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Original Article

Study of single-nucleotide polymorphism within candidate genes associated with type 2 diabetes in Western Indian population

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ARTICLE INFO

Article history:
Received 11 February 2013
Accepted 14 February 2013
Available online 5 March 2013

Keywords:
SNP
INS
INSR
TCF7L2
PPAR-γ

ABSTRACT

Aim: To evaluate linkage between single nucleotide polymorphisms within the known risk candidate genes of type 2 diabetes in Western Indian population.

Material and method: The study population was comprised of 25 normal glucose tolerant individuals (12 males and 13 females) and 25 type 2 diabetes patients (13 males and 12 females). The genes and their corresponding single-nucleotide polymorphisms that we screened were INS (rs5505), INSR (rs10500204), TCF7L2 (rs7903146) and PPAR-γ (rs1801282).

Result: The risk alleles 'T' (INS, TCF7L_2), 'C' (INSR) and 'G' (PPAR-γ) were statistically more frequent in the subjects with type 2 diabetes.

Conclusion: The findings suggest that there is a co-relation between the risk alleles and susceptibility to T2D in the present pilot study population.

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1. Introduction

There are two types of diabetes mellitus: Type 1 and Type 2. Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. While type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. It is also most common type of diabetes. Type 2 diabetes has also been loosely defined as "adult onset" diabetes. As diabetes becomes more common throughout the world, cases of T2D are being observed in younger people. The majority of individuals with type 2 diabetes are either overweight or obese. WHO predicts that by 2025, the number of diabetic people will increase to 300 million. The genes involved in this disease are poorly defined. Many genes are thought to be involved in type 2 diabetes. These genes may show subtle variation in the gene sequence and may be extremely common. Many genetic variants have been convincingly and repeatedly found to associate with the disease, each of which confers only a small increase in risk, making causality difficult to prove and also limiting the prognostic and diagnostic potential of these individual variants.[1]

Type 2 diabetes (T2D) has long been attributed to a complex interaction between an individual's genetic background and multiple environmental factors. The genetic contribution has been confirmed by twin, family and population studies.

Dissecting the genetic architecture of a complex disease such as T2D is a rather challenging task. The genetic variants

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http://dx.doi.org/10.1016/j.jopr.2013.02.013

detected, represent common variants shared by a large number of individuals but with modest effects. Each risk allele increases risk of T2D only by a small percentage. Profiling genetic variation aims to correlate biological variation (phenotype) with variation in DNA sequences (genotype). The ultimate goal of mapping genetic variability is to identify the single-nucleotide polymorphism (SNP) causing a monogenic disease or the SNPs that increase susceptibility to a polygenic disease. Approximately 10–12 SNP markers in genes like IGF2BP2, CDKAL1, TCF7L2 and PPRG have been used worldwide to determine the risk factor of T2D.[2]

Genes significantly associated with developing T2D, include TCF7L2, PPARG, FTO, KCNJ11, NOTCH2, WFS1, CDKAL1, IGF2BP2, SLC30A8, JAZF1, and HHEX and KCNJ11.[3–6] In this study, 4 prominent mutations spanning across 4 genes were investigated for their link with diabetic condition in Western Indian resource population namely Insulin Hormone (INS), Insulin Receptor (INSR), Transcription factor 7-like 2 (TCF7L2) and peroxisome proliferator-activated receptor-gamma (PPARG).

2. Subjects and methods

2.1. *Human subjects*

The study subjects were a part of an ongoing insulin resistance study being undertaken by Department of Life Sciences, University of Mumbai in association with Medical Genetics Study Centre, geneOmbio Technologies, India. The study was directed towards a sub-population living in the State of Maharashtra (India) and suffering from T2D. Blood, serum and DNA samples of 25 T2D cases (13 males and 12 females) and 25 normal glucose tolerant (NGT) (12 males, 13 females) individuals were studied. All blood samples were obtained at the baseline visit and all participants provided a written informed consent for investigations.

