Common Variants of Hepatocyte Nuclear Factor 1B are Associated with Type 2 Diabetes in a Chinese Population

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ABSTRACT

Objective: Hepatocyte nuclear factor 1β (HNF1B) is a transcription factor that is critical for pancreatic cell formation and glucose homeostasis. Previous studies have reported that common variants of HNF1B were associated with type 2 diabetes in Caucasians and West Africans. However, analysis in the subjects from the Botnia study and Malmö Preventive Project produced a conflicting result and the role for HNF1B in type 2 diabetes susceptibility has remained unclear. We therefore investigated common variants across the HNF1B gene in a Chinese population.

Research Design and Methods: Fifteen tagging SNPs were analyzed for association with type 2 diabetes in subjects with type 2 diabetes (n = 1,859) and normal glucose regulation (n = 1,785).

Results: Consistent with the initial study, we observed evidence that the risk G allele of rs4430796 in intron 2 was significantly associated with type 2 diabetes (odds ratio [OR] 1.16 [95 CI% 1.05 - 1.29], P = 0.0035, empirical P = 0.0475). Furthermore, the at-risk G allele was associated with earlier age at diagnosis in the type 2 diabetic subjects (P = 0.0228).

Conclusions: The result of this study provides evidence that variants in the HNF1B region contribute to susceptibility to type 2 diabetes in the Chinese population.
Epithocyte nuclear factor 1B (HNF1B, also known as TCF2) is a homeodomain-containing transcription factor, that functions as a homodimer or heterodimer with its homologous partner HNF1A [1]. HNF1B is widely expressed in a number of tissues where it takes a vital role in embryonic development, pancreatic cell formation, and involved in the β-cell transcription factor network [2, 3]. Heterozygous mutation of HNF1B gene was identified in an autosomal dominant, early-onset, subtype of diabetes known as maturity-onset diabetes of the young type 5 (MODY5) in white, Japanese and Chinese populations [4-6]. It is well recognized that common variants of genes that cause monogenic forms of diabetes may also have a role in common type 2 diabetes susceptibility, such as P12A polymorphism in the PPARG, E23K polymorphism in the KCNJ11 and common variants in the promoter region of HNF4A [7-9]. Recently, single nucleotide polymorphisms (SNPs) on HNF1B were identified to be associated with type 2 diabetes in Finland, Sweden, and Canada populations [10, 11]. The association signals, which were separated by recombination hotspots, located on intron 2 (rs757210) and intron 6~8 (rs1008284 and rs3110641). Moreover, a genome-wide association study on prostate cancer identified that the G allele of rs7501939 and A allele of rs4430796 (both in intron 1~2 region) were associated with increased risk of prostate cancer in Caucasians and reduced risk of type 2 diabetes in Caucasian, African but not Hong Kong Chinese [12]. However, Holmkvist’s study produced a conflicting result that common variants in HNF1B did not predict future risk of type 2 diabetes in two prospective studies [13]. Thus, extensive study especially in other ethnic group will improve our understanding of the role of HNF1B in type 2 diabetes predisposition. We therefore selected tagging SNPs spanning the HNF1B gene and performed a case-control study in a Chinese Han population.

**Research Design and Methods**

**Subjects.** All subjects were eastern Chinese Han ancestry, residing in Shanghai. All case subjects were from 2001-2005 Shanghai Diabetes Institute inpatient database and diagnosed with type 2 diabetes by the 1999 WHO criteria [14]. Type 1 diabetes as well as mitochondrial diabetes were excluded [15]. The control subjects were selected from the Shanghai Diabetes Studies, a community-based epidemiological survey for diabetes [16]. The inclusion criteria for the control subjects were: 1) over 40 years old; 2) with normal glucose regulation confirmed by a standard 75 g oral glucose tolerance test (OGTT) (fasting plasma glucose <6.1 mmol/l and 2-h plasma glucose < 7.8 mmol/l); 3) with negative family history of diabetes by a standard questionnaire. The study groups finally comprised 1,859 subjects with type 2 diabetes and 1,785 subjects with normal glucose regulation. The phenotypic characteristics of the study group are described in Table 1.

This study was approved by the institutional review board of Shanghai Jiao Tong University Affiliated Sixth People’s Hospital. Written informed consent was obtained from each participant.

