การศึกษาความแปรผันของยีน *CDKN2A/2B*, *CDKAL1*, *HHEX*, *KCNQ1*, *MTNR1B*, *SLC30A8*, *TCF7L2* และ *UBE2E2* กับการเกิดโรคเบาหวานชนิดที่สองในประชากรไทย

Study of Common Variations of *CDKN2A/2B*, *CDKAL1*, *HHEX*, *KCNQ1*, *MTNR1B*, *SLC30A8*, *TCF7L2* and *UBE2E2* and Type 2 Diabetes (T2D) in Thais

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บทคัดย่อ

จากการศึกษาหายีนที่ก่อโรคเบาหวานชนิดที่ 2 โดยใช้วิธีการศึกษาจีโนมในแนวกว้างในประชากร พบว่ามียีนหลายยีนที่อาจเป็นสาเหตุของการก่อโรค โดยมีการยืนยันผลการศึกษาดังกล่าวในหลายประชากรหลายเชื้อชาติ ทั้งนี้ ยังไม่มีการศึกษาในชาวไทย ผู้วิจัยจึงทำการศึกษาถึงผลของยีนดังกล่าวในชาวไทย ได้แก่ ยีน *CDKN2A/2B* (rs10811661), *CDKAL1* (rs7754840, rs7756992), *HHEX* (rs7923837, rs5015480, rs1111875), *KCNQ1* (rs2237892, rs2237895, rs2238228, rs2237897), *MTNR1B* (rs1387153, rs10830963), *SLC30A8* (rs13266634), *TCF7L2* (rs7903146, rs7895340, rs11196205) และ *UBE2E2* (rs6780569, rs7612463) โดยทำการตรวจ SNPs ในผู้ป่วยเบาหวานชนิดที่ 2 จำนวน 516 คน และอาสาสมัครคนปกติ จำนวน 511 คน ด้วยวิธี high resolution melting analysis (HRM) และ restriction fragment length polymorphism (RFLP) จากการศึกษาพบว่า SNPs rs2237892, rs2237895, rs2238228, rs2237897 ของยีน *KCNQ1,* SNP rs10811661 ของยีน *CDKN2A/2B,* SNPs rs7923837, rs1111875 ของยีน *HHEX,* SNP rs13266634 ของยีน *SLC30A8* และ SNP rs7903146, rs7895340, rs11196205 ของยีน *TCF7L2* มีความสัมพันธ์กับการเกิดโรคเบาหวานชนิดที่ 2 นอกจากนี้จากการวิเคราะห์ในรูปแบบ haplotype พบว่า haplotype ของยีน *KCNQ1, HHEX และ TCF7L2* มีความสัมพันธ์กับการเกิดโรคเบาหวานชนิดที่ 2 อีกด้วย สำหรับการศึกษาความสัมพันธ์ของยีนกับลักษณะทางคลินิกต่างๆ พบว่า SNPs ของยีน *KCNQ1* มีความสัมพันธ์กับระดับน้ำตาลกลูโคสในพลาสมา, ระดับน้ำตาลสะสม, ระดับไขมันโคเลสเตอรอล, ระดับไขมันแอลดีแอล ส่วน SNPs ของยีน *CDKN2A/2B* พบว่ามีความสัมพันธ์กับระดับน้ำตาลกลูโคสในพลาสมาเช่นกัน จากการศึกษาในครั้งนี้สรุปได้ว่ายีน *KCNQ1*, *CDKN2A/2B, HHEX, SLC30A8* และ *TCF7L2* อาจเป็นยีนก่อโรคเบาหวานชนิดที่ 2 ในชาวไทย

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* (rs6780569, rs7612463) and risk of T2D in Thais. We genotyped 18 single nucleotide polymorphisms (SNPs) of 8 genes in 516 patients with T2D and 511 non-diabetic controls by high resolution melting analysis (HRM) and restriction fragment length polymorphism (RFLP). SNPs of *KCNQ1*, *CDKN2A/2B, HHEX, SLC30A8* and *TCF7L2* were significantly associated with T2D. Moreover, haplotype analysis of *KCNQ1*, *HHEX* and *TCF7L2* were likewise associated with the disease. We also demonstrated the relationship between SNPs of *KCNQ1* and fasting plasma glucose (FPG), HbA1c, total cholesterol and low-density lipoprotein cholesterol (LDL-C). The correlation between SNPs of *CDKN2A/2B* and FPG were also observed. Our findings have suggested that *KCNQ1*, *CDKN2A/2B, HHEX, SLC30A8* and *TCF7L2* play a role of in the pathogenesis of T2D in Thai population.

คำสำคัญ: โรคเบาหวานชนิดที่ 2, ความแปรผัน, การศึกษาจีโนมในแนวกว้าง, การวิเคราะห์ด้วยวิธีเอชอาร์เอ็ม, อาร์เอฟแอลพี
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* (rs13266634), *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/hwal.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, rs2283228, 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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