Effect of smoking on the association of HHEX (rs5015480) with diabetes among Korean women and heavy smoking men

Jae Woong Sull 1*, Tae Yong Lee 2 and Sun Ha Jee 3

Abstract

Background: Several genome-wide association studies (GWAS) for serum fasting glucose levels have reported HHEX as possibly causal. The objective of this study was to examine the joint effect of smoking on the association of diabetes with the HHEX rs5015480 polymorphism among Korean subjects.

Methods: This replication study included a total of 4240 individuals, and multivariate linear regression and multiple logistic regression models were used. We examined the combined effect of smoking on the relationship between HHEX rs5015480 and diabetes.

Results: The rs5015480 SNP in the HHEX gene was related to the mean FBS level (effect per allele, 1.572 mg/dL, \( p = 0.0122 \)). Females with the CC genotype had a 2.68 times higher (range, 1.05–6.82 times) risk of diabetes than those with the TT/TC genotype. Although the association was stronger in female subjects (OR, 4.46; 95% CI, 1.15–17.3, \( p = 0.0304 \)) among healthy individuals (N = 2461), the association between HHEX and diabetes was much stronger in male heavy smokers (OR, 4.03; 95% CI, 1.19–13.6, \( p = 0.0247 \)) than in nonsmokers (\( p = 0.9709 \)) and ex-smokers (\( p = 0.2399 \)). The interaction of smoking was also statistically significant (P for interaction =0.0182).

Conclusions: This study clearly demonstrates that a genetic variant in HHEX influences fasting glucose levels in Korean women and male heavy smokers.

Keywords: Fasting glucose, HHEX, Polymorphisms

Background

The serum fasting glucose level is a basic indicator of diabetes [1], and several genome-wide association studies (GWAS) examining type 2 diabetes have reported that the hematopoietically expressed homeobox (HHEX) (MIM 604420) gene is a candidate causal gene [2, 3].

Recent studies have shown that the HHEX rs5015480 SNP was related to serum fasting glucose levels or type 2 diabetes [4–9]. In a recent meta-analysis, the rs5015480 SNP was associated with fasting glucose (\( p = 0.015 \)) [4]. Another recent study in the Greek-Cypriot population also reported that the rs5015480 SNP was related to type 2 diabetes (\( p = 0.002 \)) [6]. Several other studies in Chinese population reported that the rs5015480 SNP was related to type 2 diabetes [7–9]. A study in Korean populations reported that the rs5015480 SNP in HHEX was related with diabetes [10]. The HHEX gene has also been linked with fetal cardiac development [11]. A Chinese study also reported that HHEX rs5015480 was associated with diabetes and cardiovascular risk [12]. However, several other studies reported that rs5015480 was not related to type 2 diabetes [13, 14]. Previous studies did not investigate the relationship between rs5015480 and type 2 diabetes considering smoking status.

Therefore, the objective of this study was to examine the joint effect of smoking on the association of diabetes with the HHEX rs5015480 polymorphism among Korean subjects. The relationship between the HHEX rs5015480 polymorphism and cardiovascular disease was also examined.
Methods

Study population

The participants were 4294 individuals who visited Health Examination Centers from 1994 to 2012 [15]. Among 4294 individuals, 1810 individuals were Cardiovascular Disease (CVD) cases identified by the health insurance reimbursement data from the NHIC. CVD was defined according to the International classification of Disease, Tenth Revision (ICD-10) (I00-I99). In total, 54 individuals were excluded due to missing fasting blood glucose levels and SNP rs5015480 data. The final subjects were 4240 individuals. Among 4240 individuals, 2461 individuals were healthy individuals, and the other 1779 individuals were Cardiovascular Disease (CVD) patients.

Data collection

The participants were interviewed using a questionnaire about smoking status and smoking amount. Self-reported alcohol consumption data were also collected from the questionnaire. Total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglyceride, and fasting blood sugar (FBS) were measured from blood samples obtained from each subject after 12 h of fasting. Height and weight were measured with subjects lightly clothed. Detailed phenotypic data were previously described [15]. Diabetes was defined as fasting serum glucose ≥126 mg/dL or its management under medication.

