



ASSOCIATION OF UBE2E2 AND KLF14 GENE POLYMORPHISMS WITH THE RISK OF TYPE 2 DIABETES: ASIAN META-ANALYSIS

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ABSTRACT

The association of UBE2E2 rs7612463 and KLF14 rs972283 gene polymorphisms with type 2 diabetes (T2D) was recently identified in East Asian and European genome-wide association studies, respectively. However, the replication studies in various populations showed inconsistent results. The aim of the present meta-analysis is to investigate this inconsistency, especially in Asian populations.

A systemic literature search inclusive to July 2018 yielded a total of 15 potentially relevant articles with 20 eligible studies concerning the association of UBE2E2 rs7612463 and /or KLF14 rs972283 with T2D in Asian populations. The final meta-analysis was conducted for 10 studies (19248 T2D cases and 17968 controls) concerning the UBE2E2 rs7612463 and 10 studies (11165 cases T2D and 9551 controls) concerning the KLF14 rs972283 gene polymorphisms with T2D in Asian populations.

The combined overall allelic odds ratio (OR), for association of UBE2E2 rs7612463 C allele and KLF14 rs972283 G allele with T2D in Asian populations, were 1.153 (95% confidence interval 1.11 - 1.20, $p < 0.0001$) and 1.07 (95% confidence interval 1.004 - 1.133, $P = 0.036$) under fixed and random effects model, respectively.

The present meta-analysis indicated that the UBE2E2 rs7612463 and KLF14 rs972283 gene polymorphisms are significantly and nominally associated with the risk of T2D in Asian populations.

KeyWords: KLF14, UBE2E2, Type 2 diabetes, Meta-analysis, Gene polymorphism

I. INTRODUCTION

Diabetes is one of the largest global health emergencies in the twenty-first century [1]. Type 2 diabetes (T2D) is the most prevalent form of diabetes, it accounts for more than 90% of all diabetes types worldwide, and characterized by hyperglycemia resulting from impaired insulin secretion pancreatic β -cell function and a insulin resistance in target tissues [2-5]. T2D is a complex, multifactorial, chronic, endocrine and metabolic disorder, influenced by a combination of multiple genomic variants and various environmental factors [6-11]. Without careful management, T2D triggers a series of serious complications such as cardiovascular disease, kidney disease, nerve disease, and eye disease. The incidence and prevalence of T2D has reached epidemic proportions worldwide [12], and becomes one of the health-life-threatening diseases, in both developed and developing countries.

Association studies of genetics variants in the susceptibility to T2D have identified a large number of genetic loci associated with the T2D susceptibility in various populations, and most of these loci were detected initially in population samples of European ancestry [13, 14]. In addition, functional studies have shown that many of the identified T2D susceptibility loci are related to abnormal insulin secretion and insulin resistance in peripheral tissues [15] which are the hallmark of T2D. These include the two recently identified T2D susceptibility loci, rs7612463 in UBE2E2 and rs972283 in KLF14 and which first identified through two large-scale genome-wide association studies in east Asian and European populations, respectively [16, 17]. After the initial find, many replication studies concerning the association between this variant and T2DM have been conducted in different ethnic populations [13, 18-41]. However, the results from different studies were inconsistent. Therefore, we conducted a meta-analysis to assess the contributions of UBE2E2 rs1496653 and KLF14 rs972283 gene polymorphisms to the risk of T2D in Asian populations, to achieve a more comprehensive result.

II. MATERIALS AND METHODS

A. Search strategy

We searched the worldwide literature published in MEDLINE via PubMed, EMBASE, Cochrane CENTRAL, Chinese databases (CNKI, CQVIP, Wanfang databases), and Google Scholar for articles of case-control association studies of the rs7612463 polymorphism in UBE2E2 and/or rs972283 polymorphism in KLF14 with T2D, published from 2010 (when the initial study reporting the association of rs7612463 in UBE2E2 and rs972283 in KLF14 with T2D was published [16, 17] to 2018. The following search terms were used: "Ubiquitin-conjugating enzyme E2 E2"/"Kruppel-like factor 14 " "UBE2E2 rs7612463"/"KLF14 rs972283", "Gene polymorphism", "Genetic variant", "Genetic variation", "Genotype", and "Type 2 diabetes", "Type 2 diabetes mellitus" "T2D/T2DM". The research subjects were limited to human studies published in English or Chinese languages were retrieved. The reference lists of the identified articles were also searched in order to identify any other relevant articles.

