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Genetic association of *ADIPOQ* gene variants with type 2 diabetes, obesity and serum adiponectin levels in south Indian population

Kandaswamy Ramya [a,1], Kuppuswamy Ashok Ayyappa [a,2], Saurabh Ghosh [b], Viswanathan Mohan [a], Venkatesan Radha [a,*]

[a] Madras Diabetes Research Foundation-ICMR Advanced Centre for Genomics of Type 2 Diabetes and Dr. Mohan's Diabetes Specialities Centre, Gopalapuram, Chennai 600086, India
[b] Human Genetics Unit, Indian Statistical Institute, Kolkata 700 108, India

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ABSTRACT

Objective: To investigate the genetic association of eight variants of the adiponectin gene with type 2 diabetes mellitus (T2DM), obesity and serum adiponectin level in the south Indian population.
Methods: The study comprised of 1100 normal glucose tolerant (NGT) and 1100 type 2 diabetic, unrelated subjects randomly selected from the Chennai Urban Rural Epidemiology Study (CURES), in southern India. Fasting serum adiponectin levels were measured by radioimmunoassay. The variants were screened by polymerase chain reaction-restriction fragment length polymorphism. Linkage disequilibrium was estimated from the estimates of haplotype frequencies.
Results: Of the 8 variants, four SNPs namely, *+276 G/T* (*rs1501299*), *−4522 C/T* (*rs822393*), *−11365 C/G* (*rs266729*), and *+712 G/A* (*rs3774261*) were significantly associated with T2DM in our study population. The *−3971 A/G* (*rs822396*) and *−11391 G/A* (*rs17300539*) SNPs' association with T2DM diabetes was mediated through obesity (where the association with type 2 diabetes was lost after adjusting for BMI). There was an independent association of *+276 G/T* (*rs1501299*) and *−3971 A/G* (*rs822396*) SNPs with generalized obesity and +349 A/G (*rs2241767*) with central obesity. Four SNPs, *−3971 A/G* (*rs822396*), *+276 G/T* (*rs1501299*), *−4522 C/T* (*rs822393*) and *Y111H T/C* (*rs17366743*) were significantly associated with hypoadiponectinemia. The haplotypes GCCATGAAT and AGCGTGGGT conferred lower risk of T2DM in this south Indian population.
Conclusion: The adiponectin gene variants and haplotype contribute to the genetic risk towards the development of type 2 diabetes, obesity and hypoadiponectinemia in the south Indian population.

1. Introduction

The study of adipocytes has burgeoned as a result of the rising incidence of diabetes and obesity worldwide. The role of adipose tissue is of great significance where it contributes to the pathogenesis of diabetes as well as obesity by secreting a variety of secretory proteins. Among them, adiponectin is the major adipocyte secretory protein most abundantly found in human plasma with potent roles in insulin sensitivity in muscle and liver, regulating energy homeostasis and glucose tolerance (Yamauchi et al., 2001). Adiponectin, a product of the *ADIPOQ* gene (also known as *APM1*, *ACRP30* or *GBP28*) spans approximately 16 kb with three exons on chromosome 3q27 and has been linked to a susceptibility locus for metabolic syndrome, type 2 diabetes and cardiovascular disease (Francke et al., 2001; Vionnet et al., 2000). Adiponectin levels have a strong genetic component, with heritability estimated between 30% and 50% (Comuzzie et al., 2001). Several genome wide association studies (GWAS) among European and Asian populations identified *ADIPOQ* locus as the major gene for variation in the serum adiponectin levels (Heid et al., 2010; Ling et al., 2009).

A number of population based studies reported an association between single nucleotide polymorphisms (SNPs) in *ADIPOQ* gene and circulating levels of adiponectin (Hivert et al., 2008; Menzaghi et al., 2007; Vasseur et al., 2002). Low circulating levels of adiponectin and SNPs of *ADIPOQ* were reported to be associated with insulin resistance, type 2 diabetes mellitus (T2DM) and central obesity (Kadowaki et al., 2006; Peters et al., 2013). There are strong evidences to prove the genetic background of diabetes in Asian Indians (Mohan, 2004). Asian Indians have a unique phenotype characterized by increased abdominal obesity and visceral fat despite low body mass index (BMI), hyperinsulinemia

Abbreviations: BMI, Body Mass Index; SNP, Single Nucleotide Polymorphism; HWE, Hardy–Weinberg Equilibrium; T2DM, Type 2 Diabetes Mellitus; LD, Linkage Disequilibrium; ADIPOQ, Adiponectin.

