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Glucagon gene polymorphism modifies the effects of smoking and physical activity on risk of type 2 diabetes mellitus in Han Chinese

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ABSTRACT

Few genome-wide association studies have considered interactions between multiple genetic variants and environmental factors associated with disease. The interaction was examined between a glucagon gene (GCG) polymorphism and smoking, alcohol consumption and physical activity and the association with risk of type 2 diabetes mellitus (T2DM) in a case–control study of Chinese Han subjects. The rs12104705 polymorphism of GCG and interactions with environmental variables were analyzed for 9619 participants by binary multiple logistic regression. Smoking with the C-C haplotype of rs12104705 was associated with increased risk of T2DM (OR = 1.174, 95% CI = 1.013–1.361). Moderate and high physical activity with the C-C genotype was associated with decreased risk of T2DM as compared with low physical activity with the genotype (OR = 0.251, 95% CI = 0.206–0.306 and OR = 0.190, 95% CI = 0.164–0.220). However, the interaction of drinking and genotype was not associated with risk of T2DM. Genetic polymorphism in rs12104705 of GCG may interact with smoking and physical activity to modify the risk of T2DM.

1. Introduction

Type 2 diabetes (T2DM) has become one of the leading and fastest growing health problems in the world. Global estimates by the International Diabetes Federation (IDF) in 2011 were approximately 90 million Chinese and over 366 million people worldwide. By 2030, these estimates are expected to increase to more than 130 million in China and 552 million people worldwide (Whiting et al., 2011).

Although the exact etiology is unknown, T2DM is a multifactorial disease, resulting from numerous interactions between multiple genetic variants and environmental factors related to diet, physical activity, smoking, drinking alcohol and medical treatment (Mensink, 2005). In the last few years, T2DM has been the subject of genome-wide association studies (GWAS) (Hindorff et al., 2009) and candidate gene studies (Doria et al., 2008; Grant et al., 2009), which have identified variants in genes that may play a role in the etiology of T2DM. However, because of practical and statistical challenges, none of the GWAS have considered interactions among the variants and the environment, and only relatively few candidate gene studies have pursued such studies (Black et al., 2008; Cauchi et al., 2008; Miyake et al., 2009; Qi et al., 2007; Sparso et al., 2009).

Glucagon gene (GCG), located on chromosome 2q24.2, comprises 6 exons and encodes glucagon, glucagon-like peptide (GLP)-1 and GLP-2 and oxyntomodulin proteins (Holst, 1997). No large-scale sequencing findings for GCG have been published. On the basis of the crucial role of GCG in energy metabolism, we hypothesized that the frequency variants of GCG were associated with diabetes.

Here, a case–control study was performed to investigate the interaction between GCG polymorphism and the environment associated with risk of T2DM in Chinese Han subjects.

2. Methods

2.1. Samples

Subjects (N = 9619, 1842 cases and 7777 controls) were recruited from the northern Chinese Han population in northeast China and obtained blood samples from all subjects. All subjects resided in Henan Province, North China. A total of 1842 T2DM patients were consecutively recruited from the outpatient clinics of three hospitals (1032 cases) or

Abbreviations: GWAS, Genome-wide association studies; MAF, Minor allele frequency; SNP, Single nucleotide polymorphism; OR, Odds ratio; CI, Confidence interval.

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from communities (810 cases) in Henan Province. Diabetes mellitus was diagnosed by fasting blood glucose (FPG) ≥ 7.00 mmol/L or 2-h plasma glucose ≥ 11.0 mmol/L (American Diabetes Association, 2005) during an oral glucose tolerance test (OGTT) with diabetes clinical symptoms or previously diagnosed diabetes was defined if the participant (1) was receiving insulin treatment, (2) was receiving an oral hypoglycemic agent or (3) presented with a history of diabetes during the interview; all other types of diabetes were excluded on the basis of clinical data. Patients who were pregnant or lactating were excluded. All patients were unrelated and had been diagnosed with T2DM after age 20 years. Unrelated non-diabetic controls were recruited from communities in Henan Province. The participants were excluded with body mass index (BMI) < 18.50 or who were pregnant, mentally disturbed, handicapped or obese (caused by disease) or taking certain drugs or had cancer. Control subjects were included if they were Han Chinese >20 years old and had no family history of diabetes, FPG <6.1 mmol/L and normal OGTT results. Written, informed consent was obtained from each participant after a full explanation of the study, which was approved by the Ethics Committee of Zhengzhou University.

2.2. Primer design

Primers and probes were synthesized by Shanghai Generay Biotech Co.; the forward primer was 5′-ACCTTTTGGTGTCTCTACTTC-3′, and the reverse primer was 5′-TGTGTCATGTTGATTGGCTGT-3′.

