Introduction

Type 2 diabetes mellitus (T2DM) is one of the main chronic diseases and its complications have become a major cause of morbidity, mortality, and disability in the World. It has been estimated that the number of people with type 2 diabetes will double to at least 350 million worldwide by 2030 unless appropriate action is taken [1]. However, up to date, the mechanism of the disease is still not fully understood. In recent years, published data showed that genetic polymorphisms might explain individual differences in T2DM risk [2,3]. Several candidate genes are implicated in the pathogenesis of T2DM, one of which is interleukin (IL)-10.

Exel and colleagues discovered that low IL-10 production capacity is associated with the metabolic syndrome and T2DM [4]. The capacity for IL-10 production in individuals has been shown to be correlated with genetic composition of the IL-10 locus [5]. Thus, examination of genetic polymorphisms of IL-10 may explain individual differences in T2DM risk. Several molecular epidemiological studies were conducted in recent years to evaluate the risk of T2DM associated with the polymorphisms of IL-10 [5–14]. However, the results remain conflicting rather than conclusive. This meta-analysis aimed to summarize the current evidence from case-control studies that evaluated this association.

Methods: We carried out a search in Medline, EMBASE, and the Chinese National Knowledge Infrastructure (CNKI) database for relevant studies. Data were extracted using a standardized form and pooled odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to assess the strength of the association.

Results: Ten studies were included in our meta-analysis and systemic review. Our meta-analysis indicated that IL-10 −1082A/G polymorphism was associated with the risk of T2DM (GA vs. AA: OR = 1.21, 95% CI = 1.03–1.41; GA/GG vs. AA: OR = 1.22, 95% CI = 1.05–1.41), whereas there was no association between IL-10 −592C/A (CC/CA vs. AA: OR = 1.07, 95% CI = 0.59–1.93) or −819C/T (CC/CT vs. TT: OR = 0.93, 95% CI = 0.49–1.75) polymorphism and T2DM risk was found in our study.

Conclusions: This meta-analysis provides strong evidence that IL-10 −1082A/G polymorphism associated with risk of T2DM. However, no association of the IL-10 −592C/A or −819C/T polymorphism with T2DM risk was found. Additional well-designed large studies were required for the validation of our results.

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published data for estimating an odds ratio (OR) with 95% confidence interval (CI). Major reasons for exclusion of studies were (a) no control group; (b) duplicate of previous publication.

Data Extraction

Two investigators reviewed the articles independently to exclude irrelevant and overlapping studies. The results were compared, and disagreements were resolved by discussion and consensus. From each study, the following information was extracted: first author’s surname, year of publication, ethnic descent of the study population (European, Asian and African), definition of case, age, characteristics of controls, numbers of eligible cases and controls, and genotype distributions in cases and controls.

Statistical Analysis

The Hardy-Weinberg equilibrium (HWE) was utilized to compare the observed genotype frequencies with expected genotype frequencies in controls for all studies. OR and 95% CI were used to assess the strength of association between IL-10 polymorphisms and the risk of T2DM under homozygote comparison, heterozygote comparison, dominant and recessive genetic model comparison. The significance of the combined OR was determined by the Z-test, in which \( P < 0.05 \) was considered significant. Stratified analyses were performed by ethnicity, age and sources of control. The \( \chi^2 \)-based Q statistical test was used for the assessment of the between-study heterogeneity, which was considered significant for \( P < 0.1 \) [15]. In analyses, if the heterogeneity was low then we used a fixed-effect model, or else applied the random-effect model. Sensitivity analyses were also performed to test the stability of the results [16]. Funnel plots and Egger’s linear regression test were used to provide diagnosis of the potential publication bias [17]. All analyses were performed with Stata (Version10.0, Stata Corporation) and Review Manager (version 5.0.16, The Cochrane Collaboration) using two sided \( P \) values.

Results

Characteristics of Studies

The search initially identified 421 potentially eligible articles. Of these, the first screening excluded 404 citations based on abstracts or titles, leaving 17 articles for full-text review. The excluded 7 articles had no relative outcomes, no control group, and duplicate of previous publication. We finally included 10 studies in our systematic review and meta-analysis [5–14]. The detailed steps of our literature search are shown in Figure 1.