* Corresponding author at: Department of Molecular Genetics, Madras Diabetes Research Foundation & Dr. Mohan's Diabetes Specialities Centre, ICMR Advanced Centre for Genomics of Type 2 Diabetes, Kallam Anji Reddy Centre, Plot No. 20, Golden Jubilee Bio Tech Park, For Women Society, SIPCOT IT Park, Siruseri, Chennai 603 103, India. Tel.: +91 44 2747 0608, +91 44 4396 8888; fax: +91 44 2835 0935.
E-mail addresses: radharv@yahoo.co.in, drmohans@vsnl.net, mvdsc@vsnl.com (V. Radha).
[1] Present address: SRM Research Institute, SRM University, Kattankulathur, Chennai 603203, India.
[2] Present address: Centre for Nanotechnology and Advance biomaterials, SASTRA University, Tanjore 613402, India.

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(Mohan et al., 1986), insulin resistance (Sharp et al., 1987), and dyslipidemia (Mohan and Deepa, 2004), features that have been referred to as the "Asian Indian Phenotype" (Joshi, 2003) which results in increased susceptibility to T2DM (Abate and Chandalia, 2003). In particular, south Asians have greater predisposition to abdominal obesity and visceral fat (Banerji et al., 1999; Deurenberg et al., 1998; Misra et al., 2001). Furthermore, at any BMI, Indians were shown to have greater insulin resistance compared to other ethnic groups (Stratton et al., 2000). A recent study reported that though body mass index (BMI) is the most common measure of obesity, waist circumference has been shown to be a more accurate measure of the distribution of body fat (Brown, 2009; Dagan et al., 2013). Hence genetic studies on *ADIPOQ* variants gain importance in its relationship with diabetes, obesity and serum adiponectin levels in south Asians.

With this background, the present study has been performed with an objective to investigate the genetic association of the variants in the *ADIPOQ* gene with type 2 diabetes, obesity indexes (BMI and waist circumference) and serum adiponectin levels in the south Indian population.

2. Research design and methods

2.1. Subjects and study design

Using a case–control approach, a total of 2200 unrelated study subjects were recruited, comprising 1100 controls (normal glucose tolerant (NGT)) and 1100 cases (T2DM) (975 men and 1225 women, mean age 43 ± 14 years, mean BMI 24.2 ± 4.6 kg/m^2) from the Chennai Urban Rural Epidemiological Study (CURES), an ongoing epidemiological study conducted on a representative population (>20 years) of Chennai, the fourth largest city in India. The methodology of the study has been published elsewhere (Deepa et al., 2003) and is briefly outlined here. In Phase 1 of CURES, 26,001 individuals were recruited based on a systematic random sampling technique. Subjects with self-reported diabetes taking drug treatment for diabetes were classified as "known diabetes subjects." All known diabetes subjects ($n = 1529$) were invited to visit the center for detailed studies. In addition, every 10th individual of the 26,001 individuals without known diabetes was invited to undergo oral glucose tolerance tests using a 75-g oral glucose load (dissolved in 250 ml of water) (Phase 3 of CURES). Those who were confirmed by oral glucose tolerance test to have 2-h plasma glucose value ≥11.1 mmol/l (200 mg/dl) based on World Health Organization (WHO) consulting group criteria were labeled as "newly detected diabetes subjects" and those with 2-h plasma glucose value <7.8 mmol/l (140 mg/dl) as being NGT (Alberti and Zimmet, 1998).

