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Meta-analysis of the effect of *HHEX* gene polymorphism on the risk of type 2 diabetes

Yu Cai†, Jiayong Yi[1,†], Yushui Ma and Da Fu*

Department of Gastroenterology and [1]Department of Orthopaedics, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai 200032, People's Republic of China,
†Both the authors contributed equally to this work.

*To whom correspondence should be addressed. Department of Gastroenterology, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai 200032, People's Republic of China. Tel: +86 21 64041990; Fax: +86 21 54230545; Email: superdafu@hotmail.com

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In the past decade, a number of case–control studies have been carried out to investigate the relationship between the *HHEX* polymorphism and type 2 diabetes (T2D). However, the results have been inconclusive. To investigate this inconsistency, we performed a meta-analysis of all available studies dealing with the relationship between the *HHEX* polymorphism and T2D. In total, 22 association studies on two *HHEX* polymorphisms (rs1111875 and rs7923837) and risk of T2D published before April 2010, including a total of 36 695 T2D cases and 51 800 controls were included. We also explored potential sources of heterogeneity. In a combined analysis, the summary per-allele odds ratio (OR) for T2D of the rs1111875 and rs7923837 polymorphism was 1.17 [95% confidence interval (CI): 1.13–1.21] and 1.23 (95% CI: 1.18–1.28), respectively. The haplotype analysis also showed significant association in the pooled international populations with an OR of 1.19 (95% CI: 1.15–1.22). In the subgroup analysis by ethnicity, significantly increased risks were found in Asians and Caucasians for these polymorphisms in almost all genetic models. Subgroup analysis also showed that ethnicity is the main source of heterogeneity between pooled studies. This meta-analysis demonstrated that the risk allele of *HHEX* polymorphisms (rs1111875 and rs7923837) is a risk factor for developing T2D. However, additional very large-scale studies are warranted to provide conclusive evidence on the effects of the *HHEX* gene on risk of T2D.

Introduction

Type 2 diabetes (T2D) is a complex metabolic disease characterised by hyperglycemia, insulin resistance, impaired insulin secretion due to pancreatic β-cell defects and increased hepatic glucose production. It has become a global major health problem showing worldwide increasing prevalence but the underlying molecular mechanisms involved in the development of T2D remain poorly understood (1,2).

Besides the important contribution of environmental factors, including changes in dietary patterns and physical activity levels, genetic components are obviously associated with the development of T2D. Over the past decade, many efforts have been put into the search for T2D risk genes, but to identify genetic variants that explain the excess risk associated with a family history of diabetes remains a challenge. From a long list of candidate genes, only three variants have been consistently associated with T2D: *TCF7L2*, *KCNJ11* and *PPARG* (3–5). However, a number of novel genetic variants (*CDKAL1*, *IGF2BP2*, *FTO*, *HHEX*, *SLC30A8* and *WFS1*) (6–10) were shown to increase the risk of T2D susceptibility in reproducible studies.

Hematopoietically expressed homeobox (*HHEX*) gene encodes a transcription factor that is involved in Wnt signalling and is required for early development of ventral pancreas and liver (11,12). In addition, for polymorphisms within *HHEX* gene region, no phenotype except T2D and possibly impaired function of β-cell has been demonstrated (13,14). Besides impaired insulin secretion, decreased hepatic insulin degradation and insulin resistance are early and important mechanisms in T2D pathogenesis (15). Hence, it was believed to be a candidate risk gene for T2D. *HHEX* gene locus on chromosome 10q23.33 and several mutations and common single-nucleotide polymorphisms (SNPs) within or flanking the gene have been identified. Two common variants (rs1111875 and rs7923837) located near the *HHEX* gene were studied widely for their association with T2D susceptibility.

To date, many case–control studies have been carried out to investigate the role of the *HHEX* gene polymorphism in the development of T2D. However, these studies have yielded conflicting or inconclusive result. Published studies have generally been restricted in terms of sample size and ethnic diversity, and individual studies may have insufficient power to reach a comprehensive and reliable conclusion. Therefore, we performed a meta-analysis of the published studies to clarify this inconsistency and obtain summary risk estimates for the association of specific polymorphism in *HHEX* and risk of T2D.

