Research Article

Analysis of association between common variants of uncoupling proteins genes and diabetic retinopathy in a Chinese population

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Abstract

Background: The aim of this study was to explore the association between diabetic retinopathy (DR) and the variants of uncoupling proteins (UCPs) genes in a Chinese population of type 2 diabetes, in total and in patients of different glycemic status separately.

Methods: This case-control study included a total of 3107 participants from two datasets, among which 662 were DR patients (21.31%). Eighteen tag single nucleotide polymorphisms (SNPs) of UCP1, UCP2, and UCP3 were selected as genetic markers. TaqMan probes, Sequenom MassARRAY MALDI-TOF mass spectrometry platform and Affymetrix Genome-Wide Human SNP Array were used for genotyping. Online SHEsis software was used for association analysis. Bonferroni correction was used for multiple comparisons correction.

Results: Three SNPs of UCP1: rs7688743 (A allele, OR = 1.192, p = 0.013), rs3811787 (T allele, OR = 0.863, p = 0.023), and rs10011540 (G allele, OR = 1.368, p = 0.004) showed association with DR after the adjustment of glucose, but only rs10011540 was marginally significantly associated with DR when Bonferroni correction was strictly applied (padj = 0.048). In patients with uncontrolled glucose, rs7688743 (A allele, p = 0.012, OR = 1.309), rs10011540 (G allele, p = 0.033, OR = 1.432), and rs3811787 (T allele, p = 0.022, OR = 0.811) were associated with DR, while in participants with well controlled glucose, the rs2734827 of UCP3 was associated with DR (A allele, p = 0.017, OR = 0.532). Rs3811787 of UCP1 showed a protective effect to sight threatening DR (T allele, p = 0.007, OR = 0.490), and the association existed after the adjustment for environmental factors and the correction. In patients with uncontrolled glucose, the rs3811787 of UCP1 (T allele, p = 0.017, OR = 0.467) and the rs591758 of UCP3 (C allele, p = 0.026, OR = 0.103) were associated with STDR. While in those with well controlled glucose, only the rs7688743 of UCP1 showed a protective effect (A allele, p = 0.024, OR = 0.049). None of the associations remain significant when Bonferroni correction was strictly applied (all $p < 0.05$).

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Background

The age-standardized prevalence of diabetes in Chinese adult population reached 11.6% in 2013, and the number is still climbing [1]. Diabetic retinopathy (DR) is a common micro-vascular complication of diabetes, and the prevalence of DR is 25–43.1% in Chinese diabetic population [2–4]. DR is the main cause of blindness among people in productive age in several developed countries [5, 6]. The high prevalence of diabetes in China makes DR a big threat of vision loss in Chinese population.

DR is a multifactorial disease associated with both genetic and environmental factors [7, 8]. It is widely accepted that high glucose, high blood pressure and long diabetes duration are the most important environmental risk factors of DR progression [9]. Since 2003, our study group has been helping diabetes patients to control hyperglycemia and hypertension in order to prevent and control DR. However, DR still progressed in a number of patients with well controlled glucose and blood pressure [10]. This might be related to the “metabolic memory” phenomenon, which means the damages caused to cells and tissues by previous hyperglycemia persists after the normalization of glucose levels [11]. The mechanism of metabolic memory is not fully understood, but the genetic factors might be involved, because not all of the diabetes patients with hyperglycemic history develops metabolic memory.

Oxidative stress induced by hyperglycemia not only works as a vital part in the development of DR, but also plays a key role in the pathogenesis of metabolic memory [12]. The overproduction of mitochondrial reactive oxygen species (ROS) activates several molecular and biochemical pathways, and leads to long-lasting damage to tissues even after the glycemic status was controlled [13]. Uncoupling proteins (UCPs) are a group of mitochondrial anion-carrier proteins that can reduce the formation of mitochondrial ROS through reducing the proton motive force across the mitochondrial inner membrane [14]. The protective effect of UCPs in diabetic complications and the association between DR and UCPs genes were reported by several studies [15–18]; however, such association was not always verified [19, 20].

Previous researches suggested that apart from different study design, ethnicity, and synergetic effects, the interaction between genes and nutrients could be a possible reason for the inconsistency among different studies [21]. Increased glucose leads to an elevated expression of UCPs, while elevated UCP2 expression leads to a decreased insulin secretion and increased glucose [22, 23]. Thus, the interaction between UCPs and glucose level might affect the association between UCPs and DR; however, no previous study has taken the glucose controlling status into consideration.

The present case-control study is aimed to explore the association between DR and the variants of UCPs genes in a Chinese type 2 diabetes population. The relation between DR and UCPs was investigated among all the participants, and in participants with different glycemic status separately.