B. Inclusion criteria

Studies were selected based on the following inclusion criteria: case-control or cohort studies; studies that examining the association of the UBE2E2 rs7612463 and/or KLF14 rs972283 gene polymorphisms with the risk of T2D; and both cases and controls reporting genotype and/or allele frequencies; control group accord with Hardy-Weinberg equilibrium.

The exclusion criteria were: studies that did not fit within the selected conditions; studies with repetitive data.

C. Data Extraction

Data were drawn out according to a standard protocol.

Repeated publications and studies violating the inclusion criteria or providing insufficient data were excluded. Same data from

different studies were only adopted once. The extracted information from all eligible articles included: first author's surname, publication year, characteristics of study population, including country, ethnicity, sex, age, BMI, sample size "cases /controls" and number of genotypes and/or alleles frequency in case and control groups. Hardy–Weinberg equilibrium (HWE) test for the controls were included as quality assessment indicator. If the reported data were incomplete, the corresponding author was contacted to obtain complete data.

D. Statistical analysis

In the current meta-analysis, an allele-contrast model was used to investigate the associations of the UBE2E2 rs7612463 and KLF14 rs972283 gene polymorphisms with the risk of T2D. The strength of the association of each SNP and risk of T2D was determined by using odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). The pooled ORs were obtained only for allele contrast model (C vs. A) of rs7612463 polymorphism and (G vs. A) of rs972283 polymorphism because some studies lack the information for genotypes. The statistical significance of pooled ORs was determined by using the Z test, with the significance level set at $P < 0.05$.

The heterogeneity between studies was analyzed by using the chi-square test based on the Q statistic, with the significance level set at $P < 0.1$ [42] and/or heterogeneity index (I^2 , 0–100) [43]. The heterogeneity was quantified by the I^2 value [43], if no heterogeneity between the individual studies was existed, the pooled ORs were computed by using the fixed-effects method of Mantel–Haenszel (Petos method) [44]. If the significant heterogeneity between the individual studies was existed, the pooled OR was estimated using random-effects model of DerSimonian–Laird (D–L method) [45].

The potential publication bias was estimated using the funnel plot [46]. The funnel plot asymmetry was quantified using Egger's regression approach [47], on the natural logarithmic scale of the OR, with the significance level set a $P < 0.05$, which considered to indicate significant asymmetry and the existing of significant publication bias. The population-attributable risk (PAR) was calculated on the basis of estimated ORs and risk allele frequencies in cases group to get a comprehensive view of the impact of the 2 SNPs on T2D at population level, using the following formula: $(OR-1)/OR * \text{risk allele frequency}$ [48]. The statistical analyses were performed by STATA 10.1 software (Stata Corp, College Station, TX,USA).

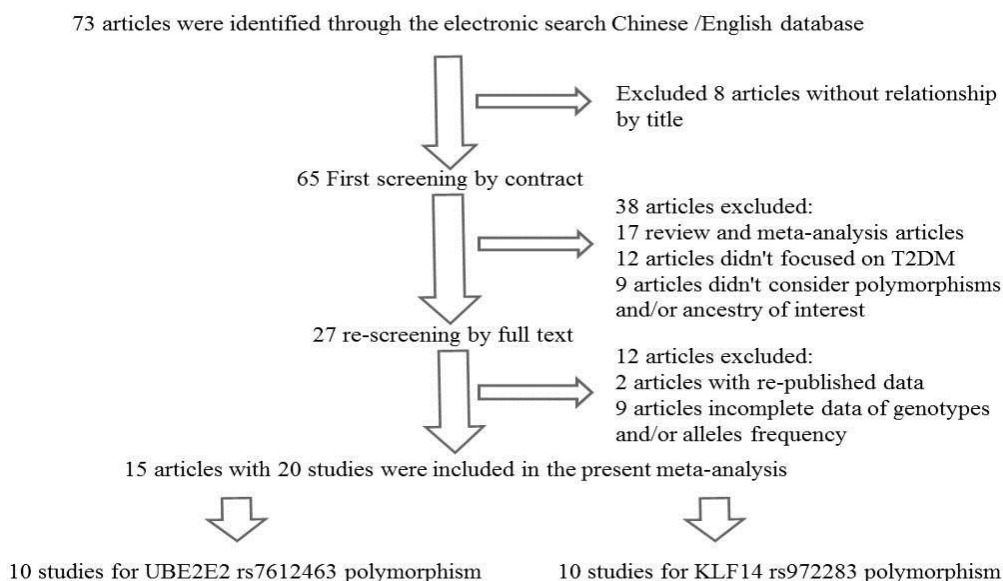


Figure 1 Flow chart of search strategy for eligible studies

Table 1 The characteristics of 20 eligible genetic association studies included in the present meta-analysis

