ABSTRACT

Several type 2 diabetes mellitus (T2D) susceptibility loci identified through genome-wide association studies (GWAS) have been replicated in various populations. However, such study has not been done in Thais. This study was to investigate the effect of CDKN2A/2B (rs10811661), CDKAL1 (rs7754840, rs7756992), HHEX (rs7923837, rs5015480, rs1111875), KCNQ1 (rs2237892, rs2237895, rs2238228, rs2237897), MTNR1B (rs1387153, rs10830963), SLC30A8 (rs13266634), TCF7L2 (rs7903146, rs7895340, rs11196205) and UBE2E2 (rs7680569, rs7612463) in Thai patients with T2D and 511 non-diabetic controls by high resolution melting analysis (HRM) and restriction fragment length polymorphism (RFLP). Our findings have suggested that SNPs of CDKN2A/2B, HHEX, KCNQ1, MTNR1B, SLC30A8, TCF7L2 and UBE2E2 play a role in the pathogenesis of T2D in Thai population.

Keywords: Type 2 Diabetes, single nucleotide polymorphism, genome wide association study, HRM analysis, RFLP
INTRODUCTION

Type 2 diabetes (T2D) is a global public health problem characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or Interaction between environmental and genetic factors are involved in the onset and development of the disease. Recently, several genome-wide association studies (GWAS) have identified genes affecting risk for type 2 diabetes in large-scale case-control samples which were replicated in various populations. Single nucleotide polymorphisms (SNPs) within or near the genes transcription factor 7-like 2 (TCF7L2), potassium voltage-gated channel, KQT-like subfamily, member 1 (KCNQ1), CDK5 regulatory subunit associated protein 1-like 1 (CDKAL1), zinc transporter, member 8 (SLC30A8), hematopoietically expressed homeobox (HHEX), exostosin 2 (EXT2), insulin-like growth factor 2 mRNA binding protein 2 (IGF2BP2), cyclin-dependent kinase inhibitor 2B (CDKN2B), fat mass and obesity associated (FTO), solute carrier family 30 (zinc transporter), member 8 (SLC30A8), melatonin receptor 1 B (MTNR1B), and ubiquitin-conjugating enzyme E2E 2 (UBE2E2) are such loci identified by GWAS. Most of the susceptibility genes identified through GWAS to date are likely to affect insulin secretion and beta-cell function, while a few are potentially involved in insulin action. In this study, we investigated the association between eighteen SNPs from eight genes using case-control study comprising 516 patients with T2D and 511 nondiabetic controls and evaluated their role in type 2 diabetes susceptibility in our population.

MATERIALS AND METHODS

1. Subjects

A total number of 511 patients with type 2 diabetes and 516 non-diabetic control subjects were enrolled in this study. Patients with diabetes were recruited at diabetic clinic Siriraj Hospital. The criteria for diagnosis of diabetes follow those described by American Diabetes Association. Control subjects were enrolled at health check-up center Department of Preventive and Social Medicine Siriraj Hospital. The inclusion criteria for the control subjects were as follows: (1) fasting plasma glucose <100 mg/dl (6.1 mmol/l), (2) no family history of type 2 diabetes, (3) age greater than 40 years old, and (4) no hypertension. Approval of the study was granted from the institutional ethic committee; the patients and control subjects gave their informed consent before participating in the study.

2. SNP genotyping

Eighteen SNPs representing 8 genes identified in recent GWAS were selected, including CDKN2A/2B (rs10811661), CDKAL1 (rs7754840, rs7756992), HHEX rs7923837, rs5015480, rs1111875), KCNQ1 (rs2237892, rs2237895, rs2238228, rs2237897), MTNR1B (rs1387153, rs10830963), SLC30A8 (rs1326634), TCF7L2 (rs7903146, rs7895340, rs11196205) and UBE2E2 (rs6780569, rs7612463). These SNPS were genotyped by HRM and PCR-RFLP.

