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Research Article

Association of Polymorphisms in Mitofusin-2 Gene with Type 2 Diabetes in Han Chinese

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MFN2 and *ESRRA* are candidate genes involved in the pathogenesis of T2D. Five tag-SNPs in *MFN2* gene and three in *ESRRA* gene were selected and genotyped with TaqMan or PCR-RFLP method in stage 1 populations (555 patients with T2D and 649 control subjects) and stage 2 populations (546 patients with T2D versus 419 control subjects) in Han Chinese. And combining our published data, we estimated the interactions between genetic variants in the *MFN2*, *ESRRA*, and *PGC-1α* genes on the T2D risk using MDR. rs873458 ($G > A$) and rs2878677 ($C > T$) in *MFN2* gene were significantly associated with T2D ($P = 0.005$ and 0.01) in stage 1 populations, and the association of other SNPs with T2D was not found. In stage 2 populations, we further confirmed the association between rs2878677 and T2D ($P = 0.01$). Combining the two stage populations, the data supported more significant effect of rs873458 and rs2878677 on T2D risk ($P = 0.003$ and 0.0001). A-C-G-T-C and G-T-C-T-C in *MFN2* had significant association with T2D ($P = 0.007$ and 0.009). The present study also provided the evidence that *MFN2* had interactions with *PGC-1α* ($P < 0.0001$) or *ESRRA* ($P < 0.0001$). This study suggested a role of *MFN2* polymorphism in the risk of T2D; however, further studies are needed.

1. Introduction

Type 2 diabetes (T2D) is a chronic disease characterized by high blood glucose which is induced by both insulin resistance and relative insulin deficiency [1], and the complications of T2D, such as cardiovascular disease, renal failure and blindness, always bring heavy burden to society. The latest studies from the International Diabetes Federation (IDF) reported that currently there were 366 million people with diabetes in the world by 2030 this number is expected to rise to 552 million with diabetes if no action is taken [2]. Some 90% of patients with diabetes have T2D, which is always as a result of a combination of lifestyle and genetic factors [3].

Peroxisome proliferator-activated receptor γ coactivator 1α (*PGC-1α*) gene is originally identified as a coactivator of PPARγ. It is a multifunctional regulatory factor involved in mitochondrial biosynthesis, β-oxidation of fatty acids, beta-cell insulin secretion, hepatic gluconeogenesis, glucose transport in muscle [4–6]. Our and other genetic association studies showed that the associations of *PGC-1α* loci with T2D were found in different populations [7–10].

Recently, it was reported that estrogen-related receptor-α (ESRRA) protein binding PGC-1α protein could regulate the expression of *Mitofusin-2* (*MFN2*) gene by stimulating the activity of the *MFN2* promoter [11]. *ESRRA* gene is located on chromosome 11q13. *ESRRA* gene is an orphan nuclear receptor (NR) belonging to the NR superfamily, group III, and it is a vital regulator involved in a wide variety of cell functions such as mitochondrial biogenesis, mitochondrial oxidative metabolism, carbohydrate metabolism, adaptive energy metabolism, and lipid metabolism [12–14]. Therefore, we hypothesize that *ESRRA* gene may be a candidate gene involved in the pathogenesis of T2D.

MFN2 gene maps on chromosome 1p36.22. MFN2 serve as a mitochondrial fusion protein regulating their morphology and distribution [15, 16]. Tissues with high energetic requirement, such as skeletal muscle and heart, show high

expression of MFN2 gene [17]. Some evidences demonstrated that it also played an important role in the oxidative metabolism [17] and ER-mitochondria juxtaposition [18], and repression of *MFN2* gene reduced glucose oxidation, mitochondrial membrane potential, cell respiration, and mitochondrial proton leak [17]. In addition, the *MFN2*-dependent mechanism of mitochondrial control is disturbed in skeletal muscle in animal or human obesity and in patients with T2D [17, 19], but no study has confirmed that *MFN2* gene was associated with T2D.

In the present study, we evaluated the associations between the *MFN2* and *ESRRA* genetic polymorphisms and T2D, and then combining our published data of *PGC-1α* [7], we estimated the interactions between genetic variants in the *MFN2*, *ESRRA*, and *PGC-1α* on the T2D risk in Han Chinese.