Methods

Setting and participants

The present study included two datasets collected from two separate studies.

The first study (Dataset 1) was a community-based cross-sectional study conducted in Xinjing community, Shanghai, China. A total of 4572 Han Chinese diagnosed with type 2 diabetes, including 528 DR patients (28.21%), were included into the study. Their mean age was 67.88 ± 9.50 years, and their mean diabetes duration was 7.17 ± 6.49 years. Seven hundred sixty-four of the participants were male (40.81%). All of the participants in the first study have glucose records of the last 3 years. They went through systematic and ophthalmic exams during a survey, and the severity of DR was classified in these participants.

The second study (Dataset 2) was a sub-study of a genome-wide association study (GWAS) [24]. Participants of dataset 2 were collected from clinic, and most of them were new diagnosed diabetes patients. A total of 1235 type 2 diabetes patients of Han Chinese were included in this study, among which 134 were diagnosed with DR (12.17%). Their mean age was 64.78 ± 9.49 years, and 482 of the participants are male (39.02%). Their diabetes duration were not collected, past glucose level not recorded, and the severity of DR not classified. The comprehensive systemic and ophthalmology exams included in dataset 1 were not provided for the participants in dataset 2.

The study population of the two studies were both Han Chinese collected from Shanghai, China. Participants in dataset 2 were younger compared with those in

Conclusions:
The rs10011540 and rs3811787 of the UCP1 gene was marginally significantly associated with DR in Chinese type 2 diabetes patients. There might be different mechanisms of DR development in patients with different glycemic status.

Keywords: Diabetic retinopathy, Uncoupling proteins, Single nucleotide polymorphisms, Type 2 diabetes
dataset 1 ($p < 0.01$), but no difference existed in gender proportion ($p = 0.32$). In dataset 2, participants with DR were older than the others (71.07 ± 7.46y vs 64.02 ± 9.42y, $p < 0.01$). Age and gender were not related with DR in dataset 1.

**Systemic and ophthalmology exams in the community-based study (dataset 1)**
The Xinjing Community Health Service Center created a health information database covering almost all of the residents in 1995, and the database has been updated annually since then. Diabetic residents have their hemoglobin A1c (HbA1c) and blood glucose monitored once every 3 months, and the results were recorded in the database. According to the glucose goal recommended by the American Diabetes Association, those patients with HbA1c under 6.5% for 3 whole years were considered glucose well controlled [25]. On the other hand, patients who didn’t meet with this standard were considered glucose uncontrolled. A comprehensive systemic and eye examinations were conducted in each of the participant. Height and weight were measured at the survey site, and body mass index (BMI) was calculated as weight (kg)/height (m)$^2$. Systolic blood pressure was measured at the survey site, and body mass index (BMI) was calculated as weight (kg)/height (m)$^2$. Systolic blood pressure was measured at the survey site, and body mass index (BMI) was calculated as weight (kg)/height (m)$^2$.

**Statistical analysis**
Online SHEsis software (http://shesisplus.bio-x.cn/SHEsis.html) was used to analyze the Hardy-Weinberg equilibrium (HWE), allele frequency, and association test for each SNP [27]. Bonferroni correction (based on the total number of markers tested) was used for multiple comparisons correction, and statistical significance was defined as $p<0.05$ (two tailed).
SAS (version 9.3, SAS Institute, Cary, NC, USA) was used for statistical analysis of the systemic and eye measurements. Continuous variables were presented as means ± standard deviation, and categorical data were presented as rates (proportions). The data distribution was examined by Kolmogorov-Smirnov Test. For normally distributed continuous variables, t test was used to compare the means between groups. For variables that didn’t follow Gaussian distribution, a Kruskal-Wallis H test was used. The categorical variables were compared with the Chi-square test. Distribution of alleles and genotypes between the case and the control groups were compared with the Chi-square test. Multivariate logistic regression analysis was used to get the odds ratios (OR) and their 95% confidential intervals (CIs) of each SNP before and after adjusting for various clinical variables. The genetic models tested in the study included additive, recessive and dominant models. Meta-analysis was used to combine the results of dataset1 and dataset2, and Cochran’s Q test was used for the heterogeneity test.

Results
The rs660339 of UCP2 and the rs1626521 of UCP3 were excluded from further analysis because of low call rates (≤95%). The rs632862 of UCP2 was excluded for MAF < 0.01, the rs668514 of UCP3 was excluded for unsuccessful genotyping, and the rs15763 of UCP3 was excluded due to disagree with HWE test (Additional file 1: TableS 1). Thus, a total of 13 SNPs were included into further analysis.

