Obesity & Weight Loss Therapy

Research Article Open Access

The *Serotonin* 2A receptor *(SER 2A)* Gene Polymorphism and its association with Obesity and Dyslipidemia in Semi Urban Subjects of Tamilnadu, South India

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Abstract

Background: Serotonin is a neurotransmitter that regulates many physiological processes such as appetite, hunger, hormone secretion and sleep. Abnormalities in the serotonin transmission pathway have been implicated in obesity but no studies in Asian Indians of South India have been conducted so far.

Objective: This case - control study *(n=374)* on semi urban subjects of Tamil Nadu, India was conducted to analyse the association of *1438 G/A* polymorphism in serotonin receptor gene with obesity, dyslipidemia and smoking.

Methodology: A detailed Questionnaire based interview was conducted between the years 2006 and 2011. Obese subjects (*n* =325) who visited the obesity unit of the hospital were recruited by purposive random sampling method, with informed and written consent. Anthropometry and clinical analysis was performed. Genotyping was carried out by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.

Results: Gender wise, in obese cases, the frequency of the GG genotype (0.49) was higher in females than males. In obese males the frequency of the GA genotype (0.43) was higher than the frequencies GG genotype (0.39) and AA genotype (0.16). Furthermore, subjects with GG genotype had eight times higher risk of developing obesity (Odds ratio: 8.06, *P* = 0.00 & 95 % Confidence interval = 4.14 – 15.69) and subjects with GG genotype for serotonin 2A receptor had four times higher risk (Odds ratio 4.6, *P* = 0.000, 95% Confidence interval = 2.32 – 9.12) for developing hypertriglyceridemia.

Discussion: Though the frequency of the AA genotype was comparably lower in obese cases than in controls *(p<0.05)*, the AA genotype had a more pronounced effect on clinical factors namely BMI, WHR and TGL levels.

Keywords: Hypertriglyceridemia; Serotonin receptor polymorphisms; Asian Indians

Introduction

Obesity as a public health problem in the Indian population demands social and medical attention [1]. The co morbidities of obesity are increasing prevalent in urban Indians [2]. Genes with known psycho physiological functions have gained much attention in the field of obesity research and a common pathophysiology has been suggested between obesity and related metabolic disorders [1,3]. The neurotransmitters - serotonin, nor adrenaline and dopamine are important in the neuroregulation of many physiological processes including hunger, energy and glucose homeostasis [4,5]. The human 5HT 2A gene is located on 13q14 – q 21 [6] and consists of three exons separated by two introns and spans over 20 kb [7]. This gene has been implicated in individual food preferences [8] abdominal obesity [9,10], fatty liver disease [11], generalized obesity, type two diabetes [12], and hypertension [13] but the results have been confounding between different populations and ethnic groups. There are no previous reports on this polymorphism in association with obesity and dyslipidemia in Asian Indians from south India. Thus in this case control study, the possible association of serotonin 2A receptor gene polymorphism with obesity and dyslipidemia was tested in semi urban subjects of Coimbatore, South India. Further, the association of this polymorphism with smoking in obese men of the study group was also analysed.

Methodology

The study was approved by the human ethics committee of "Kovai medical center and hospital", Coimbatore, Tamil Nadu. The population of this city largely consists of people who have migrated from rural to urban areas for economic prospects. A detailed Questionnaire based interview was conducted between the years 2006 and 2011. Obese subjects (*n* =325) who visited the obesity unit of the hospital were recruited by purposive random sampling method, with informed and written consent. The interview recorded details of demographic profile, socio-economic data, and dietary habits. Out of 325 obese subjects, 208 (64%) subjects consented for genetic analysis. The cases *(n*=208*)* and controls (n=166) for the study were determined in accordance to the Asia pacific guidelines for defining obesity [14]. The controls comprised of non obese, non diabetic individuals who performed intensive physical workouts for at least one hour daily in fitness centres with standard exercise equipment. A subject was defined as a smoker or alcoholic if a history of smoking or alcoholism was recorded for at least one year during the period of study.