2. Materials and Methods

2.1. Subjects. The stage 1 populations are summarized in Table 1. The total of stage 1 populations comprised 1204 unrelated subjects, including 555 patients with T2D and 649 nondiabetic control subjects, ascertained from Han Chinese. All subjects were randomly enrolled from three top-grade hospitals in Beijing in 2010. Total case subjects were diagnosed according to the World Health Organization criteria (WHO 1998). As total control subjects, the age was between 35 and 70 years old, and they had normal fasting plasma glucose levels (5.0 ± 0.5 mmol/L) without diabetic record or T2D family history. The genomic DNA was extracted from peripheral blood using QIAGEN kit. The clinical characteristics of stage 2 populations are also summarized in Table 1; it included 546 unrelated patients with T2D and 419 nodiabetic control subjects and of Han Chinese origin, as described previously [20]. All subjects provided written informed consent under protocols approved by the local ethics committee.

2.2. Single-Nucleotide Polymorphism (SNP) Selection and Genotyping. International HapMap Project SNP database (http://www.hapmap.org/) was implemented to search for SNPs in *MFN2* and *ESRRA* gene. Using tagging with an $r^2 > 0.8$ and minor allele frequency (MAF) of >5% and sequencing data in 20 nondiabetic control subjects, we selected 5 tag SNPs (rs873458, rs2878677, rs2236058, rs3766742, rs3766741) in *MFN2* gene and 3 tag SNPs (rs731703, rs650008, rs11600990) in *ESRRA* gene; they were all located in intron region and could capture 100% of common variations across *MFN2* and *ESRRA* gene region (containing 21 HapMap SNPs). 7 of 8 SNPs were successfully genotyped using the TaqMan method on a Bio-Rad iQ5 system. Primers and probes of rs873458C_8861262_10, rs3766741C_25606040_10 and rs650008 AHWR23M were purchased from Applied Biosystems, the others from Shanghai GeneCore BioTechnologies (primers showed in Table 2). Only one SNP (rs3766742) in the *MFN2* was genotyped with PCR-restriction fragment-length polymorphism (PCR-RFLP) method, using the following primers (5′-AGCAGGACATGATAGGTTAG-3′and 5′-CACAGCTTGTCACAGTTTAG-3′) and PCR Tm was 57°C, PCR product length is about 900 bp, and restriction enzyme (Hha I) provided by MBI Fermentas. A 10 μL aliquot of PCR product was digested overnight at 37 in a 20 μL reaction containing 5 units of Hha I. After overnight digestion, the products were separated on a 3% agarose gel stained with ethidium bromide. TT genotypes were represented by a DNA band with a size of 900 bp, TC genotypes were represented by DNA bands with sizes of 900, 700, and 200 bp, and CC genotypes were represented by DNA bands with sizes of 700 and 200 bp. Call rates for all SNPs were >99%. 20 samples randomly selected from the whole sample bank were sequenced for 8 tag SNPs and the results showed excellent correspondence between sequencing and TaqMan genotyping or PCR-RFLP technique.

2.3. Statistical Methods. Genotype distributions for all studied SNPs were tested for Hardy-Weinberg equilibrium (HWE) by chi-square tests and no significant deviation was found in control subjects. Allele frequencies and genotype distributions, linkage disequilibrium (LD), and haplotypes were tested using the online software SHEsis (http://analysis.bio-x.cn/myAnalysis.php). Logistic regression analysis with additive model was used to adjust for sex, age, and body mass index (BMI), and the analysis was performed by SPSS (version 16.0). Tests for association between genotypes and quantitative traits were performed in control subjects using Kruskal-Wallis analysis of ranks for traits with non-normal distribution or, alternatively, ANOVA for normally distribution in SPSS. We examined the analyses of gene-gene interactions on the risk of T2D using multifactor dimensionality reduction (MDR) version 2.0 beta 6 (http://www.epistasis.org/). P value <0.05 is nominally significant.

