Genetic Variants in the *UCP2-UCP3* Gene Cluster and Risk of Diabetes in the Women's Health Initiative Observational Study

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OBJECTIVE—Mitochondrial uncoupling proteins (UCPs) are involved in body weight regulation and glucose homeostasis. Genetic variants in the *UCP2-UCP3* gene cluster, located on chromosome 11q13, may play a significant role in the development of type 2 diabetes.

RESEARCH DESIGN AND METHODS—We conducted a comprehensive assessment of common single nucleotide polymorphisms (SNPs) at the 70-kb *UCP2-UCP3* gene cluster in relation to type 2 diabetes risk in a prospective, case-control study nested in the Women's Health Initiative Observational Study, an ethnically diverse cohort of postmenopausal women including Caucasian, African, Hispanic, and Asian American subjects. We genotyped 14 tag SNPs in 1,584 incident type 2 diabetes case and 2,198 control subjects matched by age, ethnicity, clinical center, time of blood draw, and length of follow-up.

RESULTS—We identified a haplotype set (rs591758-rs668514-rs647126-rs1800006, spanning the *UCP2-UCP3* intergenic and *UCP3* regions) as significantly associated with greater type 2 diabetes risk (nominal $P = 0.0011$, permutation $P = 0.046$) in Caucasian women, especially among overweight Caucasians (BMI >25 kg/m^2) (nominal $P = 0.0006$, permutation $P = 0.032$). Compared with the most common haplotype (h1010 as the referent), haplotype h0001 (19.5% in control subjects) had odds ratios of 2.0 (95% CI 1.13–3.37) in Caucasians and 3.8 (1.44–9.93) in Caucasian overweight women. Similar haplotype–type 2 diabetes association was also observed among Hispanic women who were overweight.

CONCLUSIONS—These findings suggest a role of *UCP2-UCP3* gene cluster haplotypes in diabetes; in particular, the effects of the high-risk haplotypes were more apparent in overweight Caucasian women. These data warrant further confirmation in future prospective and experimental studies. ***Diabetes*** **57: 1101–1107, 2008**

Uncoupling proteins (UCPs) are members of the super family of anion carrier proteins located in the inner membrane of mitochondria (1). Among the five UCP homologues, *UCP1–UCP5* (2), *UCP2* and *UCP3* are closely located (supplemental Fig. 1, available in an online appendix at http://dx.doi.org/10.2337/db07-1269) on chromosome 11q13 (3). At the amino acid sequence level, UCP2 and UCP3 are ~73% identical to each other (2). In humans, significant linkage has been reported for markers at the *UCP2-UCP3* gene cluster with resting metabolic rate (D11S911, $P = 0.000002$) (4), type 2 diabetes (5), and fasting insulin (6). This region is also homologous to a region of chromosome 7 in mice that has been linked to both hyperinsulinemia and obesity (7). Therefore, both *UCP2* and *UCP3* have been implicated as important biological candidate genes for type 2 diabetes and obesity.

To date, several genetic variants in the *UCP2-UCP3* gene cluster have been examined in multiple studies, including −866 G/A (rs659366), Ala55Val (rs660339), a 45-bp insertion/deletion (I/D) in the 3′ untranslated region (UTR) of exon 8 in *UCP2*, and the −55 C/T (rs1800849) polymorphism in *UCP3*. Briefly, the −866 G/A variant was associated with higher *UCP2* mRNA level, reduced insulin secretion, or increased risk of type 2 diabetes in Austrian (8), Italian (9), and Japanese (10) samples. The Ala55Val and the 45–base pair (bp) I/D polymorphisms in *UCP2* were both associated with metabolic rates during sleep (11). The −55 C/T polymorphism in *UCP3* resides 6 bp upstream from the TATA box in the core promoter and was associated with the skeletal muscle mRNA level in nondiabetic subjects (12). Despite strong functional evidence for the *UCP2* −866 G/A and *UCP3* −55 C/T polymorphisms, previous association studies of both *UCP2* and *UCP3* gene variants have not been consistent. Several studies found that these functional variants exhibited significantly increased (8,9,13) or decreased (14–16) type 2 diabetes risk, but others did not report any significant associations (10,17). Such discrepancies may be attributable to differences in study design (population based vs. hospital based), selection and ascertainment schemes, sample sizes, or statistical analysis strategies. It also remains possible that the −866 G/A and −55 C/T polymorphisms are in linkage disequilibrium (LD) with an unidentified causative variant located in the proximity of both genes on chromosome 11q13. However, no prospective studies have comprehensively assessed variants in the entire region of *UCP2–3* in relation to type 2 diabetes risk.

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CVD, cardiovascular disease; I/D, insertion/deletion; LD, linkage disequilibrium; LOD, logarithm of odds; MAF, minor allele frequency; SNP, single nucleotide polymorphism; tSNP, tag SNP; UCP, uncoupling protein; UTR, untranslated region; WHI-OS, Women's Health Initiative Observational Study.

TABLE 1
The SNP dbSNP IDs, gene names, physical locations, function annotations, major and minor alleles, and the relative distances of the 14 tSNPs (genotyped at stage II) in the *UCP2-UCP3* gene cluster

dbSNP ID	Gene symbol*	Physical location†	Function annotation‡	Major/minor allele	Relative distance (bp)§
rs637028	*DNAJB13*	3′ flanking	—	T/G	(origin)
rs653263	*DNAJB13*	Exon 3	His93His	C/T	10099
rs622064	*DNAJB13*	Intron 3	—	A/C	1929
rs2306820	*DNAJB13*	Promoter	—	T/C	21158
rs673494	*UCP2*	3′ flanking	—	C/T	1162
rs655717	*UCP2*	3′ flanking	—	C/T	1706
rs643064	*UCP2*	3′ UTR	—	G/A	500
rs660339	*UCP2*	Exon 4	Ala55Val	C/T	4389
rs659366	*UCP2*	Promoter	−866 G/A (A: risk allele)	G/A	5650
rs591758	*UCP2–3*	Intergenic region	—	C/G	3306
rs668514	*UCP3*	3′ flanking	—	C/T	8454
rs647126	*UCP3*	3′ UTR	—	G/A	5506
rs1800006	*UCP3*	Exon 3	Tyr99Tyr	T/C	5234
rs1800849	*UCP3*	Promoter	−55 C/T (T: risk allele)	C/T	2911

