Clinica Chimica Acta 411 (2010) 167–171

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

The serotonin transporter promoter polymorphism (5-HTTLPR) is associated with type 2 diabetes

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ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form 26 October 2009
Accepted 26 October 2009
Available online 2 November 2009

Keywords:
Serotonin
5-HTTLPR polymorphism
5-HTT gene
Type 2 diabetes mellitus
Obesity

ABSTRACT

Background: The serotonergic system contributes substantially to the regulation of glucose homeostasis and feeding. 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 with type 2 diabetes mellitus and obesity.
Methods: Study population consisted of 252 subjects diagnosed with Type 2 DM and 211 non-diabetic subjects, all Caucasians of Greek ethnic origin. Genomic DNA was extracted from peripheral blood and analyzed for 5-HTTLPR polymorphism with a novel PCR protocol.
Results: The frequency of SS and SL genotypes of HTTLPR was significantly higher in the diabetic group (77.0%) than in the non-diabetic group (61.6%) ($P<0.001$). The genetic risk of Type 2 DM for subjects carrying at least one S allele was increased compared to non-diabetic subjects (OR = 2.08, 95% CI = 1.39–3.12). When subjects were divided according to BMI status, the frequency of S allele carriers was similar in obese and non-obese subjects.
Conclusions: The S allele of 5-HTTLPR is strongly associated with the presence of Type 2 DM. This association appears to be direct and not dependent on obesity status. Therefore, 5-HTTLPR LL genotype might be protective for development of Type 2 DM.

1. Introduction

Type 2 diabetes mellitus is a common metabolic disorder affecting millions of people worldwide defined by insulin resistance and an insulin secretory defect resulting in impaired glucose tolerance and hyperglycaemia. Its pathogenesis appears to involve complex interactions between genetic and environmental factors. On the other hand, obesity is a major risk factor for Type 2 DM associated with insulin resistance.

A growing number of studies suggest that central neural pathways may play an important role in regulation of glucose homeostasis. Serotonin (5-hydroxytryptamine, 5-HT) is one of the most important neurotransmitters implicated in the regulation of energy balance through both central modulation of the activity of various downstream neuropeptide systems and autonomic pathways and also peripheral mechanisms [1].

Serotonin transporter (5-HTT or SERT) is the major factor responsible for the inactivation of serotoninergic transmission after serotonin release in the synaptic cleft [2]. It is a sodium-dependent transporter that regulates the entire serotoninergic system and its receptors as it is responsible for the active transport of serotonin into neurons, enterochromaffin cells, platelets, and other cells modulating extracellular fluid serotonin concentrations [3]. Human 5-HTT gene spans 37.8 kb on chromosome 17q11.2 and is composed of 14 exons encoding a protein of 630 amino acids [4]. Transcriptional activity of human 5-HTT gene is modulated by a repetitive element of varying length in the 5' flanking region located ~1.4 kb upstream of the transcription start site, termed 5-HTT gene-linked polymorphic region (5-HTTLPR). 5-HTTLPR is only present in humans and higher non-human primates and a typical insertion/ deletion of 44 bp results in the presence of two alleles, the "long" (L) comprising 16 copies of a 20–23 bp repeat unit and the "short" (S) comprising 14 copies [5].

Human 5-HTT gene is differentially modulated by the allelic variants of the 5-HTT gene promoter at both transcriptional and translational levels modifying 5-HTT function [6]. S allele is dominant and its presence is associated with lower expression of 5-HTT gene, resulting in a reduced capability to take up and release 5-HT [5,7]. In contrast, the L variant was associated with an almost threefold increased transcription of the 5-HTT gene [5].

A possible association of 5-HTTLPR with Type 2 DM has been implied in diabetic animals that exhibit altered neurotransmitters in

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doi:10.1016/j.cca.2009.10.022

brain monoaminergic systems. Particularly, it was suggested that altered monoamine transporter gene expression may contribute to the observed dysfunctions in brain monoamine transmission in chronic diabetes in mice [8]. Additionally, in a study with 264 Japanese women not on medication for diabetes, hypercholesterolemia or hypertension, subjects with SS genotype of 5-HTTLPR showed larger change in fasting blood glucose between beginning and end of the study in comparison with other genotypes [9].

Polymorphisms in 5-HTT gene have been shown to influence satiety and sensory aspects of energy balance [10]. To the best of our knowledge, no studies have been published on the association of 5-HTTLPR with Type 2 DM. Thus, the aim of the present study was to investigate the possible association of the 5-HTTLPR polymorphism with Type 2 DM in a human population of Caucasian origin. In addition we assessed the relation of 5-HTTLPR with obesity in this population.

