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Is there an association between T102C polymorphism of the serotonin receptor 2A gene and urinary incontinence?

C.H.A. Schwanke[1], L. Bittencourt[1], J.A.P. Noronha[2], S.A.J. Augustin[3], I.E. Jung[4] and I.B.M. Cruz[5]

[1]Faculdade de Biociências, [2]Faculdade de Medicina, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brasil
[3]Universidade Luterana do Brasil, Canoas, RS, Brasil
[4]Universidade Regional do Noroeste do Estado do Rio Grande do Sul, Ijuí, RS, Brasil
[5]Departamento de Morfologia, Centro de Ciências da Saúde, Universidade Federal de Santa Maria, Santa Maria, RS, Brasil

Abstract

The regulation of bladder function is influenced by central serotonergic modulation. Several genetic polymorphisms related to serotonin control have been described in the literature. T102C polymorphism of the serotonin receptor 2A gene (5-HT2A) has been shown to be associated with certain diseases such as non-fatal acute myocardial infarction, essential hypertension, and alcoholism. In the present study, we examined the association between 5-HT2A gene polymorphism and urinary incontinence in the elderly. A case-control study was performed in 298 elderly community dwellers enrolled in the Gravataí-GENESIS Project, Brazil, which studies gene-environmental interactions in aging and age-related diseases. Clinical, physical, biochemical, and molecular analyses were performed on volunteers. 5-HT2A genotyping was determined by PCR-RFLP techniques using the *Hpa*II restriction enzyme. The subjects had a mean age of 68.05 ± 6.35 years (60-100 years), with 16.9% males and 83.1% females. The C allele frequency was 0.494 and the T allele frequency was 0.506. The CC genotype frequency was 21.78%, the CT genotype frequency was 55.24% and the TT genotype frequency was 22.98%. We found an independent significant association between the TT genotype (35.7%) and urinary incontinence (OR = 2.06, 95%CI = 1.16-3.65). Additionally, urinary incontinence was associated with functional dependence and systolic hypertension. The results suggest a possible genetic influence on urinary incontinence involving the serotonergic pathway. Further investigations including urodynamic evaluation will be performed to better explain our findings.

Key words
- T102C polymorphism of the serotonin receptor 2A gene
- 5-HT2A gene polymorphism
- Urinary incontinence
- Elderly patients

Correspondence
C.H.A. Schwanke
Faculdade de Biociências, PUCRS
Av. Ipiranga, 6690-219
90610-000 Porto Alegre, RS
Brasil
Fax: +55-51-3320-5075
E-mail: chashwanke@uol.com.br

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Introduction

The prevalence of urinary incontinence in persons over the age of 60 years living in the community can be as high as 15-35%, resulting in elderly institutionalization and increased risk of urinary tract diseases. Aging causes a number of changes in urinary tract physiology that can affect continence: a) a decrease in bladder elasticity, which decreases bladder capacity and requires the older adult to void more frequently, b) a

decrease in the strength of the detrusor muscle, resulting in incomplete bladder emptying, c) an increase in spontaneous detrusor muscle contractions, d) a decrease in the ability to postpone urination, e) a decrease in urethral closing pressure, and f) a decrease in the ability of kidneys to concentrate urine, causing an increase in urine volume. Also, some risk factors can precipitate urinary incontinence, such as female gender (women are twice as likely as men to suffer from the condition), iatrogenic causes of incontinence (the use of some medications that affect α- and ß-adrenergic and cholinergic receptors and diuretics), immobility, and cognitive impairment (1). Urinary system physiology shows that the normal micturition cycle requires a complex interaction between various anatomical structures, where the central nervous system (spinal and supraspinal centers) controlling urine storage and voiding (2).

Neurochemical studies have shown that sphincter motor neurons exhibit high concentrations of various neurotransmitters and receptors. The Onuf nucleus (segments S1-S2 or S2-S3 of the sacral spinal cord), which innervates the urethral striated muscle (rhabdosphincter), has remarkable associations with serotonin (5-HT) and norepinephrine (NE). Compared with other areas in the ventral horn of the sacral spinal cord, the Onuf nucleus shows dense staining of noradrenergic and serotonergic terminals and also contains neurotransmitter receptors such as 5-HT1 and 5-HT2 receptors (2).