The association between UCPs and DR in all participants
A total of 3107 type 2 diabetic participants were included in the whole study, including 1872 from dataset 1 and 1235 from dataset 2. Three SNPs of UCP1: rs7668743, rs10011540, and rs3811787, showed association with DR (Table 1). The A allele of rs7668743 (p = 0.013, OR = 1.192) and the G allele of rs10011540 (p = 0.004, OR = 1.368) were risk factors of DR, while the T allele of rs3811787 was less frequent in DR patients (p = 0.023, OR = 0.863). Only rs10011540 showed marginally significant association with DR when Bonferroni correction was strictly applied (p_adj = 0.048). No difference was observed in the association results between the two datasets (all Q > 0.05).

The association between the candidate SNPs genotypes and DR was presented in Table 2. The genotype of GG and GT of rs10011540 showed significantly association with DR in the additive model (GG vs GT vs TT: p = 0.004, p_adj = 0.048, OR = 1.373) and in the Dominant model (GG + GT vs TT: p = 0.003, p_adj = 0.039, OR = 1.410). Additionally, the genotype analysis of rs3811787 (p = 0.027, OR = 0.868), rs7668743 (p = 0.014, OR = 1.196) and rs3811790 (p = 0.037, OR = 0.828) all showed association with DR, but not significant after the Bonferroni correction. No positive associations between DR and SNPs was found in the recessive model. No other SNP genotypes was found to be associated with DR.

The interaction effect of the rs10011540 and environmental factors on DR was further tested in dataset 1 participants by logistic regression analysis (Table 3), which used DR status as the dependent variables, and the rs10011540 and environmental features as the independent variables. It was suggested that rs10011540 was associated with DR after the adjustment for age, gender, BMI, high blood pressure, hypercreatininemia, hypercholesterolemia, hyperlipidemia and HbA1c (p = 0.025, OR = 1.360). While diabetes duration might have interaction with rs10011540.

The association between UCPs and DR in participants with well controlled and uncontrolled glucose
There were 1016 participants with uncontrolled high glucose in dataset 1, among which 356 had DR (35.04%). DR patients had significantly higher glucose and longer diabetes duration compared with those with no DR, while no other difference was found (Table 4). DR was associated with rs7668743 (A allele, p = 0.012, OR = 1.309), rs10011540 (G allele, p = 0.033, OR = 1.432), and rs3811787 (T allele, p = 0.022, OR = 0.811). However, none of those association remains when Bonferroni correction is applied (all p_adj > 0.05) (Table 5). After adjusted by HbA1c level, rs7668743 (p = 0.009, OR = 1.332), rs10011540 (p = 0.003, OR = 1.458), and rs3811787 (p = 0.044, OR = 0.827) were still associated with DR.

Among the 856 participants with well controlled low glucose in dataset 1, 172 had DR (20.09%). DR patients had longer diabetes duration compared with the control group, while no other difference was observed (Table 4). DR was associated with rs2734827 of UCP3 (A allele, p = 0.017, OR = 0.532), and the association still existed after adjusted by HbA1c level (p = 0.045, OR = 0.531). However, the association didn’t remain when Bonferroni correction is applied (all p_adj > 0.05) (Table 5). No association between UCP1 and DR was discovered in this population.

The association between UCPs and STDR
Among all the participants in dataset1, a total of 127 participants with STDR (case group) and 1268 DM patients with neither DR nor DME (control group) were included into the sub-group analysis. The case group included 29 patients with PDR, 41 with severe NPDR, and 82 with CSME in at least one eye (25 of the 82 patients also had PDR or severe NPDR). The STDR prevalence in patients with well controlled glucose is significantly lower than that with uncontrolled glucose (3.38% vs 14.54%, p < 0.01). In those with well controlled glucose,
Table 1 The association between uncoupling proteins genes variants and diabetic retinopathy