Materials and methods

Literature search

The literature included in our analysis was selected from PubMed, EMBASE and Chinese National Knowledge Infrastructure with keywords relating to the relevant genes (e.g. 'Hematopoietically expressed homeobox gene' or '*HHEX*') in combination with words related to T2D (e.g. 'Type 2 diabetes' or 'Type 2 diabetes mellitus') and 'polymorphism'. Genetic association studies published before April 2010 on T2D and polymorphisms in the *HHEX* gene described above were retrieved, and their references were checked to identify other relevant publications. All relevant reports identified were included without language restriction.

For inclusion, studies had to meet all of the following criteria: (i) original papers containing independent data, (ii) case–control or cohort studies, (iii) identification of T2D was confirmed pathologically and (iv) sufficient data to calculate the odds ratio (OR) with a confidence interval (CI) and *P*-value. The major reasons for exclusion of studies were (i) overlapping data, (ii) case-only studies and (iii) review papers.

Data extraction

The following information was independently extracted from each report by two participants in the meta-analysis: the first author, publication year, study

Table I. Characteristics of the studies included in the meta-analysis

First author	Year	Ethnicity	Country	Genotyping method	No. of cases	No. of controls	Control source
Tan *et al.* (24)	2010	Singaporean	Singapore	MassARRAY	1015	2145	PB
Hu *et al.* (25)	2009	Chinese	China	MassARRAY	1828	1757	PB
Pivovarova *et al.* (26)	2009	German	Germany	TaqMan	221	470	PB
Tabara *et al.* (27)	2009	Japanese	Japan	TaqMan	490	396	PB
Lyssenko *et al.* (28)	2008	Finn	Sweden	TaqMan	2063	10 064	PB
Rong *et al.* (29)	2008	Indian	USA	SNPlex	1374	1748	PB
Lee *et al.* (30)	2008	Korean	South Korea	TaqMan	856	501	HB
Wu *et al.* (31)	2008	Chinese	China	GenomeLab SNPstream	413	1850	PB
Sanghera *et al.* (32)	2008	Indian	USA	TaqMan	514	367	PB
Horikawa *et al.* (33)	2008	Japanese	Japan	TaqMan	1848	1563	PB
Ng *et al.* (34)	2008	Chinese, Korean	China	MassARRAY; TaqMan	3140	3678	PB
Lewis *et al.* (35)	2008	American	USA	MassARRAY	982	1040	PB
Hertel *et al.* (36)	2008	Norwegian	Norway	MassARRAY	1618	1845	PB
Ezzidi *et al.* (37)	2008	Tunisian	Tunisia	TaqMan	795	504	PB
Takeuchi *et al.* (38)	2008	Japanese	Japan	Infinium Infinium; MassARRAY	7225	7952	PB
van Vliet-Ostaptchouk *et al.* (39)	2008	Dutch	Netherlands	TaqMan	490	908	PB
Furukawa *et al.* (40)	2008	Japanese	Japan	TaqMan	405	340	HB
Horikoshi *et al.* (41)	2007	Japanese	Japan	ABI Big Dye	860	860	HB
Zeggini *et al.* (42)	2007	Scot	UK	Affymetrix; TaqMan	3597	5033	PB
Schulze *et al.* (43)	2007	German	Germany	TaqMan	686	2267	PB
Scott *et al.* (9)	2007	Finn	USA	Illumina Infinium; MassARRAY	2325	2360	PB
Sladek *et al.* (10)	2007	French	Canada	Illumina Infinium; MassARRAY	3950	4152	PB

HB, hospital based; PB, population based.