Neither NE nor 5-HT has a direct effect on motor neurons; instead, these neurotransmitters only seem to facilitate the effects of the neurotransmitter glutamate, which can directly activate motor neurons. Glutamate appears to be the principal "on" switch for urine storage, whereas 5-HT and NE largely appear to have modulating functions (2).

Physiological evidence shows that 5-HT plays a role in the regulation of urinary control (3,4). Recently, the regulation of bladder function via central serotonergic modulation of these sensory pathways has been the focus for the development of pharmacological treatments of bladder dysfunction (5). de Groat (3) reviewed the influence of the central serotonergic mechanism on lower urinary tract function and concluded that, in experimental animals, spinal reflex circuits involved in voiding exhibit: a) a dense serotonergic innervation, b) multiple 5-HT receptors, and c) sensitivity to 5-HT receptor agonists and antagonists and to 5-HT reuptake inhibitors. The latter author also postulated that although there is some evidence for serotonergic facilitation of voiding in the rat, the great majority of experiments in both the rat and cat indicate that activation of the central serotonergic system can suppress voiding by enhancing the efferent control of the urethral outlet and by inhibiting parasympathetic excitatory input to the urinary bladder. Cohen (6), in a review article, concluded that there is marked species variability in responsiveness to serotonin and indicated that contraction in response to serotonin in the canine bladder is mediated by activation of the 5-HT2 receptor.

If this notion is true, 5-HT receptors, as well as 5-HT2A receptors, could have a role in the etiology of urinary incontinence. The human 5-HT2A receptor is a post-synaptic receptor present in many neocortical areas, but scarcely present in the hippocampus and completely absent in the raphe (7). Many actions of serotonergic agents are attributed to the 5-HT2A receptor, which appears to play a role in several physiological functions such as thermoregulation and sleep (5). Chronic administration of tricyclic or monoamine oxidase-inhibiting antidepressants results in the down-regulation of 5-HT2 receptors. Drug classes that have a similar function, such as imipramine which is used as an agent that blocks the reuptake of 5-HT at nerve terminals, have been shown to increase the bladder volume threshold by activating the spinal micturition reflex in the

anesthetized rat. These results suggested that 5-HT inhibitory mechanisms are involved in the modulatory effect of imipramine on the spinal micturition reflex pathway (8).

On the other hand, smooth muscle fiber contraction and platelet activation are known to be 5-HT2A-mediated physiological functions. Therefore, the 5-HT2A receptor has been implicated in the pathogenesis of acute myocardial infarction (AMI) (9).

These lines of evidence led to a molecular approach in the search for a possible association between serotonergic gene polymorphism and neural, psychiatric and cardiovascular functions or disorders. The 5-HT2A receptor gene is located on chromosome 13 (13q14-21) and consists of three exons spanning more than 20 kb. Polymorphisms of the 5-HT2A genes have been extensively investigated in psychiatric disorders, such as the silent T/C mutation in the amino acid 102 (T102C polymorphism) (10). Warren et al. (11) described this polymorphism, identifying two alleles (C and T).

There have been reports of a correlation between the C allele and psychiatric disorders (10), while the T allele has been shown to be associated with myocardial infarction (9). A Japanese case-control study conducted on 255 non-fatal AMI patients and 255 control subjects showed an association between T102C polymorphism of the 5-HT2A receptor gene and non-fatal AMI, independent of other risk factors in males under 65 years old (9).

In view of the extensive evidence that serotonergic pathways control bladder function, we investigated the possible association between 5-HT2A polymorphism and urinary incontinence in the elderly (4).

Subjects and Methods

Subjects

The study population comprised 298 free-living subjects (102 urinary incontinent and 196 continent subjects), who were involved in an epidemiological study of aging and non-transmissible disease (GENESIS Research Program) conducted since 1998 in the city of Gravataí located in Southern Brazil.

The study was structured considering the checklist for the reporting and appraising of gene-disease associations proposed by Little et al. (12).

