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ARTICLE

UCP2 −866G/A and Ala55Val, and *UCP3* −55C/T polymorphisms in association with type 2 diabetes susceptibility: a meta-analysis study

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Abstract
Aims/hypothesis A meta-analysis was performed to assess the association between the *UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms and type 2 diabetes susceptibility.
Methods A literature-based search was conducted to identify all relevant studies. The fixed or random effect pooled measure was calculated mainly at the allele level to determine heterogeneity bias among studies. Further analyses were performed that stratified for ethnicity.
Results We examined 17 publications. Stratified analysis for ethnicity and sensitivity analysis revealed that there was no heterogeneity between studies for these variants. Using an additive model, no significant association of the *UCP2* −866G/A polymorphism with type 2 diabetes risk was observed, either in participants of Asian (OR 1.05, 95% CI 0.96, 1.16) or of European (OR 1.03, 95% CI 0.99, 1.07) descent. Neither the *UCP2* Ala55Val nor the *UCP3* −55C/T polymorphism showed any significant association with type 2 diabetes risk in Europeans (OR 1.04, 95% CI 0.98, 1.09 for Ala55Val; OR 1.04, 95% CI 1.00, 1.09 for −55C/T). In contrast, a statistically significant association was observed for both polymorphisms in participants of Asian descent (OR 1.23, 95% CI 1.12, 1.36 for Ala55Val; OR 1.15, 95% CI 1.03, 1.28 for −55C/T).
Conclusions/interpretation Our meta-analysis suggests that the *UCP2* −866G/A polymorphism is unlikely to be associated with increased type 2 diabetes risk in the populations investigated. In contrast, our results indicate that the *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms may indeed be risk factors for susceptibility to type 2 diabetes in individuals of Asian descent, but not in individuals of European descent. This conclusion warrants confirmation by further studies.

Keywords Meta-analysis · Polymorphism · Type 2 diabetes · UCP2 · UCP3

Abbreviations

DIAGRAM	Diabetes Genetics Replication and Meta-analysis
FEM	Fixed effect model
FPRP	False-positive report probability
GWAS	Genome-wide association study/studies
HWE	Hardy–Weinberg equilibrium
REM	Random effect model
SNP	Single nucleotide polymorphism
UCP	Uncoupling protein

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Introduction

Uncoupling proteins (UCPs), a family of mitochondrial transporter proteins, uncouple the transport of protons across the inner mitochondrial membrane from electron transport and the synthesis of ATP from ADP [1]. Among the five UCP homologues (*UCP1* to *UCP5*), *UCP2* and *UCP3* are located adjacent to one another on human chromosome 11q13 [2, 3] and are 73% identical to each other at the amino acid sequence level [4]. Studies indicate that UCP2, as a key component of the beta cell glucose-sensing mechanism that regulates glucose-stimulated insulin secretion [5–7], is a critical link between beta cell dysfunction and type 2 diabetes [8]. It has also been observed that lower *UCP3* mRNA levels are present in the skeletal muscle of type 2 diabetes patients [9].

A number of studies have examined the association between genetic variability in the *UCP2–UCP3* gene cluster and the risk of type 2 diabetes, with most studies focusing on three common single nucleotide polymorphisms (SNPs) [10]. These are a SNP located in a multifunctional *cis*-regulatory site of the *UCP2* promoter region (−866G/A, rs659366), a missense variant in exon 4 of *UCP2* (Ala55Val, rs660339) and a SNP 6 bp upstream from the TATA box in the core promoter region of *UCP3* (−55C/T, rs1800849). The −866G/A polymorphism, which acts as a binding site for the pancreatic transcription factors Paired box-containing 6 and Insulin promoter factor 1 [11, 12], has been associated with higher *UCP2* mRNA levels, reduced insulin secretion and increased type 2 diabetes risk [13–15]. The Ala55Val polymorphism has been associated with a lower degree of uncoupling, lower energy expenditure [16] and a higher risk of obesity, as well as a higher incidence of diabetes [17, 18]. Similarly, the T allele of the *UCP3* −55C/T polymorphism has been associated with a reduced risk of type 2 diabetes and higher plasma total cholesterol and LDL-cholesterol [19].

Despite strong functional evidence for the involvement of these three SNPs in the regulation of uncoupling, the results of the genetic association studies on association with type 2 diabetes remain inconclusive. To further examine the potential role of these three SNPs in influencing type 2 diabetes susceptibility, we performed a meta-analysis on eligible case–control studies. Our aim was to estimate the effect of these SNPs in populations of Asian and European descent. Our results suggest that the *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms may have a selective effect on the development of type 2 diabetes in individuals of Asian descent.

