or waist-to-hip ratio, consistent with decreased function, in French and German Caucasians, South Asian Indian parent-offspring trios, South Asian Indians, and the British Diabetic Association Warren 2 trios collection (29,31,33,48). However, one study showed a lower BMI in TT subjects in a U.K. sample (31), and increased function is also suggested with the association of protection from diabetic neuropathy (22). In these studies association with type 2 diabetes was examined only once, and a relationship was not found. The variant allele was found to be protective against type 2 diabetes in two French cohorts, although it was also associated with an atherogenic lipid profile (30). The reason for this difference is not clear; the French sample does not appear to be very different to the NPHSII sample, although the NPHSII sample is nearly three times larger, which may be important when the impact of an individual variant is small.

The overall prevalence of type 2 diabetes in this sample

TABLE 5
Risk of development of type 2 diabetes associated with *UCP2-866/UCP3-55* haplotypes

Haplotype	Frequency	10-year HR (95% CI)	*P*	15-year HR (95% CI)	*P*
GC	0.50	1		1	
GT	0.13	0.74 (0.47–1.17)	0.19	0.73 (0.5–1.05)	0.09
AC	0.28	1.09 (0.76–1.55)	0.64	0.94 (0.72–1.22)	0.64
AT	0.09	1.63 (1.12–2.36)	0.01	1.27 (0.93–1.73)	0.13

is 2.5% at baseline and 8.1% after 15 years of follow-up. This is lower than that reported by the Joint Health Surveys Unit (49); however, the increase in prevalence of both diagnosed and undiagnosed diabetes between the age range 55–64 years and 65–74 years is similar, from 10.4 to 18.8%. The lower prevalence is in part attributable to the exclusion of those with diabetes on treatment at the beginning of the study. The method of identification of the men with type 2 diabetes (by medical record search) is unlikely to include any false-positive diagnosis, but in the absence of a full recall for fasting glucose testing, some subjects may be missed. This would then result in a small underestimate of the 15-year incidence of type 2 diabetes, but it would not confound the genetic association seen. This is the first prospective study to look at risk of type 2 diabetes, and, overall, prospective gene association studies are more powerful than the case-control design (50), but further replication is required to resolve these apparent discrepancies.

This study has shown that variation in the *UCP2-UCP3* gene cluster is associated with an increased risk of type 2 diabetes. This underlines the importance of the UCPs in human metabolism, but further investigation is required to define their exact role in fuel oxidation and mitochondrial function. The results also raise the possibility that type 2 diabetes is a disease at least in part caused by mitochondrial dysfunction and that UCP2 and UCP3 could serve as targets for drug development to prevent and treat type 2 diabetes.

ACKNOWLEDGMENTS

NPHSII was supported by the U.K. Medical Research Council, the U.S. National Institutes of Health (National Heart, Lung, and Blood Institute Grant 33014), and DuPont Pharma (Wilmington, DE). J.W.S. was supported by a clinical training fellowship from Diabetes UK (BDA: RD01/0001357). D.R.G., J.A.C., and S.E.H. were supported by the British Heart Foundation (FS/04/012:RG2000/015).

The following general practices in the U.K. collaborated in the study: the Surgery, Aston Clinton, Camberley; the Health Centre, Carnoustie; Whittington Moor Surgery, Chesterfield; the Market Place Surgery, Halesworth; the Health Centre, Harefield; Potterells Medical Centre, North Mymms; Rosemary Medical Centre, Parkstone, Poole; and the Health Centre, St. Andrews.

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