3. Results

In stage 1 populations, we found that the A allele and C allele frequencies of rs873458 and rs2878677 of *MFN2* gene in the diabetic group were lower than in the control group ($P = 0.005$, OR = 0.79, 95% CI= 0.67–0.93; $P = 0.01$, OR = 0.81, 95% CI = 0.68–0.95) (Table 3); it showed A allele and C allele of rs873458 and rs2878677 of *MFN2* gene confered protection agasist T2D. After adjusting for age, sex, and body mass index by using analysis of logistic regression with additive model, the results showed that the genotype distributions of rs873458 and rs2878677 of *MFN2* gene also were significantly different in case-control subjects ($Pc = 0.01$, OR = 0.80, 95% CI = 0.67–0.95; $Pc = 0.02$, OR = 0.83, 95% CI = 0.71–0.97) (Table 3).

Replicating the two associated SNPs in stage 2 populations, data suggested that the C allele of rs2878677 of *MFN2* gene in the diabetic group was lower than in the control group ($P = 0.01$, OR = 0.79, 95% CI = 0.65–0.94) (Table 3). And it revealed that there was a borderline association between genotype distributions of rs873458 of *MFN2* gene and type 2 diabetes (adjusted $Pc = 0.08$, OR = 0.84, 95% CI = 0.69–1.02) (Table 3). Additionally, analyses in the combined populations showed that the allele frequencies and genotype distributions of rs873458 and rs2878677 of *MFN2* gene had

Table 1: Characteristics of the study populations.

Characteristic	Stage 1 ($n = 1204$)		Stage 2 ($n = 965$)	
	T2D	Control	T2D	Control
Total (male %)	555 (55.9)	649 (46.4)*	546 (50.7)	419 (48.7)
Age (years)	53.6 (8.2)	50.5 (8.5)*	58.8 (11.8)	63.2 (8.2)*
BMI (kg/m^2)	25.8 (3.4)	25.4 (3.3)*	24.9 (3.5)	24.6 (3.4)
DBP (mmHg)	82.7 (11.4)	79.7 (11.8)*	80.3 (11.0)	75.6 (10.1)*
SBP (mmHg)	129.6 (18.5)	128.3 (16.9)	131.4 (19.2)	127.7 (17.2)
FPG (mmol/L)	9.0 (2.9)	5.0 (0.5)*	9.2 (3.2)	5.0 (0.5)*
TG (mmol/L)	2.1 (2.3)	1.6 (1.1)*		
IFCC-HbA1c (mmol/mol)	63 (19.0)	41 (3.0)*		
DCCT-HbA1c (%)	7.9 (1.7)	5.9 (0.3)*		

Data are means (SD), or male (%), unless otherwise indicated. *$P < 0.05$. BMI: body mass index; DBP: diastolic blood pressure; SBP: systolic blood pressure; FPG: fasting plasma glucose; TG: triglycerides; HbA1c: glycated haemoglobin A_{1c}.

Table 2: Primers and probes of all SNPs.

SNPs ID	Primer (5′-3′)	Probe (5′-3′)
rs2878677	F: GTGGAATAAAAGTTTTATGAATGGAG	P1: FAM-ATTCCACTTACGTGAGACaTCTAGAATAGTC-BHQ
	R: TAGCCATTAAACAATAACTCCCTG	P2: HEX-TCCACTTACGTGAGACgTCTAGAATAGTC-BHQ
rs2236058	F: GGTGAGGAGGGCGGCT	P1: FAM-CTTCATCACCCCACCTGGTCTGTG-BHQ
	R: TGTGTTCAGGCTCTGAGAATGG	P2: HEX-CTTCATCACCCGACCTGGTCTGTG-BHQ
rs731703	F: AGCCAGAGTCCCTGTTCGG	P1: FAM-TTGCTGTCCTGGCCAGTGTTCC-BHQ
	R: TGCTTCAGCCCCAGCTATG	P2: HEX-TTTGCTGTCCTGACCAGTGTTCCAG-BHQ
rs11600990	F: AAGTCATTGTTCTTCCCTAAGCCTC	P1: FAM-TGCTACCGTCACTGAG-MGB
	R: GAGGTGTCTCCGTAAGGTCTTCA	P2: HEX-TGCTACCATCACTGAGT-MGB

more significant effects on type 2 diabetes susceptibility ($P = 0.002$, OR = 0.83, 95% CI = 0.73–0.93; $P = 0.0001$, OR = 0.79, 95% CI = 0.69–0.89 and adjusted $Pc = 0.002$, OR = 0.82, 95% CI = 0.72–0.93; $Pc = 0.0004$, OR = 0.81, 95% CI = 0.72–0.91) (Table 3). No significant association of other SNPs in *MFN2* or *ESRRA* gene with type 2 diabetes was found (See Supplementary Table 1 in Supplementary material available online at doi:10.1155/2012/205752).

