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Genetic association analysis of serotonin transporter polymorphism (5-HTTLPR) with type 2 diabetes patients of Pakistani population

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ABSTRACT

Aims: It is well established that the serotonergic system contributes to the regulation of glucose homeostasis and feeding and therefore it has been presumed to contribute to the biological susceptibility to type 2 diabetes mellitus (T2DM) and body-mass index (BMI). 5-*HTTLPR* is a serotonin transporter (5-*HTT*) gene-linked polymorphic region that regulates the transcriptional activity of 5-*HTT*. Our aim was to investigate the possible association of 5-*HTTLPR* polymorphism (L and S alleles) in the promoter region of the serotonin transporter gene with T2DM and/or higher BMI in Pakistani population.

Methods: In this study, 574 subjects diagnosed with T2DM and 402 unrelated normal controls from the general Pakistani population were genotyped for 5-*HTTLPR* polymorphism by PCR amplification and agarose gel electrophoresis. The genotyping data (S/S, S/L and L/L) were recorded and analysed statistically using various software and online available tools.

Results: In the total sample, patients with type 2 diabetes and controls without diabetes, genotypes were distributed according to Hardy–Weinberg equilibrium, and S allele frequency was 61.52% (0.61). There was no statistical association between 5-*HTTLPR* polymorphism and the development of T2DM in this Pakistani population ($p = 0.12$).

Conclusions: No significant statistical association of 5-*HTTLPR* polymorphism with type 2 diabetes and obesity in Pakistani population shows that 5-*HTTLPR* polymorphism is not a major factor in determining type 2 diabetes and obesity in Pakistan.

1. Introduction

Type 2 Diabetes mellitus (T2DM) is a common metabolic disorder, affecting 85–90% of all people with diabetes Over the past few decades the rate of obesity and type T2DM has increased alarmingly worldwide. Obesity and T2DM are a growing epidemic in South Asian countries with a higher prevalence. Pakistan was ranked amongst the top 10 countries of the world with the highest number of people with diabetes in 2004 and an estimated 14.5 million Pakistanis will have diabetes by the year 2025. According to a recent national survey, 35% people over the age of 45 years have diabetes in Pakistan. Similar observations of higher prevalence of T2DM amongst immigrant South Asians have also been made. Obesity and T2DM frequently co-occur, indicating that these conditions may share common pathological mechanisms, including complex interactions between genetic and environmental factors [8]. Therefore, both environmental as well as genetic factors contribute to the incidence of this disease [10].

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Table 1 – Major clinical characteristics of the analyzed patients and control subjects.

Clinical characteristics		Type 2 diabetic patients ($n = 574$)	Non-diabetic controls ($n = 402$)	Total	p Value
Age (Years)	Mean ± SD	51.4 ± 11.3	53.1 ± 11.6	976	0.0224
	<40	71(12.4%)	18 (4.5%)	89	<0.0001
	41–60	390 (68%)	294 (73%)	684	0.0812
	>61	113 (19.6%)	90 (22.5)	203	0.3055
Gender	Male	187 (32.5%)	212 (53%)	399	<0.0001
	Female	387 (67.5)	190 (47%)	577	<0.0001
BMI (Kg/M^2)	Mean ± SD	27.6 ± 6.0	24.37 ± 6.0	976	0.0001
	<25 (Non-Obese)	188 (33%)	254 (63%)	442	<0.0001
	>25 (Obese)	386 (67%)	148 (37%)	534	<0.0001