**Clinical measurements.** Anthropometric parameters such as height, weight, waist and hip circumference and blood pressure were measured as previously described [16, 17]. For the case subjects, fasting and postprandial plasma glucose and serum insulin were measured. For the control subjects, blood samples were obtained at 0 and 120 min during the OGTT and plasma glucose as well as serum insulin levels were examined. Insulin resistance and pancreatic β-cell function were assessed by homeostasis model assessment (HOMA) [18].

**Genotyping.** We initially selected 20 SNPs
spanning the HNF1B region including tagging SNPs selected from HapMap Phase II Chinese Han database that capturing all the common variants (minor allele frequency over 0.1) from 3kb upstream to 1kb downstream of the gene based on an $r^2$ of $\geq 0.7$ and SNPs previously reported to be associated with type 2 diabetes. SNPs were genotyped by matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectroscopy (MassARRAY Compact Analyzer, Sequenom, San Diego, CA, USA). Genotyping data underwent a series of quality control checks and cleaned data were used in the further association analyses. Fifteen SNPs had call rate $\geq 95\%$ while 5 SNP were excluded because of low genotyping call rate (rs1016990, rs7407025, rs1008284, rs3110640 and rs11263755). SNPs with Hardy-Weinberg equilibrium test $P$ values $< 0.05$ in the cases or controls were excluded. No SNP was excluded due to inconsistent with Hardy-Weinberg expectations. The average reproducibility rate among 1500 duplicate genotype pairs were 100%. The overall call rate for the remaining 15 SNPs was 99.1%.

**Statistical methods.** Hardy-Weinberg equilibrium test was performed in case and control groups separately for each variant using $\chi^2$ test. Pairwise linkage disequilibrium (LD) was computed from the combined data of cases and controls by calculating $|D'|$ and $r^2$ in Haplovew (v 4.1) [19]. Sliding windows consisting of two or three adjacent SNPs were generated for haplotype analyses using PLINK (v 0.99) [20]. Allele, genotype and haplotype frequencies for cases and controls were compared using the $\chi^2$ test or Fisher’s exact test. Odds ratio (OR) with 95% confidence interval (95%CI) were presented. Correction of multiple testing was performed in Haplovew (v 4.1) through 10,000 permutations. Empirical $P$ value of logistic regression analysis was calculated through 500 permutations which generated simulation data by randomly shuffling case and control status. In the control group, continuous diabetes-related traits were analyzed and quantitative traits were transformed to reduce skewness. All statistical analyses were performed by SAS (version 8.0; SAS Institute Inc., Cary, NC, USA) unless specified otherwise. A two-tailed $P$ value $< 0.05$ was considered significant.

**RESULTS**

We examined 15 tagging SNPs spanning the HNF1B region. Figure 1 showed the pairwise linkage disequilibrium and haplotype block structure among these SNPs. The association of single-SNP with type 2 diabetes risk was shown in table 1. Six of the 15 SNPs including three previously reported SNPs had a nominal $P < 0.05$. Three previously reported SNPs (rs4430796, rs757210 in intron 2 and rs3110641 in intron 8) were significantly associated with type 2 diabetes susceptibility (OR = 1.13 - 1.16, $P = 0.0035 - 0.0178$). Three additional SNPs (two SNPs in intron 8 and one SNP in the 5' end of the gene region) were also showed nominal significance (OR = 1.15 - 1.25, $P = 0.0046 - 0.0178$). Logistic regression analysis of type 2 diabetes with these six SNPs showed that G allele of rs4430796 and A allele of rs11656817 tended to confer independent risks of type 2 diabetes (adjusted OR 1.135 and 1.211, $P = 0.0162$ and 0.0203, respectively). However, after adjustment for multiple testing by permutation, only the risk G allele of rs4430796 (in intron 2) showed significantly associated increased risk of type 2 diabetes (OR =1.16, 95CI% 1.05 - 1.29, $P = 0.0035$, empirical $P = 0.0475$). Logistic regression analysis adjusting for age, sex, and BMI also indicated that the association with SNP rs4430796 statistically significant.
(OR = 1.18, 95% CI 1.06 - 1.31, \( P = 0.0025 \), empirical \( P = 0.034 \)). Sliding windows haplotype analysis found windows containing rs4430796 showed strongest association to type 2 diabetes which merely confirmed the finding by single SNP association analysis (Figure S1).