The characteristics of 10 included studies are summarized in Table 1. There are eight case-control studies concerning \(-592C\!\!/A\) polymorphism [5–12], four case-control studies concerning \(-819C\!\!/T\) polymorphism [5–8], and six case-control studies concerning \(-1082A\!\!/G\) polymorphism [6–9,13,14]. Controls were selected from healthy population in all the studies and most studies used frequency-matched controls to the cases by age, sex, residence, or ethnicity. The genotype distributions among the controls of all studies were in agreement with HWE except for three studies for the \(-592C\!\!/A\) [8,10,12], one study for the \(-819C\!\!/T\) [8], and two for the \(-1082A\!\!/G\) [8,13].

Quantitative Synthesis

**IL-10 -592C/A.** The evaluation of association between IL-10 polymorphisms and T2DM risk was presented in Table 2. Overall, no significant association was found between IL-10 polymorphisms and the risk of T2DM under homozygote comparison, heterozygote comparison, dominant and recessive genetic model comparison. The significance of the combined OR was determined by the Z-test, in which \( P < 0.05 \) was considered significant. Stratified analyses were performed by ethnicity, age and sources of control. The \( \chi^2 \)-based Q statistical test was used for the assessment of the between-study heterogeneity, which was considered significant for \( P < 0.1 \) [15]. In analyses, if the heterogeneity was low then we used a fixed-effect model, or else applied the random-effect model. Sensitivity analyses were also performed to test the stability of the results [16]. Funnel plots and Egger’s linear regression test were used to provide diagnosis of the potential publication bias [17]. All analyses were performed with Stata (Version10.0, Stata Corporation) and Review Manager (version 5.0.16, The Cochrane Collaboration) using two sided \( P \) values.

**Table 1.** Study characteristics of included studies in this meta-analysis.

| Author         | Year | Country | Ethnicity | Source of controls | SNPs studied | Sample size | HWE     |
|----------------|------|---------|-----------|-------------------|-------------|------------|---------|
| Chang [5]      | 2005 | China   | Asian     | Healthy Control   | –592C/A, –819C/T | 370/175    | 0.86, 0.87 |
| Ezzidi [6]     | 2009 | Tunisia | African   | Healthy Control   | –592C/A, –819C/T, –1082G/A | 917/748   | 0.41, 0.41, 0.14 |
| Tsiavou [7]    | 2004 | Greece  | European  | Healthy Control   | –592C/A, –819C/T, –1082G/A | 31/39     | 0.38, 0.38, 0.82 |
| Kung [8]       | 2010 | China   | Asian     | Healthy Control   | –592C/A, –819C/T, –1082G/A | 47/25     | <0.01, <0.01, <0.01 |
| Scarpelli [9]  | 2006 | Italy   | European  | Healthy Control   | –592C/A, –1082G/A | 551/1131  | 0.21, 0.68 |
| Saxena [10]    | 2012 | India   | Asian     | Healthy Control   | –592C/A      | 406/168   | 0.01    |
| Arababadi [11] | 2012 | Iran    | Asian     | Healthy Control   | –592C/A      | 200/100   | 0.32    |
| Wang [12]      | 2010 | China   | Asian     | Healthy Control   | –592C/A      | 224/275   | 0.04    |
| Kolla [13]     | 2009 | India   | Asian     | Healthy Control   | –1082G/A     | 198/202   | <0.01   |
| Erdogan [14]   | 2012 | Turkey  | Asian     | Healthy Control   | –1082G/A     | 91/112    | 0.68    |

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subgroup analysis by ethnicity and obtained statistically similar results in European descendents and Asian descendents. However, a statistically significant association of IL-10 -592C/A polymorphism and T2DM risk were found in African descendents (CC/CA vs. AA: OR = 0.58, 95% CI = 0.41–0.82), IL-10 -819C/T. There was no statistically significant differences between IL-10 -819C/T polymorphism and T2DM risk (GG/CT vs. TT: OR = 0.93, 95% CI = 0.49–1.75; Figure 2B). In the stratified analyses for the -819C/T polymorphism, there was a significantly increased risk was observed among African

**Figure 2.** Meta-analysis with a random-effect model for the ORs of type 2 diabetes mellitus risk associated with interleukin-10 polymorphisms in dominant genetic model comparison. (A: -592C/A; B: -819C/T; C: -1082A/G). doi:10.1371/journal.pone.0066568.g002