Study	Population	Groups	Sex (M/F %)	Age (Years)	BMI (kg/m ²)	Sample size	UBE2E2 rs7612463			KLF14 rs972283					
							CC/CA/AA	C/A F	RAF %	HWE -P	GG/GA/AA	G/A F	RAF %	HWE-P	
Yamauchi et al. 2010	Japanese 1	case		65.8±10.0	24.1±3.8	4338		7365/1311	0.849						
		control		52.5±15.2	22.5±3.8	3071		5067/1075	0.825						
	Japanese 2	case		63.8±11.0	24.2±4.0	2613		4494/732	0.860						
		control		63.5±11.1	23.8±3.4	3073		5130/1016	0.835						
	Japanese 3	case		65.1±10.9	24.0±3.9	3492		5976/1008	0.856						
		control		58.6±16.6	23.0±3.4	2244		3761/727	0.838						
Yamakawa-K et al. 2012	East Asiana	case				4143		6834/1452	0.825	> 0.05					
		control				4062		6592/1532	0.811						
	Japanese	case	100/0	54.4±6.4	25.0±3.6	333		532/101	0.84	> 0.05					
Iwata et al. 2012	Japanese	case		53.7±5.1	23.1±2.7	417		658/135	0.83						
		control		62.3/37.7	64.9±11.1	27.4±4.3	724	536/173/15	1245/203	0.860	0.972				
Alharbi et al. 2014	Saudi	case		47.05/52.9	72.5±9.0	24.7±3.1	762	538/207/17	1283/241	0.842	0.855				
		control		59.8/40.2	50.6±10.4	29.5±5.9	376	348/18/10	714/38	0.95					
Kazakova et al. 2015	Chinese	case		53.2/46.8	46.1±7.7	29.2±5.5	380	357/15/8	729/31	0.96	> 0.05				
		control		61.4/38.6	46.1±12.5	25.8±3.6	993	320/637/36	1594/392	0.80	> 0.05				
Xu et al. 2016	Chinese	case		58.8/41.2	42.96±11.7	23.3±3.4	964	339/563/62	1465/463	0.76	> 0.05				
		control		61.8±8.5	25.2±3.5	1736	1107/561/68	2775/697	0.80	0.769					
Plengvidhya et al. 2018	Thai	case		55.1±9.0	23.6±3.0	2495	1513/852/130	3878/1112	0.78	0.481					
		control		32.8/67.2	57.2±12.2	27.3±5.0	500	346/134/20	826/174	0.83					
Mastouri . 2011	Lebanese	case		28.8/71.2	53.0±8.4	24.1±3.3	500	339/141/20	819/181	0.82		41/144/128	226/400	0.361	
		control		59.0/41.0	62.0±9.78	30.2±5.8	313					45/123/112	213/347	0.380	
Ohshige et al. 2011	Japanese 1	case		60.0/40.0	61.5±11.6	23.7±3.9	1630					920/570/95	2410/760	0.760	0.36
		control		64.9/35.1	44.3±9.9	22.9±3.1	716					342/265/52	949/369	0.720	0.82
	Japanese 2	case		62.3/37.7	64.9±11.1	24.5±3.9	724					401/266/45	1068/356	0.750	0.995
		control		47.1/52.9	72.5±9.0	22.7±3.3	763					388/304/55	1080/414	0.723	0.911
	Japanese 3	case		59.4/40.6	64.2±11.5	24.9±4.6	485					265/179/38	709/255	0.735	
		control		29.1/70.9	35.6±10.3	21.6±3.0	646					348/251/45	947/341	0.735	
Shi et al 2011	Chinese	case		55.5/44.5	53.2±10.4	308						146/33/129	325/291	0.472	
		control		47.0/53.0	52.8±9.7	234						101/25/108	227/241	0.515	
Kong et al 2015	Chinese	case				5166						7269/2827	0.720	0.677	
		control				4560						6566/2554	0.720	0.326	
Qian et al. 2015	Chinese	case		39.8/60.2	57.4±9.8	24.9±3.4	1200					1646/722	0.695		
		control		39.8/60.2	56.4±8.0	22.6±2.9	1200					1627/741	0.687	> 0.05	
Gao et al. 2016	Chinese	case		57.9/42.1	52.50	28.58	721					379/286/56	1044/398	0.724	
		control		42.2/57.8	47.0	23.5	757					389/297/71	1075/439	0.710	0.1942
O'Beirne et al. 2016	Qataris	case				518						207/226/85	640/396	0.62	
		control				295						112/137/46	361/229	0.61	
Sharif et al. 2018	Palestinian	case		100/00	35-50	30.3±4.6	100					46/38/16	130/70	0.65	
		control		100/00	35-50	27.9±3.98	100					29/55/16	113/87	0.565	0.234