As rs4430796 exhibited strong association with diabetes in our population, further analyses focused on this SNP for diabetes-related traits. We first treated age at diagnosis as a quantitative trait and analyzed its association with rs4430796 in the type 2 diabetic subjects. There was evidence that the G allele of rs4430796 was associated with younger age at diagnosis (52.40 ± 11.18 years of age for G/G, 53.94 ± 11.85 years of age for G/A, and 54.61 ± 11.97 years of age for A/A, \( P = 0.0228 \)). When treating age at diagnosis as a dichotomous trait to stratify the type 2 diabetic patients and compared the allele frequencies of rs4430796 with control subjects, we also found that the association signal was slightly stronger in the early-onset (age of diagnosis < 45 years) subgroups (OR = 1.27, 95% CI 1.07 - 1.49, \( P = 0.0051 \), Table 3).

In the subjects with normal glucose regulation, this SNP was not associated with plasma glucose levels, adiposity-related anthropometrics such as BMI, waist circumference and waist to hip ratio, lipid profile, insulin sensitivity and insulin secretion (supplementary Table S1).

**DISCUSSION**

We replicated previous finding that a SNP (rs4430796) in intron 2 of \( HNF1B \) was significantly associated with type 2 diabetes in our Chinese population. Our results are consistent with the deCODE study [12], and the effect size of the at-risk allele G for rs4430796 in our Chinese subjects (OR = 1.16) is quite similar to the deCODE findings (for the G allele, overall OR = 1.10). Notably the allele frequency for the SNP rs4430796 is quite different between different ethnic groups. The at-risk allele G for rs4430796 had a frequency of 0.25-0.30 in the Chinese population, but a frequency of 0.47-0.54 and 0.70-0.73 in Caucasians and West Africans, respectively [11, 12]. Although the minor allele G identified in the Chinese and Caucasian populations is the major allele in the West Africans, this allele showed the same direction of its effect for type 2 diabetes. In the deCODE study, the rs4430796 was not significantly associated with type 2 diabetes in the Hong Kong Chinese, although the effect sizes between the Shanghai and Hong Kong samples were similar (for the risk allele G, OR = 1.16 and 1.12, respectively) and in the same direction. One potential explanation of the discrepancy between the current findings and deCODE results in Hong Kong population is the difference in the control ascertainment. The Shanghai control subjects were over 40 years of age and measured both fasting plasma glucose and 2-h plasma glucose. In contrast to our study, the Hong Kong control subjects only tested fasting plasma glucose. In addition, more than one third these controls were adolescents. All these may raise the possibility of misclassification in the Hong Kong control samples and consequently may underestimate the effect size.

In Winckler’s study, they observed that other SNPs in intron 2 (rs757210) and intron 8 (rs3110641) were associated with type 2 diabetes in a large Caucasian sample (combined OR = 1.12 and 1.10, \( P = 5 \times 10^{-6} \) and 0.0006, respectively) [11]. SNP rs757210 is correlated with rs4430796 (\(|D'| = 0.87 \) and \( r^2 = 0.71 \) in our population). In the present study, although none of the two SNPs reached significance after correcting for multiple testing, these two SNPs showed similar trend in the same direction in our samples. In Holmkvist’s study, the three previously reported SNPs (including rs1008284,
rs3110641 and rs757210) did not predict future risk. However, our study suggests that the strongest effect for type 2 diabetes susceptibility was at rs4430796, and further study to follow up and replicate the association is still necessary.

Our study is the first to demonstrate that the at-risk G allele for rs4430796 was also associated with an earlier onset of type 2 diabetes. Interestingly, previous studies provide evidence that common variants in the MODY1 gene (HNF4A) also associated with type 2 diabetes and age at diagnosis [21, 22]. Nevertheless, rs4430796 was in the intron region of HNF1B with no obvious functional effect reported, thus we could not exclude that other, as yet unknown or untyped, SNPs in LD with rs4430796 may be the causal variant(s).

There are several limitations of this study. First, we noticed that although the age of diagnosis of diabetes was determined by physician-documented medical record, we cannot exclude the possibility that the case subjects had an earlier age of onset. Secondly, the SNPs we successfully genotyped can only capture 60% of common SNPs in HNF1B gene region under the threshold of \( r^2 \geq 0.7 \) based on Phase 3 HapMap Chinese data. We may inevitably miss some common variants contribute to diabetes in this region.

In summary, our data replicated the previous finding by Gudmundsson et al [12] and support the notion that genetic variation in the HNF1B was associated with type 2 diabetes. Further studies in other populations as well as more SNPs at this gene will be required to further elucidate the role of HNF1B variation in the pathogenesis of type 2 diabetes.