Genotyping assays

The rs5015480 HHEX gene SNP was genotyped using the TaqMan reaction [16], with genotyping success rates of more than 98% and repeatability rates of more than 99%.

Statistical analysis

Data are expressed as means ± standard deviation. Most statistical analyses were performed using PLINK and SAS ver. 9.2 (SAS Institute, Cary, NC, USA). The linear regression under the additive genetic model was used to assess the association of HHEX rs5015480 with fasting blood glucose levels considering age and sex as covariates. We also used multiple logistic regression analysis under the recessive model to examine the combined effect of smoking on the relationship between HHEX rs5015480 and diabetes. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to investigate the relationship between HHEX SNP and diabetes. In the logistic model, we tested for interactions by assessing the statistical significance of the interaction term in models

Table 1: General characteristics of the study population

| Subjects | All | Men | Women |
|----------|-----|-----|-------|
| N        | 4240| 2887| 1353  |
| Mean ± SD|     |     |       |
| Age, year| 52.2±10.2 | 51.9±10.2 | 52.7±10.2 |
| Waist circumference, cm | 84.1±9.0 | 87.1±7.6 | 78.6±8.7 |
| Body mass index, kg/m² | 24.4±2.9 | 24.8±2.7 | 23.5±3.2 |
| Fasting blood sugar, mg/dL | 97.0±22.7 | 98.9±24.1 | 93.1±18.7 |
| Systolic blood pressure, mmHg | 121.9±14.4 | 123.3±13.7 | 118.9±15.3 |
| Diastolic blood pressure, mmHg | 78.3±10.8 | 79.8±10.6 | 75.0±10.5 |
| HDL cholesterol, mg/dL | 50.9±11.6 | 48.2±10.2 | 56.7±12.4 |
| LDL cholesterol, mg/dL | 116.8±31.8 | 116.6±31.7 | 117.1±31.9 |
| Triglyceride, mg/dL | 143.8±96.5 | 157.2±100.9 | 114.9±78.9 |
| % | % | % |
| Smoking status | Ex | 28.4 | 40.1 | 2.4 |
| Current | 27.0 | 37.4 | 3.8 |
| Cardiovascular disease | 41.9 | 44.7 | 36.1 |
| Diabetes | 9.0 | 10.2 | 6.3 |
| Family history of diabetes | 14.5 | 14.2 | 15.3 |

SD standard deviation

Data were defined as fasting serum glucose ≥126 mg/dL or medication

Table 2: Association between the rs5015480 single nucleotide polymorphism in the HHEX gene and fasting blood sugar levels based on a linear regression model

| Phenotypes | Genotypes | TT Mean ± SD | TC Mean ± SD | CC Mean ± SD | Effect (β) | P-value |
|------------|-----------|--------------|--------------|--------------|------------|---------|
| All subjects | (N = 2827) | (N = 1278) | (N = 135) |
| Fasting blood sugar, mg/dL | 96.5±21.7 | 97.8±24.1 | 100.1±28.1 | 1.572 | 0.0122 |
| Systolic blood pressure, mmHg | 121.6±14.0 | 122.6±15.2 | 121.6±14.9 | 0.721 | 0.0676 |
| Diastolic blood pressure, mmHg | 78.3±10.7 | 78.5±11.1 | 75.6±10.9 | −0.341 | 0.2541 |
| Healthy subjects | (N = 1672) | (N = 720) | (N = 69) |
| Fasting blood sugar, mg/dL | 94.0±18.9 | 95.9±21.8 | 94.8±19.6 | 1.420 | 0.0545 |
| Systolic blood pressure, mmHg | 119.2±12.9 | 119.9±13.8 | 119.3±12.4 | 0.544 | 0.2585 |
| Diastolic blood pressure, mmHg | 78.7±10.8 | 79.0±10.7 | 76.9±10.0 | −0.044 | 0.9103 |

Estimated effect size (β) and p-value in the multiple linear regression model considered age and sex in the additive model.
that included the main effects. A two-sided significance level of $\alpha = 0.05$ was used.