*Based on NCBI Entrez database. *DNAJB13*, DnaJ (Hsp40) related, subfamily B, member 13; *UCP2*, mitochondrial, proton carrier); *UCP3*, mitochondrial, proton carrier. †Based on NM_153614 (*DNAJB13*), NM_003355 (*UCP2*), and NM_003356 (*UCP3*). ‡Provided only for coding SNPs (amino acid residues for the two alleles) and for SNPs in the 5′ flanking region (the nucleotide positions relative to the transcription start sites in the promoter regions are indicated). §Relative distance of an SNP is defined as its physical distance with its 5′ adjacent SNP based on the contig positions of contig NT_033927.

To provide a comprehensive assessment of the associations between common variation in *UCP2–3* cluster and type 2 diabetes risk, we conducted a large prospective case-control study nested in the Women's Health Initiative Observational Study (WHI-OS), an ethnically diverse cohort of postmenopausal women aged >50 years including Caucasian, African, Hispanic, and Asian American subjects.

RESEARCH DESIGN AND METHODS

Study subjects. The WHI-OS is a longitudinal study designed to examine the association between clinical, socioeconomic, behavioral, and dietary risk factors with subsequent incidence of health outcomes, including cardiovascular disease (CVD) and diabetes. Details regarding the scientific rationale, eligibility, and other design aspects have previously been described (18). The study has been reviewed and approved by human subjects review committees at each participating institution, and signed informed consent was obtained from all women enrolled.

Incident diabetes cases were identified based on postbaseline self-report of first-time use of hypoglycemic medication (oral hypoglycemic agents or insulin) during a median follow-up period of 5.9 years. Approximately 82,069 subjects had no prior history of CVD and/or diabetes at baseline. Following the principle of risk-set sampling, for each new case, control subjects were selected randomly from women who remained free of CVD and/or diabetes at the time the case was identified during follow-up. Control subjects were matched to case subjects by age (±2.5 years), racial/ethnic group (Caucasian, African, Hispanic/Latino, and Asian/Pacific Islander), clinical center (geographic location), time of blood draw (±0.10 h), and length of follow-up. In our current study, each of the 968 Caucasian case subjects were randomly chosen and matched with one control subject. Of 616 incident cases among ethnic minority women, 366 case subjects were African American, 152 Hispanic, and 98 Asian/Pacific Islander. The 1:2 matching ratio was used for minorities to strengthen the power in these smaller sample sizes of cases (19).

Tag single nucleotide polymorphism selection and genotyping. We undertook a two-stage approach. The first stage consists of comprehensive common single nucleotide polymorphism (SNP) discovery by genotyping a total of 21 SNPs in 244 samples randomly selected from the WHI-OS source population. The second stage involved genotyping a total of 14 tag SNPs (tSNPs) in the entire case-control samples according to LD patterns defined during the first stage.

Briefly, we first surveyed all common genetic variants available from the National Center for Biotechnology Information dbSNP database. In total, an initial set of 21 SNPs were selected based on the following criteria: *1*) functionality priority (nonsynonymous coding SNPs [cSNPs] and splicing-site SNPs were kept following the order of coding SNPs > splicing-site SNPs > 5′ UTR SNPs >3′ UTR SNPs > synonymous SNPs > intronic SNPs); *2*) minor allele frequency (MAF) ≥5% in at least one ethnic group; and *3*) relatively even spacing across the genomic region (20). The initial set of SNPs was genotyped using the high-throughput Illumina BeadArray platform (21) at Illumina (San Diego, CA) in a multiethnic panel of 244 women randomly chosen from the entire case-control sample (n = 61 for each group of Caucasian, African, Hispanic, and Asian-American subjects). A graphical depiction of the physical locations of these SNPs is shown in supplemental Fig. 1 (available in an online appendix at http://dx.doi.org/10.2337/db07-1269).

In the second stage, we selected tSNPs based on the LD patterns of those 21 SNPs. Pairwise LDs between SNPs were assessed using Lewontin's D′ statistic and the squared correlation statistic r^2. The Haploview program was used to calculate the LD coefficients and define haplotype blocks (22). We chose all common tSNPs with special focus on Caucasian and African-American samples, since Hispanic and Asian-American subjects only constitute relatively small proportions in either case or control groups. Using the r^2-based Tagger program (22), tSNPs in African Americans were chosen by finding the minimum set of tSNPs with pairwise r^2 ≥0.80 and MAF ≥5%. We then added additional SNPs to reach a minimum set of tSNPs for Caucasian-American samples to ensure a sufficient and yet nonredundant parsimonious set of tSNPs. From the initial dense set of 21 SNPs, a total of 14 tSNPs were selected and genotyped in all case-control samples (Table 1). In the HapMap II dataset, the estimated coverage ($r^2 \geq 0.8$) of the *UCP2-UCP3* genetic variation by these 14 genotyped SNPs is 76% for Caucasians and 80% for Asians. The estimated coverage when $r^2 \geq 0.5$ is 86% for Caucasians and 96% for Asians. The untaggable SNPs genotyped in HapMap dataset are located evenly across the whole 70-kb *UCP2-UCP3* genome region. Genotyping was performed using the TaqMan allelic discrimination method in the molecular epidemiology laboratory (S.L., principal investigator) at the University of California, Los Angeles (19). A total of 138 randomly selected replicate samples were genotyped, and the consistency rate was 99% for each of the 14 tSNPs. The average genotyping dropout rate was 1.8% (from 1.3 to 2.5%).

Statistical methods. MAF in the control samples was estimated for each ethnic group. The Hardy-Weinberg equilibrium test for each of the 14 tSNPs was performed using the χ^2 test (1 d.f.) (SAS version 9.1; SAS Institute, Cary, NC). We also tested for heterogeneity of genotype distributions across ethnicities by the χ^2 test (3 d.f.).