Methods

Search strategy PubMed and Embase were searched systematically to identify all available relevant articles. The most-studied SNPs (*UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T) were investigated using combinations of the following search terms: ‘diabetes and UCP2’, ‘UCP3’, ‘uncoupling protein 2’, ‘uncoupling protein 3’, ‘variant’, and ‘polymorphism’. The search was limited to English language papers and completed on June 10, 2011. We also used the PubMed option ‘Related Articles’ for each research article to retrieve additional potentially relevant articles. All of the included articles were also hand-searched to identify any other relevant citations. No restriction was set on the source of control participants (general population, clinic or hospital).

Inclusion and exclusion criteria To determine whether an individual study was eligible for inclusion in the meta-analysis, all of the studies identified were carefully reviewed by two investigators working independently, any discrepancies being resolved by discussion and, when necessary, adjudicated by a third reviewer. The inclusion and exclusion criteria were as follows: First, each case–control study had to have been published as an original study designed to evaluate the association. Second, numbers in case and control groups had to be reported for each allele or genotype. Third, case–control studies had to have sufficient published data to estimate an OR with 95% CI or to provide raw data that allowed us to calculate them. Fourth, if the data were duplicated and had been published more than once, the most recent and complete study was chosen. Fifth, studies were excluded if the genotype distribution of the controls deviated from Hardy–Weinberg equilibrium (HWE). Sixth, the following were excluded: animal studies, review articles, abstracts, editorials, reports with incomplete data, studies based on pedigree data, studies on other type of diabetes (type 1 diabetes, gestational diabetes, etc.) and prospective studies.

Data extraction Data were independently extracted by two investigators who reached a consensus on all of the items. Information extracted from each study was considered as follows: name of first author, publication year, ethnic origin of the population studied, number of participants in case and control groups, genotype and allele frequency by case/control status, and OR (95% CI). Not all papers reported the necessary statistics directly, so in some instances we transformed and estimated an OR from the reported data [20]. We did not define a minimum number of patients for a study to be included in our meta-analysis.

Statistical analysis HWE of the genotype distribution of controls was tested by a goodness-of-fit χ^2 analysis. The distribution was considered to have deviated from HWE at $p<0.05$. Pooled ORs with 95% CI were used to assess the strength of association in the additive, dominant and recessive models, respectively. Pooled estimates of the OR

were obtained by calculating a weighted average of ORs from each study, with the statistical significance of the pooled OR being determined by the Z test.

To examine the possibility of heterogeneity across the studies, a statistical test for heterogeneity was performed. This was based on the χ^2-based Q statistic and I^2 metric, and quantifies between-study heterogeneity irrespective of the number of studies. Heterogeneity was considered significant at $p<0.05$ for the Q statistic and $I^2>50\%$ for the I^2 metric. In the presence of substantial heterogeneity, the DerSimonian and Laird random effect model (REM) was adopted as the pooling method; otherwise the fixed effect model (FEM) was used [21, 22]. Meta-regression and sensitivity analysis were conducted to evaluate the key studies with a substantial impact on between-study heterogeneity. Influence analysis was performed to assess the stability of the results, with a single study in the meta-analysis being deleted each time to reflect the influence of the individual data set on the pooled OR.

The statistical power for each of the three SNPs was calculated by power and sample size software [23], and the false-positive report probability (FPRP) test of Wacholder et al. [24] was applied to address the issue of false-positive SNP associations. All genetic variants were analysed using the Begg and Egger tests for potential publication bias [25]. The significance of the intercept was determined by the t test suggested by Egger, with $p<0.10$ considered representative of statistically significant publication bias. All statistical analyses were conducted using STATA version 11.0 (Stata, College Station, TX, USA).

Results

Characteristics of study The trial flow is summarised in Fig. 1 of the electronic supplementary material (ESM). A total of 17 published articles [11, 13, 14, 19, 26–38] with 28 outcomes met the inclusion and exclusion criteria. All were case–control studies and most were population-based. The allele and genotype distributions in the studies included are summarised in Tables 1, 2, and 3 for the *UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms respectively. The association of the *UCP2* −866G/A polymorphism, the *UCP2* Ala55Val polymorphism and the *UCP3* −55C/T polymorphism with type 2 diabetes risk was examined in 13, 7 and 8 studies respectively. Other characteristics (sex, age, etc.) are summarised in ESM Table 1.