Clinical assessment

Subjects who were enrolled in this study were instructed to report to the clinic in fasting state and in light clothing. Further they were

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Received May 29, 2013; **Accepted** June 19, 2013; **Published** June 21, 2013

Citation: Shajithanoop S, Tamilselvi periyasamy, Rathinavel SM, Usha Rani MV (2013) The *Serotonin* Receptor 2A *(SER 2A)* Gene Polymorphism and its association with Obesity and Dyslipidemia in Semi Urban Subjects of Tamilnadu, South India. J Obes Weight Loss Ther 3: 178. doi:10.4172/2165-7904.1000178

instructed to void urine prior to clinical assessment. Male and female nurses recorded anthropometric measurements for the subjects of respective genders. Body weight was recorded to the nearest 0.1 kg using a weighing machine calibrated for accuracy. The height was recorded to the nearest 0.1 cm. BMI was expressed in kg/m^2 Waist circumference was measured using a non stretchable measuring tape with the participant standing erect, abdomen relaxed, arms at the side and feet together with weight divided equally over both legs. Participants were told to breathe normally and to breathe out gently at the time of measurement. Hip circumference was measured at the level of the maximal protrusion of the gluteal muscles. It was expressed as centimetres (cms). Waist hip ratio (WHR) was calculated by the formula waist (cms)/Hip (cms). Body mass Index [BMI] was calculated by the formula - Weight (kg)/Height (m^2) and expressed as kg/m^2 [15]

Metabolic parameters

5 ml of venous blood samples for biochemical analysis were obtained after an 8 hour overnight fast. The samples were stored on ice and transported to the laboratory immediately. Serum was obtained by centrifugation at 1600 rpm for 20 minutes at 4°C and an aliquot of serum was tested for total cholesterol (TC) and Triglycerides (TGL) using a semi automated analyzer using commercial reagents. (Medsource one biomedicals Private Ltd., India). Total cholesterol was measured by Cholesterol oxidase - peroxidase (CHOD – POD) method. Fasting blood glucose (FBG) was measured by Glucose oxidase – Peroxidase method (GOP–POD). High density lipoprotein cholesterol [HDL-C] was determined by precipitation with phosphotungstic acid and magnesium chloride as per the instructions of the manufacturer. Low density lipoprotein cholesterol [LDL-C] was estimated by Friedewald formula [16]. Very low density lipoprotein cholesterol was abbreviated as VLDL-C. All biochemical values were expressed in milligrams per decilitre (mg/dl).

Clinical standards

Asian Indians have higher percentage of body fat, abdominal obesity at lower or similar BMI levels as compared to white Caucasians. Therefore obesity in this study was defined in accordance to the Asia Pacific guidelines. High WHR in males is defined as >0.95 and >0.80 in females [15]. Dyslipidemia was defined by the criteria formulated by the National cholesterol education program, Adult treatment panel 111 (NCEP–ATP III) [17]. Dysglycemia was defined in accordance to IDF definition [18].

Genotype analysis

Five milliliter of fasting venous bloods from consenting cases and controls were collected in sterile EDTA coated vaccutainers and coded appropriately. DNA was isolated by a non enzymatic method [19] and was amplified by PCR using primers as mentioned by Sorlí et al. [20] with initial denaturation at 94°C for 3 minutes, final denaturation at 94°C for 15 seconds, annealing at 55°C for 15 seconds, initial extension at 72°C for 30 seconds and final extension at 72°C for ten minutes, to obtain a 497 base pair amplicon. A total of 35 cycles was essential for amplification. Restriction digestion of PCR amplicon with *Msp I* restriction enzyme (New England Biolabs, USA) resulted in two fragments of 236 and 261 base pairs for the G allele whereas the allele A was undigested. The fragments were separated by electrophoresis on a 2% agarose gel.

Data analysis

Demographic and clinical factors were entered into an Excel spread sheet and double checked for errors. The comparison of mean differences between cases and controls, smokers and non smokers, was performed by student's *'t'* test and one way ANOVA. The correlation between anthropometric factors and bio-chemical parameters was tested by Pearson's two tailed correlation test. Allelic frequencies were determined by chi square test and the association of genotypes with obesity and elevated triglycerides was determined by Univariate logistic regression analysis. A *p* value less than or equal to 0.05 was considered significant. Linear regression analysis was performed to test the association of genotypes with smoking in males subjects of this study. Statistical analyses were performed by SPSS (version 11, Stat corp USA.)

