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

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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 parallel 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

<table>
<thead>
<tr>
<th>First author</th>
<th>Year</th>
<th>Ethnicity</th>
<th>Country</th>
<th>Genotyping method</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Control source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tan et al. (24)</td>
<td>2010</td>
<td>Singaporean</td>
<td>Singapore</td>
<td>MassARRAY</td>
<td>310</td>
<td>3678</td>
<td>PB</td>
</tr>
<tr>
<td>Hu et al. (25)</td>
<td>2009</td>
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<td>China</td>
<td>MassARRAY</td>
<td>1828</td>
<td>1757</td>
<td>PB</td>
</tr>
<tr>
<td>Pivovarova et al. (26)</td>
<td>2009</td>
<td>German</td>
<td>Germany</td>
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<td>221</td>
<td>470</td>
<td>PB</td>
</tr>
<tr>
<td>Tabara et al. (27)</td>
<td>2009</td>
<td>Japanese</td>
<td>Japan</td>
<td>TaqMan</td>
<td>490</td>
<td>396</td>
<td>PB</td>
</tr>
<tr>
<td>Lysenkov et al. (28)</td>
<td>2008</td>
<td>Finnish</td>
<td>Sweden</td>
<td>TaqMan</td>
<td>2063</td>
<td>10 064</td>
<td>PB</td>
</tr>
<tr>
<td>Rong et al. (29)</td>
<td>2008</td>
<td>Indian</td>
<td>USA</td>
<td>SNPlex</td>
<td>1374</td>
<td>1748</td>
<td>PB</td>
</tr>
<tr>
<td>Lee et al. (30)</td>
<td>2008</td>
<td>Korean</td>
<td>South Korea</td>
<td>TaqMan</td>
<td>856</td>
<td>501</td>
<td>HB</td>
</tr>
<tr>
<td>Wu et al. (31)</td>
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<td>Chinese</td>
<td>China</td>
<td>GenomeLab SNPstream</td>
<td>413</td>
<td>1850</td>
<td>PB</td>
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<td>Indian</td>
<td>USA</td>
<td>TaqMan</td>
<td>514</td>
<td>367</td>
<td>PB</td>
</tr>
<tr>
<td>Horikawa et al. (33)</td>
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<td>Japanese</td>
<td>Japan</td>
<td>TaqMan</td>
<td>1848</td>
<td>1563</td>
<td>PB</td>
</tr>
<tr>
<td>Ng et al. (34)</td>
<td>2008</td>
<td>Chinese, Korean</td>
<td>China</td>
<td>MassARRAY; TaqMan</td>
<td>3140</td>
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<td>PB</td>
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<tr>
<td>Lewis et al. (35)</td>
<td>2008</td>
<td>American</td>
<td>USA</td>
<td>MassARRAY</td>
<td>982</td>
<td>1040</td>
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<tr>
<td>Hertel et al. (36)</td>
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<td>Norwegian</td>
<td>Norway</td>
<td>MassARRAY</td>
<td>1618</td>
<td>1845</td>
<td>PB</td>
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<td>Ezzidi et al. (37)</td>
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<td>Tunisian</td>
<td>Tunisia</td>
<td>TaqMan</td>
<td>795</td>
<td>504</td>
<td>PB</td>
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<tr>
<td>Takeuchi et al. (38)</td>
<td>2008</td>
<td>Japanese</td>
<td>Japan</td>
<td>Infinium; MassARRAY</td>
<td>7225</td>
<td>7952</td>
<td>PB</td>
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<tr>
<td>van Vliet-Oostapchouk et al. (39)</td>
<td>2008</td>
<td>Dutch</td>
<td>Netherlands</td>
<td>TaqMan</td>
<td>490</td>
<td>908</td>
<td>PB</td>
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<td>Funukawa et al. (40)</td>
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<td>Japanese</td>
<td>Japan</td>
<td>TaqMan</td>
<td>405</td>
<td>340</td>
<td>HB</td>
</tr>
<tr>
<td>Horikoshi et al. (41)</td>
<td>2007</td>
<td>Japanese</td>
<td>Japan</td>
<td>ABI Big Dye</td>
<td>860</td>
<td>860</td>
<td>HB</td>
</tr>
<tr>
<td>Zeggini et al. (42)</td>
<td>2007</td>
<td>Scottish</td>
<td>UK</td>
<td>Affymetrix; TaqMan</td>
<td>3597</td>
<td>503</td>
<td>PB</td>
</tr>
<tr>
<td>Schulze et al. (43)</td>
<td>2007</td>
<td>German</td>
<td>Germany</td>
<td>TaqMan</td>
<td>686</td>
<td>2267</td>
<td>PB</td>
</tr>
<tr>
<td>Scott et al. (44)</td>
<td>2007</td>
<td>Finnish</td>
<td>USA</td>
<td>Illumina Infinium; MassARRAY</td>
<td>2325</td>
<td>2360</td>
<td>PB</td>
</tr>
<tr>
<td>Sladek et al. (10)</td>
<td>2007</td>
<td>French</td>
<td>Canada</td>
<td>Illumina Infinium; MassARRAY</td>
<td>3950</td>
<td>4152</td>
<td>PB</td>
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</tbody>
</table>

HB, hospital based; PB, population based.

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

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Overall association</th>
<th>Sub-group analysis by ethnicity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>Asian</td>
</tr>
<tr>
<td></td>
<td>Test for heterogeneity $P, I^2$</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>C allele</td>
<td>1.17</td>
<td>(1.13–1.21)</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>1.16</td>
<td>(1.12–1.20)</td>
</tr>
<tr>
<td>Homozygous</td>
<td>1.36</td>
<td>(1.27–1.46)</td>
</tr>
<tr>
<td>G allele</td>
<td>1.23</td>
<td>(1.18–1.28)</td>
</tr>
<tr>
<td>Heterozygous</td>
<td>1.17</td>
<td>(1.02–1.34)</td>
</tr>
<tr>
<td>Homozygous</td>
<td>1.70</td>
<td>(1.25–2.32)</td>
</tr>
</tbody>
</table>

NA, not available.
**Results**

**Characteristics of studies**

The combined search yielded 54 references. Thirty-two articles were excluded because they clearly did not meet the criteria for overlapping references. Finally, a total of 22 case–control studies concerning rs7923837 and 13 398 T2D cases and 17 396 controls concerning rs1111875 were excluded because they clearly did not meet the criteria or sample size required for 80% power to detect risk allele is ~3300 and 2700 for Asian and Caucasian, respectively.

**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, I² = 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 \textit{HHEX} rs1111875 polymorphism (C versus T).

Fig. 3. Forest plot from the meta-analysis of T2D risk and \textit{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 to 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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References