III. RESULTS

A. Characteristics of included studies

A total of 15 potentially relevant articles with 20 eligible studies were included in the present meta-analysis (Fig. 1) describing an association of the two genes polymorphism and T2D. Ten studies (19248 cases and 17968 controls) concerning the association between UBE2E2 rs7612463 and T2D [16, 18, 19, 21-23, 37] and ten studies (11165 cases and 9551 controls) concerning the association between KLF14 rs972283 and T2D [26, 29, 31, 35, 36, 38, 40, 41]. Table 1 lists the main characteristics of the 15 eligible articles for our meta-analysis. No study was excluded for deviating from the Hardy-Weinberg equilibrium (HWE). Egger regression analysis indicated no publication bias for the two gene polymorphisms, UBE2E2 rs7612463 and KLF14 rs972283 which indicated reliability of the pooled results (t=-0.93, P=0.379, 95%CI -2.37~1.101, t=1.72, P=0.124, 95%CI -0.419~2.88, respectively) (data not shown).

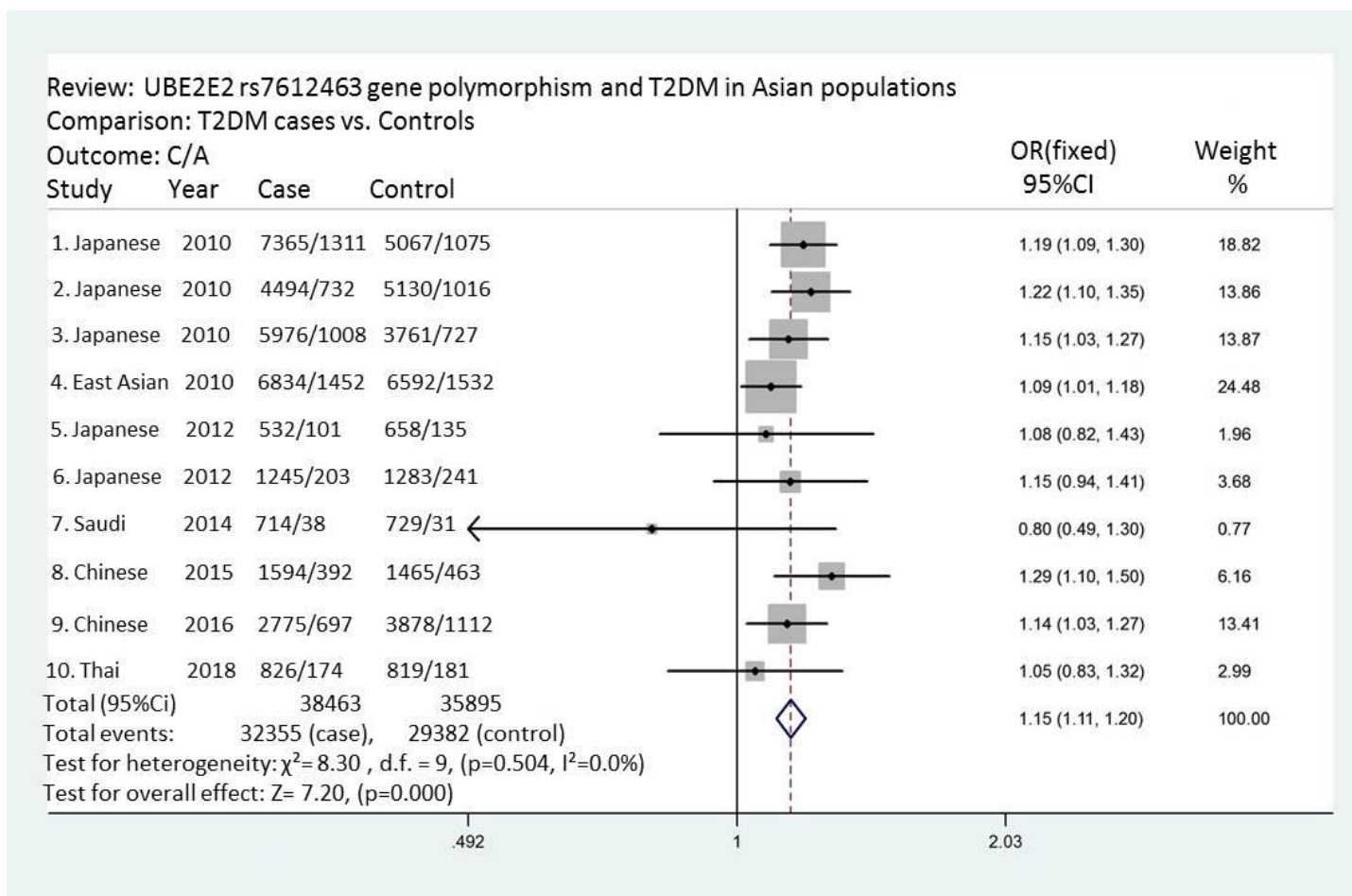


Figure 2 Forest plot of association between UBE2E2 rs7612463 gene polymorphism and risk of T2D in Asian populations under allele contrast model comparison. For each study, the estimate of OR and its 95% CI is plotted with a closed square and horizontal line. The size of the black squares is proportional to the weight that the study has in calculating the summary effect estimate (diamond). The center of the diamond indicates the pooled OR and the ends of the diamond correspond to the 95% CI. A dashed line is plotted vertically through the combined odds ratio. This line crosses the horizontal lines of all individual studies.