We excluded first- or second-degree relatives of subjects previously included to avoid genetic frequency bias. Parra et al. (13) have described the ancestry of the population in Brazil's southern region, emphasizing the extensive inter-ethnic crosses occurring in the 500 years of Brazilian history between people from three continents, European colonists, autochthonous Amerindians and African slaves. These investigators also noted that large urban areas such as Porto Alegre's metropolitan area (3.5 million inhabitants), where Gravataí city is located, do not have significant numbers of isolated ethnic groups. Parra et al. (13) estimated the ancestry of the mtDNA lineage for this population and found a quite homogeneously miscegenated population formed ancestrally by 0.66 European, 0.22 Amerindian and 0.12 African mtDNA. Therefore, we consider the sample source to be a unique population, known as the Gaucho population. For this reason, no population stratification is presented here. To test for possible genetic or genotypic frequency differences in the two first samples, we used the chi-square test. We did not find any statistical differences and assumed that these populations had the same genetic contributions.

Ethics

The project was submitted to the Research Ethics Committee of Pontifícia Universidade Católica do Rio Grande do Sul affiliated with Conselho Nacional de Saúde and the study protocol was approved (documents number

226/01 and 797/2001). Written informed consent was obtained from all participants.

Clinical variables

Details of clinical data collection have been described by Schwanke et al. (14). The following variables were analyzed: a) urinary incontinence, individuals with a history of involuntary loss of urine; b) body mass index, calculated by squaring the ratio of weight to height (kg/m^2); c) diabetes mellitus, individuals with glucose levels above 126 mg/dL and those using glucose-lowering drugs; d) systolic hypertension, individuals with systolic blood pressure levels >140 mmHg and those using antihypertensive drugs; e) cardiovascular disease, individuals with a previous diagnosis of AMI, angina, intermittent claudication, and stroke; f) functional dependence, assessed according to the activity of daily living scale; g) depressive disorder, individuals who answer yes to the question "do you feel sad or depressed frequently?", and h) cognitive impairment, individuals with a mini-mental state examination score <24.

Molecular analysis

The 5-HT2A receptor polymorphism was determined from DNA isolated from lymphocytes using an extraction kit. Genotyping of the T102C polymorphism was performed by the polymerase chain reaction (PCR) method proposed by Warren et al. (11), with minor modifications performed by do Prado-Lima et al. (15). Briefly, standard PCR was carried out in a 25-µL volume containing 100 ng genomic DNA, 200 µM of each dNTP, 1.5 mM $MgCl_2$, 250 nM of the sense (5'-TGTGCTACAAG TTCTGGCTT-3') and anti-sense (5'-GTGCA GTTTTTCT CTAGGG-3') primers, and 0.6 U DNA *Taq* polymerase. The PCR products were digested with *Hpa*II (BM). The digestion products were separated by electrophoresis and visualized under UV light. The 102T allele PCR products remained uncut, with a single-DNA band of 342 bp, whereas the 102C allele showed two bands of 216 bp and 126 bp. Figure 1 shows the different genotypes (CC, TT, and TC).

The genotyping data were subjected to the following quality measures: a) before the sample analysis was performed, a pilot genotyping analysis (N = 50) was conducted to verify the reliability of the two investigators in charge of these analyses in the study, which showed 100% concordance; b) all genotyping data were recorded in a numbered notebook; c) when the image conditions were unsatisfactory, the genotyping sample was prepared anew. Furthermore, to improve the accuracy of the interpretation of our genotyping analyses, we used the Image Master VDS and Fuji Film Photography System FTI-500 (Pharmacia Biotech, Uppsala, Sweden).

Statistical analysis

The allele and genotype frequencies were tested for Hardy-Weinberg equilibrium. The significance of allele frequency or genotype distribution among case-control volunteers was examined by the non-parametric chi-square test or the Fischer exact test (two-tailed). Multivariate analyses, including sex and age effects, were conducted with multiple logistic regression methods and estimates of conditional relative risk and 95% confidence interval. Statistical analyses were performed using the SPSS/PC Statistical Package Version 11.0 (SPSS Inc., Chicago, IL, USA). All P values were two-tailed. A value of $P < 0.05$ was considered to be statistically significant. To test for intervening factors we performed a multivariate analysis using the Forward Wald logistic regression.

Results

The baseline characteristics of case-control subjects regarding urinary incontinence

are shown in Table 1. Only dependence and systolic hypertension showed significant differences between case-control subjects, both more prevalent among incontinent individuals.