**Results**

The majority of subjects examined were middle-aged individuals (Table 1). Mean FBS level was significantly higher in males (98.9 mg/dL) than in females (93.1 mg/dL) ($p < 0.0001$). About 9.0% of the subjects were diabetes patients, and 14.5% of the subjects had a family history of diabetes. Of the sample dataset, 37.4% of males and 3.8% of females were current smokers, and 44.7% of males and 36.0% of females were cardiovascular disease patients. Table 2 indicates linear regression results, after adjusting for age and sex. The rs5015480 SNP in the HHEX gene was related to a mean FBS level (effect per allele, 1.572 mg/dL, $p = 0.0122$).

The relationship of the HHEX gene SNP rs5015480 with diabetes was examined (Table 3). Females with the CC genotype had a 2.68 times higher (range, 1.05–6.82-fold) risk of diabetes than those with the TT/TC genotype. For healthy individuals ($n = 2461$), the relationship was stronger in female subjects (OR, 4.46; 95% CI, 1.15–16.3, $p = 0.0304$). In contrast, a relationship between HHEX and diabetes was not found in male subjects. The relation of the HHEX gene SNP rs5015480 with cardiovascular disease was also examined (Table 4). Females with the CC genotype had a 2.14 times higher (range, 1.05–6.80-fold) risk of cardiovascular disease than those with the TT/TC genotype.

The analysis according to smoking status in males is presented in Table 5. The relationship between HHEX and diabetes was much stronger in male heavy smokers (OR, 4.03; 95% CI, 1.19–13.6, $p = 0.0247$) than in non-smokers ($p = 0.9709$) and ex-smokers ($p = 0.2399$). Table 6 presents the age-adjusted odds ratios (ORs) for diabetes according to HHEX (rs5015480) genotype in strata of smoking status among Korean men. When compared with non-, ex-, or light smokers with the TT/TC genotype, the ORs (95% confidence interval (CI)) were 4.95 (1.51–16.3) in heavy smokers (P for interaction =0.0182).

**Discussion**

In this study of 4240 individuals, the rs5015480 polymorphism in the HHEX was related to serum glucose level, which is similar to the results of previous studies. Our study found that HHEX polymorphism had a stronger relation to fasting glucose levels in women than in men. However, the interaction between sex and genotypic covariates was not significant ($p$ for interaction $= 0.1359$) (Data not shown). A study in Korea also reported that the HHEX rs5015480 polymorphism was associated with the risk of diabetes in women ($p < 0.005$), but not in men ($p > 0.005$).
Another recent study suggested the association of the HHEX gene rs5015480 polymorphism with risk of gestational diabetes mellitus in women [17]. In this study, females with the CC genotype had a 2.14 times higher (range, 1.05–6.80-fold) risk of cardiovascular disease than those with the TT/TC genotype. However, an association between HHEX and cardiovascular disease was not found in male subjects.

Smoking is also highly related to type 2 diabetes [18–20]. In the present study, 37.4% of males and 3.8% of females were current smokers, which is similar to the results of Korean national data (44.6% male and 4.6% female current smokers) [21]. We also found the association between HHEX SNP and fasting glucose was stronger in heavy smokers than in nonsmokers. Some studies have reported that fasting glucose is regulated by smoking [22, 23]. A recent study reported that genetic polymorphism in glucagon may be modified by smoking for the risk of type 2 diabetes [22]. Another recent study reported that the AMPKα1 polymorphism may have the joint effects with cigarette smoking for the risk of coronary artery disease in the Chinese people [23]. A recent study also suggested interactions of well-known obesity-related polymorphisms with smoking [24].

HHEX encode homeobox transcription factors that are involved in organogenesis of liver and pancreas [17, 25]. A recent study reported that HHEX was related to insulin processing and secretion [4]. The frequency of rs5015480 C allele is 56.8% in Europeans and 56.7% in sub-Saharan Africans while the frequency in East Asians was 21.1% in Han Chinese in Beijing and 20.0% in Japanese, as shown in the HapMap data (NCBI website). Our study found a C allele frequency of 18.2%.