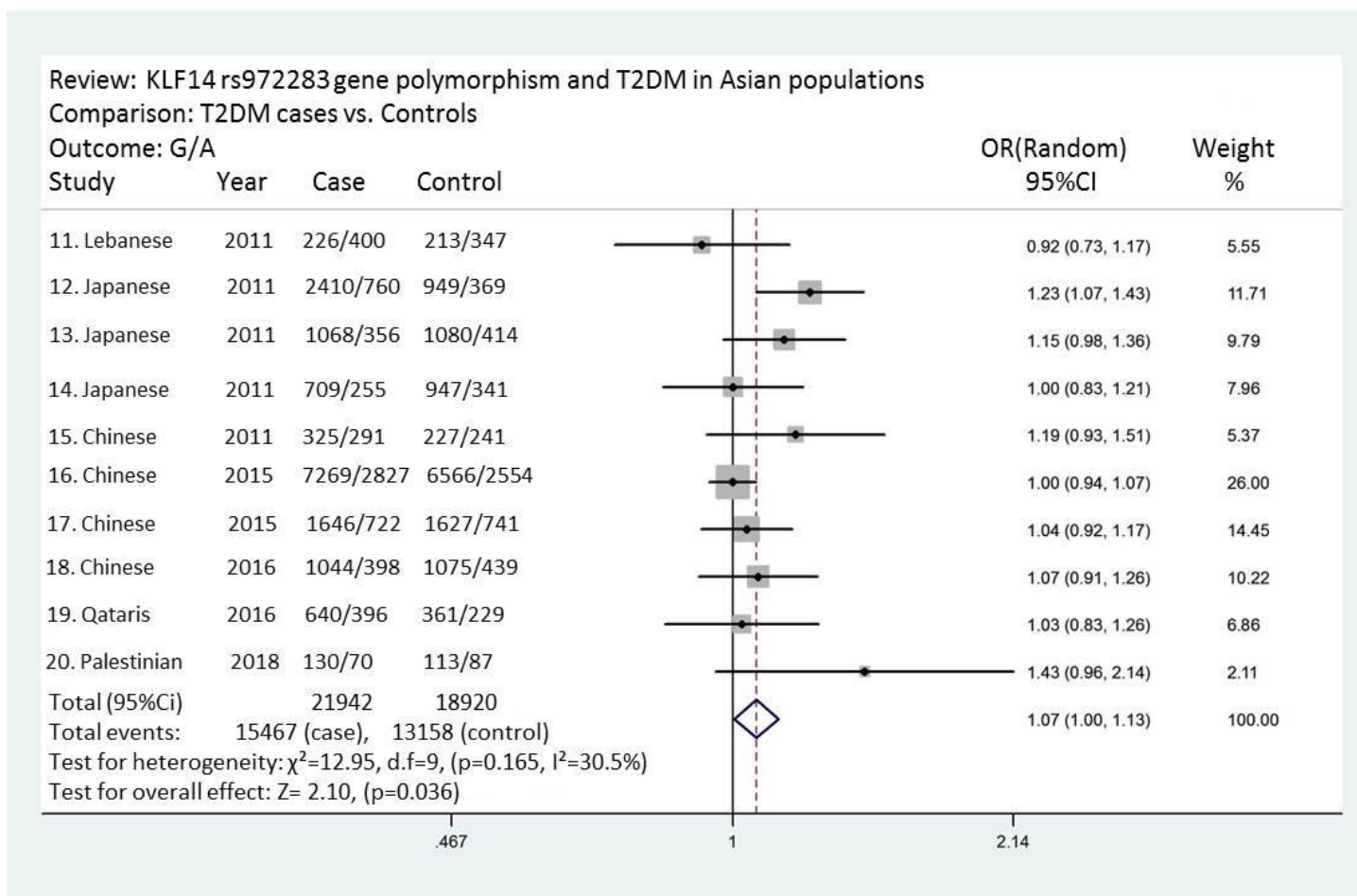


Figure 3 Forest plot of the association between KLF14 rs972283 gene polymorphism and risk of T2D in Asian populations under allele contrast model comparison.

B. UBE2E2 rs7612463 and type 2 diabetes

Figure 2 represents the forest plot of risk allele OR of an individual studies and meta-analysis for association between UBE2E2 rs7612463 and T2D in a total of 19248 T2D patients and 17968 control subjects from the ten studies. Nine studies showed a trend of elevated OR for the risk allele C. One study from Saudi ancestry [21] showed a trend in the opposite direction. The overall frequency of the risk allele C was to 84.12% in cases and 81.9% in controls. No heterogeneity was found between studies (P=0.504, I²=0.0%). A fixed effect model was performed and generated a combined allelic OR of 1.153 (95%CI 1.11 - 1.20, P=0.000) for the rs7612463 C allele. The population attributable risk (PAR) of T2D related to this polymorphism was 11.2%.

C. KLF14 rs972283 and type 2 diabetes

Figure 3 represents the forest plot of risk allele OR of an individual study and meta-analysis for association between KLF14 rs972283 and T2D in a total of 11165 T2D patients and 9551 control subjects from the ten studies. Seven studies showed a trend of elevated OR for the risk allele G. Two studies, Japanese [29] and Chinese [35], showed no association. One study from Lebanon [26] showed a trend in the opposite direction. The overall frequency of the risk allele G was to 70.5% in cases and 69.5% in controls. A weak between-study heterogeneity was observed (P=0.165, I²=30.5%). A random effect model was performed and generated a combined allelic OR of 1.07 (95%CI 1.004 - 1.133, p=0.036) for the rs972283 G allele. The population attributable risk (PAR) of T2D related to

this polymorphism was 4.64%.

IV. DISCUSSION

Multiple lines of evidence support the view that genetic components play an important role in the pathogenesis of T2D [49]. Genetic association in particular, Genome-wide association studies (GWAS) have discovered more than 100 genetic regions associated with modified risk for T2D [50]. European and East Asian GWAS have revealed multiple risk-associated loci for T2D, and some of them have been confirmed and shown to be common across different ethnic groups [51]. Recently, two independent GWA studies were performed in European and east Asian populations identified the rs7612463 in UBE2E2 and rs972283 in KLF14 as a T2D susceptibility loci, respectively [16, 17]. After that, a number of replication studies concerning the association between this variants and T2D have been conducted in different ethnic populations. However, the results from different studies were inconsistent. The inconsistency may be due to chance, insufficient power of limited sample size, ethnic diversity or phenotypic heterogeneity. Meta-analysis is a powerful tool for summarizing the results from different studies to estimate the major effect with enhanced precision. To the best of our knowledge, the present meta-analysis is the first largest study reported to date on the association of these two gene polymorphisms and T2D in Asian populations.