### Table A

| Study or Subgroup | Patient Events | Patient Total | Control Events | Control Total | Weight | M-H. Random. 95% CI | Odds Ratio M-H. Random. 95% CI |
|-------------------|----------------|---------------|----------------|---------------|--------|---------------------|-------------------------------|
| Arababadi 2012    | 190            | 200           | 77             | 100           | 13.9%  | 5.68 [2.58, 12.48]  |
| Chang 2005        | 200            | 353           | 62             | 134           | 17.0%  | 1.52 [1.02, 2.26]   |
| Ezzidi 2009       | 820            | 917           | 701            | 748           | 17.2%  | 0.57 [0.39, 0.81]   |
| Kung 2010         | 40             | 47            | 25             | 25            | 3.4%   | 0.11 [0.01, 1.93]   |
| Saxena 2012       | 390            | 406           | 163            | 168           | 11.9%  | 0.75 [0.27, 2.07]   |
| Scarpelli 2006    | 527            | 551           | 1064           | 1131          | 16.4%  | 1.38 [0.86, 2.23]   |
| Tsaiou 2004       | 30             | 31            | 37             | 39            | 4.4%   | 1.62 [0.14, 18.76]  |
| Wang 2010         | 188            | 224           | 251            | 275           | 15.9%  | 0.50 [0.23, 0.87]   |
| **Total (95% CI)**| **2729**       | **2620**      | **100.0%**     |               |        | **1.07 [0.59, 1.93]** |

Total events: 2385

Heterogeneity: $\text{Tau}^2 = 0.49; \text{Chi}^2 = 42.89, df = 7 (P < 0.00001); I^2 = 84%$

Test for overall effect: $Z = 0.23 (P = 0.82)$

### Table B

| Study or Subgroup | Patient Events | Patient Total | Control Events | Control Total | Weight | M-H. Random. 95% CI | Odds Ratio M-H. Random. 95% CI |
|-------------------|----------------|---------------|----------------|---------------|--------|---------------------|-------------------------------|
| Chang 2005        | 200            | 370           | 84             | 175           | 48.7%  | 1.27 [0.89, 1.83]   |
| Ezzidi 2009       | 855            | 917           | 716            | 746           | 45.3%  | 0.62 [0.40, 0.98]   |
| Kung 2010         | 47             | 47            | 25             | 25            | 3%     | Not estimable       |
| Tsaiou 2004       | 30             | 31            | 37             | 39            | 6.0%   | 1.62 [0.14, 18.76]  |
| **Total (95% CI)**| **1365**       | **987**       | **100.0%**     |               |        | **0.93 [0.49, 1.75]** |

Total events: 1132

Heterogeneity: $\text{Tau}^2 = 0.18; \text{Chi}^2 = 6.49, df = 2 (P = 0.04); I^2 = 69%$

Test for overall effect: $Z = 0.22 (P = 0.82)$

### Table C

| Study or Subgroup | Patient Events | Patient Total | Control Events | Control Total | Weight | M-H. Fixed. 95% CI | Odds Ratio M-H. Fixed. 95% CI |
|-------------------|----------------|---------------|----------------|---------------|--------|---------------------|-------------------------------|
| Erdogan 2012      | 69             | 91            | 68             | 112           | 4.6%   | 2.03 [1.10, 3.74]   |
| Ezzidi 2009       | 796            | 917           | 642            | 748           | 29.4%  | 1.09 [0.82, 1.44]   |
| Kolla 2009        | 74             | 198           | 54             | 202           | 10.5%  | 1.64 [1.07, 2.50]   |
| Kung 2010         | 45             | 47            | 25             | 25            | 0.5%   | 0.38 [0.02, 7.72]   |
| Scarpelli 2006    | 332            | 551           | 646            | 1131          | 52.9%  | 1.14 [0.93, 1.40]   |
| Tsaiou 2004       | 21             | 31            | 22             | 39            | 2.0%   | 1.62 [0.61, 4.34]   |
| **Total (95% CI)**| **1835**       | **2257**      | **100.0%**     |               |        | **1.22 [1.05, 1.42]** |

Total events: 1337

Heterogeneity: $\text{Chi}^2 = 6.52, df = 5 (P = 0.26); I^2 = 23%$

Test for overall effect: $Z = 2.65 (P = 0.008)$
Table 2. Total and stratified analyses of the interleukin-10 polymorphisms on type 2 diabetes mellitus risk.