Table 1 Characteristics of the *UCP2* −866G/A polymorphism allelic and genotype distribution for type 2 diabetes risk in studies included in the meta-analysis

Study details			Cases (*n*) by total and genotype				Controls (*n*) by total and genotype				G allele frequency (%)		
Reference	Year	Ethnicity	Total	GG	GA	AA	Total	GG	GA	AA	Cases	Controls	OR (95% CI)[b]
Krempler et al. [11]	2002	European	201	65	106	30	391	186	156	49	0.587	0.675	0.68 (0.53, 0.88)[c]
Wang et al. [26]	2003	European	131	ND	ND	ND	118	ND	ND	ND	0.67	0.58	1.45 (1.01, 2.09)[c]
D'Adamo et al. [13]	2004	European	483	222	197	64	563	247	266	50	0.664	0.673	0.95 (0.79, 1.14)[c]
Sasahara et al. [14]	2004	Asian	413	116	205	92	172	50	90	32	0.529	0.553	0.91 (0.71, 1.17)[c]
Ji et al. [27]	2004	Asian	184	53	94	37	134	37	69	28	0.543	0.534	1.04 (0.76, 1.43)[c]
Bulotta et al. [28]	2005	European	746	374	317	55	327	142	144	41	0.713	0.654	1.32 (1.08, 1.60)[c]
Pinelli et al. [29]	2006	European	342	167	145	30	305	147	124	34	0.700	0.685	1.07 (0.85, 1.36)[c]
Hsu et al. [30]	2008	European	968	ND	ND	ND	968	ND	ND	ND	ND	0.646	1.20 (0.80, 1.70)
Hsu et al. [30]	2008	African	366	ND	ND	ND	732	ND	ND	ND	ND	0.573	0.90 (0.60, 1.40)
Hsu et al. [30]	2008	Asian	98	ND	ND	ND	195	ND	ND	ND	ND	0.586	0.80 (0.20, 2.70)
Hsu et al. [30]	2008	European	152	ND	ND	ND	303	ND	ND	ND	ND	0.533	1.00 (0.50, 2.10)
Hsu et al. [30]	2008	Mixed	1584	ND	ND	ND	2,198	ND	ND	ND	ND	0.604	1.00 (0.80, 1.30)
Lee et al. [31]	2008	Asian	761	ND	ND	ND	632	ND	ND	ND	0.531	0.492	1.17 (1.01, 1.36)
Beitelshees et al. [32]	2010	European	107	37	56	14	341	132	151	58	0.607	0.608	1.00 (0.73, 1.36)[c]
Heidari et al. [33]	2010	Asian	75	27	41	7	75	29	38	8	0.633	0.64	0.97 (0.61, 1.56)[c]
Voight et al. [34][a]	2010	European	8,130	ND	ND	ND	38,987	ND	ND	ND	ND	ND	1.02 (0.98, 1.06)
Vimaleswaran et al. [35]	2011	Asian	487	185	239	63	919	358	432	129	0.62	0.63	1.00 (0.85, 1.18)[c]

ND, no data (no genotype data available)

[a] This was a DIAGRAM study, which included eight GWAS on type 2 diabetes

[b] Data analysed under additive model; [c] Calculated from the reported genotypes

Table 2 Characteristics of the *UCP2* Ala55Val polymorphism allelic and genotype distribution for type 2 diabetes risk in studies included in the meta-analysis

Study details			Cases (*n*) by total and genotype				Controls (*n*) by total and genotype				C allele frequency (%)		
Reference	Year	Ethnicity	Total	CC	CT	TT	Total	CC	CT	TT	Cases	Controls	OR(95% CI)[a]
Kubota et al. [36]	1998	Asian	210	60	107	43	218	64	97	57	0.54	0.516	1.10 (0.84, 1.44)[b]
Wang et al. [26]	2003	European	131	ND	ND	ND	118	ND	ND	ND	0.37	0.45	0.71 (0.50, 1.03)[b]
Cho et al. [37]	2004	Asian	500	158	227	115	133	30	76	27	0.54	0.51	1.14 (0.87, 1.49)
Hsu et al. [30]	2008	European	968	ND	ND	ND	968	ND	ND	ND	ND	0.604	1.20 (0.80, 1.70)
Hsu et al. [30]	2008	African	366	ND	ND	ND	732	ND	ND	ND	ND	0.566	0.90 (0.60, 1.50)
Hsu et al. [30]	2008	Asian	98	ND	ND	ND	195	ND	ND	ND	ND	0.562	0.90 (0.30, 3.40)
Hsu et al. [30]	2008	European	152	ND	ND	ND	303	ND	ND	ND	ND	0.543	1.40 (0.70, 2.90)
Hsu et al. [30]	2008	Mixed	1,584	ND	ND	ND	2,198	ND	ND	ND	ND	0.581	1.10 (0.80, 1.40)
Lee et al. [31]	2008	Asian	761	ND	ND	ND	632	ND	ND	ND	0.536	0.498	1.16 (1.00, 1.35)
Voight et al. [34]	2010	European	8,130	ND	ND	ND	38,987	ND	ND	ND	ND	ND	1.04 (0.98, 1.09)
Vimaleswaran et al. [35]	2011	Asian	487	264	198	25	919	408	412	99	0.75	0.67	1.45 (1.22, 1.73)[b]