For the association between UBE2E2 rs7612463 and T2D, the results of the present meta-analysis revealed that the C allele of rs7612463 in UBE2E2 was significantly associated with the susceptibility of T2D in Asian population (OR =1.153, P<0.0001). The result was inconsistent with the other studies in Europeans [16, 32]. Interestingly, the large scale GWAS meta-analysis which did not detect any association between rs7612463 variant and T2D in Europeans, but instead they identified that rs1496653 in UBE2E2 was significantly associated with T2D in European population [32], suggested that the functional biology of the population-specific causal variants may differ among different ethnic groups. The overall PAR for the risk allele C of rs7612463 in UBE2E2 was 11.2%.

UBE2E2 gene, located on the short (P) arm of chromosome 3 at position p24.2 [52], it was reported to be expressed in human pancreas, liver, muscle and adipose tissue, as well as in a cultured insulin-secreting cell line [6, 53], and encodes ubiquitin-conjugating enzyme E2 E2 (UBE2E2), which plays an important role in the synthesis, secretion and signaling of insulin in pancreatic β -cells [54, 55], and has been linked with various insulin-related diseases other than T2D, including obesity and atherosclerosis [56,57], gestational diabetes mellitus (GDM) [58], and the risk of non-small cell lung cancer, which has been proposed as being one of the biomarkers of lung adenocarcinoma metastasis [59].

Recent studies have reported that the risk allele C of rs7612463 in UBE2E2 was significantly associated with lower level of homeostasis in β -cells (HOMA- β) in Japanese population, and suggested that this variant may have a role in the reduction of insulin secretion in T2D patients [16].

Assessed by fasting-based homeostasis model, recently studies indicated that rs7612463 was not associated with HOMA- β or HOMA-IR [19, 23]. However, when further applying OGTT-derived indexes, the risk allele C of rs7612463 in UBE2E2 was found to be significantly associated with insulin release indices in Han Chinese population, suggested that the UBE2E2 rs7612463 may mediate its diabetogenic impact on insulin response, which highly depends on the impairment of β -cell function [23].

For the association between KLF14 rs972283 and T2D, the results of the present meta-analysis revealed that the G allele of rs972283 in KLF14 was nominally associated with the susceptibility of T2D in Asian population, OR of 1.07 (95%CI 1.004 - 1.133, P=0.036), with similar effect size to that seen in European populations "1.07 (1.05–1.10)" [17] and Japanese "1.07 (1.01–1.14)". The result of the present meta-analysis was consistent with the study by Adeyemo et al. [39] in Africans (OR=0.76, P=0.035), and study by Ohshige et al. [29] in Japanese samples as the association becomes nominal (not remained significant) when he was combined it's study (2,839

cases and 2,125 controls) data with the data from previously performed Japanese GWAS (4,470 cases and 3,071 controls) OR of 1.07 (95%CI 1.01–1.14, P=0.017) [29].

Similarly, the opposite allele, A, of this SNP was nominally associated with T2D in non-Kashmiri sample of Pakistan population, OR of 1.162 (95%CI 1.02–1.32, P=0.0222) [24], also with the same direction of effect sizes as that seen for G risk allele in the present study, European populations and Kashmiri simple of Pakistan population "1.14 (1.03–1.27)" [28]. Suggested that the association of opposite allele of the same SNP in European and Pakistani populations indicates that yet to be identified functional variant exist in the KLF14 genes [24]. However, the significant association of the KLF14 rs972283, G allele with T2D was previously reported in European population, P=4.4x10⁻¹⁰ [17] and South Asians "Kashmiri simple of Pakistan population, P=9.66x 10⁻⁰³" [28]. Suggested that the nominal P value in present study may have been related to the sample size, and so a more precise result will need to be obtained in the future by using a larger sample. The overall PAR for the risk allele G of rs972283 of KLF14 was 4.64%.