| Chr. | rs ID | Position (hg19) | A1 | Dataset 1 (528 cases vs 1344 controls) | Dataset 2 (134 cases vs 1101 controls) | Meta-analysis |
|------|-------|-----------------|----|---------------------------------------|----------------------------------------|--------------|
|      |       |                 |    | OR [95% CI]                            | OR [95% CI]                            |              |
|      |       |                 |    | P                                     | P                                      |              |
|      |       |                 |    | P adj                                 | P adj                                  |              |
|      |       |                 |    | Q                                       |                                        |              |
| 4    | rs6818140 | 141,487,955     | G  | 1.028 [0.852–1.240]                    | 1.190 [0.870–1.629]                    | 1.068 0.421  1.000 0.4326 |
| 4    | rs7688743 | 141,488,795     | A  | 1.152 [0.982–1.351]                    | 1.322 [0.999–1.750]                    | 1.192 0.013  0.173 0.4028 |
| 4    | rs100111540 | 141,489,996  | G  | 1.312 [1.031–1.670]                    | 1.571 [1.012–2.438]                    | 1.368 0.004  0.048 0.4814 |
| 4    | rs381787 | 141,490,419     | T  | 0.861 [0.747–0.993]                    | 0.870 [0.657–1.151]                    | 0.863 0.023  0.293 0.9501 |
| 4    | rs381790 | 141,491,576     | A  | 0.946 [0.816–1.098]                    | 0.938 [0.718–1.225]                    | 0.944 0.387  1.000 0.9494 |
| 4    | rs381791 | 141,491,773     | C  | 0.973 [0.823–1.150]                    | 0.874 [0.642–1.190]                    | 0.949 0.488  1.000 0.5499 |
| 4    | rs1472268 | 141,492,943     | A  | 1.007 [0.873–1.161]                    | 0.991 [0.769–1.277]                    | 1.003 0.961  1.000 0.9130 |
| 4    | rs1800592 | 141,493,961     | T  | 0.996 [0.864–1.148]                    | 1.015 [0.787–1.308]                    | 1.001 0.993  1.000 0.9013 |
| 11   | rs659366 | 73,694,754      | T  | 0.998 [0.862–1.156]                    | 1.249 [0.965–1.617]                    | 1.055 0.041  1.000 0.1394 |
| 11   | rs591758 | 73,698,060      | C  | 0.989 [0.854–1.145]                    | 1.206 [0.932–1.561]                    | 1.038 0.567  1.000 0.1896 |
| 11   | rs1685356 | 73,712,859      | T  | 1.053 [0.913–1.215]                    | 0.910 [0.699–1.185]                    | 1.019 0.770  1.000 0.3415 |
| 11   | rs374135 | 73,713,960      | A  | 1.022 [0.882–1.184]                    | 1.305 [1.003–1.698]                    | 1.083 0.223  1.000 0.1123 |
| 11   | rs2734827 | 73,716,277     | A  | 0.990 [0.743–1.320]                    | 0.868 [0.605–1.247]                    | 0.941 0.597  1.000 0.5774 |

Chr., chromosome; A1, effect allele; OR, odds ratio; CI, confidence interval; P adj, Bonferroni correction based on the total number of markers tested (n = 13); P<0.05 in the meta-analysis was marked in bold.
Table 2 The association of uncoupling proteins genotypes and diabetic retinopathy

| rsID   | Genotype | Dataset 1 | Dataset 2 | Meta-analysis |
|--------|----------|-----------|-----------|---------------|
|        |          | DR       | NDR      | OR [95% CI]   | P  | DR       | NDR      | OR [95% CI]   | P  | OR     | P   | P_adj | Q  |
|        |          |          |          |              |    |          |          |              |    |        |     |       |    |
| rs10011540 | GG     | 4        | 7        | Add:1.312 [1.026–1.676] | 0.030 | 0        | 3        | Add:1.607 [1.021–2.527] | 0.040 | 1.373  | 0.004 | 0.048  | 0.440 |
|         | GT      | 101      | 204      | Dom:1.332 [1.027–1.726] | 0.030 | 26       | 138      | Dom:1.685 [1.066–2.664] | 0.025 | 1.410  | 0.003 | 0.039  | 0.380 |
|         | TT      | 423      | 1132     |              |    | 100      | 914      |              |    |        |     |       |    |
| rs3811787 | TT     | 121      | 342      | Add:0.867 [0.753–0.998] | 0.047 | 23       | 238      | Add:0.870 [0.657–1.151] | 0.329 | 0.868  | 0.027 | 0.351  | 0.985 |
|         | GT      | 246      | 657      | Dom:0.787 [0.630–0.983] | 0.035 | 56       | 477      | Dom:0.859 [0.553–1.334] | 0.499 | 0.801  | 0.029 | 0.377  | 0.729 |
|         | GG      | 161      | 345      |              |    | 31       | 241      |              |    |        |     |       |    |
| rs7688743 | AA     | 38       | 76       | Add:1.151 [0.977–1.355] | 0.092 | 9        | 57       | Add:1.351 [1.009–1.808] | 0.043 | 1.196  | 0.014 | 0.182  | 0.347 |
|         | AG      | 219      | 528      | Dom:1.162 [0.950–1.422] | 0.145 | 63       | 425      | Dom:1.481 [1.027–2.136] | 0.129 | 1.229  | 0.022 | 0.286  | 0.256 |
|         | GG      | 271      | 740      |              |    | 58       | 575      |              |    |        |     |       |    |
| rs3811790 | AA     | 80       | 168      | Add:0.952 [0.821–1.104] | 0.517 | 17       | 133      | Add:0.937 [0.715–1.227] | 0.634 | 0.949  | 0.425 | 1      | 0.916 |
|         | AC      | 213      | 644      | Dom:0.817 [0.666–1.001] | 0.051 | 58       | 522      | Dom:0.864 [0.603–1.243] | 0.043 | 0.828  | 0.037 | 0.481  | 0.785 |
|         | CC      | 235      | 532      |              |    | 59       | 466      |              |    |        |     |       |    |