The recruited members of the resource population were above the age of 25 years with an average of (mean $\pm$ SD) 44.6 ± 10.42 and 49.6 ± 12.5 years for control and T2D group respectively. The diagnosis of T2D was confirmed by analyzing medical records for symptoms, use of medication, and measuring of fasting glucose levels following the guidelines of American Diabetes Association (Diabetes Care, December 29, 2009; January 2010, Supplement).

Primary inclusion criteria comprised a medical record indicating either 1) a fasting plasma glucose level of $\geq$126 mg/dL or $\geq$7.0 mM after a minimum of 12 h fasting or 2) a 2-h post-glucose level [2-h oral glucose tolerance test (OGTT)] of $\geq$200 mg/dL or $\geq$11.1 mM on more than one occasion with symptoms of diabetes.

Impaired glucose tolerance was defined as a fasting plasma glucose level of $\geq$100 mg/dL (5.6 mM) but $\leq$126 mg/dL (7.0 mM) or a 2-h OGTT of $\geq$140 mg/dL (7.8 mM) but $\leq$200 mg/dL (11.1 mM).

In cases where a medical report was not readily available, self-reported T2D cases were confirmed by performing a 2-h OGTT. The 2-h OGTTs were performed according to the WHO criteria (75 g oral load of glucose). Body mass index (BMI) was computed as weight (kg)/height (meter) while waist-to-hip ratio (WHR) was calculated as the ratio of abdomen or waist circumference to hip circumference. Details of the NGT and T2D population mentioned in Tables 1 and 2.

The NGT subjects that participated in this study were from the same subpopulation group from Maharashtra. All protocols were reviewed and approved by the project authorities at geneOmbio Technologies in Pune and a memorandum of understanding and material transfer agreements for sample sharing were signed between the two collaborating Institutes.

2.2. *Metabolic assays*

Quantification of HbA1c was done from whole blood. HbA1c levels were determined by turbidometric inhibition immunoassay (Tina Quant; Roche).

The homeostatic model assessment (HOMA) was used to quantify insulin resistance and beta-cell function. HOMA-IR value of T2D population was 4.6 ± 0.75 as compared to control group 2.7 ± 0.44. The HOMA-B mean value in control and diabetic population was 196.6 ± 180.17 and 28.7 ± 7.15 respectively. Thus indicating insulin resistance and reduction in beta-cell function in T2D population.

2.3. *SNP genotyping*

DNA was extracted from blood cells using standardized SDS–phenol/chloroform method described by Sambrook et al (1989).[7] Genotyping of samples for single-nucleotide polymorphisms (SNPs) within INS (rs5505), INSR (rs10500204), TCF7L2 (rs7903146) and PPAR-γ (rs1801282) were done according to Halsall et al (2004), Bennermo et al (2004), Marquezine et al (2008), and Romeo et al (2001) respectively.[8–11] For quality control, 2 replicates of positive controls and 1 replicate of negative controls were included in each PCR run to match the concordance. The discrepancy in the concordance was <0.01%. Genotyping success rate was 100% for all the investigated SNPs.

2.4. *Statistical analysis*

The Hardy–Weinberg equilibrium was used with a one-degree of freedom goodness-of-fit test separately among cases and controls with the help of the Pearson chi-square test. Allelic frequencies between test and control samples were done using the chi-square test or the Fisher exact probability test, wherever appropriate.

Unconditional logistic regression was used, before and after adjusting for gender, age and other variants for statistical analysis of genetic effects measured by the odds ratio (OR) and its corresponding 95% confidence limits. Association analyzes were performed for each polymorphism using the 'SNPassoc' software.[12]

3. Result and discussion

All samples, including those with T2D ($N = 25$) and normal glucose tolerant ($N = 25$), were genotyped for 4 SNP within 4 genes of interest.

A total of 4 genes and 4 SNPs were identified for genotyping analysis within each of the samples from the resource

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