2. Patients and methods

A total of 463 subjects were included in this study. The diabetic study population consisted of 252 subjects diagnosed as having Type 2 DM (122 males and 130 females) aged 67.4 ± 8.8 years. The control (non-diabetic) study population consisted of 211 subjects (118 males and 93 females) aged 66.3 ± 12.8 years. Matching of subjects was performed on a frequency basis. All subjects were of Caucasian ethnic origin, Greek citizens residing in Alexandroupolis urban area but with origins from all parts of Greece. From November 2006 to December 2007, 314 consecutive diabetic patients were presented in the Outpatient Diabetic Clinic of the Internal Medicine Department of the Academic General Hospital of Alexandroupolis. From these patients, 43 did not meet inclusion criteria and 19 refused enrollment. Inclusion of the remaining 252 subjects in the diabetic group was based on the criteria recommended by WHO for Type 2 DM (http://www.aafp.org/afp/981015ap/mayfield.html). According to these criteria, diagnosis of Type 2 DM was based on two measurements of fasting plasma glucose levels of 126 mg/dl (7.0 mmol/l) or higher or two casual glucose readings of 200 mg/dl (11.1 mmol/l) or higher or if Type 2 diabetes was controlled by medication. Subjects included in the control population had been at the Internal Medicine Department of the same hospital in the same period for reasons unrelated to Type 2 DM and had received a thorough medical examination, including specific evidence (medical and drug history taken by a specialist clinician, two measurements of fasting plasma glucose <126 mg/dl) to exclude the presence of Type 2 DM. They were age and sex-matched with the diabetic group. No subjects diagnosed with psychiatric diseases or on treatment with 5-HT agonists or antagonists (such as typical and atypical antipsychotic agents) were included in this study. Furthermore, patients with pulmonary hypertension or known cardiovascular disease were excluded from the study population.

Subjects were also divided to obese and non-obese groups on the basis of their BMI. Of the total 463 subjects, 182 (80 males and 102 females) were classified as obese (BMI ≥ 30 kg/m^2) and 281 (160 males and 121 females) were classified as non-obese (BMI < 30 kg/m^2). Glucose levels, urea, creatinine and lipid profile (total cholesterol, HDL-cholesterol, and triglycerides) were calculated by standard enzymatic methods, while LDL was calculated by Friedewald equation. Blood samples were obtained after an overnight fast. Blood pressure was measured with a mercury sphygmomanometer. Hypertension was defined as known or newly diagnosed hypertension according to current national guidelines (Systolic BP / Diastolic BP > 140/90 mmHg) or if hypertension was controlled by medication. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). The study was approved by the Scientific Council and the Ethics Committee of the Academic General Hospital of Alexandroupolis, Greece and was conducted according to the Declaration of Helsinki. All subjects participated after being informed about the study by their attending clinician and giving written consent.

Genomic DNA was extracted from white blood cells in peripheral venous blood by Puregene DNA Purification System (Gentra, Minnesota, MI, USA) and analyzed for the 5-HTTLPR polymorphism, taking into account previous reports presenting the difficulties in genotyping this polymorphism concerning Mg concentrations [11,12]. A novel set of primers was designed with the aid of the Oligo-6 software (NBI, Plymouth, USA) at very high stringency conditions. Using polymerase chain reaction (PCR), DNA was amplified with the following primer set: [5 -GTTTTGTGTTGCCCTTGCCTAT - 3] and [5 - CACCGCCCCTTGTACTTG - 3] to generate 705- or 749-bp fragments. The PCR reaction was performed in a 50 μl aliquot of the reaction mixture with 0.5 μg genomic DNA; 0.2 mM dNTP mix; 60 pmol of each primer; 1.2 mM $MgCl_2$ and 2.5 IU of Taq polymerase. All reagents for PCR amplification were supplied by Invitrogen (Carlsbad, CA, USA). After an initial denaturation step at 94 °C for 10 min, the cycling parameters were 42 cycles with denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. All PCR amplifications were carried in the PCR-engine apparatus PTC-200 of MJ Research (Watertown, Mass., USA). The PCR products were visualized on a 1.5% agarose gel stained with ethidium bromide to determine each subject's genotype. The L allele of 5-HTTLPR was denoted by the presence of 749 bp fragment and the S allele by the presence of 705 bp fragment. All genotype determinations were carried out in duplicate with identical results for all 463 subjects genotyped.

All quantitative data are presented as mean ± standard deviation (SD). Relative frequencies of genotypes and alleles were calculated for each group and a chi-square analysis was conducted comparing the distribution of genotypes and alleles between diabetic and non-diabetic subjects as well as between obese and non-obese subjects. Comparisons for continuous or categorical data between two groups were conducted using an independent *t*-test or chi-square test, respectively. To estimate the risk of Type 2 DM or obesity associated with 5-HTTLPR polymorphism, odds ratios (OR) were calculated using logistic regression analysis before and after adjustment for other factors known to affect these conditions. A $P < 0.05$ was considered statistically significant. Analyses were carried out with the use of SPSS software package (15.0 for Windows).

3. Results

Demographic and clinical variables of diabetic and non-diabetic subjects, as well as obese and non-obese subjects are shown in Table 1. Subjects were age matched and the frequency of male and female subjects did not differ significantly between study groups. As expected, subjects in the diabetic group had significantly higher blood glucose levels than subjects in the non-diabetic group. Furthermore, in the diabetic and obese groups, subjects had higher body weight and BMI than subjects in the non-diabetic and non-obese group, respectively. Regarding other clinical variables, subjects in the non-diabetic group had significantly higher total cholesterol and LDL levels than subjects in the diabetic group ($P < 0.005$). Similarly, subjects in the non-obese group had lower triglycerides and higher HDL levels ($P < 0.05$) compared to the subjects in the obese group. The higher total cholesterol and LDL levels observed in non-diabetic vs. diabetic subjects could possibly be attributed to their controlled diet and treatment for dyslipidemia of diabetic patients. However, no associations were found between frequencies of 5-HTTLPR alleles and these clinical variables (data not shown).

Frequencies of 5-HTTLPR genotypes and alleles in diabetic and non-diabetic group are shown in Table 2. Genotype and allele frequencies, tested using conventional x^2 test, were in Hardy-Weinberg equilibrium both in diabetic and non-diabetic group. The