* OR odds ratio, CI confidence interval, *P* adj Bonferroni correction based on the total number of markers tested (n = 13), *Q* Cochran’s Q statistic *P* the SNP with corrected *P* < 0.05 in the meta-analysis was marked in bold; Add, the additive model; Dom, the dominant model.
STDR patients were older and more often to have hyperlipidemia. While in those with uncontrolled glucose, STDR patients tend to have longer diabetes duration, lower BMI, higher HbA1c, and more often with hypercreatininemia, hypercholesterolemia, hyperlipidemia. While in those with well controlled glucose, STDR patients were older and more often to have hypercreatininemia, hypercholesterolemia, hyperlipidemia.

When all participants were included, rs3811787 of UCP1 showed a protective effect to STDR (T allele, \( p = 0.007, \text{OR} = 0.490 \)) (Table 6), and the association existed after the adjustment for age, gender, BMI, high blood pressure, hypercreatininemia, hypercholesterolemia, hyperlipidemia and HbA1c (T allele, \( p = 0.034, \text{OR} = 0.732 \)) (Table 3). The protective effect of genotype TT and GT was shown in the additive model (TT vs GT vs GG: \( \text{OR} = 0.715, \ p = 0.034 \)) and in the recessive model (TT vs GT + GG: \( \text{OR} = 0.565, \ p = 0.021 \)), but not in the dominant model (Additional file 2: Table S2).

In patients with uncontrolled glucose, the rs3811787 of UCP1 (T allele, \( p = 0.017, \text{OR} = 0.467 \)) and the rs591758 of UCP3 (C allele, \( p = 0.026, \text{OR} = 0.103 \)) were associated with STDR. While in those with well controlled glucose, only the rs7688743 of UCP1 showed a protective effect (A allele, \( p = 0.024, \text{OR} = 0.049 \)) (Table 6). None of the associations remain significant when Bonferroni correction was strictly applied (all \( p < 0.05 \)).

**Discussion**

UCPs are a group of anion-carrier proteins located in the inner mitochondrial membrane with similar structure and different tissue distributions. Their roles is to mediate regulated proton leaking across the inner mitochondrial membrane. The UCP1 gene is located in the 4th chromosome, and it is mainly expressed in brown adipose tissue, but the protein and mRNA of UCP1 were also detected in retinal cells. The UCP2 and UCP3 genes are located within 8 kb of each other in a gene cluster in the 11th chromosome. UCP2 is widely distributed in the whole body, while UCP3 is mainly expressed in the skeletal muscle.

UCPs are candidate genes for the pathology of hypertension, cardiac injuries, obesity, and diabetes. Its mechanism is adjusting energy homeostasis though thermogenesis and regulating ROS release. The association between UCPs and hypertension was discovered in lab studies, and was verified in Asian population. Molecular epidemiology studies found UCP2 polymorphism alters the risk of developing CAD in some populations, while the relation between UCPs and obesity was widely recognized in several populations.

The association between the UCPs genes and diabetes has been investigated by many studies, and the most often studied SNPs are the rs1800592 (−3826A/G) of the UCP1 gene, the rs659366 (−866G/A), rs660339 (Ala55-Val), and Ins/Del of the UCP2 gene, and the rs1800849 (−55C/T) of the UCP3 gene. Lab and molecular epidemiology studies found UCPs polymorphisms are associated with diabetic micro-vascular complications, including diabetic neuropathy, nephropathy, and DR. However, there are some controversial findings about the association between UCPs variants and DR.

The present study included 3107 Chinese type 2 diabetic patients of Han race, and the results suggested that the rs7688743, rs10011540, and rs3811787 of UCP1 were associated with DR; however, only the rs10011540 (−122 A/C) of UCP1 was still marginally significantly associated with DR under Bonferroni correction, and the G allele was a risk factor of DR in additive model and in dominant model. The association between the rs10011540 and type