In both single-SNP and haplotype-based analyses, conditional multivariable logistic regression models were employed to estimate the odds ratios (ORs) (95% CI) for each genetic variant of type 2 diabetes risk with adjustments for multiple confounding variables including matching factors (age, clinical center, time of blood draw, and ethnicity), BMI, ln(fasting glucose), ln(fasting insulin), cigarette smoking (never, past, and current), alcohol intake (never, past, and current), postmenopausal hormone therapy (never, past, and current), type 2 diabetes family history (presence/absence), and total MET value (the energy expended by a person at rest; 1 MET = 1 kcal · kg^{-1} body weight · h^{-1} [23]) from recreational physical activity per week at baseline. In single-SNP analyses, each SNP was coded as additive, dominant, and reces-

TABLE 2
MAFs of the 14 tSNPs (genotyped at stage II) in the *UCP2-UCP3* gene cluster

dbSNP ID	Function annotation*	Major/minor allele	MAF (%)† Caucasian American	African American	Asian American	Hispanic American	Combined	$P_{heterogeneity}$‡
n	—	—	968	732	303	195	2,198	
rs637028	—	T/G	17.4	39.8	16.1	17.1	25.1	<0.0001
rs653263	His93His	C/T	54.4	20.3	45.1	50.6	41.0	<0.0001
rs622064	—	A/C	22.6	36.5	32.9	28.4	29.2	<0.0001
rs2306820	—	T/C	30.0	19.6	36.7	20.4	26.3	<0.0001
rs673494	—	C/T	38.3	63.7	43.5	45.4	48.4	<0.0001
rs655717	—	C/T	54.2	22.2	49.3	52.7	42.4	<0.0001
rs643064	—	G/A	13.8	40.1	12.6	25.3	23.6	<0.0001
rs660339	Ala55Val	C/T	39.6	43.4	43.8	45.7	41.9	0.0416
rs659366	−866 G/A	G/A	35.4	42.7	41.4	46.7	39.6	<0.0001
rs591758	—	C/G	62.2	25.4	55.7	53.4	48.0	<0.0001
rs668514	—	C/T	22.5	27.5	31.9	21.0	25.4	<0.0001
rs647126	—	G/A	50.1	23.4	47.3	50.6	40.4	<0.0001
rs1800006	Tyr99Tyr	T/C	24.1	59.1	19.2	26.7	35.6	<0.0001
rs1800849	−55 C/T	C/T	22.6	13.7	15.0	27.6	18.9	<0.0001

*Provided only for coding SNPs (amino acid residues for the two alleles) and for SNPs in the 5′ flanking region (the nucleotide positions relative to the transcription start sites in the promoter regions are indicated). †MAF was estimated in the control subjects only. ‡*P* value was estimated based on a χ^2 test (3 d.f.) for genotype distribution across the four ethnicities.

sive genetic models for estimations of ORs (95% CI) using conditional logistic regressions for each genetic model, respectively.

In haplotype-based analyses, haplotypes were estimated from the unphased genotype data using the HAPPY macro in SAS, version 9.1 (24,25), and the haplotype frequency estimates were then validated via HAPLOTYPER v2, a Bayesian haplotype inference algorithm (26). We reported haplotype frequencies for the haplotypes with frequency ≥2.5% in control subjects but pooled those rare haplotypes (<2.5%) together as one haplotype category, z_{pooled}. In all tests performed, the frequency of z_{pooled} was found to be <10% in either the control group or the case group, respectively. To account for the uncertainty of haplotype phasing, we used the expectation-substitution approach treating the expected haplotype scores under the additive model as observed covariates in the conditional logistic regression model (24). To increase the genomic coverage, we employed a sliding window (window width 4 SNPs) haplotype-based analysis. For each window, an omnibus likelihood ratio test was used, which was a χ^2 test (d.f. = number of haplotypes in a particular window − 1). The test was based on the difference of the logarithmic likelihood of two conditional logistic regression models: the reduced model, which does not contain the haplotype covariates, and the full model, which contains the haplotype covariates. As a priori, stratified analyses by overweight status (BMI >25 kg/m^2, overweight subgroup) were also performed.

To adjust for the single-point significance level for multiple testing with corrected type I error, we reported empirical *P* values based on global random permutation tests. We randomly permuted the case-control status of each subject, performed the same set of analyses (including single SNP analyses, sliding window analyses, stratified analyses by ethnic groups, and stratified analyses by overweight status), and record the minimal *P* value for each permutation dataset. The distribution of the minimal *P* values obtained from 10,000 permutation datasets was used to derive the empirical significance of the observed test statistic ($P_{permutation}$). The adjusted global-wide *P* values were determined as $P_{adjusted} = P(P_{observed} = < P_{permutation})$. All reported *P* values are from two-sided tests. To address the issue of undiagnosed diabetes at baseline and assess the robustness of our findings, we also conducted sensitivity analyses excluding case and control subjects who had a one-time measure of fasting glucose ≥126 mg/dl at baseline.

RESULTS

Estimation of MAF of the 14 tSNPs and LD structures in the *UCP2-UCP3* gene cluster among control subjects. The characteristics of the 14 tSNPs are shown in Table 1. None of the tSNPs had genotype distributions deviating from Hardy-Weinberg equilibrium at $P < 0.01$ levels. The estimated MAFs in control subjects stratified by ethnicity were shown in Table 2. With the exceptions of *UCP2* Ala54Val (rs660339) ($P = 0.04$), the genotype distributions of all tSNPs varied significantly across different ethnicities (Table 2). In particular, the MAFs in the African-American women differed significantly from those of all other ethnic groups for the majority of the tSNPs.