Results

Gender wise, in obese cases, the frequency of the GG genotype (0.49) was highest among obese females than males. In obese males the frequency of the GA genotype (0.43) was high followed by GG genotype (0.39) and AA genotype (0.16) (Table 1a and 1b).The frequencies were significantly in Hardy Weinberg equilibrium with Chi square p value of 0.03. In non obese controls, the frequency of the GA genotype (0.53) was higher in males than females. The frequency of the GG genotype was lowest (0.15) in females than males (0.20). The chi square p value was 0.24 and thus insignificant. Univariate regression analysis revealed that subjects with GA genotype for serotonin 2A receptor had two times higher risk of developing obesity (Odds ratios 2.9, p = 0.00, and 95% Confidence Interval 1.59 -5.32) than subjects of AA genotype. On the other hand subjects with GG genotype had eight times higher risk of developing obesity (Odds ratio: 8.06, p = 0.00 and 95% Confidence interval = 4.14 – 15.69).

Logistic regression analysis with TGL as the outcome variable revealed that subjects with GG genotype for serotonin 2A receptor had four times higher risk (Odds ratio 4.6, p = 0.000, 95% Confidence interval = 2.32 – 9.12) for developing hypertriglyceridemia than subjects with GA genotype (Odds ratio 2.76, p = 0.00, 95% Confidence interval = 1.43 – 5.43). Clinical factors associated with elevated TGL levels were LDL, VLDL, BMI and WHR. A positive two tailed correlation was observed between BMI and WHR, TGL and LDL but a negative correlation was observed between BMI and HDL. The mean BMI, WHR and TGL levels were highest among obese cases of AA genotype than subjects of GG and GA genotypes (Tables 2a-2c). Among non obese control subjects, the lowest BMI was recorded in subjects of GG genotype. Likewise the mean TGL and LDL levels were lowest in subjects of GG genotype. Pearson's two tailed correlation test was applied to test the association of BMI with clinical factors. Among subjects of GG genotype, BMI was positively associated with WHR, TC, TGL and LDL. A negative correlation was observed between BMI and HDL which indicates that as BMI increases, the levels of HDL decrease.

Table 1 (a): Frequency distribution of genotypes in obese cases for *SER2A* receptor.

Obese cases	AA genotype	GG genotype	GA genotype
Male	0.16	0.39	0.43
Female	0.03	0.49	0.47

Table 1(b): Frequency distribution of genotypes in non obese controls for *SER2A* receptor.

Non Obese controls	AA genotype	GG genotype	GA genotype
Male	0.25	0.20	0.53
Female	0.37	0.15	0.46

Values are presented as mean and S.D with 95% CI.

Table 2 (a): Anthropometric and biochemical features of GG homozygotes.

Clinical Variable	*Controls (n=31)*	*Cases (n=95)*	*P Value* *
BMI (kg/m2)	22.53 ± 3.89	30.04 ± 2.62	0.00
WHR	0.87 ± 0.04	0.93 ± 0.08	NS
FBG (mg/dl)	105.51 ± 10.8	109.36 ± 19.06	NS
TC (mg/dl)	162.60 ± 10.11	180.75 ± 15.12	0.00
TGL (mg/dl)	123.13 ± 18.91	176.24 ± 17.24	0.00
HDL (mg/dl)	45.72 ± 8.03	29.04 ± 8.18	0.00
LDL (mg/dl)	93.47 ± 11.7	134.04 ± 14.16	0.00
VLDL (mg/dl)	25.33 ± 5.91	27.60 ± 11.82	NS
LDL/HDL Ratio	2.05 ± 0.05	4.82 ± 1.02	0.00

Table 2 (b): Anthropometric and biochemical profile of AA homozygotes.

Clinical Variable	*Controls (n=50)*	*Cases (n=19)*	*P Value*
BMI (kg/m2)	22.96 ± 4.03	32.37 ± 3.50	0.00*
WHR	0.85 ± 0.08	0.97 ± 0.08	0.00*
FBG (mg/dl)	100.89 ± 15.68	104.12 ± 7.7	NS
TC (mg/dl)	167.75 ± 15.9	168.78 ± 19.63	0.000
TGL (mg/dl)	108.73 ± 14.8	185.21 ± 16.73	0.000
HDL (mg/dl)	52.79 ± 9.7	35.06 ± 13.4	0.000
LDL (mg/dl)	92.06 ± 9.0	137.00 ± 15.31	NS
VLDL (mg/dl)	23.42 ± 4.92	24.31 ± 8.31	0.000
LDL/HDL Ratio	1.74 ± 0.47	4.46 ± 1.5	0.00

Table 2 (c): Anthropometric and biochemical features of GA homozygotes.