| Variables | Cases/ | Homozygote OR(95%CI) | Heterozygote OR(95%CI) | Dominant Model OR(95%CI) | Recessive Model OR(95%CI) |
|-----------|--------|----------------------|------------------------|--------------------------|---------------------------|
| -592C/A   | 8      | 1.17[0.54,2.55]      | 1.05[0.66,1.68]        | 1.07[0.59,1.93]          | 1.13[0.79,1.61]           |
| European  | 2      | 1.36[0.86,2.13]      | 1.38[0.87,2.18]        | 1.37[0.88,2.12]          | 1.02[0.84,1.25]           |
| Asian     | 5      | 1.27[0.32,2.50]      | 1.08[0.52,2.23]        | 1.11[0.44,2.80]          | 1.39[0.65,2.96]           |
| African   | 1      | 0.53[0.37,0.75]      | NA                     | NA                       | NA                        |
| -819C/T   | 4      | 0.99[0.37,2.69]      | 1.02[0.77,1.36]        | 0.93[0.49,1.75]          | 0.93[0.49,1.78]           |
| European  | 1      | 1.72[0.17,17.75]     | NA                     | NA                       | NA                        |
| Asian     | 2      | 1.63[0.87,3.03]      | NA                     | NA                       | NA                        |
| African   | 1      | 0.54[0.35,0.82]      | NA                     | NA                       | NA                        |
| -1082A/G  | 6      | 1.37[0.84,2.25]      | 1.21[1.03,1.14]        | 1.22[1.05,1.41]          | 1.25[0.76,2.03]           |
| European  | 2      | 1.22[0.88,1.69]      | 1.14[0.92,1.41]        | 1.15[0.94,1.41]          | 1.14[0.84,1.55]           |
| Asian     | 3      | 0.56[0.01,29.63]     | 1.60[0.81,3.17]        | 1.69[1.21,2.38]          | 0.38[0.00,47.72]          |
| African   | 1      | 1.03[0.76,1.39]      | NA                     | NA                       | NA                        |

a number of studies; b P value of Q-test for heterogeneity test; c Random-effects model was used when P value for heterogeneity test <0.10; otherwise, fixed-effects model was used; 0.00 means value <0.01; NA not applicable.

Publication Bias
We used Funnel plot and Egger’s regression asymmetry test to access the publication bias of literatures. The data suggested that there was no evidence of publication bias in dominant genetic model comparison (t = 0.74, P = 0.486 for -592C/A; t = 0.80, P = 0.507 for -819C/T; t = 1.49, P = 0.209 for -1082A/G; Figure 3).

Discussion
In the present study, a meta-analysis was performed to examine the association between three IL-10 polymorphisms and T2DM risk, by critically reviewing 8 studies on IL-10 -592C/A polymorphism (2,729 patients and 2,620 controls), 4 studies on IL-10 -819C/T polymorphism (1,365 patients and 987 controls), and 6 studies on IL-10 -1082A/G polymorphism (1,935 patients and 2,257 controls). To the best of our knowledge, this is the first comprehensive meta-analysis to date investigating the association between IL-10 -819C/T and -1082A/G polymorphisms and T2DM risk. In addition, more studies were included in our study than a recently published meta-analysis concerning -592C/A polymorphisms and T2DM risk [10].

The findings from our study indicated that IL-10 -1082A/G polymorphism associated with risk of T2DM. However, no association of the IL-10 -592C/A or -819C/T polymorphism with T2DM risk was found. There was evidence of heterogeneity between studies in our analyses. We then assessed the source of heterogeneity for homozygote comparison by ethnicity and sample size. As a result, for -592C/A and -819C/T, ethnicity (x^2 = 12.32, P = 0.002; x^2 = 0.46, P = 0.01; respectively), but not sample size (x^2 = 1.10, P = 0.29; x^2 = 0.73, P = 0.39; respectively), was found to contribute to substantial heterogeneity. However, neither ethnicity (x^2 = 1.77, P = 0.41) nor sample size(x^2 = 2.34, P = 0.13) was found to contribute to substantial heterogeneity for -1082A/G.

Sensitivity Analysis
Although the distribution of genotypes in the controls in some studies did not follow HWE, the corresponding pooled OR and between-study heterogeneity were not significant altered without these studies (CC vs. AA: OR = 1.37, 95% CI = 0.66–5.30, Phetogeneity <0.01 for -592C/A; CC vs. CT/TT: OR = 1.00, 95% CI = 0.51–1.96, Phetogeneity = 0.02 for -819C/T). However, sensitivity analysis indicated the studies by Kung et al. and Kolla et al. were the main origin of heterogeneity for -1082A/G. The heterogeneity significantly decreased when excluding the two studies (Phetogeneity = 0.13), while the value of pooled OR was not significantly altered without the two studies (GG vs. AA OR = 1.11, 95% CI = 0.74–1.65).
language studies were included in this meta-analysis might have contributed to this ethnic difference. However, there are only one study included in the analysis of African descendents with limited sample sizes, the result should be interpreted with caution, therefore, more studies based on larger population should be conducted to further examine those associations.