2.3. Single nucleotide polymorphism (SNP) selection and genotyping

We selected one tagging SNP (rs12104705) from the Hap Map Phase 3 Chinese population using Haploview version 4.1 (Barrett et al., 2005). The criterion for screening was minor allele frequency >0.1 in Han Chinese and $r^2 \geq 0.8$. Genomic DNA was isolated from whole blood by use of a blood genome DNA extraction kit (Yaneng BIO). Polymorphic regions were amplified by PCR, then rs12104705 genotyping involved ligase detection reactions (LDRs) performed in a 200-μl PCR tube, with a final volume of 10 μl containing 2 μl PCR products (100 ng/μl), 1 μl 10* Taq DNA ligase buffer, 1 μl LDR probes (12.5 pmol/μl), 6 μl sterile H_2O and 0.125 μl Taq DNA ligase (40 U/μl). The reactions involved heating for 2 min at 95 °C, then 30 thermal cycles of 95 °C for 30 s (denaturation) and 56 °C for 2 min (annealing and ligation). The LDR products were analyzed by electrophoresis with use of the ABI PRISM 3100 DNA sequenator.

2.4. Environmental factors

Smoking status included current smoker and not current smoker. Participants who currently smoked and/or had smoked at least 100 cigarettes during their lifetime were classified as current smokers if they answered affirmatively to the questions, “Do you smoke cigarettes now?” and “Have you smoked at least 100 cigarettes during your lifetime?” Self-reported alcohol consumption data were also collected from the questionnaire using the following question: “Considering all types of alcoholic beverages, how many times during the past 30 days did you drink?” Alcohol drinkers consuming 100 ml liquor in 30 days were included in the study. Physical activity level for each individual was classified as low, moderate or high based on the International Physical activity Questionnaire (IPAQ) [www.ipaq.ki.se].

2.5. Data analysis

Categorical variables are shown as number (percentage) and were analyzed by chi-square test. Continuous variables are shown as median (range) for data with skewed distribution. The Mann–Whitney–Wilcoxon test and the Kruskal–Wallis rank test were used to assess differences between cases and controls. Haploview version 4.2 was used to test for Hardy Weinberg equilibrium independently among cases and controls. Logistic regression models (SAS version 9.1.3) were used to assess the effects of the SNP on T2DM after adjusting for sex, age, anthropometric measurements (BMI, waist circumference and blood pressure) and environment (smoking, drinking and physical activity) with calculation of odds ratios (ORs), 95% confidence intervals (95% CI) and corresponding p values for risk of T2DM. The interaction between the SNP and the environment was modeled by use of logistic regression multiplicative interaction models. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Subject characteristics

BMI and waist circumference were significantly greater in cases than controls ($P < 0.001$), cases featured more males than did controls ($P < 0.001$), and the median age was higher for cases than controls ($P < 0.001$) (Table 1). Moderate and high physical activities were lower among cases than controls, with no difference between groups in alcohol drinking.

3.2. Genotype frequencies and allelic estimates for GCG SNP

The SNP rs12104705 was in the Hardy–Weinberg equilibrium independently among cases and controls ($P > 0.1$). Genotyping results did not differ between cases and controls and had a minor allele (T allele) frequency of 7.13 % (Table 2). The rs12104705 genotyping was not associated with disease susceptibility ($P > 0.05$) even after adjusting for sex, age, anthropometric measurements (BMI, waist circumference and blood pressure) and environment (smoking, drinking and physical activity) (Table 3).

3.3. Association of gene–environment interaction and T2DM risk

With non-smoking and the C-C haplotype of rs12104705 as the reference, smoking with the C-C haplotype was associated with increased risk of T2DM (OR = 1.174, 95% CI = 1.013–1.361). As compared with low physical activity with the C-C genotype, moderate and high physical activity with the genotype was associated with decreased risk of T2DM (OR = 0.251, 95% CI = 0.206–0.306 and OR = 0.190, 95% CI = 0.164–0.220, respectively), high physical activity with the T-T haplotype was associated with increased risk of T2DM (OR = 7.748, 95% CI = 1.408–42.635). The interaction of alcohol drinking and rs12104705 was not associated with risk of T2DM (Table 4).

4. Discussion

In the present study, a case–control study of 9619 Chinese Han subjects was examined the association of the interaction of one SNP, rs12104705, in GCG with environmental factors and risk of T2DM. The allelic frequencies of rs12104705 were identical between cases and controls. We found the interaction between smoking and physical activity and rs12104705 associated with risk of T2DM.

The GCG gene, encoding glucagon, GLP-1 and GLP-2 and oxyntomodulin proteins, for crucial for energy metabolism, is involved in multiple steps of metabolism. Blood glucose and serum insulin levels were lower and insulin sensitivity higher in GCG-knockout than wild-type mice (Hayashi et al., 2009). The GCG polymorphism rs12104705 is a newly discovered, single genetic variant possibly associated with risk of T2DM, but only 1 study has investigated the association of a GCG variant and risk of T2DM, in a Danish population (Torekov et al., 2011): the Ile158Val variant was associated with risk of T2DM (OR = 1.83 [1.05–3.9]), but rs4664447 and rs7581952 were not. The inclusion of more than 9000 subjects conferred sufficient power to separate the 3 genotypes, but the results were not significant. The binary logistic modeling in this study revealed no association of rs12104705 of GCG and risk of T2DM even when adjusting for sex, age,