The LD (D' was used as the pairwise LD metric) structures and haplotype blocks across the 70-kb *UCP2-UCP3* gene cluster are shown in supplemental Fig. 2. Overall, four visually discernable blocks with slightly varying boundaries across different ethnicities were defined, including block one, which covers the *DNAJB13* gene and the 5′ upstream region of the *UCP2-UCP3* gene cluster; block 2, the *DNAJB13-UCP2* intergenic region; block 3, *UCP2* and 3′ UTR of *UCP3*; and block 4, the *UCP3* region. The −866 G/A (rs659366) and the Ala55Val (rs660339) in *UCP2* and the −55 C/T (rs1800849) and the Tyr99Tyr (rs1800006) in *UCP3* were in high LD ($D' > 0.95$ and logarithm of odds [LOD] ≥2) for all four ethnic groups. However, the −866 G/A and Ala55Val polymorphisms of *UCP2* were not in high LD ($D' < 0.50$ and LOD ≥2 for Caucasian, African, and Hispanic-American samples; $D' < 0.80$ and LOD ≥2 for the Asian-American sample) with either the −55 C/T or the Tyr99Tyr polymorphism of *UCP3*.

Single-SNP analyses. The association of each tSNP with type 2 diabetes risk in each ethnic group or in the combined samples was evaluated under the additive, dominant, and recessive genetic models. As shown in Table 3 (using the additive genetic model), nominally significant (i.e., $P < 0.05$) associations were found for rs2306820 (OR 1.8 [95% CI 1.1–3.1]), rs668514 (1.8 [1.1–3.0]), and Tyr99Tyr (rs1800006) (0.6 [0.4–0.9]) among African-American women. In the combined multiethnic sample, a nominally significant association was found between rs653263 and type 2 diabetes (0.7 [0.6–0.9]). However, none of these associations remained significant after adjustment for multiple testing. Similarly, no significant association was found under either the dominant or the recessive genetic model for any tSNP after further adjustment for multiple testing (data not shown). Leaving out baseline fasting glucose and insulin in multivariable models did not materially change these findings.

TABLE 3
Single SNP association studies of the 14 tSNPs (genotyped at stage II) in the *UCP2-UCP3* gene cluster with type 2 diabetes*

DbSNP ID	Function annotation	Caucasian American	African American	Asian American	Hispanic American	Combined	$P_{interaction}$†
rs637028	—	0.9 (0.6–1.5)	1.1 (0.7–1.7)	0.2 (0.03–1.5)	1.3 (0.5–3.5)	1.0 (0.8–1.4)	0.86
rs653263	His93His	0.9 (0.6–1.3)	0.6 (0.4–1.1)‡	0.2 (0.03–2.5)	0.6 (0.3–1.4)	0.7 (0.6–0.9)§	0.56
rs622064	—	1.1 (0.7–1.7)	0.9 (0.6–1.3)	3.3 (0.7–16.7)	1.4 (0.7–2.7)	1.1 (0.9–1.4)	0.40
rs2306820	—	1.1 (0.7–1.7)	1.8 (1.1–3.1)§	1.4 (0.3–6.0)	1.6 (0.7–4.0)	1.3 (1.0–1.7)‡	0.46
rs673494	—	1.0 (0.7–1.5)	1.5 (1.0–2.4)‡	0.9 (0.3–3.4)	1.4 (0.6–2.9)	1.2 (0.9–1.5)	0.75
rs655717	—	1.2 (0.8–1.7)	0.7 (0.4–1.2)	1.1 (0.3–4.4)	0.5 (0.2–1.2)	0.9 (0.8–1.2)	0.63
rs643064	—	1.0 (0.6–1.6)	0.8 (0.5–1.1)	0.7 (0.2–2.9)	1.0 (0.4–3.0)	0.9 (0.7–1.1)	0.60
rs660339	Ala55Val	1.2 (0.8–1.7)	0.9 (0.6–1.5)	0.9 (0.3–3.4)	1.4 (0.7–2.9)	1.1 (0.8–1.4)	0.55
rs659366	−866 G/A	1.2 (0.8–1.7)	0.9 (0.6–1.4)	0.8 (0.2–2.7)	1.0 (0.5–2.1)	1.0 (0.8–1.3)	0.65
rs591758	—	0.9 (0.6–1.4)	0.8 (0.5–1.4)	1.1 (0.3–3.8)	1.0 (0.5–2.0)	0.9 (0.7–1.2)	0.99
rs668514	—	1.1 (0.7–1.8)	1.8 (1.1–3.0)§	2.8 (0.5–16.9)	1.2 (0.5–2.5)	1.3 (1.0–1.7)‡	0.47
rs647126	—	0.8 (0.5–1.1)	0.8 (0.5–1.4)	0.8 (0.3–2.5)	0.7 (0.3–1.5)	0.8 (0.6–1.0)	0.93
rs1800006	Tyr99Tyr	1.1 (0.7–1.8)	0.6 (0.4–0.9)§	0.7 (0.2–2.5)	1.2 (0.5–2.9)	0.9 (0.7–1.1)	0.04¶
rs1800849	−55 C/T	1.2 (0.7–2.0)	0.7 (0.4–1.3)	0.7 (0.2–2.5)	1.1 (0.4–2.7)	1.0 (0.7–1.3)	0.08¶

*Adjusted OR (95% CI) for each tSNP was estimated under the additive genetic model, using conditional logistic regression models with adjustments for multiple confounding variables including matching factors (age, clinical center, time of blood draw, and ethnicity), BMI, ln(fasting glucose), ln(fasting insulin), cigarette smoking (never, past, and current), alcohol intake (never, past, and current), postmenopausal hormone therapy usage (never, past, and current), type 2 diabetes family history (presence/absence), and total MET value (the energy expended by a person at rest: 1 MET = 1 kcal · kg^{-1} body weight · h^{-1} [ref. 37]) from recreational physical activity per week at baseline. For Asian Americans, the model was adjusted for all the covariates above except the type 2 diabetes family history and the total metabolic equivalent value. †*P* value was estimated for the interaction of genotype with ethnicity (including all 4 ethnicities; likelihood ratio test with 3 d.f.). ‡$0.05 < P < 0.10$. §$P < 0.05$. ¶*P* value was estimated for the interaction of genotype with ethnicity (only Caucasian and African-American ethnicities).