Many studies reported, no significant association between KLF14 rs972283 variant and T2D, in Indo-European population [20], Lebanese population [26], Henan Han Chinese [31, 36, 38], African Americans [33], Saudi Arabian [34], Qatari population [40], Palestinian population [41]. This may be due to the small sample size in the individual studies, corresponding low statistical power to detect this association, as this locus was identified through a meta-analysis of GWAS, including large study populations.

The Kruppel-like factor 14 (KLF14) is located on the long (q) arm of chromosome 7 at position q32.3, and belong to the large family of zinc-finger transcription factors (KLF family) which a group of 17 members of the transcription factors that regulate a diverse array of cellular processes, including cellular proliferation, differentiation, development and apoptosis in normal or in pathological situations [60, 61].

A biological study has demonstrated that KLF14 could participate in the metabolism as a transcriptional activator via regulating the gene networks involved in lipid metabolism [61]. KLF14 is a master trans-regulator of multiple genes that are associated with metabolic phenotypes in adipose tissue (e.g., LDL-c, HDLc, TG and BMI) [30], It was previously reported that the KLF14 gene may play a central regulator of lipid and lipoprotein metabolism [62], and it was recently referred to as a "conductor of the metabolic syndrome orchestra" [63], so that it is thought to be candidates for conferring susceptibility to T2D and metabolic diseases. The results of large-scale GWAS indicated that KLF14 may play a role in the pathogenesis of insulin resistance and T2D. Yang et al. (2015) found reduced KLF14 mRNA and protein in both muscle and adipose tissue of T2D patients [64]. Reduced KLF14 expression was accompanied of low adiponectin expression, a known insulin sensitizer, thus increased insulin resistance and the KLF14 overexpression in Hepa1-6 cells partly prevented the inhibition of glucose uptake induced by high glucose and high insulin [64]. In addition, the risk allele G of rs972283 in KLF14 was significantly associated with obesity (BMI, WHR), TC, HOMA-IR, HOMA-β, blood pressure and Triglycerides among T2D patients in Chinese population [31, 35], metabolic disease in Japanese population [30].

Furthermore, this G allele was significantly associated with reduced insulin sensitivity and it appears to have a primary effect on insulin action, which is not driven by obesity [17], indicating that the primary effect on diabetes-risk is mediated by decreased peripheral insulin sensitivity.

The present meta-analysis limited to Asian populations, albeit it is still revealed a weak between-study heterogeneity for rs972283 polymorphism (P=0.165, I²=30.5%). The source of between-study heterogeneity may be due to: (1) Ethnicity difference. Studies were conducted in different geographical regions and ethnic, and the factors that play a leading role across populations may be different. (2) Difference in the sample size. Some are thousands in a large sample size, and some only a few hundreds or hundred. (3) Differences in sample selection (age, gender). For example, mean age of control groups is younger than that of case groups in the

two Japanese studies of Ohshige et al. [29], the mean age of case groups are younger than that of control groups the other two studies of Mastouri [26] and Ohshige et al. [29], also the mean age of control group is much younger than that of the other control groups in the study of Ohshige et al. [29] (Table 1). (4) Hardy-Weinberg equilibrium is the principal law in population genetic studies. Generally, meeting Hardy-Weinberg equilibrium suggests that samples have representation. The genotypic distributions of these SNPs were in Hardy-Weinberg equilibrium in both T2D patients and control groups in all selected studies for our meta-analysis. Sometimes Hardy-Weinberg equilibrium was met, but the genotype frequency was not always consistent to that of the local population.

V. CONCLUSION

To our knowledge, the present study is the first meta-analysis to evaluate the association of the rs7612463 in UBE2E2 and rs972283 in KLF14 with T2D in Asian population. Our meta-analysis demonstrated that the rs7612463 in the UBE2E2 gene was significantly associated with the susceptibility of T2D in the Asian population. However, the association of rs972283 in the KLF14 gene was found to be nominally significant in the present study population, which may be related to the relatively sample size. Additional studies with larger sample sizes are needed for more precise result with the determination of the molecular mechanisms of these variants to confirm this association.

Author contributions

Mustafa Abdo Saif Dehwa designed the study, analysed the data, prepared the manuscript and revised critical data.;

Shuang Zhang and Emad Najeep Ali searched the literature

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