| Characteristics | Number | rs10011540 and DR (OR [95% CI]) | p | rs3811787 and STDR (OR [95% CI]) | p |
|-----------------|--------|-------------------------------|---|--------------------------------|---|
| unadjusted      |        | 1.312 [1.026–1.676]          | 0.030 | 0.715 [0.553–0.926]               | 0.011 |
| age             |        | 1.312 [1.026–1.676]          | 0.030 | 0.713 [0.550–0.924]               | 0.011 |
| age + gender    |        | 1.312 [1.026–1.676]          | 0.030 | 0.713 [0.550–0.924]               | 0.011 |
| age + gender+BMI |       | 1.307 [1.018–1.677]          | 0.036 | 0.720 [0.553–0.936]               | 0.014 |
| age + gender+BMI + HBP |     | 1.311 [1.011–1.700]          | 0.041 | 0.755 [0.574–0.992]               | 0.044 |
| age + gender+BMI + HBP + hypercreatininemia | | 1.310 [1.011–1.699]          | 0.042 | 0.756 [0.575–0.994]               | 0.045 |
| age + gender+BMI + HBP + hypercreatininemia + hypercholesterolemia | | 1.310 [1.011–1.699]          | 0.042 | 0.756 [0.575–0.994]               | 0.045 |
| age + gender+BMI + HBP + hypercreatininemia + hypercholesterolemia + hyperlipidemia | | 1.310 [1.011–1.699]          | 0.042 | 0.756 [0.575–0.994]               | 0.045 |
| age + gender+BMI + HBP + hypercreatininemia + hypercholesterolemia + hyperlipidemia + HbA1c | | 1.360 [1.039–1.779]          | 0.025 | 0.732 [0.548–0.977]               | 0.034 |
| age + gender+BMI + HBP + hypercreatininemia + hypercholesterolemia + hyperlipidemia + HbA1c + DD | | 1.259 [0.941–1.686]          | 0.121 | 0.770 [0.565–1.040]               | 0.098 |

*BMI body mass index, HBP high blood pressure, HbA1c glycosylated haemoglobin, DD diabetes duration
Table 4: The association between environmental factors and diabetic retinopathy in participants with and without controlled glucose

| Diabetic retinopathy | Well controlled glucose | Uncontrolled glucose | Sight threatening diabetic retinopathy | Well controlled glucose | Uncontrolled glucose |
|---------------------|-------------------------|----------------------|---------------------------------------|-------------------------|----------------------|
|                     | Case | Control | p | Case | Control | p | Case | Control | p | Case | Control | p |
| n                   | 172  | 684     |   | 356  | 660     |   | 23   | 657     |   | 104   | 611     |   |
| age (year)          | 67.04 ± 9.89 | 67.32 ± 9.34 | 0.73 | 67.97 ± 9.13 | 68.62 ± 9.13 | 0.32 | 71.87 ± 6.38 | 67.11 ± 9.22 | 0.01 | 69.58 ± 10.10 | 68.29 ± 9.08 | 0.19 |
| male                | 66 (38.37) | 254 (37.13) | 0.76 | 159 (44.66) | 285 (43.18) | 0.65 | 8 (3478) | 243 (36.99) | 0.83 | 51 (49.04) | 264 (43.21) | 0.27 |
| diabetes duration   | 5.88 ± 6.00 | 4.45 ± 4.88 | 0.01 | 1161 ± 7.06 | 7.95 ± 6.07 | < 0.01 | 7.11 ± 7.63 | 4.11 ± 4.64 | 0.13 | 13.25 ± 7.29 | 7.72 ± 6.10 | < 0.01 |
| BMIa                | 24.62 ± 2.86 | 24.46 ± 3.08 | 0.25 | 2488 ± 3.45 | 25.08 ± 3.38 | 0.6 | 24.65 ± 3.05 | 24.81 ± 3.16 | 0.82 | 24.25 ± 3.56 | 25.15 ± 3.27 | 0.01 |
| high blood pressure | 125 (72.67) | 472 (69.01) | 0.4 | 286 (80.34) | 515 (7803) | 0.42 | 17 (77.27) | 448 (75.17) | 0.82 | 93 (89.42) | 519 (84.94) | 0.23 |
| hypercreatininemia  | 8 (4.65) | 37 (5.41) | 0.69 | 28 (7.87) | 33 (500) | 0.07 | 0 (0.00) | 34 (5.18) | 0.26 | 11 (10.58) | 28 (4.59) | 0.01 |
| hypercholesterolemia| 68 (39.53) | 295 (43.13) | 0.39 | 167 (46.91) | 277 (4203) | 0.14 | 9 (39.13) | 288 (43.84) | 0.65 | 48 (46.15) | 261 (42.03) | 0.52 |
| hyperlipidemia      | 40 (23.26) | 198 (28.95) | 0.14 | 134 (37.64) | 245 (37.18) | 0.88 | 2 (8.7) | 191 (29.07) | 0.03 | 34 (32.69) | 232 (38.03) | 0.3 |
| HbA1c (%)           | 5.93 ± 0.38 | 592 ± 0.38 | 0.89 | 827 ± 1.46 | 7.69 ± 108 | < 0.01 | 6.06 ± 0.36 | 5.92 ± 0.37 | 0.08 | 8.44 ± 1.57 | 7.7 ± 1.09 | < 0.01 |

aBMI body mass index, bHbA1c hemoglobin A1c
2 diabetes has been reported in two previous researches in Asian population. A study including 1800 Asian Indians indicated that a haplotype, the -3826A/ -122C/-4902 T diabetes has been reported in two previous researches in