Haplotype-based analyses. The sliding window (window width 4 SNPs) was employed to analyze haplotype-disease associations, which generated a total of 11 window frames for the 14 tSNPs. Figure 1 shows the most common haplotype and number of common haplotypes in each window. *P* values in Fig. 1 indicate the overall differences in haplotype frequencies from the omnibus likelihood ratio test. Two overlapping 4-tSNP windows (rs591758-rs668514-rs647126-rs1800006 [$\chi^2 = 24.14$; 7 d.f.; nominal $P = 0.00107$; $P_{adjusted} = 0.046$] and rs668514, rs647126, rs1800006, and rs1800849 [$\chi^2 = 19.26$; 5 d.f.; nominal $P = 0.00171$; $P_{adjusted} = 0.063$]) were found to be associated with a higher type 2 diabetes risk in Caucasian women. The latter was also found to be significantly associated with incident diabetes in the combined multiethnic sample ($\chi^2 = 20.19$; 5 d.f.; nominal $P = 0.00115$; $P_{adjusted} = 0.048$). A marginal association was found between the haplotype set rs591758-rs668514-rs647126-rs1800006 and type 2 diabetes risk in Hispanic women ($\chi^2 = 13.47$; 7 d.f.; nominal $P = 0.0614$; $P_{adjusted} = 0.591$).

This haplotype set (rs591758-rs668514-rs647126-rs1800006) was also found to be associated with an even higher risk of type 2 diabetes in overweight (BMI >25 kg/m^2) Caucasian ($\chi^2 = 25.57$; 7 d.f.; nominal $P = 0.0006$; and permutation $P = 0.032$) and Hispanic ($\chi^2 = 19.17$; 7 d.f.; nominal $P = 0.0077$; permutation $P = 0.174$) women. Haplotype set rs591758-rs668514-rs647126-rs1800006 spans the *UCP2-UCP3* intergenic and *UCP3* regions. Using the most common haplotype, h1010, as the referent group, the haplotype-specific ORs for type 2 diabetes were 2.0 (95% CI 1.13–3.37) for haplotype h0001 specifically (19.5% in controls) in Caucasian women and 3.8 (1.44–9.93) in Caucasian overweight women. Without adjustment of baseline fasting glucose and insulin, the haplotype h0001–specific ORs for type 2 diabetes were 1.3 (1.01–1.75) in all Caucasian women and 3.3 (1.35–7.75) in Caucasian overweight women. The effect of this particular haplotype on risk of type 2 diabetes seems to be independent from baseline fasting glucose and insulin. We also did not find a significant interaction effect on type 2 diabetes risk between haplotype h0001 and BMI.

We performed haplotype analyses for BMI in healthy control and diabetic case subjects for the diabetes-associated haplotype set rs591758-rs668514-rs647126-rs1800006. No significant association was found between haplotype and BMI in either control or case subjects. The diabetes-associated haplotype was found to be associated with overweight status (BMI >25 kg/m^2) only in Hispanic-American diabetic case subjects (nominal *P* value = 0.005). We did not observe a significant association with obesity status (BMI >30 kg/m^2).

Secondary analyses. To address the issue of undiagnosed diabetes at baseline and assess the robustness of our findings, we conducted sensitivity analyses excluding 630 case subjects who had fasting glucose ≥126 mg/dl at baseline. Significant associations with type 2 diabetes risk were found for the haplotype set rs591758-rs668514-rs647126-rs1800006 in Caucasian women (nominal $P = 0.0013$) and the overweight subgroup (nominal $P = 0.0005$). As a result of small sample size, marginally significant associations with incident diabetes were also found for this haplotype set in Hispanic women (nominal $P = 0.0754$) and the overweight subgroup (nominal $P = 0.0322$). Compared with those who carried the most common haplotype (h1010), Caucasian women with haplotype h0001 had a 1.9-fold (95% CI 1.10–3.38) higher risk of developing incident diabetes, and the risk increased to 3.3-fold (1.25–8.94) for Caucasian overweight women.

DISCUSSION

In this large prospective study of postmenopausal women with diverse ethnicity, we found that Caucasians who were carriers of haplotypes defined by rs591758, rs668514, rs647126, and rs1800006 that covered *UCP3* and the *UCP2-UCP3* intergenic region had a significant higher type 2

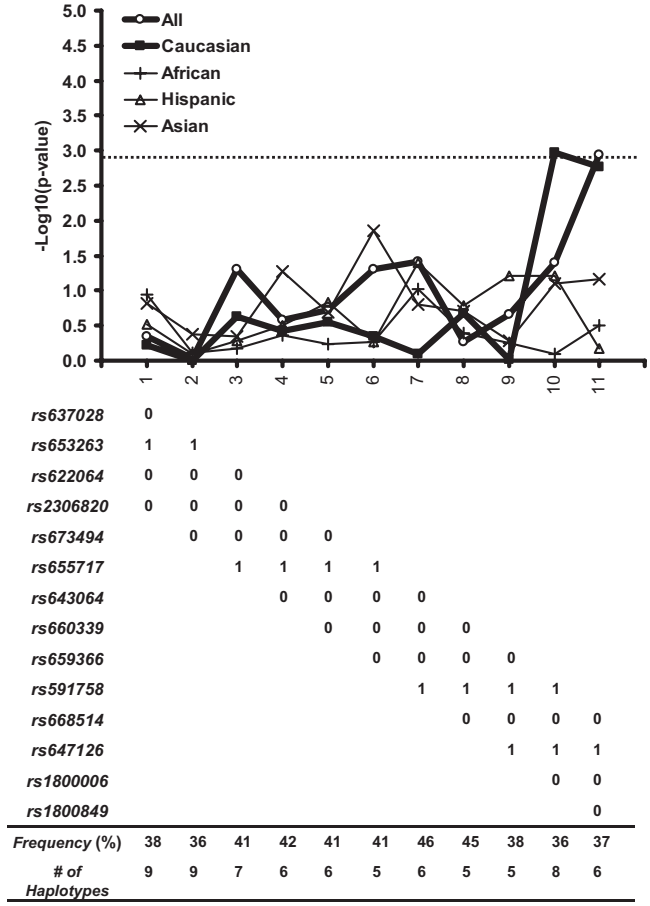

FIG. 1. Sliding window (window width 4) haplotype-based studies of 14 tSNPs. For each window frame, an omnibus likelihood ratio test was used (a χ^2 test with d.f. = number of haplotypes in a particular window − 1). *P* values indicate the overall differences in haplotype frequencies. The most frequent haplotype for each window frame was indicated below the graph, and its estimated frequency in pooled controls was reported along with the total number of haplotypes (defined as those with frequencies ≥2.5%). At each SNP locus, 0 and 1 denote the major and minor alleles for each SNP included. A $-\log_{10} P > 2.93$ ($P < 0.00118$) was used as the global significance threshold by a permutation procedure for all performed tests.