There are some limitations of this meta-analysis should be acknowledged. Firstly, detailed information, such as environmental exposures and gene-gene interactions, were unavailable in most studies, which limited our further assessment of those confounding factors at the patient level, and were incorporated into the analysis. Secondly, some studies with small sample size appear to overestimate the true association due to lack of sufficient power to detect such an association. Thirdly, only English and Chinese language studies were included in this meta-analysis might have led to bias.

IL-10 Polymorphisms and T2DM Risk

Even though, our meta-analysis has some advantages. Firstly, the search and selection studies were conducted very strictly, which significantly increased statistic power of this meta-analysis. Secondly, all the studies included in this meta-analysis were case-control researches and contained available genotype frequency, which met our including criterion well. Third, controls in all the included studies were healthy population, which avoided occurring population stratification bias.

In conclusion, our meta-analyses suggested that IL-10 -592C/A or -819C/T polymorphism had no association with T2DM risk in all examined patients, whereas there was an association between IL-10 -1082A/G polymorphism and risk of T2DM. However, additional large studies are warranted to validate our findings. Future studies should include multi-ethnic groups and use standardized unbiased genotyping methods, different grades of asthma patients, and well-matched controls.

Author Contributions

Conceived and designed the experiments: YH JS. Performed the experiments: YH JS YX XY. Analyzed the data: YH JS YS. Contributed reagents/materials/analysis tools: YH JS YX XY. Wrote the paper: YH JS. Study supervision: YS YX XY.

References

1. Screening for Type 2 Diabetes: Report of a World Health Organization and International Diabetes Federation meeting. Available: http://www.who.int/diabetes/publications/en/screening_mmc03.pdf. Accessed 2002 May 9–11.
2. Biswas D, Vetrivelvi V, Choudhury J, Joshiimalar R (2011) Adiponectin gene polymorphism and its association with type 2 diabetes mellitus. Indian J Clin Biochem 26: 172–177.
3. Perez-Luque E, Malacara JM, Garay-Sevilla ME, Fajardo ME (2012) Association of the TNF-α -308G/A polymorphism with family history of type 2 diabetes mellitus in a Mexican population. Clin Biochem 45: 12–15.
4. van Exel E, Gussekloo J, de Craen AJ, Frolich M, Bootsma-Van Der Wiel A, et al. (2002) Low production capacity of interleukin-10 associates with the metabolic syndrome and type 2 diabetes: the Leiden 85-Plus Study. Diabetes 51: 1080–1089.
5. Chang YH, Huang GN, Wu CY, Shiao MY (2005) Association of interleukin-10 -592C/T and -819C/T polymorphisms with type 2 diabetes mellitus. Hum Immunol 66: 1238–1246.
6. Ezzedi I, Mtrraoui N, Kacem M, Mallat SG, Mohamed MB, et al. (2009) Interleukin-10-592C/A, -819C/T and -1082A/G promoter variants affect the susceptibility to nephropathy in Tunisian type 2 diabetes (T2DM) patients. Clin Endocrinol (Oxf) 70: 401–407.
7. Tsivron A, Hazitazitaki E, Chatzifragkou A, Manginas A, Koniziotou K, et al. (2004) TNF-alpha, TGF-beta1, IL-10, IL-6, gene polymorphisms in late autoimmune diabetes of adults (LADA) and type 2 diabetes mellitus. J Clin Immunol 24: 591–599.
8. Kung WJ, Lin CC, Liu SH, Chaung HC (2010) Association of interleukin-10 polymorphisms with cytokines in type 2 diabetic nephropathy. Diabetes Technol Ther 12: 809–813.
9. Scarpelli D, Cardellini M, Andreozzi F, Laratta E, Hirbal ML, et al. (2006) Variants of the interleukin-10 promoter gene are associated with obesity and insulin resistance but not type 2 diabetes in caucasian italian subjects. Diabetes 55: 1529–1533.
10. Saxena M, Agrawal CG, Bid HK, Banerjee M (2012) An Interleukin-10 Gene Promoter Polymorphism (-592A/C) Associated with Type 2 Diabetes: A Nor. In Indian Study. Biochem Genet In Press.