Table II. Meta-analysis of the *HHEX* polymorphism on T2D risk

Polymorphism	Overall association		Sub-group analysis by ethnicity							
			Asian		Caucasian		Indian		African American	
	OR (95% CI)	Test for heterogeneity P, I^2	OR (95% CI)	Test for heterogeneity P, I^2	OR (95% CI)	Test for heterogeneity P, I^2	OR (95% CI)	Test for heterogeneity P, I^2	OR (95% CI)	Test for heterogeneity P, I^2
					rs1111875					
C allele	1.17 (1.13–1.21)	$P = 0.0001$, $I^2 = 55\%$	1.20 (1.16–1.24)	$P = 0.14$, $I^2 = 29\%$	1.16 (1.10–1.23)	$P = 0.0003$, $I^2 = 67\%$	1.06 (0.97–1.16)	$P = 0.89$, $I^2 = 0\%$	1.05 (0.90–1.21)	P = NA, I^2 = NA
Heterozygous	1.16 (1.12–1.20)	$P = 0.10$, $I^2 = 26\%$	1.19 (1.14–1.24)	$P = 0.30$ $I^2 = 14\%$	1.13 (1.06–1.20)	$P = 0.12$, $I^2 = 32\%$	1.06 (0.92–1.21)	$P = 0.61$, $I^2 = 0\%$	0.84 (0.56–1.27)	P = NA, I^2 = NA
Homozygous	1.36 (1.27–1.46)	$P = 0.0005$, $I^2 = 51\%$	1.46 (1.35–1.57)	$P = 0.24$ $I^2 = 19\%$	1.33 (1.19–1.48)	$P = 0.001$, $I^2 = 63\%$	1.14 (0.94–1.37)	$P = 0.99$, $I^2 = 0\%$	0.94 (0.63–1.41)	P = NA, I^2 = NA
					rs7923837					
G allele	1.23 (1.18–1.28)	$P = 0.11$, $I^2 = 34\%$	1.26 (1.19–1.33)	$P = 0.25$ $I^2 = 24\%$	1.22 (1.15–1.29)	$P = 0.09$, $I^2 = 50\%$	—	—	1.19 (0.94–1.50)	P = NA, I^2 = NA
Heterozygous	1.17 (1.02–1.34)	$P < 0.00001$, $I^2 = 79\%$	1.28 (1.18–1.37)	$P = 0.36$ $I^2 = 8\%$	0.98 (0.79–1.22)	$P = 0.006$, $I^2 = 72\%$	—	—	1.90 (0.57–6.32)	P = NA, I^2 = NA
Homozygous	1.70 (1.25–2.32)	$P < 0.00001$, $I^2 = 80\%$	1.56 (1.33–1.83)	$P = 0.43$ $I^2 = 0\%$	4.08 (1.32–12.57)	$P < 0.00001$, $I^2 = 94\%$	—	—	2.17 (0.67–7.09)	P = NA, I^2 = NA

NA, not available.

design, ethnicity of the study population, definition and numbers of cases and controls, source of control, genotyping method, number of genotypes in cases and controls. The results were compared and any disagreement was discussed and resolved with consensus. Where essential information was not presented in articles, every effort was made to contact the authors.

Statistical analysis

Deviation from Hardy-Weinberg equilibrium (HWE) was examined by Chi-square tests. If controls of studies were found not to be in HWE, sensitivity analyses were performed with and without these studies to test the robustness of the findings. OR with 95% CIs was used to assess the strength of association between the *HHEX* gene polymorphism and T2D risk. The per-allele OR of the risk allele (C of rs1111875, G of rs7923837) was compared between cases and controls. Then, we estimated the risks of the heterozygous and homozygote genotypes on T2D compared with the wild-type homozygote.

Heterogeneity was assessed with the Cochrane Q test and I^2 test, which describes the proportion of variation in the log ORs that is attributable to genuine differences across studies rather than to random error (16). If heterogeneity existed, the random effects model (the DerSimonian and Laird method) (17), which yields wider confidence intervals, was adopted to calculate the overall OR value. Otherwise, the fixed effects model (the Mantel–Haenszel method) was used (18). In addition, sources of heterogeneity were investigated by stratified meta-analyses based on ethnicity (Asian, Caucasian, Indian and African American). The 95% CIs were constructed using Woolf's method (19). The significance of the overall OR was determined by the Z-test.