ND, no data (no genotype data available). [a] Data were analysed under additive model; [b] Calculated from the reported genotypes

Quantitative synthesis Results of pooled analyses are summarised in detail in Table 4. Our meta-analysis showed no significant association between the *UCP2* −866G/A polymorphism and risk of type 2 diabetes, either by additive (REM OR 1.03, 95% CI 0.95, 1.11), dominant (FEM OR 1.03, 95% CI 0.90, 1.18) or recessive (REM OR 1.00, 95% CI 0.84, 1.18) models. Moreover, no significant association was observed when an additive model was used after stratification for ethnicity (Asian descent FEM OR 1.05, 95% CI 0.96, 1.16; European descent REM OR 1.04, 95% CI 0.92, 1.17) (Fig. 1).

For the *UCP2* Ala55Val polymorphism, the C allele was found to be significantly associated with an increased risk of type 2 diabetes when using a recessive model (FEM OR 1.39, 95% CI 1.16, 1.66), but not when using additive (REM OR 1.11, 95% CI 0.98, 1.26) or dominant (REM OR 1.38, 95% CI 0.80, 2.37) models. However, after stratification by ethnicity, a significant association was revealed by

Table 3 Characteristics of the *UCP3* −55C/T polymorphism allelic and genotype distribution for type 2 diabetes risk in studies included in the meta-analysis

Study details			Cases (*n*) by total and genotype				Controls (*n*) by total and genotype				C allele frequency (%)		OR(95% CI)[a]
Reference	Year	Ethnicity	Total	CC	CT	TT	Total	CC	CT	TT	Cases	Controls	
Meirhaeghe et al. [19]	2000	European	49	36	13	0	894	542	312	40	0.867	0.78	1.84 (1.01, 3.33)[b]
Meirhaeghe et al. [19]	2000	European	171	116	49	6	124	70	46	8	0.822	0.75	1.54 (1.03, 2.29)[b]
Dalgaard et al. [38]	2001	European	455	253	169	33	521	280	192	49	0.742	0.722	1.11 (0.91, 1.35)[b]
Cho et al. [37]	2004	Asian	499	251	204	44	132	62	59	11	0.71	0.69	1.07 (0.80, 1.44)
Pinelli et al. [29]	2006	European	342	240	94	8	305	224	78	3	0.835	0.86	0.83 (0.61, 1.13)[b]
Hsu et al. [30]	2008	European	968	ND	ND	ND	968	ND	ND	ND	ND	0.774	1.20 (0.70, 2.00)
Hsu et al. [30]	2008	African	366	ND	ND	ND	732	ND	ND	ND	ND	0.863	0.70 (0.40, 1.30)
Hsu et al. [30]	2008	Asian	98	ND	ND	ND	195	ND	ND	ND	ND	0.85	0.70 (0.20, 2.50)
Hsu et al. [30]	2008	European	152	ND	ND	ND	303	ND	ND	ND	ND	0.724	1.10 (0.40, 2.70)
Hsu et al. [30]	2008	Mixed	1,584	ND	ND	ND	2,198	ND	ND	ND	ND	0.811	1.00 (0.70, 1.30)
Lee et al. [31]	2008	Asian	740	ND	ND	ND	647	ND	ND	ND	0.709	0.694	1.07 (0.91, 1.26)[b]
Voight et al. [34]	2010	European	8,130	ND	ND	ND	38,987	ND	ND	ND	ND	ND	1.03 (0.99, 1.08)
Vimaleswaran et al. [35]	2011	Asian	487	185	239	63	919	358	432	129	0.62	0.63	1.00 (0.85, 1.18)[b]

ND, no data (no genotype data available). [a] Data were analysed under additive model; [b] Calculated from the reported genotypes

Table 4 Pooled measures for the association between the *UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms and susceptibility to type 2 diabetes