Analyses of association between haplotypes and phenotypes were performed in nondiabetic control subjects, for most patients with T2D included in the present study had medical treatments, which may affect the real parameters. We found that the genotype distributions of SNPs in *MFN2* and *ESRRA* gene in control subjects displayed no significant association with quantitative traits of type 2 diabetes including body mass index, diastolic blood pressure, systolic blood pressure, fasting plasma glucose, triglycerides, and glycated haemoglobin A1c (data not shown).

We further examined the degree of linkage disequilibrium of SNPs in *MFN2* and *ESRRA* gene (Supplementary Tables 2 and 3). To test whether haplotypes represent the causal variants better than single SNPs, we estimated the frequencies of haplotypes between the case-control subjects in stage 1 populations (all those frequencies <0.03 were ignored). It was showed that A-C-G-T-C and G-T-C-T-C in *MFN2* gene were significantly associated with T2D ($P = 0.007$, OR = 0.79, 95% CI = 0.66–0.94; $P = 0.009$, OR = 1.26, 95% CI = 1.06–1.49) (Table 4), while the frequencies of haplotypes in *ESRRA* gene did not significantly differ in case-control subjects (data not shown).

The results of the exhaustive MDR analysis are summarized in Table 5. The model was considered to be statistically significant with a testing accuracy >50% and $P < 0.05$ via permutation test of 1000 iterations. The rs2878677/rs3766741/rs731703/rs11600990 model ($P < 0.0001$) in *MFN2-ESRRA* from stage 1 populations, the rs2878677/rs3774923/rs7656250 model ($P < 0.0001$) and the rs2878677/rs3774923/rs7656250/rs13131226 model ($P < 0.0001$) in *MFN2-PGC-1α* gene from stage 2 populations (shown in Table 5), data of *PGC-1α* gene from our previously study [7], suggest that there are interactions between genetic variants in the *MFN2*, *ESRRA*, and *PGC-1α* gene.

4. Discussion

In the present study, we examined eight common variants in *MFN2* and *ESRRA* genes and confirmed that G allele of rs873458 and T allele of rs2878677in *MFN2* gene are associated with higher T2D risk compared with A and C allele, respectively. Consistently, in haplotypes analyses of *MFN2* gene, results show that individuals carrying G-T-C-T-C present increased T2D risk compared with those carrying A-C-G-T-C.

Because rs873458 and rs2878677 map within intron 2 and 3, it is difficult for us to shed light on their downstream consequences, given our rudimentary knowledge of the

Table 3: Allele frequencies and genotype distributions of rs873458 and rs2878677 in *MFN2* gene among stage 1 and stage 2 populations.

	SNP	Genotype				Allele				
			T2D (%)	Control (%)	*P*/*Pc*	OR (95%CI)		T2D (%)	Control (%)	*P*	OR (95% CI)
Stage 1	rs873458		555	638							
		A/A	50 (9.0)	105 (16.5)	0.0007/0.01	0.80 (0.67–0.95)	A	382 (34.4)	510 (40.0)	0.005	0.79 (0.67–0.93)
		A/G	282 (50.8)	300 (47.0)			G	728 (65.6)	766 (60.0)		
		G/G	223 (40.2)	233 (36.5)							
	rs2878677		549	643							
		T/T	124 (22.6)	108 (16.8)	0.03/0.02	0.83 (0.71–0.97)	T	487 (44.4)	503 (39.1)	0.01	0.81 (0.68–0.95)
		C/T	239 (43.5)	287 (44.6)			C	611 (55.6)	783 (60.9)		
		C/C	186 (33.9)	248 (38.6)							
Stage 2	rs873458		539	412							
		A/A	72 (13.4)	58 (14.1)	0.21/0.08	0.84 (0.69–1.02)	A	386 (35.8)	321 (39.0)	0.16	0.87 (0.72–1.05)
		A/G	242 (44.9)	205 (49.8)			G	692 (64.2)	503 (61.0)		
		G/G	225 (41.7)	149 (36.2)							
	rs2878677		545	417							
		T/T	135 (24.8)	78 (18.7)	0.05/0.02	0.80 (0.67–0.97)	T	516 (47.8)	348 (41.8)	0.01	0.79 (0.65–0.94)
		C/T	248 (45.5)	192 (46.0)			C	564 (52.2)	484 (58.2)		
		C/C	162 (29.7)	147 (35.3)							
Combinded	rs873458		1094	1050							
		A/A	122 (11.2)	163 (15.5)	0.005/0.002	0.82 (0.72–0.93)	A	768 (35.1)	831 (39.6)	0.002	0.83 (0.73–0.93)
		A/G	524 (47.9)	505 (48.1)			G	1420 (64.9)	1269 (60.4)		
		G/G	448 (41.0)	382 (36.4)							
	rs2878677		1089	1059							
		T/T	259 (23.8)	186 (17.6)	0.0006/0.0004	0.81 (0.72–0.91)	T	1003 (46.1)	851 (40.2)	0.0001	0.79 (0.69–0.89)
		C/T	485 (44.5)	479 (45.2)			C	1183 (53.9)	1267 (59.8)		
		C/C	345 (31.7)	394 (37.2)							