3. Statistical analysis

The distribution of genotypes was tested for deviation from the Hardy-Weinberg equilibrium using web-based program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). Difference in genotype frequencies of 18 SNPs of 8 genes between type 2 diabetic patients and non-diabetic control subjects and haplotype analysis were done by SNPstats (http://bioinfo.iconcologia.net/SNPstats) Linear regression model was used to analyze continuous variables adjusted for covariate variables of age, gender, BMI and drugs where appropriate were done by using SPSS version13. A p value of less than 0.05 was considered statistically significant.
RESULTS AND DISCUSSION

This was the first study reported on the contribution and importance of diabetes-susceptibility loci identified through GWAS in Thais population. Significant association with T2D were demonstrated for CDKN2B (rs10811661) (OR 0.56, 95%CI (0.34-0.93) p=0.010), KCNQ1(rs2237892) (OR 0.82, 95%CI (0.63-0.93) p=0.018), KCNQ1(rs2238228) (OR 0.74, 95%CI (0.58-0.95) p=0.018), KCNQ1(rs2237897) (OR 0.68, 95%CI (0.53-0.87) p=0.002), SLC30A8 (rs13266634)(OR 0.70, 95%CI (0.54-0.92) p=0.010), HHEX (rs7923837) (OR 1.56, 95%CI (1.00-2.43) p=0.049), HHEX (rs1111875) (OR 1.54, 95%CI (1.03-2.30) p=0.032), TCF7L2 (rs7903145) (OR 1.54, 95%CI (1.03-2.29) p=0.0031), TCF7L2 (rs7895340) (OR 10.16, 95%CI (1.30-79.63) p=0.0033), TCF7L2 (rs11196205) (OR 5.57, 95%CI (1.23-25.24) p=0.0087) which were in agreement with previous reports. However, we failed to replicate the results for SNPs of CDKAL1, UBE2E2 and MTNR1B.

We also examined the association between haplotypes of KCNQ1, HHEX and TCF7L2 and T2D. Four haplotypes of KCNQ1 (TAAT, CCCC, TCAT and TCAC; SNPs rs2237892, rs2238228, rs2237895 and rs2237897) were associated with T2D (OR 10.61, 95%CI (5.77-19.51), p<0.0001) and (OR 5.30(2.67-10.53, p<0.0001), (OR 0.39, 95%CI (0.27-0.57), p<0.0001) and (OR 0.24 (0.80-0.66, p<0.0001), respectively). This finding was similar to study in Malaysians. In addition, TCF7L2 haplotype (TGG; SNPs rs7903146, rs7895340, rs11196205) was also significantly associated with T2D (OR 1.82 95%CI1.18-2.81 p=0.007). HHEX haplotype (ATG; rs7923837, rs5015480, rs1111875) reveal association with T2D (OR 1.4895%CI1.02-2.14, p=0.037).

We studied the impact of these SNPs on certain clinical parameters. The risk allele C of KCNQ1 SNPs rs2237892, rs2237895 and rs2237897 were associated with higher FPG (p=0.008, 6.82x10-6 and 0.003, respectively). Moreover, the risk allele C of rs2237895 exhibited association with HbA1c (p=0.003). The risk allele C of rs2237892 was associated with total cholesterol (p=0.025) while rs2237897 was associated with total cholesterol and LDL-C (p=0.004 and 0.013, respectively). Furthermore, the risk allele T of CDKN2A/2B (rs10816661) was associated with higher FPG (p=0.015). Our findings suggested that KCNQ1 and CDKN2A may participate in glucose and lipid metabolism.

CONCLUSION

Variants of CDKN2A/B, KCNQ1, HHEX, SLC30A8 and TCF7L2 were associated with T2D in Thai population. Our results supported that these genes conferred susceptibility to T2D across different ethnicities. Moreover, certain genes may influence lipid metabolism.

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