It is complex or a multi-gene disorder with a number of genes and their polymorphisms contributing to disease pathogenesis have been identified [9]. The genes of the serotonergic system have been included in the candidate gene list due to their function in the brain and gastrointestinal tract. The serotonergic system contributes substantially to the regulation of feeding and glucose homeostasis. 5-*HTTLPR* is serotonin transporter (5-*HTT*) gene-linked polymorphic region that regulates the transcriptional activity of 5-*HTT* gene. The human 5-*HTT* gene (SLC6A4) spans 37.8 kb and is located on chromosome 17q11.1-12. The gene is composed of 14 exons and encodes a protein of 630 amino acid residues. Transcriptional activity of 5-*HTT* gene is modulated by a repetitive element of varying length in the 5′ flanking region located approximately 1.4 kb upstream of the transcription start site, termed as 5-*HTT* gene linked polymorphic region (5-*HTTLPR*). A 44 bp insertion/deletion has been identified in the promoter region, which results into long (L) and short (S) alleles. *In vitro* transfection studies have demonstrated that S allele is dominant over the long allele (L) and its presence suppresses the expression of long allele resulting in lower expression of 5-*HTT* gene, thus reducing the capability to take up and release serotonin transporter. In contrast, L allele has an almost three fold transcription rate of 5-*HTT* gene [5,6]. This suggests that 5-*HTTLPR* is associated with an altered response of the serotonin system. In previous studies a possible association of 5-*HTTLPR* with obesity and T2DM has been reported [1,7]. Keeping in view previous reports, this study was aimed to compare the frequencies of different alleles of 5-*HTT* gene between patients with type 2 diabetes and the normal controls and to find out association of 5-*HTT* gene polymorphism with T2DM or BMI in Pakistani population.

2. Material and methods

A total of 574 unrelated subjects diagnosed with T2DM (187 males and 387 females) and 402 unrelated controls (212 males and 190 females) from Pakistani population were collected and studied. All individuals were recruited from various hospitals located in Islamabad and in its vicinity. Inclusion of subjects in the group with diabetes was based on medical diagnosis, according to the criteria recommended by the WHO for T2DM [2]. Subjects with either diagnosis or a first-degree family history of type T2DM were excluded from the control sample. BMI was calculated as the ratio between weight and the square of the height (kg/m^2). Subjects were also divided into overweight/obese (BMI > 25) and non-obese (BMI < 25) groups. The other clinical parameters of the patients recorded were age of onset of the diabetes, systolic/diastolic blood pressures, fasting/random blood glucose, HbA1C are shown in Table 1. The study protocol for human experimentation was approved by the institutional ethics committee. Informed consent was obtained from each subject before participation in the study. Genomic DNA was isolated from peripheral blood with standard organic method of DNA extraction.

2.1. 5-*HTTLPR polymorphism genotyping*

Polymerase Chain Reaction (PCR) for 5-*HTTLPR* polymorphism genotyping was performed as previously described Cook et al. (1997), with small modifications. The polymorphic region was amplified by using 5-*HTT*_F, 5′-TGAATGCCAGCACCTAACCC-3′, and 5-*HTT*_R, 5′-TTCTGGTGCCACCTAGACGC-3′ as forward and reverse primers respectively. This set of primers generated Amplicons of 406 bp for S allele and Amplicons of 450 bp for L allele. Each reaction was carried out in a total volume of 25 μl containing 200 μM of dNTP, 1 unit of *Taq DNA* polymerase (Fermentas), 1.5 mM of MgSO4, 5% DMSO and 10 mM of Tris-HCl buffer.

PCR amplification was performed in a GeneAmp® PCR system 9700 System (Applied Biosystem) Thermal Cycler. PCR amplification was carried out for 40 cycles consisting of 30 s at 95 °C, 30 s at 61 °C, and 1 min at 71 °C, followed by 10 min at 72 °C. PCR products (Amplicons) were separated by electrophoresis on a 6% polyacrylamide gel at 120 V for 3 h. The gels were stained with ethidium bromide, visualised under UV-transillumination and photographed. Genotyping was performed by classifying subjects into three genotypes: individuals homozygous for the short allele SS, those heterozygous for the short and long allele LS, and those homozygous for the long allele LL.

2.2. *Statistical analysis*

Hardy–Weinberg equilibrium (HWE) was analysis, genotype distribution and allele frequencies between T2DM patients and controls without diabetes were determined using online available statistical tools, such as SNPStats [12], Vasser stats, HWE calculator for two alleles and SPSS software. The clinical data is shown as means and standard deviation (SD). Deviation from Hardy-Weinberg equilibrium was confirmed by χ^2 test analysis of genotype distribution. The association between 5-*HTTLPR* and T2DM/BMI, was defined by logistic regression analysis, assuming an additive model. Considering gender, age and T2DM as the appropriate covariates, associations were adjusted accordingly. The odds ratios (ORs) with

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