P had no adjustment, whereas *Pc* were adjusted for age, sex, and BMI.

Table 4: Common haplotypes in the *MFN2* gene.

Haplotype	T2D (%)	Control (%)	*P*	OR (95% CI)
A-C-G-T-C	347.95 (31.9)	474.88 (37.7)	0.007	0.79 (0.66–0.94)
G-C-C-C-C	66.82 (6.1)	100.87 (8.0)	0.10	0.76 (0.55–1.05)
G-C-C-T-C	35.18 (3.2)	40.09 (3.2)	0.90	1.03 (0.65–1.63)
G-C-G-T-G	114.87 (10.5)	111.74 (8.9)	0.14	1.23 (0.94–1.62)
G-T-C-T-C	443.08 (40.6)	454.77 (36.2)	0.009	1.26 (1.06–1.49)
others	84.1 (7.7)	75.65 (6.0)		
Total	1092 (100%)	1258 (100%)		

Haplotype analysis was conducted with rs873458, rs2878677, rs2236058, rs3766742, rs3766741. *P* had no adjustment.

Table 5: Models of multi-loci interaction on the risk of T2D by MDR.

Gene-gene	Combination of multiloci	*P value	CV consistency	Testing accuracy (%)
MFN2-ESRRA	rs873458/rs3766742	0.0007	7/10	56.1
	rs873458/rs2878677/rs3766741	<0.0001	4/10	56.2
	rs2878677/rs3766741/rs731703/rs11600990	<0.0001	3/10	57.7
MFN2-PGC-1α	rs7656250/rs13131226	0.0002	7/10	56.4
	rs2878677/rs3774923/rs7656250	<0.0001	10/10	59.9
	rs2878677/rs3774923/rs7656250/rs13131226	<0.0001	9/10	63.9

Rs873458, rs2878677, rs3766742, rs3766741 located in *MFN2*, rs731703 and rs11600990 located in *ESRRA*rs7656250, rs13131226, and rs3774923 located in *PGC -1α*. *P* value based on 1,000 permutations.

mechanics of gene regulation. In multifactorial disease, most susceptibility variants, mapping outside the coding regions of genes, are assumed to influence transcript regulation rather than gene function [21]. Locations of rs873458 and rs2878677 are surrounded by the regions coding the GTPase domain of MFN2 which drive GTP hydrolysis to provide energy for the mitochondrial fusion activity induced by MFN2 [16, 22]. In addition, *MFN2* gene is the first structural tether identified gene in the molecular basis of ER-mitochondria juxtaposition, it has a role in the intercommunication during Ca^{2+} signaling [18]. A study revealed that ablation or silencing of *MFN2* gene in mouse embryonic fibroblasts or HeLa cell created confusion in ER morphology and increased distance between the ER and mitochondria, hence retarding the mitochondrial Ca^{2+} uptake [18], which may lead to insulin secretion disorder and insulin resistance. Besides, positive correlation was observed between the expression of *MFN2* gene and insulin sensitivity [23]. In addition, the *MFN2*-dependent mechanism of mitochondrial control is disturbed in skeletal muscle in animal or human obesity, and in patients with T2D [17, 19]. It is possible that the associated SNPs may be in strong linkage disequilibrium with unidentified causal variant(s) that may regulate the expression or function of *MFN2* gene.