Inherited model[a]	Data	Before sensitivity analysis								After sensitivity analysis						
		n			I^2 (%)	FEM OR (95% CI)	*p* value	REM OR (95% CI)	*p* value	*n*			I^2 (%)	OR (95% CI)	*p* value	Excluded[b]
		St[c]	Ca[d]	Co[e]						St[c]	Ca[d]	Co[e]				
−866G/A																
Additive	Overall	13	13,644	45,162	51.8	1.03 (0.99, 1.06)	0.172	1.03 (0.95, 1.11)	0.465	12	13,443	44,771	24.9	1.03 (1.00, 1.07)	0.071	[11]
Additive	Asian	6	2,018	2,127	0	1.05 (0.96, 1.16)	0.294	1.05 (0.96, 1.16)	0.294	–	–	–	–	–	–	–
Additive	European	8	11,260	42,152	62.8	1.02 (0.98, 1.06)	0.255	1.04 (0.92, 1.17)	0.511	7	11,059	41,912	38.0	1.03 (0.99, 1.07)	0.106	[11]
Dominant	Overall	10	3,799	3,859	43.7	1.03 (0.90, 1.18)	0.648	1.04 (0.86, 1.26)	0.688	–	–	–	–	–	–	–
Recessive	Overall	10	3,799	3,859	61.5	1.03 (0.94, 1.14)	0.522	1.00 (0.84, 1.18)	0.996	9	3,598	3,468	5.9	1.09 (0.99, 1.21)	0.090	[11]
Ala55Val																
Additive	Overall	7	11,803	43,205	68.7	1.07 (1.03, 1.12)	0.002	1.11 (0.98, 1.26)	0.095	6	11,672	43,087	27.6	1.05 (1.00, 1.10)	0.043	[34]
Additive	Asian	5	2,056	2,097	23.7	1.23 (1.12, 1.36)	5.4×10^{-5}	1.22 (1.09, 1.38)	0.001	–	–	–	–	–	–	–
Additive	European	3	9,281	40,376	43.6	1.04 (0.98, 1.09)	0.189	1.01 (0.82, 1.24)	0.924	–	–	–	–	–	–	–
Dominant	Overall	3	1,197	1,270	76.0	1.42 (1.10, 1.84)	0.007	1.38 (0.80, 2.37)	0.243	–	–	–	–	–	–	–
Recessive	Overall	3	1,197	1,270	44.9	1.39 (1.16, 1.66)	2.8×10^{-4}	1.34 (1.02, 1.76)	0.034	–	–	–	–	–	–	–
−55C/T																
Additive	Overall	8	12,457	44,727	47.8	1.05 (1.01, 1.10)	0.012	1.11 (1.00, 1.21)	0.047	–	–	–	–	–	–	–
Additive	Asian	4	1,824	1,893	2.7	1.15 (1.03, 1.28)	0.013	1.15 (1.02, 1.29)	0.016	–	–	–	–	–	–	–
Additive	European	5	10,267	42,103	40.6	1.04 (1.00, 1.09)	0.073	1.10 (0.96, 1.26)	0.183	–	–	–	–	–	–	–
Dominant	Overall	5	2,003	2,895	10.0	1.33 (1.02, 1.73)	0.04	1.30 (0.96, 1.76)	0.088	–	–	–	–	–	–	–
Recessive	Overall	5	2,003	2,895	41.0	1.19 (1.04, 1.36)	0.009	1.20 (1.00, 1.44)	0.056	–	–	–	–	–	–	–

Sensitivity analysis was conducted to reduce heterogeneity by omitting studies if STATA gave $I^2 \geq 50\%$

[a] Per SNP; [b] reference number; [c] St, Studies; [d] Ca, Cases; [e] Co, Controls