Sensitivity analyses were performed to assess the stability of the results, namely, a single study in the meta-analysis was deleted each time to reflect the influence of the individual data set to the overall OR. Publication bias was assessed using Egger's test (20) and Begg's funnel plots (21). Sample size required for 80% power (nominal $a = 0.05$) was calculated with the pooled OR estimate from different ethnicity, assuming an equal number of cases and controls, allele frequency in controls (according to different ethnicity). All P values are two-sided at the $P = 0.05$ level. All the statistical analyses were carried out using the Review Manager software package (version 5.0; The Cochrane Collaboration, Oxford, UK) and SAS (version 9.1; SAS Institute, Cary, NC, USA).

The linkage disequilibrium (LD) structure of a 100-kb region on chromosome 10q23.33 including the human *HHEX* gene was constructed

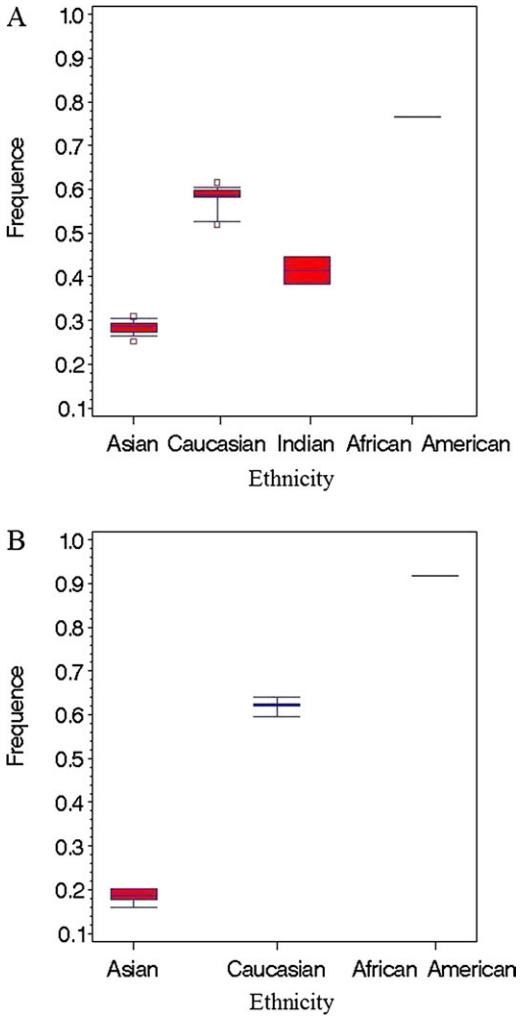

Fig. 1. Frequencies of the risk alleles of *HHEX* among controls stratified by ethnicity. (**A**) rs1111875. (**B**) rs7923837. The '□' represent outlier.

using 29 SNPs. Genotype data were downloaded from the International HapMap Project website (www.hapmap.org) for 30 Centre d'Etude du Polymorphisme Humain (CEPH) trios. The multiallelic *D'* was computed by performing a series of pairwise *D'* calculations using each haplotype in turn as an allele with all other haplotypes at the locus serving as the other allele. This was then repeated for each haplotype at each locus and averaged by haplotype frequency (22). Maximum likelihood haplotype blocks were calculated using expectation maximization algorithm (23).

Results

Characteristics of studies

The combined search yielded 54 references. Thirty-two articles were excluded because they clearly did not meet the criteria or overlapping references. Finally, a total of 22 case–control studies were retrieved based on the search criteria for T2D susceptibility related to the *HHEX* polymorphisms. The main study characteristics were summarised in Table I. There are 21 studies with 35 883 T2D cases and 53 359 controls concerning rs1111875 and 12 studies with 13 398 T2D cases and 17 396 controls concerning rs7923837.

Meta-analysis results

As shown in Table II and Figures 2 and 3, significant associations between the two *HHEX* polymorphisms and T2D susceptibility were found.