The association between UCPs and diabetic retinopathy in participants with and without controlled glucose indicated that a haplotype, the -3826A/ -122C/-4902 T diabetes has been reported in two previous researches in

Table 5 The association between uncoupling proteins and diabetic retinopathy in participants with and without controlled glucose

| Chr. | rs ID | Position (hg19) | A1β | Well controlled glucose (172 cases vs 684 controls) | Uncontrolled glucose (356 cases vs 660 controls) |
|------|-------|----------------|-----|--------------------------------|--------------------------------|
| 4    | rs6818140 | 141,487,955 | G   | 0.857 [0.617–1.190] | 0.357 [1.064–1.493] |
| 4    | rs7688743 | 141,488,795 | A   | 0.935 [0.709–1.232] | 0.634 [1.061–1.615] |
| 4    | rs10011540 | 141,489,996 | G   | 1.129 [0.745–1.711] | 0.367 [1.045–1.963] |
| 4    | rs3811787 | 141,490,419 | T   | 0.959 [0.760–1.210] | 0.724 [0.677–0.971] |
| 4    | rs3811790 | 141,491,576 | A   | 0.978 [0.766–1.250] | 0.862 [0.795–1.163] |
| 4    | rs3811791 | 141,491,773 | C   | 1.121 [0.855–1.470] | 0.408 [0.274–1.343] |
| 4    | rs1472268 | 141,492,943 | A   | 1.098 [0.870–1.387] | 0.432 [0.302–1.774] |
| 4    | rs1800592 | 141,493,961 | T   | 1.105 [0.874–1.396] | 0.404 [0.274–1.343] |
| 4    | rs659366  | 73,694,754  | T   | 1.065 [0.829–1.368] | 0.624 [0.419–1.692] |
| 4    | rs591758  | 73,698,060  | C   | 1.092 [0.851–1.400] | 0.491 [0.302–1.343] |
| 4    | rs1685356 | 73,712,859  | T   | 0.927 [0.731–1.176] | 0.534 [0.302–1.343] |
| 4    | rs3741135 | 73,713,960  | A   | 1.139 [0.888–1.461] | 0.305 [0.202–1.343] |
| 4    | rs2734827 | 73,716,277  | A   | 0.532 [0.286–0.989] | 0.017 [0.532–1.502] |

* Chr. chromosome, β A1 effect allele, a OR odds ratio, c CI confidence interval

The mRNA and protein concentrations of UCP1 were induced by insulin at physiological dose, thus this polymorphism may affect the affinity of transcriptional factors for the IRS of UCP1, result in impaired promoter activity, decreased UCP1 protein producing, increased ROS production and energy storage. Mori et al. measured the transfecting activity of this polymorphism by using COS cells by transfecting promoter-reporter constructs with or without the polymorphism [43]. They found that the transcriptional activity of a fragment of the 5' UTR of the mutant G allele decreased to approximately 40% compared with that of the corresponding

Table 6 The association between UCPs and sight threatening diabetic retinopathy in participants with and without controlled glucose

| rs ID | A1β | All sub-group participants (127 cases vs 1,268 controls) | Well controlled glucose (23 cases vs 657 controls) | Uncontrolled glucose (104 cases vs 611 controls) |
|-------|-----|--------------------------------------------------------|---------------------------------------------------|-----------------------------------------------|
| rs6818140 | G   | 2.773 [0.636–12.085] | 0.178 | 11.773 [0.832–166.650] | 0.068 |
| rs7688743 | A   | 0.241 [0.058–1.005] | 0.051 | 0.049 [0.004–0.672] | 0.024 |
| rs10011540 | G   | 2.612 [0.592–11.528] | 0.205 | 18.758 [1.006–349.653] | 0.050 |
| rs3811787 | T   | 0.490 [0.293–0.822] | 0.007 | 0.505 [0.158–1.617] | 0.250 |
| rs3811790 | A   | 1.111 [0.687–1.798] | 0.667 | 1.919 [0.612–6.015] | 0.264 |
| rs3811791 | C   | 0.689 [0.385–1.234] | 0.211 | 0.704 [0.187–2.644] | 0.603 |
| rs1472268 | A   | 0.906 [0.005–166.826] | 0.970 | 0.001 [0.01–1.00] | 0.982 |
| rs1800592 | T   | 1.282 [0.007–236.211] | 0.926 | > 999 [> 999–1000] | 0.981 |
| rs659366  | T   | 2.790 [0.533–14.599] | 0.224 | 0.506 [0.066–3.899] | 0.513 |
| rs591758  | C   | 0.222 [0.040–1.237] | 0.086 | 1.244 [0.125–12.341] | 0.852 |
| rs1685356 | T   | 1.004 [0.699–1.443] | 0.981 | 0.592 [0.262–1.340] | 0.209 |
| rs3741135 | A   | 1.565 [0.838–2.924] | 0.160 | 1.182 [0.343–4.068] | 0.791 |
| rs2734827 | A   | 0.881 [0.481–1.615] | 0.683 | 0.381 [0.050–2.922] | 0.354 |