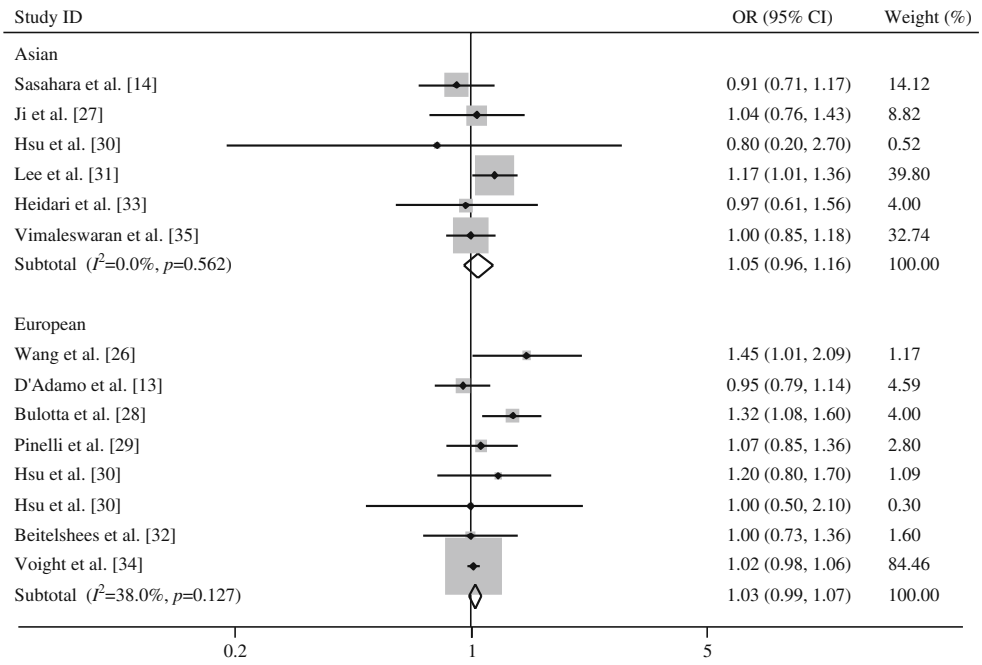

Fig. 1 Stratified analysis pooled ORs for the association between the *UCP2* −866G/A polymorphism and susceptibility to type 2 diabetes by ethnicity. The area of the squares reflects the study-specific weight. The diamond shows the summary fixed-effects OR estimate from 12 studies

an additive model in populations of Asian descent (FEM OR 1.23, 95% CI 1.12, 1.36), but not in those of European descent (FEM OR 1.04, 95% CI 0.98, 1.09) (Table 4 and Fig. 2).

Our meta-analysis also showed a significant overall association between the *UCP3* −55C/T polymorphism and increased risk of type 2 diabetes in all models (additive FEM OR 1.05, 95% CI 1.01, 1.10; dominant FEM OR 1.33, 95% CI 1.02, 1.73; recessive FEM OR 1.19, 95% CI 1.04, 1.36). Surprisingly, when stratified by ethnicity, the significant association between the *UCP3* −55C/T polymorphism and risk of type 2 diabetes was most evident in individuals of Asian descent (FEM OR 1.15, 95% CI 1.03, 1.28), with only a marginal significance observed in persons of European descent (FEM OR 1.04, 95% CI 1.00, 1.09) (Table 4 and Fig. 3).

Heterogeneity and sensitivity analyses As shown in Table 4, significant heterogeneity was observed among studies of the *UCP2* −866G/A and Ala55Val polymorphisms in the overall populations, but no heterogeneity was found in the inherited models for the *UCP3* −55C/T polymorphism. To investigate this further, the following covariates were considered: publication year, sex (ratio of males in cases

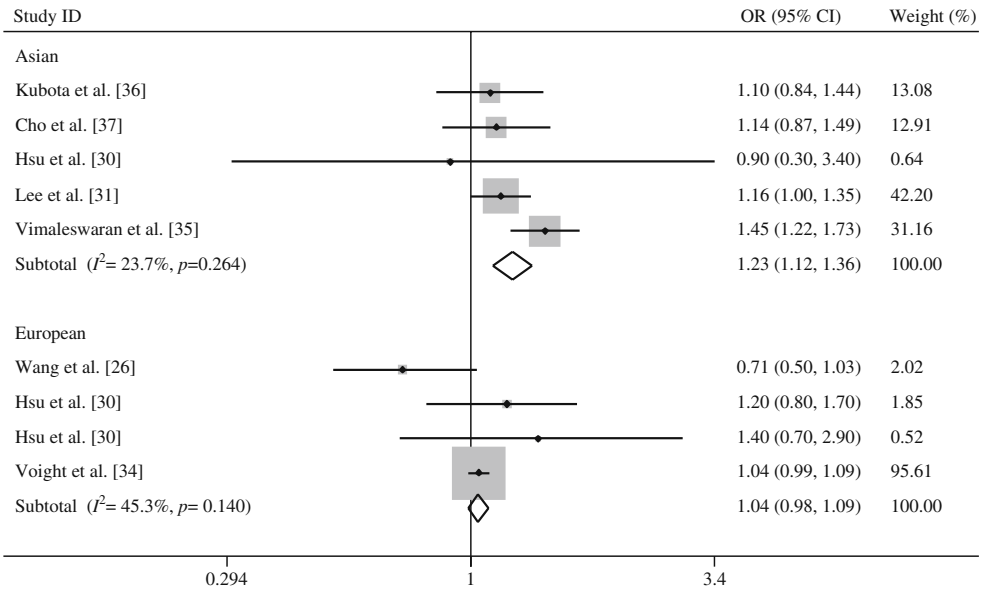

Fig. 2 Stratified analysis pooled ORs for the association between the *UCP2* Ala55Val polymorphism and susceptibility to type 2 diabetes mellitus by ethnicity. The area of the squares reflects the study-specific weight. The diamond shows the summary fixed-effects OR estimate from seven studies