HHEX rs1111875. There was a wide variation in the C allele frequency of the rs1111875 polymorphism among the controls across different ethnicities, ranging from 0.25 to 0.76 (Figure 1A). For Asian controls, the C allele frequency was 0.28 (95% CI: 0.27–0.29), which was lower than that in Caucasian controls (0.58; 95% CI: 0.56–0.59), Indian controls (∼0.39) and African American controls (∼0.76).

Overall, significantly increased T2D risks were found for C versus T (OR = 1.17; 95% CI: 1.13–1.21; Figure 2), CT versus TT (OR = 1.16; 95% CI: 1.12–1.20) and CC versus TT (OR = 1.36; 95% CI: 1.27–1.46). In addition, stratification by ethnicity indicated that the rs1111875 was significantly associated with T2D for Asians and Caucasian in all genetic models. However, no such association was detected in Indian or African American. Sample size required for 80% power to detect risk allele is ∼3300 and 2700 for Asian and Caucasian, respectively.

HHEX rs7923837. The G allele frequency in the three major ethnicities was 0.18 (95% CI: 0.16–0.20) for Asians, 0.62 (95% CI: 0.60–0.64) for Caucasians and ∼0.92 for African American (Figure 1B), indicating a significant difference among Asians as compared with Caucasians ($P < 0.00001$).

In the overall analysis, the risk allele of rs7923837 was significantly associated with elevated T2D (Figure 3). Significant associations were also found for heterozygous (OR = 1.17; 95% CI: 1.02–1.34) and homozygous (OR = 1.70; 95% CI: 1.25–2.32) when compared with wild genotype. In the stratified analysis by ethnicity, significantly increased risks were also found among Asian and Caucasian populations in all genetic models except for heterozygote comparison in Caucasian. However, these similar significant associations were still not observed for African American. Sample size required for 80% power to detect risk allele is ∼2400 and 1700 for Asian and Caucasian, respectively.

Haplotype analysis. Association between T2D and rs1111875 and rs7923837 polymorphism is supported by the LD analyses. Overall, the studies showed a significant *P*-value of $<10^{-5}$ with an overall OR = 1.19 (95% CI: 1.15–1.22), but significant heterogeneity was found between studies ($P < 10^{-4}$, $I^2 = 54\%$). Using the block definition that defines 'strong LD' if the one-sided upper 95% confidence bound on $D' > 0.98$ (22), we find that there are two regions of strong LD in which the rs1111875 and rs7923837 polymorphism of *HHEX* gene are in the same block.

Sensitivity analyses and Publication bias

All studies indicated that the frequency distributions of genotypes in the controls were consistent with HWE. In addition, the results of both allelic and genotypic analysis were consistent and were not changed substantially by the removal of any data set, suggesting that the results of this meta-analysis are stable (data not shown).

Begg's funnel plot and Egger's test were performed to evaluate the publication bias of literatures. The shape of the funnel plots seemed symmetrical for both polymorphisms, suggesting no publication bias among the studies included. The statistical results still did not show publication bias ($P > 0.05$, for all).

Discussion

Large sample and unbiased epidemiological studies of predisposition genes polymorphisms could provide insight into the