The allelic, genotype and dose-effect genotype of T102C polymorphism of the 5-HT2A gene comparisons are described in Table 2. The distributions of the T102C genotype were in Hardy-Weinberg equilibrium ($\chi^2 = 0.509$, P = 0.77).

We found a significant correlation between TT genotype and urinary incontinence. The results showed that subjects with the TT genotype had a 2-fold greater risk for urinary incontinence than subjects with other genotypes.

The multivariate model equation included 5-HT2A polymorphism, sex, age, systolic hypertension, and diabetes. The results showed that 5-HT2A polymorphism is an independent risk factor for urinary incontinence.

Discussion

The results described here indicate an association between T102C 5-HT2A polymorphism and urinary incontinence in elderly subjects. This association could be of physiological and clinical importance, since 5-HT has been recognized for almost 50 years as an effector in various tissue types, including those involved in lower urinary tract function (3,5).

Several studies have provided insights into the mechanism involved in the serotonergic control of voiding, and consequently could be related to the etiology of urinary incontinence. Neurophysiological investigations have shown that neurons in the raphe nucleus could be activated by bladder distension, indicating that the raphe nucleus could be involved in a spinobulbospinal negative-feedback circuit (16,17). This idea was corroborated by studies using 5-HT antagonists and agonists. Intrathecal administra-

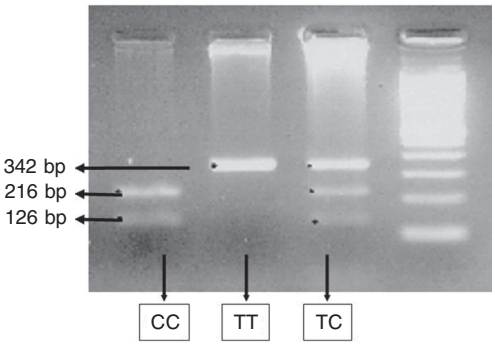

Figure 1. Genotypes of 5-HT2A gene polymorphism. The digestion products were visualized under UV light.

Table 1. Baseline characteristics and common geriatric problems among urinary incontinence case-control subjects.

Variables	Controls (N = 196)	Incontinent (N = 102)
Age (years)	67.7 ± 6.6	68.8 ± 6.8
Gender		
Male	34 (17.3%)	18 (17.9%)
Female	162 (82.7%)	84 (82.1%)
BMI (kg/m^2)	29.0 ± 4.6	28.8 ± 4.1
Dependence (ADL)	0 (0%)	11 (10.8%)*
Diabetes	46 (23.5%)	15 (14.3%)
Systolic hypertension	123 (62.8%)	81 (79.8%)*
Depressive disorder	73 (40.3%)	48 (47.4%)
Cognitive impairment	88 (44.8%)	49 (48.0%)
Cardiovascular disease	59 (30.1%)	29 (28.6%)

Data are reported as means ± SD or as number with percent in parentheses. BMI = body mass index; ADL = activity of daily living.
Frequencies were compared by the chi-square test and means by the Student *t*-test.
*P < 0.05 compared to controls (chi-square test).

Table 2. Comparison of 5-HT2A allelic, genotype and dose-effect genotype frequency between urinary incontinent and control subjects.

Genetic	Controls (N = 196)	Incontinent (N = 102)	OR	95%CI (min-max)
Genotypes				
TT	39 (20.1%)	36 (35.7%)*		
CC	42 (21.4%)	17 (16.4%)		
TC	115 (58.5%)	49 (47.9%)		
Alleles				
T	0.4970	0.4411		
C	0.5030	0.5589		
Dose-effect				
TT	41 (20.9%)	36 (35.7%)*	2.06	1.16-3.65
TC + CC	155 (79.1%)	66 (64.3%)		
CC	42 (21.4%)	17 (16.7%)	0.94	0.86-1.06
TC + TT	154 (78.6%)	85 (83.3%)		

OR = odds ratio; 95%CI = confidence interval at 95% from logistic regression multivariate analysis.
*P < 0.05 compared to controls (chi-square test).

tion of methysergide, a 5-HT1/2 receptor antagonist, decreased micturition volume threshold in cats, implying that serotonergic pathways tonically depress the afferent limb of the micturition reflex (18). Kim et al. (19) tested the *in vitro* acute effects of serotonin on rat bladder contractility, specifically investigating the effects of serotonin on the rat detrusor and determined the drugs that could inhibit the serotonin-induced detrusor contractions. The results showed that the 5-HT2 antagonist blocked the effect of serotonin-induced contractions and that the 5-HT2 receptor was mainly responsible for serotonin-induced contractions of the rat detrusor.