In this study, the rs5015480 SNP was related to a mean FBS level ($p = 0.0122$). When the population is restricted to healthy subjects, the significant association was not found ($p = 0.0545$). One of the possible reasons for the difference is that the mean FBS level was lower in the healthy subjects (94.6 mg/dL) than in all subjects (97.0 mg/dL). This study has several other limitations. The available data do not allow us to classify participants by diabetes type. However, the proportion of type I diabetes in Korea is low, at 1% of diabetes cases [26]. In addition, we did not consider other major genes that contribute to type 2 diabetes susceptibility.

### Conclusion
Genetic backgrounds in Western populations were different for Asian populations [27]. Thus, results of this study may not be generalized to all populations. However, our study showed that the HHEX gene on chromosome 10 is related to serum glucose levels in Korean women and male heavy smokers.

### Table 5
Odds ratios (OR) of polymorphic rs5015480 HHEX genotypes for diabetes$^a$ in Korean men ($n = 2887$)

| Subjects          | Genotype | Normal | Diabetes$^a$ | OR (95% CI$^b$) | $P$-value |
|-------------------|----------|--------|--------------|-----------------|-----------|
|                  |          | N (%)  | N (%)        |                 |           |
| Non smokers      | TT/TC    | 533 (97.3) | 68 (97.1) | 1.00 (reference) |           |
|                  | CC       | 15 (2.7) | 2 (2.9)     | 1.03 (0.23–4.67) | 0.9709   |
| Ex smokers       | TT/TC    | 958 (97.2) | 113 (99.1) | 1.00 (reference) |           |
|                  | CC       | 28 (2.8) | 1 (0.9)     | 0.30 (0.04–2.24) | 0.2399   |
| Light smokers    | TT/TC    | 409 (94.0) | 36 (94.7) | 1.00 (reference) |           |
|                  | CC       | 26 (6.0) | 2 (5.3)     | 0.83 (0.19–3.69) | 0.8040   |
| Heavy smokers    | TT/TC    | 452 (97.8) | 50 (92.6) | 1.00 (reference) |           |
| (1–19/day)       | CC       | 10 (2.2) | 4 (7.4)     | 4.03 (1.19–13.6) | 0.0247   |

$^a$Adjusted for age  
$^b$CI, confidence interval  
$^c$Diabetes were defined as fasting serum glucose ≥126 mg/dL or medication

### Table 6
Age-adjusted odds ratios (OR) for diabetes$^a$ according to HHEX (rs5015480) genotypes in strata of smoking status in Korean men ($n = 2887$)

| Subjects                        | No. of subjects by genotypes | OR (95% CI$^b$) | $P$ for interaction |
|---------------------------------|------------------------------|-----------------|---------------------|
|                                 | TT/TC                        |                 |                     |
| Non/Ex/Light smokers            | 2117/74                      | 1.00 (reference) | 0.65 (0.26–1.64)    |
| Heavy smokers                   | 502/14                       | 1.21 (0.87–1.69) | 4.95 (1.51–16.3)    |

$^a$Adjusted for age  
$^b$CI, confidence interval
Abbreviations
CI: Confidence intervals; CVD: Cardiovascular Disease; FBS: Fasting blood sugar; GWAS: Genome-wide association studies; HDL: High density lipoprotein; HHEX: Hematopoietically expressed homeobox; ICD: International classification of Disease; LDL: Low density lipoprotein; OR: Odds ratios; SD: Standard deviation; SNP: Single nucleotide polymorphism

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Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions
Study design: JWS, SHJ. Subjects recruitment and acquisition of data: SHJ. Analysis and interpretation of data: JWS, TYL, SHJ. Revision of manuscript: all authors. Approving final version of manuscript: all authors.

Ethics approval and consent to participate
The Severance Medical Ethics Committee approved the study, and all authors. Approving final version of manuscript: all authors.

Competing interests
The authors declare that they have no competing interests.

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