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Table 1. Clinical characteristics of the study subjects

|                        | Cases (n = 1,859) | Controls (n = 1,785) |
|------------------------|-------------------|----------------------|
| Sex (M/F)              | 973/886           | 739/1,046            |
| Ages (years)           | 61.16±12.63       | 57.37±12.33          |
| Body mass index (kg/m²)| 24.08±3.53        | 23.63±4.23           |
| Fasting plasma glucose (mmol/l) | 13.04±5.19     | 4.99±0.50            |
| Age at diagnosis (years) | 54.10±11.85      | ---                  |
Table 2. Allele frequencies and association results for SNPs in the HNF1B gene

| SNP      | Position | HNF1B region | Major/minor allele | Risk allele | Risk allele frequency | OR (95%CI)   | $P_{SNP}$ | $P_{gene}$ | Adjusted $P_{SNP}^*$ |
|----------|----------|--------------|--------------------|-------------|-----------------------|--------------|----------|------------|----------------------|
| rs17626423 | -3492    | 5'           | T, C               | T           | 0.886                 | 0.885        | 1.01     | 0.8804     | 1                    | 0.4552 |
| rs3760511  | -1438    | 5'           | C, A               | A           | 0.368                 | 0.336        | 1.15     | 0.0046     | 0.0624               | 0.0117 |
| rs4430796  | 6836     | intron2      | A, G               | G           | 0.312                 | 0.280        | 1.16     | 0.0035     | 0.0475               | 0.0025 |
| rs757210   | 8361     | intron2      | G, A               | A           | 0.289                 | 0.264        | 1.13     | 0.0178     | 0.2129               | 0.6318 |
| rs3744763  | 13991    | intron4      | C, T               | C           | 0.557                 | 0.545        | 1.05     | 0.3065     | 0.9874               | 0.5032 |
| rs3786127  | 17002    | intron4      | C, G               | C           | 0.837                 | 0.834        | 1.02     | 0.7708     | 1                    | 0.7296 |
| rs2107131  | 18187    | intron4      | C, T               | T           | 0.375                 | 0.364        | 1.05     | 0.3403     | 0.9934               | 0.3711 |
| rs12450628 | 22455    | intron4      | C, T               | C           | 0.651                 | 0.646        | 1.02     | 0.6989     | 1                    | 0.8704 |
| rs11649743 | 29897    | intron4      | G, A               | A           | 0.328                 | 0.323        | 1.02     | 0.6373     | 1                    | 0.8719 |
| rs2158254  | 39381    | intron5      | G, A               | A           | 0.266                 | 0.257        | 1.05     | 0.4075     | 0.9981               | 0.2062 |
| rs2189303  | 44771    | intron7      | C, T               | T           | 0.397                 | 0.390        | 1.03     | 0.5362     | 1                    | 0.9497 |
| rs11656817 | 47977    | intron8      | A, G               | A           | 0.916                 | 0.898        | 1.25     | 0.0067     | 0.0873               | 0.0210 |
| rs11868513 | 52184    | intron8      | G, A               | G           | 0.721                 | 0.719        | 1.01     | 0.8353     | 1                    | 0.8193 |
| rs1859211  | 53504    | intron8      | A, G               | A           | 0.911                 | 0.894        | 1.21     | 0.0178     | 0.2138               | 0.0344 |
| rs3110641  | 57459    | intron8      | C, T               | C           | 0.770                 | 0.745        | 1.14     | 0.0139     | 0.1742               | 0.0307 |

* Age, gender and BMI were adjusted.
Table 3. Allelic association analysis of rs4430796 among type 2 diabetes and controls stratified according to age at diagnosis of type 2 diabetes

|                           | Allele frequencies | OR (95%CI)     | P vs. control subjects |
|---------------------------|--------------------|----------------|------------------------|
|                           | A      | G      |                    |                       |
| Type 2 diabetic patients  | 0.688  | 0.312  | 1.16 (1.05-1.29)   | 0.0035                |
| Early-onset (n = 395)     | 0.670  | 0.330  | 1.27 (1.07-1.49)   | 0.0051                |
| Late-onset (n = 1,461)    | 0.693  | 0.307  | 1.14 (1.02-1.26)   | 0.0211                |
| Control subjects (n = 1,781) | 0.720  | 0.280  | 1                   | ---                   |

Early-onset type 2 diabetes age of diagnosis < 45 years; late-onset type 2 diabetes age of diagnosis ≥ 45 years
Figure 1. LD patterns of the 15 typed SNPs in the Chinese population.
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B