diabetes risk (nominal $P = 0.00115$, $P_{\text{adjusted}} = 0.048$), especially among those who were overweight (nominal $P = 0.0006$, permutation $P = 0.032$). Compared carriers of the most common haplotype (h1010), carriers of haplotype h0001 had a twofold (95% CI 1.13–3.37) higher risk of developing type 2 diabetes, and the risk increased to 3.8-fold (1.44–9.93) for those carriers who were also overweight. However, after adjustment for multiple comparisons, we did not observe any significant association in Hispanic, African, or Asian-American ethnicity.

Human *UCP3* gene encodes a mitochondrial transmembrane carrier protein (3,27,28), and as an uncoupler of oxidative phosphorylation, UCP3 is thought to play an important role in maintenance of energy balance and body weight (29). *UCP3* is predominantly expressed in skeletal muscle (27,30), a major tissue contributing to nonshivering thermogenesis in humans (28). Krook et al. (31) reported lower *UCP3* mRNA levels in type 2 diabetic patients, whereas increased *UCP3* mRNA levels were also observed by others (32). More recently, Vidal et al. (33) showed that obese type 2 diabetic patients had three- to fourfold higher *UCP3* mRNA levels than obese control subjects. However, mRNA levels do not necessarily reflect protein content. Relatively little is known about the protein levels of UCP3 in humans, even though overexpression of *UCP3* in transgenic mice resulted in increased glucose tolerance and reduced fasting plasma glucose levels (34).

In the current study, we found that overweight Caucasian women with the high-risk haplotype (h0001, C-C-G-C) had a 3.8-fold increased risk of type 2 diabetes. Exactly what specific variants in this region may account for this effect are not known. Haplotype h0001 is in high LD with both −866 A- and −55 T-alleles. This may indicate that previous significant finding for −866 G/A and −55 C/T polymorphisms may be due to some as yet unidentified variants covered by this diabetes-associated haplotype. A 45-bp I/D variant in the 3′UTR of *UCP2* gene has been reported to be associated with BMI (15,17) and 24-h metabolic rate (35). This 45-bp I/D variant is not genotyped in the HapMap project or in our study. However, based on a study by Wang et al. (15) of a North European–ancestry Caucasian population, this 45-bp I/D polymorphism seems to be in high LD with the *A55V* polymorphism ($D' = 0.97$; $r^2 = 0.82$) and with −866 G/A polymorphism ($D' = 0.75$, $r^2 = 0.55$). Since haplotype h0001 is in high LD with −866 A- and −55 T-alleles, this 45-bp I/D variant may also be covered by the diabetes-associated haplotype set rs591758-rs668514-rs647126-rs1800006. In a 15-year follow-up study of healthy middle-aged Caucasian men, Gable et al. (13) found that those with both *UCP2* −866 AA and *UCP3* −55 TT genotypes had an OR of 4.2 (95% CI 1.70–10.37), and this association was particularly strong among men with a BMI >30 kg/m^2 (OR 19.2 [95% CI 5.6–63.7]). Taken together, these finding indicate that the adverse effect of these genetic variants on type 2 diabetes risk might be exacerbated by adiposity. One possible explanation for our findings is that being overweight could exacerbate the extent of insulin resistance as a consequence of an impairment of mitochondrial fat oxidation and accumulation of intramyocellular lipid due to reduced UCP3 protein expression by high-risk haplotypes (13). Recently, Schrauwen et al. (36) found that type 2 diabetic patients had a 50% lower UCP3 protein content compared with age-matched control subjects. UCP3 protein content was also found to be inversely correlated with plasma glucose and insulin levels (36). Thus, high levels of UCP3 may protect against the development of type 2 diabetes (36,37). This hypothesis is in accordance with the observation that mice overexpressing *UCP3* were resistant to development of diet-induced diabetes (38). If, as observed in our study, the diabetes-associated haplotype *UCP2-UCP3* is a true positive association, then this haplotype would be expected to be associated with decreased UCP3 protein levels in skeletal muscle. Thus, it would be warranted to assess the direct haplotypic effects on mRNA transcriptional efficiency of *UCP3* and its protein activity in experimental settings.

Contributions of *UCP2-UCP3* genetic variants to type 2 diabetes risk may differ significantly in different ethnicities. It has been suggested that population stratification may lead to false-positive results (39) because participants of different ethnicities/geographic locations might have different type 2 diabetes risk mainly because of their different environmental exposures or different allele frequencies for at-risk SNPs or haplotypes. To address any potential bias from population stratification, we carefully selected the control samples to be representative of the WHI-OS source population and also performed ethnicity-stratified analyses. However, in these ethnicity-stratified analyses, our study might be underpowered to detect

associations of *UCP2* −866 G/A (rs659366) and *UCP3* −55 C/T (rs1800849) polymorphisms with type 2 diabetes.