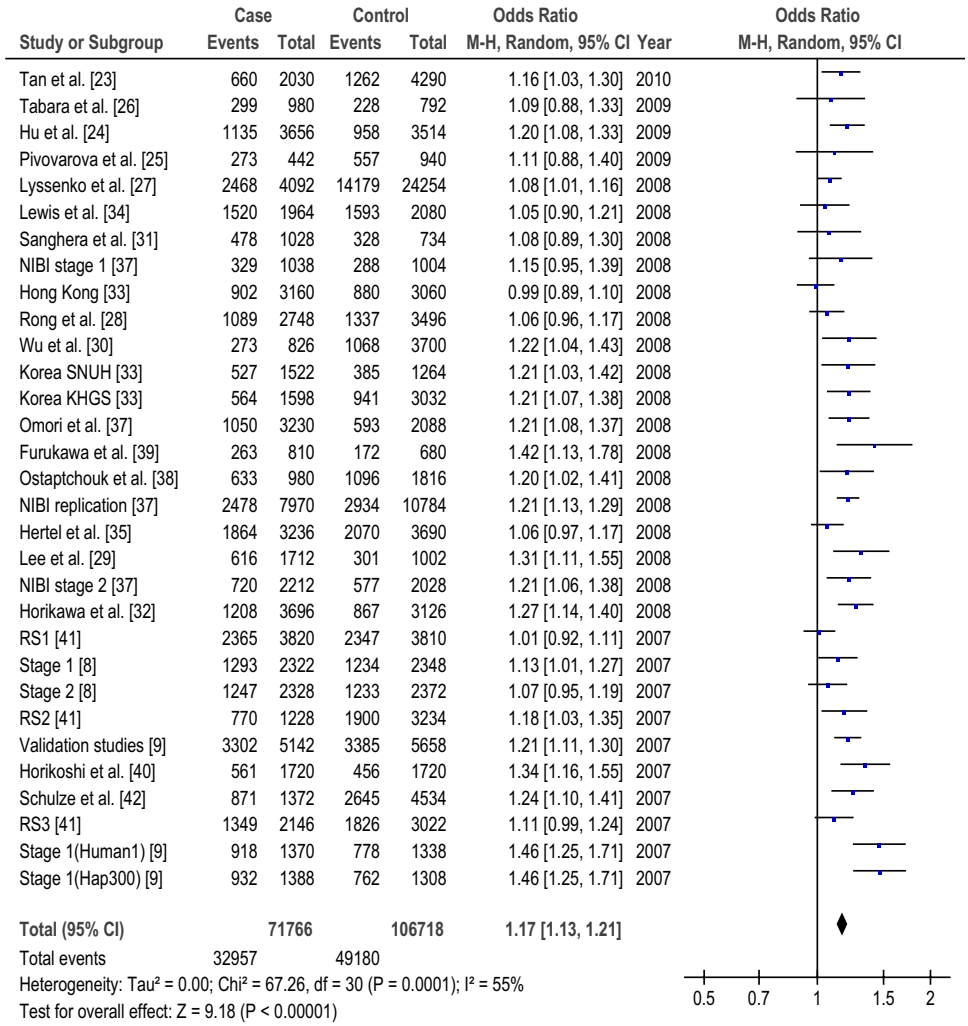

Fig. 2. Forest plot from the meta-analysis of T2D risk and *HHEX* rs1111875 polymorphism (C versus T).

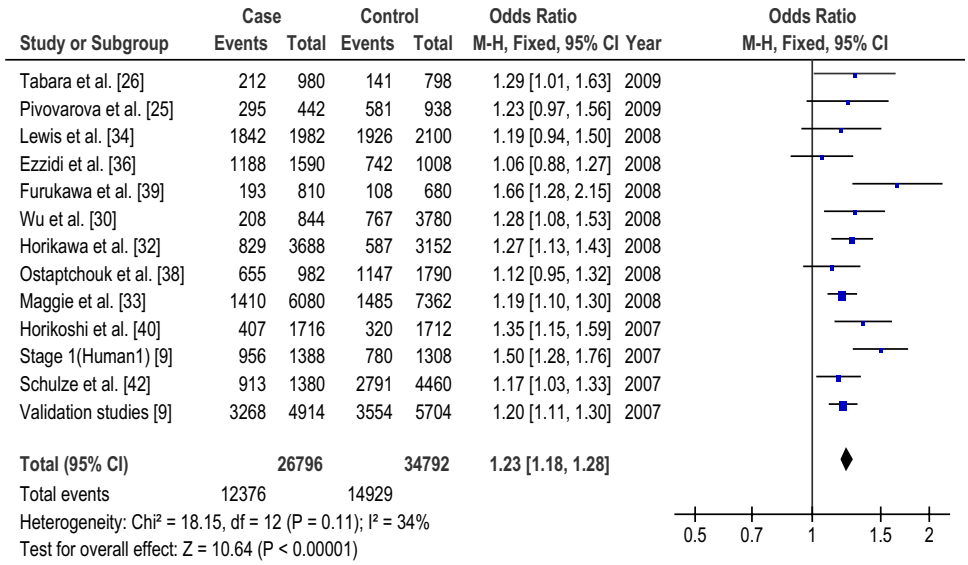

Fig. 3. Forest plot from the meta-analysis of T2D risk and *HHEX* rs7923837 polymorphism (G versus A).