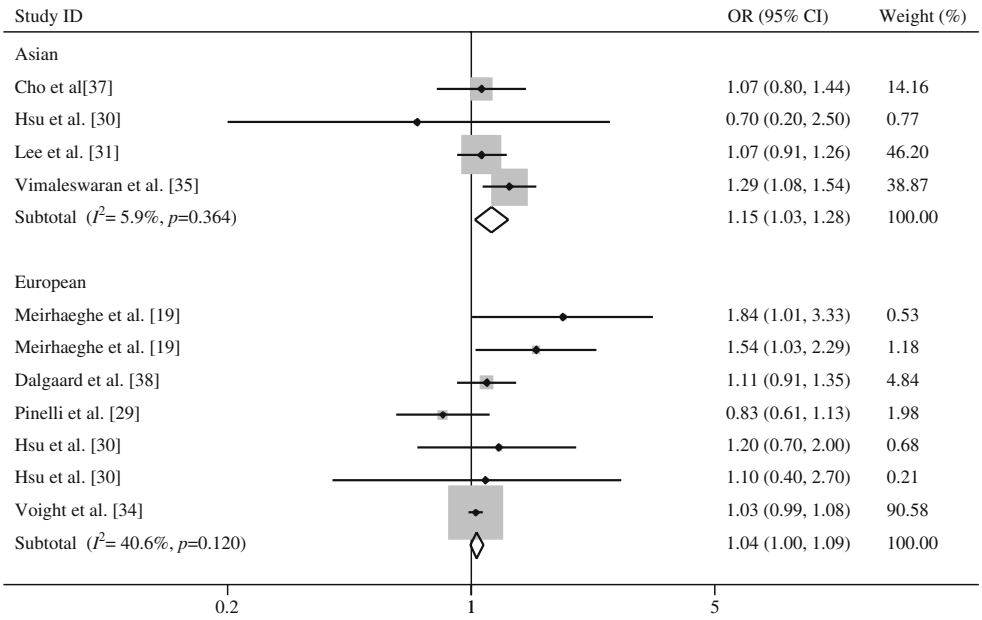

Fig. 3 Stratified analysis pooled ORs for the association between the *UCP3* −55C/T polymorphism and susceptibility to type 2 diabetes mellitus by ethnicity. The area of the squares reflects the study-specific weight. The diamond shows the summary fixed-effects OR estimate from eight studies

to that in controls), age (ratio of the mean age in cases to that in controls) and sample size. However, univariate meta-regression analysis showed that none of the tested covariates could by themselves explain the observed between-study heterogeneity. To identify the studies with the greatest impact on the overall between-study heterogeneity, sensitivity analyses were conducted in the overall population. The results indicated that two studies [11, 35] were mainly responsible for the observed heterogeneity. Moreover, when the data were stratified by ethnicity and an additive model used, the heterogeneity between the studies of the *UCP2* Ala55Val polymorphism was significantly decreased or eliminated in populations of Asian and European descent (Table 4). Similarly, the heterogeneity was also effectively removed from the studies of the *UCP2* −866G/A polymorphism in participants of Asian descent, but still existed in studies investigating individuals of European descent.

Influence analysis To assess the degree to which each individual study affected the overall OR estimates, influence analysis was conducted by repeating the meta-analysis sequentially excluding one study at a time. As shown in Table 4, only one study [35] was found to have an excessive influence on the pooled effect. This was limited to analysis of the *UCP2* Ala55Val polymorphism in the overall population using an additive model (FEM OR 1.05, 95% CI 1.00, 1.10). Otherwise no single study excessively influenced the analyses.

Publication bias As expected, no significant publication bias was detected in the inherited models for any of the polymorphisms examined (ESM Table 2), confirming that our results are statistically robust.

Discussion

Results from several genome-wide association studies (GWAS) in a variety of populations have identified 37 replicating type 2 diabetes susceptibility loci [34, 39–44]. However, the biological pictures revealed by GWAS remain incomplete. Thus, many of the associations identified by GWAS do not involve previously identified type 2 diabetes candidate genes, and many of the associated markers are in genomic locations containing genes whose function is currently unknown. Recently, several studies suggested an association between the *UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms and type 2 diabetes risk. Despite strong functional evidence for the relevance of these three SNPs, the results for association with type 2 diabetes show significant between-study variation. To obtain a more definitive conclusion, we conducted a meta-analysis of 17 published articles with 28 outcomes from populations of different ethnic origins [11, 13, 14, 19, 26–38]. We believe such a meta-analysis has a much greater possibility of reaching reasonably strong conclusions.