Clinical Variable	*Controls (n=85)*	*Cases (n=94)*	*P Value*
BMI (kg/m2)	23.81 ± 1.4	29.11 ± 2.63	0.000*
WHR	0.87 ± 0.06	0.92 ± 0.07	0.000
FBG (mg/dl)	103.61 ± 9.89	110.10 ± 18.69	0.005
TC (mg/dl)	172.10 ± 8.5	183.28 ± 12.84	0.018
TGL (mg/dl)	131.93 ± 18.4	177.44 ± 22.4	0.000
HDL (mg/dl)	49.6 ± 6.86	29.98 ± 10.89	0.000
LDL (mg/dl)	97.2 ± 6.33	132.57 ± 15.73	0.000
VLDL (mg/dl)	26.60 ± 6.2	26.08 ± 4.99	NS
LDL/HDL Ratio	2.03 ± 0.057	4.77 ± 1.2	0.000

Discussion

In this case control study, the possible association between the *-1438 G/A* polymorphism and obesity was assessed in semi urban subjects of Tamil Nadu, India. Among the GG, AA and GA genotypes obtained for the serotonin 2A receptor, the AA genotype was associated with higher BMI and WHR than GG and GA genotypes. Though the frequency of the AA genotype was comparably lower in obese cases than in controls *(P<0.05)*, the AA genotype had a more pronounced effect on clinical factors namely BMI, WHR and TGL levels. This is similar to the observations of Santos et al. [21] wherein a lower percentage (17.7%) of AA genotype was observed than AG and GG genotypes, In a case control study between obese and non obese controls, Rosmond et al. [9] observed a higher frequency of the G/A genotype (51.6%) followed by A/A (35.6%) and G/G genotype (12.9%) in obese men, which are similar to the observations of the present study. Gender wise, the frequency of the G/G allele was higher in obese females than in obese males. The mean WHR (0.92) in obese subjects of A/A genotype in the present study was higher than the observations of Rosmond et al. [9,10] indicating the possible role of the A/A genotype with abdominal obesity and hypertriglyceridemia in the study subjects. Sorli et al. [20] reported that the A/A genotype was associated with lower BMI and WHR, but the present study observed the association of the AA genotype with elevated BMI, WHR and TGL levels. This could be probably due to ethnic differences of the study population with those of previous reports. In frequency distribution, the GA genotype (45.67%) and GG (45.19%) were more predominant than the AA genotype. It can be postulated that the GA and GG genotype are highly associated with obesity in concordance to earlier reports. The association of genotypes with BMI and TGL levels was tested in separate linear regression models. The AA genotype predisposes obese individuals to hyper triglyceridemia than GA and GG genotypes. Rosmond et al. [9,10] observed elevated triglyceride levels in obese subjects of GA genotype and Muldoon et al. [22] revealed an association between reduced central serotonergic response and the metabolic syndrome in overweight, insulin-resistant and dyslipidemic individuals. Conversely, Hinney et al. [23] did not find any association between the 5-HTT variant and body weight, although the study was focused on the allele distribution in relation to *anorexia nervosa* patients and extremely obese individuals. Other studies [24-26] added evidence to original finding that the frequency of the A allele of the -1438A/G polymorphism was significantly higher in patients with eating disorders in control subjects.

The -1438 *G/A* polymorphism in the serotonin 2A receptor is a functional SNP and the presence of the A allele increases the transcriptional activity of the gene [27]. This polymorphism also influences individual predisposition to smoking and alcohol dependence. The probable association of this SNP in male obese smokers was also analysed. In this study group, only male subjects were smokers and alcoholics. Among obese males, the AA genotype was associated more with smoking and alcoholism (r^2=0.835) than GA and GG genotypes. These subjects reported a history of chronic addiction to smoking and alcoholism for more than four years. Pearson's two tailed correlation test revealed a positive two tailed correlation with alcoholism and smoking only in obese subjects of AA genotype similar to the observations of Polina et al. [27] in brazilian smokers. On the contrary, Terayama et al. [28] found no association between the A-1438G polymorphism and smoking in the Japanese population. Smoking and alcoholism initiate the risk for arthrosclerosis and metabolic disorders such as type 2 diabetes and hypertriglyceridemia. It is evident that if obese men of the study group do not adhere to lifestyle modification, may develop type two diabetes and cardiovascular diseases. Among numerous molecular markers, genetic screening of obese smokers and alcoholics for polymorphisms in the serotonin *2A* receptor assumes significance in view of the need for appropriate clinical treatment of obesity due to genetic etiology.

Acknowledgement

The authors are thankful to the subjects who participated in this study. Financial support as Major Research Project from University Grants Commission, New Delhi, India, to the corresponding author is gratefully acknowledged.

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