Potential biases due to the inclusion of some women with undiagnosed diabetes need careful consideration. To avoid potential bias associated with the timing of outcome definition, we have adopted a well-established strategy: *1*) utilizing standard and identical protocols to define case and control subjects; *2*) excluding all of the prevalent case subjects from our original case-control sampling space; *3*) matching each case-control pair on age, ethnicity, clinical center, time of blood draw, and follow-up time; *4*) performing analyses by adjusting for obesity/insulin resistance indexes such as baseline fasting glucose, insulin levels, or HOMA-IR in the multivariable models; and *5*) conducting secondary analyses after excluding those with fasting glucose ≥126 mg/dl or further excluding all cases occurring in the first year of follow-up. Therefore, we conducted sensitivity analyses by adjusting for baseline fasting glucose and insulin levels. It is possible that individuals with risk SNPs in *UCP2–3* may have increased glucose or insulin concentrations already at baseline; thus, the absolute marginal effect of this gene on type 2 diabetes risk would be underestimated by the adjustment for baseline glucose and insulin levels in our model. However, we believe that adjusting for glucose and insulin would allow us to examine conditional effects of this gene on diabetes risk that may be independent of glycemica or insulinemia. We recognize that by adopting such a stringent criterion for diagnosis, we may pick up the more severe cases and therefore misclassify some less severe cases of diabetes into the nondiabetic groups. Nevertheless, our strategy serves to minimize false positive, which is a major threat to validity. The consistency between the results from our secondary analyses and our primary results speaks to the robustness of our findings.

Finally, our findings among postmenopausal women were likely to be generalizable only to women. Because our study included ethnically diverse women from 40 U.S. states, these findings may be generalizable to women of similar age and ethnically diverse background.

In summary, for each of the 14 tSNPs across the genomic region of the *UCP2-UCP3* gene cluster, we did not observe significant effects on type 2 diabetes. However, Haplotype-based analyses suggest that a haplotype set defined by rs591758, rs668514, rs647126, and rs1800006 was significantly associated with type 2 diabetes risk in Caucasian women only, especially among those who were overweight. These data warrant confirmation in future prospective and experimental studies.

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REFERENCES

1. Fisler JS, Warden CH: Uncoupling proteins, dietary fat and the metabolic syndrome. *Nutr Metab (Lond)* 3:38, 2006
2. Krauss S, Zhang CY, Lowell BB: The mitochondrial uncoupling-protein homologues. *Nat Rev Mol Cell Biol* 6:248–261, 2005
3. Gong DW, He Y, Karas M, Reitman M: Uncoupling protein-3 is a mediator of thermogenesis regulated by thyroid hormone, beta3-adrenergic agonists, and leptin. *J Biol Chem* 272:24129–24132, 1997