Heterogeneity is potentially a significant problem when interpreting the results of any meta-analysis of genetic association studies [45]. Our meta-analysis also showed significant between-study heterogeneity in most of the models that we used to examine the associations of the *UCP2* −866G/A and Ala55Val polymorphisms. Many of

the variables that varied between the various studies might be responsible for this observed heterogeneity, including the source of the controls, sex bias, ethnicity, etc. Initial inspection of the data did not immediately identify any likely candidate variable or study that was significantly impacting on our overall results. Thus, to explore this matter further, meta-regression and 'leave one out' sensitivity analyses were performed [46], revealing that ethnicity was the only covariate likely to have made an important contribution to the overall between-study heterogeneity. The reason for this is unclear, but it may be that populations of different ethnicity also have environmental differences that affect their sensitivity to particular genomic variants. Similarly, based on sensitivity analyses using I^2>50% as the cut-off criteria, two studies [11, 35] were identified as the principal outliers in our analyses.

The study by Voight et al. [34], which is a Diabetes Genetics Replication and Meta-analysis (DIAGRAM) study and includes eight GWAS on type 2 diabetes, also met our inclusion criteria. After confirming by sensitivity analysis that it would not contribute to overall heterogeneity, we combined this with the other studies included. This additional analysis indicated that the *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms, but not the *UCP2* −866G/A polymorphism were significantly associated with type 2 diabetes risk in the overall population. As heterogeneity still existed and the DIAGRAM study was from populations of European descent, we again stratified our analysis by ethnicity. The results indicated that no obvious heterogeneities among the stratified studies existed and that the *UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms had no significant association with type 2 diabetes risk in populations of European descent, a finding consistent with the conclusions of the DIAGRAM study. Interestingly, the results from the studies examining populations of Asian descent conflicted with this conclusion and indicated that the association with type 2 diabetes was statistically significant for the *UCP2* Ala55Val and *UCP3* polymorphisms, but not for the *UCP2* −866G/A polymorphism. Although our analysis of Asian populations had a relatively small sample size, we nevertheless had 80% power at a 0.05 significance level to detect an OR of 1.5 or greater (statistical power 0.996 and 0.793 for *UCP2* Ala55Val and *UCP3* −55C/T respectively). The FPRP value for the *UCP3* −55C/T polymorphism suggested a <20% chance of the result being a false positive when assigned a relatively high prior probability range (i.e. 0.01–0.1) (data not shown). In contrast, the FPRP value for the *UCP2* Ala55Val polymorphism remained below 0.2 even for a prior probability of 0.001, suggesting that the FPRP value is quite robust and that *UCP2* may contain one or more genetic variants that increase type 2 diabetes risk in individuals of Asian descent.

The results of the present meta-analysis should also be interpreted within the context of its limitations. Thus previous studies have also indicated that the *UCP2* −866G/A, *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms are associated with obesity [10], and that the *UCP2* −866G/A and Ala55Val polymorphisms are associated with proliferative diabetic retinopathy in type 2 diabetes patients or decreased risk of coronary artery disease in men with type 2 diabetes [47, 48]. However, the number of studies providing this clinical information was too low for us to take these covariates into account by meta-regression. Similarly, besides ethnicity, other potential environment × gene interactions may well be contributors to the observed disease-effect unconformity, but we had insufficient data to perform an evaluation of such interactions. Furthermore, one single study found that overweight white women with a potential high-risk haplotype (in high linkage disequilibrium with 866A- and 55T-alleles) had a 3.8-fold increased type 2 diabetes risk [30]. We again had insufficient data to confirm this association, but on the basis of our meta-analysis, we propose that this may be due to some other, as yet unidentified variants also contained within this diabetes-associated haplotype.

In conclusion, our results indicate that the *UCP2* −866G/A polymorphism is not a candidate for susceptibility to type 2 diabetes in any ethnic population. However, our results do support the hypothesis that the *UCP2* Ala55Val and *UCP3* −55C/T polymorphisms are type 2 diabetes susceptibility loci in populations of Asian, but not European descent. We suggest that additional larger studies allowing stratification for other gene × environment interactions should be performed to further clarify the possible roles of the three *UCP2* and *UCP3* genetic variants in the aetiology of type 2 diabetes.

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Contribution statement K.X. was involved in conception and design, analysis and interpretation of data, drafting of the article and revising it critically for important intellectual content. M.Z., D.C., Y.F., L.Q. and R.G. worked on collection and interpretation of data, and critical revision of the manuscript for important intellectual content. M.W., C.S. and R.Y. were involved in analysis and interpretation of

data and critical revision of the manuscript for important intellectual content. T.Y. was involved in conception and design, and critical revision of the manuscript for important intellectual content. All the co-authors gave final approval of the version to be published.

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