* A1 effect allele, a OR odds ratio, c CI confidence interval
fragment of the wild type T allele, which indicated the important role of this IRS in the transcription of UCP1. Moreover, Fukuyama et al. found that the G allele of rs10011540 may contribute to the accumulation of hepatic lipid content, and the development of insulin resistance [44].

The sub-group analysis of STDR showed that rs3811787 of UCP1 had a protective effect to STDR. Previous researches have reported that rs3811787 is associated with obesity, abdominal fat accumulation, and diabetic nephropathy [45–47]. Our study revealed its association with another diabetic microvascular complication-DR. Rs3811787 locates at the −412 position upstream from the transcription start site, and is in moderate LD ($r^2 = 0.53$) with rs1800592, which was associated with several diabetic complications. Former study indicated that rs3811787 is located adjacent to the binding site for the c-Rel transcription factor, this could be a possible operating mechanism.

When all the participants in dataset 1 were included, the association between DR/STDR and SNPs was affected by HbA1c level, which indicated the status of glucose control is a significant confounder. In uncontrolled glucose and well controlled glucose groups, the SNPs of UCPs were independently associated with DR/STDR after the adjustment of HbA1c. The difference between the results of the uncontrolled and the controlled glucose subgroups in the present study suggested that there might be different mechanisms in patients with different glucose control status during the development of DR/STDR; however, the result was limited by small sample size, because it is hard to collect participants with years of glucose records into the study. Although three SNPs of UCP1 were associated with DR in participants of poor glucose control, and one SNP of UCP3 was found to be associated with DR in diabetes patients with well controlled hyperglycemia, the significance did not remain after Bonferroni correction was applied. A similar situation also happen to the association between SNPs and STDR in participants with and without controlled glucose. In patients with uncontrolled glucose, rs3811787 of UCP1 and rs591758 of UCP3 were associated with STDR, while in those with controlled glucose, the rs75688743 of UCP1 was related to STDR. Therefore, our study suggested that UCPs could be related to metabolic memory, and future studies should consider glucose status when exploring the association between UCPs genes and DR.

Small sample size, especially in the subgroups of participants with glucose history records, is the main shortage of the present study. Moreover, other diabetic microvascular complications such as nephropathy and neuropathy was not explored in our study. Our study group is going to conduct another survey in the same population to screen for DR in those without DR currently, and the main purpose is to confirm the association between the potential risk SNPs of UCPs genes found in the present study and DR development.

Conclusions

In conclusion, the present study indicated that the rs10011540 of the UCP1 gene is marginally significantly associated with DR, and rs3811787 is marginally significantly associated with STDR in Chinese type 2 diabetes patients. There might be different mechanisms in patients with different glucose control status during the development of DR.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s12881-020-0956-y.

Additional file 1: Table S1. The characteristics of 18 single nucleotide polymorphisms of the uncoupling proteins genes.

Additional file 2: Table S2. The association of uncoupling proteins genotypes and sight threatening diabetic retinopathy.

Abbreviations

BMI: Body mass index; DR: Diabetic retinopathy; GWAS: Genome-wide association study; HbA1c: Hemoglobin A1c; MAF: Minor allele frequency; ROS: Reactive oxygen species; SNPs: Single nucleotide polymorphisms; UCPs: Uncoupling proteins

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Authors’ contributions

PJ and ZL carried out the molecular genetic studies, participated in the genotyping and drafted the manuscript. XX1, JH, XD, XB, BZ, XH, LL, and JZ carried out the systemic and ophthalmology exams. JC, YS and ZL participated in the GAWS study. PJ and HZ participated in the design of the study and performed the statistical analysis. HZ and XX2 conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. Please contact Haidong Zou: Email: zouhaidong@hotmail.com; Phone number: 86–21–63240090-6833; Fax number: 86–21–33011075; Address: No.100 Haining Road, Shanghai, China 200080.

Ethics approval and consent to participate

This study was conducted according to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Shanghai...
General Hospital, Shanghai Jiao Tong University. All of the participants understood the study protocol and provided written informed consent for this study.

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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