Although many genomewide association studies (GWASs) of T2D are now emerging, *MFN2* gene is not involved. Indeed, the effect sizes of the known, common variants influencing the risk of type 2 diabetes are modest, and the proportion of overall predisposition explained is approximately 5 to 10% for T2D [24]. It has been found that T2D loci identified by linkage analyses and GWAS lack the correlation of T2D [25]. Additionally, most of them were conducted in Caucasians. Differences in genetic background, risk-factor profile, environment, and study design may lead to ethnic differences in susceptibility loci [26]. Different allele frequencies of rs873458 and rs2878677 among the HapMap populations indicate that differences in genetic architecture may play a role (Supplementary Table 4). Therefore, further studies including GWAS are needed to confirm our findings.

ESRRA is an orphan nuclear receptor, it could regulate a number of downstream genes to control energy balance in animals. *ESRRA* gene KO mice showed altered expression of several target genes implicated in the regulation of adipogenesis and energy metabolism [27]. However, in the present study, no association between the phenotypes of T2D, such as BMI, was found. Our data also showed that there were three common variants (rs731703, rs650008, rs11600990) in *ESRRA* in Han Chinese populations, while only one common variant rs11600990 was found in Danish populations [28]. The analysis of rs11600990 in the two populations is consistent. Three tag SNPs could capture 100% of common variations (MAF > 5%) across *ESRRA* gene region, but we cannot exclude rare causal genetic polymorphisms in *ESRRA* gene, for the rare variations may be responsible for disease [29–31].

PGC-1α is an important transcriptional coactivator involved in the regulation of genes related to energy metabolism [32]. Mice lacking *PGC-1α* developed fasting hepatic steatosis which may lead to lower rates of fatty acid oxidation that might play a causative role to develop insulin resistance [33, 34]. In addition, PGC-1α maintained higher number of active mitochondria and OXPHOS proteins that were reduced in T2D subjects. As it relates to the human genetics, several SNPs of *PGC-1α* gene in certain populations were associated with T2D in certain populations [7–10]. The analysis of association between *PGC-1α* gene and T2D has been published in our previous paper [7].

In addition, it was found that the interactions between genetic variants of the *MFN2-ESRRA* and *MFN2-PGC-1α* in the pathogenesis of type 2 diabetes. Moreover, a positive result of association analyses regarding single SNP rs2878677 in *MFN2* gene indicates *MFN2* gene may be the main factor during the interaction and has an important role in the pathogenesis of T2D. It is consistent with the study that it proved the interactions between MFN2, ESRRA, and PGC-1α proteins, which revealed the stimulatory effect of PGC-1α on the activity of *MFN2* gene needed to integrate the ESRRA, ESRRA, and PGC-1α caused a synergic effect on activity of the *MFN2* gene expression, and the stimulatory effect of *PGC-1α* gene on mitochondrial membrane potential can be weakened by *MFN2* loss of function [11]. Therefore, further studies are needed to explore the functions and interactions of the *MFN2*, *ESRRA*, and *PGC-1α* genes.

In most patients, T2D is due to alterations of many genes, each of which has a partial and additive effect [35]. Consequently, we hypothesize that these three genes may serve as partial or additive factors leading to T2D, while the intrinsic mechanism is unknown. Functional studies, multiplex-gene analyses, and computational biology are needed to further characterization of their interactions in the pathogenesis of T2D.

However, some limitations should be noted in the present study. First, the effect of gene-environment interactions was not evaluated; environmental risk factors may influence the effect estimates. Second, given the study samples were relatively small, we need to replicate our finding in additional samples in future studies. Third, clinical characteristics were not defined strictly.

In conclusion, the present analysis shows that *MFN2* gene has significant associations with T2D in Han Chinese. It may represent a potential explanation for T2D. But more studies with different ethnic populations are needed to verify this finding and the intrinsic mechanism is expected to be uncovered.

Conflict of Interests

The authors have no conflict of interests to declare.

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