4. Bouchard C, Perusse L, Chagnon YC, Warden C, Ricquier D: Linkage between markers in the vicinity of the uncoupling protein 2 gene and resting metabolic rate in humans. *Hum Mol Genet* 6:1887–1889, 1997
5. Ghosh S, Watanabe RM, Valle TT, Hauser ER, Magnuson VL, Langefeld CD, Ally DS, Mohlke KL, Silander K, Kohtamäki K, Chines P, Balow Jr J, Birznieks G, Chang J, Eldridge W, Erdos MR, Karanjawala ZE, Knapp JI, Kudelko K, Martin C, Morales-Mena A, Musick A, Musick T, Pfahl C, Porter R, Rayman JB: The Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) study. I. An autosomal genome scan for genes that predispose to type 2 diabetes. *Am J Hum Genet* 67:1174–1185, 2000
6. Watanabe RM, Ghosh S, Langefeld CD, Valle TT, Hauser ER, Magnuson VL, Mohlke KL, Silander K, Ally DS, Chines P, Blaschak-Harvan J, Douglas JA, Duren WL, Epstein MP, Fingerlin TE, Kaleta HS, Lange EM, Li C, McEachin RC, Stringham HM, Trager E, White PP, Balow Jr J, Birznieks G, Chang J, Eldridge W: The Finland-United States Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) study. II. An autosomal genome scan for diabetes-related quantitative-trait loci. *Am J Hum Genet* 67:1186–1200, 2000
7. Fleury C, Neverova M, Collins S, Raimbault S, Champigny O, Levi-Meyrueis C, Bouillaud F, Seldin MF, Surwit RS, Ricquier D, Warden CH: Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat Genet* 15:269–272,1997
8. Krempler F, Esterbauer H, Weitgasser R, Ebenbichler C, Patsch JR, Miller K, Xie M, Linnemayr V, Oberkofler H, Patsch W: A functional polymorphism in the promoter of UCP2 enhances obesity risk but reduces type 2 diabetes risk in obese middle-aged humans. *Diabetes* 51:3331–3335, 2002
9. D'Adamo M, Perego L, Cardellini M, Marini M, Frontoni S, Andreozzi F, Sciacqua A, Lauro D, Sbraccia P, Federici M: The −866A/A genotype in the promoter of the human uncoupling protein 2 gene is associated with insulin resistance and increased risk of type 2 diabetes. *Diabetes* 53:1905–1910, 2004
10. Sasahara M, Nishi M, Kawashima H, Ueda K, Sakagashira S, Furuta H, Matsumoto E, Hanabusa T, Sasaki H, Nanjo K: Uncoupling protein 2 promoter polymorphism −866G/A affects its expression in β-cells and modulates clinical profiles of Japanese type 2 diabetic patients. *Diabetes* 53:482–485, 2004
11. Walder K, Norman RA, Hanson RL, Schrauwen P, Neverova M, Jenkinson CP, Easlick J, Warden CH, Pecqueur C, Raimbault S: Association between uncoupling protein polymorphisms (UCP2-UCP3) and energy metabolism/obesity in Pima indians. *Hum Mol Genet* 7:1431–1435, 1998
12. Schrauwen P, Xia J, Walder K, Snitker S, Ravussin E: A novel polymorphism in the proximal UCP3 promoter region: effect on skeletal muscle UCP3 mRNA expression and obesity in male nondiabetic Pima Indians. *Int J Obes Relat Metab Disord* 23:1242–1245, 1999
13. Gable DR, Stephens JW, Cooper JA, Miller GJ, Humphries SE: Variation in the *UCP2-UCP3* gene cluster predicts the development of type 2 diabetes in healthy middle-aged men. *Diabetes* 55:1504–1511, 2006
14. Meirhaeghe A, Amouyel P, Helbecque N, Cottel D, Otabe S, Froguel P, Vasseur F: An uncoupling protein 3 gene polymorphism associated with a lower risk of developing type II diabetes and with atherogenic lipid profile in a French cohort. *Diabetologia* 43:1424–1428, 2000
15. Wang H, Chu WS, Lu T, Hasstedt SJ, Kern PA, Elbein SC: Uncoupling protein-2 polymorphisms in type 2 diabetes, obesity, and insulin secretion. *Am J Physiol* 286:E1–E7, 2004
16. Bulotta A, Ludovico O, Coco A, Di Paola R, Quattrone A, Carella M, Pellegrini F, Prudente S, Trischitta V: The common -866G/A polymorphism in the promoter region of the UCP-2 gene is associated with reduced risk of type 2 diabetes in Caucasians from Italy. *J Clin Endocrinol Metab* 90:1176–80, 2005
17. Cassell PG, Saker PJ, Huxtable SJ, Kousta E, Jackson AE, Hattersley AT, Frayling TM, Walker M, Kopelman PG, Ramachandran A: Evidence that single nucleotide polymorphism in the uncoupling protein 3 (UCP3) gene influences fat distribution in women of European and Asian origin. *Diabetologia* 43:1558–1564, 2000
18. Anderson GL, Manson J, Wallace R, Lund B, Hall D, Davis S, Shumaker S, Wang CY, Stein E, Prentice RL: Implementation of the Women's Health Initiative study design. *Ann Epidemiol* 13:S5–S17, 2003
19. Song Y, You NC, Hsu YH, Sul J, Wang L, Tinker L, Eaton CB, Liu S: Common genetic variation in calpain-10 gene (CAPN10) and diabetes risk in a multi-ethnic cohort of American postmenopausal women. *Hum Mol Genet* 16:2960–71, 2007
20. Wang L, Liu S, Niu T, Xu X: SNPHunter: a bioinformatic software for single nucleotide polymorphism data acquisition and management. *BMC Bioinformatics* 6:60, 2005
21. Oliphant A, Barker DL, Stuelpnagel JR, Chee MS: BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. *Biotechniques* Suppl.:56–61, 2002
22. de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D: Efficiency and power in genetic association studies. *Nat Genet* 37:1217–1223, 2005
23. Kupari M, Koskinen P, Virolainen J: Correlates of left ventricular mass in a population sample aged 36 to 37 years: focus on lifestyle and salt intake. *Circulation* 89:1041–50, 1994
24. Zaykin DV, Westfall PH, Young SS, Karnoub MA, Wagner MJ, Ehm MG: Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. *Hum Hered* 53:79–91, 2002
25. Kraft P, Cox DG, Paynter RA, Hunter D, De Vivo I: Accounting for haplotype uncertainty in matched association studies: a comparison of simple and flexible techniques. *Genet Epidemiol* 28:261–72, 2005
26. Niu T, Qin ZS, Xu X, Liu JS: Bayesian haplotype inference for multiple linked single-nucleotide polymorphisms. *Am J Hum Genet* 70:157–169, 2002
27. Boss O, Samec S, Paoloni-Giacobino A, Rossier C, Dulloo A, Seydoux J, Muzzin P, Giacobino JP: Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett* 408:39–42, 1997
28. Argyropoulos G, Brown AM, Willi SM, Zhu J, He Y, Reitman M, Gevao SM, Spruill I, Garvey WT: Effects of mutations in the human uncoupling protein 3 gene on the respiratory quotient and fat oxidation in severe obesity and type 2 diabetes. *J Clin Invest* 102:1345–1351, 1998
29. Vidal-Puig A, Solanes G, Grujic D, Flier JS, Lowell BB: UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem Biophys Res Commun* 235:79–82, 1997
30. Solanes G, Vidal-Puig A, Grujic D, Flier JS, Lowell BB: The human uncoupling protein-3 gene: genomic structure, chromosomal localization, and genetic basis for short and long form transcripts. *J Biol Chem* 272:25433–25436, 1997
31. Krook A, Digby J, OqRahilly S, Zierath JR, Wallberg-Henriksson H: Uncoupling protein 3 is reduced in skeletal muscle of NIDDM patients. *Diabetes* 47:1528–1531, 1998
32. Bao S, Kennedy A, Wojciechowski B, Wallace P, Ganaway E, Garvey WT: Expression of mRNAs encoding uncoupling proteins in human skeletal muscle: effects of obesity and diabetes. *Diabetes* 47:1935–1940, 1998
33. Vidal H, Langin D, Andreelli F, Millet L, Larrouy D, Laville M: Lack of skeletal muscle uncoupling protein 2 and 3 mRNA induction during fasting in type-2 diabetic subjects. *Am J Physiol Endocrinol Metab* 277:E830–E837, 1999
34. Clapham JC, Arch JR, Chapman H, Haynes A, Lister C, Moore GB, Piercy V, Carter SA, Lehner I, Smith SA, et al: Mice overexpressing human uncoupling protein-3 in skeletal muscle are hyperphagic and lean. *Nature* 406:415–418, 2000
35. Kovacs P, Ma L, Hanson RL, Franks P, Stumvoll M, Bogardus C, Baier LJ: Genetic variation in UCP2 (uncoupling protein-2) is associated with energy metabolism in Pima Indians. *Diabetologia* 48:2292–2295, 2005
36. Schrauwen P, Hesselink MK, Blaak EE, Borghouts LB, Schaart G, Saris WH, Keizer HA: Uncoupling protein 3 content is decreased in skeletal muscle of patients with type 2 diabetes. *Diabetes* 50:2870–2873, 2001
37. Schrauwen P, Mensink M, Schaart G, Moonen-Kornips E, Sels JP, Blaak EE, Russell AP, Hesselink MK: Reduced skeletal muscle uncoupling protein-3 content in prediabetic subjects and type 2 diabetic patients: restoration by rosiglitazone treatment. *J Clin Endocrinol Metab* 91:1520–1525, 2006
38. Li B, Nolte LA, Ju JS, Han DH, Coleman T, Holloszy JO, Semenkovich CF: Skeletal muscle respiratory uncoupling prevents diet-induced obesity and insulin resistance in mice. *Nat Med* 6:1115–1120, 2000
39. Lander ES, Schork NJ: Genetic dissection of complex traits. *Science* 265:2037–2048, 1994