in vivo relationship between candidate genes and complex diseases. This is the first meta-analysis, which comprise a total of 36 678 T2D cases and 53 863 controls from 22 case–control studies, examining the association of two commonly studied polymorphisms of *HHEX* (rs1111875 and rs7923837) with T2D risk. Our results indicated that the risk allele of *HHEX* polymorphisms (rs1111875 and rs7923837) is a risk factor for developing T2D.

In meta-analysis, heterogeneity evaluation was always conducted. Thus, subgroup meta-analyses were performed according to ethnicity. In racial subgroups, no statistically significant association between *HHEX* polymorphisms and T2D appeared in Indian or African American. Such result could be due to limited number of studies, which had insufficient statistical power to detect a slight effect or may have generated a fluctuated risk estimate. While significant associations were observed both in Asians and Caucasians in almost all genetic models. In addition, subgroup analyses show that ethnicity is the main source of heterogeneity.

The haplotype block structure of the *HHEX* gene region in CEPH samples shows that the 100-kb region can be described by two blocks of strong LD, where the rs1111875 and rs7923837 polymorphism localised in a strong LD region. The structure was consistent with the current results of meta-analysis.

Previously published studies give evidence of different alteration in insulin secretion by variants of *HHEX* gene including decreased acute insulin response after tolbutamide challenge or oral glucose tolerance test (OGTT) and decreased insulin secretion after intravenous glucose challenge or OGTT (13,14,44). Recently, Pivovarova *et al.* (26) found that the risk allele of rs1111875 and rs7923837 within the *HHEX* gene were associated with reduced β-cell secretion capacity, which was measured as the first and second phase of insulin response in the OGTT. Detailed mechanisms of the influence of *HHEX* variants on the insulin secretion remain a matter of speculation. However, Tanaka *et al.* (45) demonstrated that *HHEX* may regulate β-cell development and/or function through the activation of hepatocyte nuclear factor 1α. Research on *HHEX*-knockout mouse showed alterations in the embryonic organogenesis of the ventral pancreas (11). Based on these research, one plausible hypothesis is that the association with risk allele is mediated through decreased β-cell secretory capacity or decreased β-cell mass. Recently presented evidence showed that the common risk alleles in *HHEX* gene were associated with reduced birth weight through a predominant effect of foetal genotype and this result supports our hypothesis (46).

Although it has been known for decades that both type 2 diabetes and obesity have a genetic basis (47), remarkable few susceptibility genes with robust and reproducible effects have been identified for these diseases. Unfortunately, almost all the studies included in current meta-analysis did not explore the interaction between *HHEX* genotype and obesity. The mechanism by which it relates to obesity and T2D in humans is still unclear.

Several limitations of this meta-analysis should be addressed. Firstly, the subgroup meta-analyses dealing with interactions between the *HHEX* genotype and Indian or African American population are based on the small number of studies where such information is available. As studies among the Indians and Africans are currently limited, further studies including a wider spectrum of subjects should be carried to investigate the role of these variants in different populations. Secondly, our results were based on unadjusted estimates, while a more precise analysis should be conducted if all individual raw data were available, which would allow for the adjustment by other co-variants including age, drinking status, obesity, cigarette consumption and other lifestyle.

Despite these limitations, this meta-analysis suggests that *HHEX* polymorphisms may increase the risk of T2D for Asians and Caucasians but no significant effect for Indian or African American population. For future association studies, strict selection of patients, much larger sample size will be required. More studies should also be carried out to examine the impact of *HHEX* on T2D risk, especially in Indian and African American. Moreover, gene–gene and gene–environment interactions should also be considered in future studies.

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