2.2. Phenotype measurements

Anthropometric measurements including weight, height, and waist were obtained using standardized techniques. The BMI was calculated as weight (in kg) divided by the square of height (in m). Biochemical analyses were done on a Hitachi-912 Auto Analyzer (Hitachi, Mannheim, Germany) using kits supplied by Roche Diagnostics (Mannheim). Fasting plasma glucose (glucose oxidase–peroxidase method), serum cholesterol (cholesterol oxidase-phenol-4-amino-antipyrene peroxidase method), serum triglycerides (glycerol phosphatase oxidase-phenol-4-amino-antipyrene peroxidase method), and high-density lipoprotein cholesterol (direct method; polyethylene glycol-pretreated enzymes) were measured. Low-density lipoprotein cholesterol was calculated using the Friedewald formula (Friedewald et al., 1972). Glycated hemoglobin (HbA1c) was estimated by high-performance liquid chromatography using a Variant™ machine (Bio-Rad, Hercules, CA, USA). Serum insulin concentration was estimated using an enzyme-linked immunosorbent assay (Dako, Glostrup, Denmark). Total serum adiponectin was measured by radioimmunoassay (cat. no. HADP-61 HK; Linco Research, St. Charles, MO, USA) and the intra- and inter-assay coefficients of variation were 0.38 and 0.74, respectively, and the lower detection limit was 1 ng/ml.

Informed consent was obtained from all study participants, and the study was approved by the Madras Diabetes Research Foundation Institutional Ethics Committee.

2.3. Definition of risk factors

In the present study, associations between SNPs in the *ADIPOQ* gene and measures of obesity (BMI and waist circumference) as generalized (BMI) and central obesity (waist circumference) were investigated to evaluate the contribution of the SNPs towards the genetic risk for obesity. Generalized obesity was defined according to the World Health Organization Asia Pacific Guidelines for Asians as non-obese (BMI < 25 kg/m^2) and obese (BMI ≥ 25 kg/m^2) (Health Communications Australia Pty Ltd., 2000) and the central obesity was defined according to the same guidelines as low risk (men < 90 cm; women < 80 cm) and high risk (men ≥ 90 cm; women ≥ 80 cm) (Health Communications Australia Pty Ltd., 2000; Mohan et al., 2007).

2.4. Genetic analysis

Genomic DNA was extracted from the whole blood by the phenol-chloroform method of DNA extraction (Fritsch et al., 1982). Eight *ADIPOQ* SNPs namely, *−11391 G/A* (*rs17300539*), *−11365 C/G* (*rs266729*) in the promoter region, *−4522 C/T* (*rs822393*), *−3971 A/G* (*rs822396*) in intron 1, *+276 G/T* (*rs1501299*), *+349 A/G* (*rs2241767*), *+712 G/A* (*rs3774261*) in intron 2, and *Y111H T/C* (*rs17366743*) in exon 3 were selected based on a careful literature review and were included in the study based on their previous significant association with type 2 diabetes, obesity and serum adiponectin levels. The SNPs were identified in the NCBI database (http://www.ncbi.nlm.nih.gov/SNP) and were genotyped by polymerase chain reaction on a GeneAmp® PCR system 9700 thermal cycler (Applied Biosystems, Foster City, CA) followed by restriction enzyme digestion (New England Biolabs, Inc., Beverly, MA). Primers, restriction enzymes and the rs numbers with relative positions of the SNPs are shown in Supplementary Table 1. The resulting products were electrophoresed on a 3% agarose gel. To ensure that the genotyping was of adequate quality, we performed random duplicates in 20% of the samples. The assays were performed by a technician who was masked to the phenotype, and there was 99% concordance in the genotyping. Furthermore, a few variants were confirmed by direct sequencing with an ABI Prism® 3500 genetic analyzer (Applied Biosystems, Foster City, CA).

2.5. Statistical analysis

Statistical Package for Social Sciences for Windows version 17.0 (SPSS, Chicago, IL) was used for statistical analysis. The effects of the variants on quantitative and categorical variables were analyzed. Allele frequencies were estimated by gene counting. Agreement with Hardy–Weinberg expectations (HWE) was tested using a χ^2 goodness-of-fit test. Comparison of the means between the two groups was analyzed by Student's *t* test. The χ^2 test was used to compare the proportions of genotypes or alleles. Analyses for T2DM and NGT are given for an "additive" model in which homozygotes for the major allele (0), heterozygotes (1), and homozygotes for the minor allele (2) were coded. One-way analysis of variance was used to compare groups for continuous variables. Logistic regression analysis was used to identify the risk of the genotype combinations for T2DM and obesity. T2DM or obesity was taken as the dependent variable, and the genotypes were used as the independent variable. As subjects with diabetes were older and had higher BMI, we adjusted for age, sex, and BMI in all the logistic regression analyses. It is possible that some of our controls, who are younger, will develop diabetes because the prevalence of diabetes increases with age. In order to circumvent this problem, age was adjusted for in