Investigations examining dysfunctions or diseases have also suggested a major role for serotonin in the regulation of the lower urinary tract, such as in the study by Kodama and Takimoto (20). These investigators examined changes in the detrusor muscle in response to 5-HT and its receptor mechanisms in the pathologic bladder of 8-week-old male diabetic Wistar rats. The rats received an intraperitoneal injection of streptozotocin to induce diabetes mellitus. After treatment, bladder strips were taken from the animals and subjected to a tension of 1.0 g in an organ bath to measure isotonic contractile responses to 5-HT or relaxation responses to 5-HT antagonists. The results obtained suggested that the increased contractile response to 5-HT of the diabetic rat bladder was related to smooth muscle hypertrophy and/or hyperplasia, and indicated that this effect could be mediated by the activation of 5-HT2A receptors.

Considering all the above findings, what possible physiological explanation could there be for the association between TT genotype and risk for urinary incontinence? Regardless of being a silent polymorphism, the T102C seems to be functional. Polesskaya and Sokolov (21) developed a method to determine the effect of the T and C alleles of this polymorphism on gene expression. 5-HT2A receptor mRNA transcribed from the T or C alleles was determined in heterozygous (C/T) subjects. This method does not involve the comparison of different individuals, making it possible to avoid effects of variation in demographics and tissue sampling. The results obtained revealed that the expression of the C allele in the temporal cortex of normal heterozygous individuals was significantly lower than the expression of the T allele. Total levels of 5-HT2A mRNA and protein in normal individuals with the C/C genotype were lower than in individuals with the T/T genotype, thereby confirming the decreased expression of the C allele. The authors also demonstrated that the expression of the C allele was ~20% lower than the expression of the T allele.

Further studies could be conducted to better answer some questions left open in this study. Thus, the findings reported here could be considered preliminary, having some limitations. One limitation is related to population stratification. Although the investigation was conducted on an admixed population, it is possible that ethnic admixture could have biased the study results. However, recent studies have suggested that the extent of bias attributable to population stratification is minimal (22). In order to rule out population stratification and to validate the present findings, family studies of 5-HT2A and other candidate genes are warranted. The limited sample size could be considered, as well as the lack of urodynamic analyses. In further investigations, the examination of a larger number of subjects and the inclusion of urodynamic studies could help elucidate the association described here, if found to be relevant in clinical urology. It is clinically relevant because stress urinary incontinence is highly prevalent in women between the ages of 18 and 90 years, affecting their quality of life. Since serotonin-norepinephrine reuptake inhibitors such as duloxetine are being used in the treatment of incontinence (23), additional studies considering genetic effects could be useful. There-

fore, if the association described here is confirmed, pharmacogenetic studies will be needed to test new treatments of detrusor overactivity and urinary incontinence.

In short, some possibilities to explain our findings are: a) there is different expression of the 5-HT2A receptor due to polymorphism in the cortex (~20% lower expression of the C allele than T allele) (21); b) activation of the central serotonergic system suppresses voiding by enhancing the efferent control of the urethral outlet and by inhibiting parasympathetic excitatory input to the urinary bladder (1); c) 5-HT1A, 5-HT2 and 5-HT3 receptors may be involved in the serotonergic activity in the spinal cord (they appear to inhibit detrusor function and to enhance rhabdosphincter function) (7); d) duloxetine hydrochloride is a dual-action serotonin and noradrenaline reuptake inhibitor, and in animal studies it has increased bladder capacity and sphincter muscle activity by a central nervous system action which appears to be mediated via both motor and sensory afferent modulation. This drug can promote urine storage by relaxing the bladder and increasing urethral resistance and may be beneficial in the management of stress urinary incontinence in women (24).

An association between the TT genotype of the T102C 5-HT2A gene and urinary incontinence among elderly individuals is described here. However, the biological significance of this association remains to be clarified. Further investigations including urodynamic analysis, pharmacogenetic assays, and longitudinal investigation will be performed to contribute in clinical treatment of urinary incontinence.

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