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# Rs 6313 Polymorphism in 5-Hydroxytryptamine Receptor 2A Gene Association With Polysymptomatic Primary Nocturnal Enuresis

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*Background*: Tricyclic antidepressants (TCA) were used to treat nocturnal enuresis (NE) for decades of years although their real mechanisms are unknown. Recently, some case studies demonstrated the efficacy of selective serotonin reuptake inhibitors (SSRI) in the treatment of NE. Both TCA and SSRI have similar influences on serotonin transmission. This study was aimed at evaluating whether 5-hydroxytryptamine receptor 2A (5HTR2A) gene is associated with NE. *Methods*: We analyzed rs6313 polymorphism in 5HTR2A gene of 213 Taiwanese children (116 NE cases and 97 healthy control subjects) using polymerase chain reaction-restriction fragment length polymorphism. *Results*: There were no significant differences when comparing the genotypes and allelic frequencies of rs6313 polymorphism in 5HTR2A gene between patients with NE and control subjects. However, when subsequently comparing 5HTR2A genotypes and allelic frequencies in NE child with different phenotypes, genotypes TT and TC appeared higher risks of polysymptomatic NE compared with CC (odds ratio (OR) = 10.71, 95% confidence interval (CI) = 2.66–43.12; OR = 2.68, 95% CI = 0.67–10.75, respectively; $P = 0.0002$); and allele T also revealed higher frequencies of polysymptomatic NE compared with allele C (OR = 3.7, 95% CI = 2.01–6.79, $P = 0.000015$). *Conclusions*: This is the first study that shows the association between 5HTR2A gene polymorphisms and polysymptomatic NE. These results provide further evidence suggesting that genetic variations at 5HTR2A may influence NE treatment response. J. Clin. Lab. Anal. 24:1–5, 2010. © 2010 Wiley-Liss, Inc.

Q1 **Key words:** nocturnal enuresis; serotonin



## INTRODUCTION

Primary nocturnal enuresis is a common disorder with an estimated prevalence of 5–10% at the age of 7 years and a spontaneous cure rate 15% per year (1–5). However, 1–2% of enuresis children continue to be wet in adulthood. Nocturnal enuresis (NE) comprises a heterogeneous group of disorders and clinicians usually divide NE into two forms: mono- and polysymptomatic NE. Polysymptomatic NE is bed-wetting associated with severe urgency, severe frequency, or other signs of an unstable bladder. Monosymptomatic NE is bed-wetting associated with normal daytime urination. Children who have polysymptomatic NE are far more difficult to treat. The exact etiology of NE has not been fully elucidated. In about 75% of affected children, there is a strong family history of enuresis (6). It is generally accepted that genetic factors play an

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important role in NE. The molecular genetic approach of enuresis have been performed since 1995 and several highly positive markers has been found on chromosone12q, 13q13–q14.3 (ENUR1), and 22q11 (ENUR3) by linkage analyses (7–11). These research findings also demonstrate genetic heterogeneity of NE. Multigenerational linkage analyses seem to be difficult to perform in our country because most families are small households. On the basis of our previous experiences, we used single nucleotide polymorphisms (SNP) as a tool and found several genetic markers for some complex diseases of polygenic traits. Therefore, we attempted to use SNP to search the genetic markers of NE and to assess the influence of these polymorphisms in the susceptibility of NE. Tricyclic antidepressants (TCA) were used to treat NE for decades of years although their real mechanisms are unknown. Recently, some case studies demonstrated the efficacy of selective serotonin reuptake inhibitors (SSRI) in the treatment of NE. Both TCA and SSRI have similar influences on serotonin transmission. Linkage studies indicate that 13q contains a locus or loci for NE, and 5-hydroxytryptamine receptor 2A (5HTR2A) gene was mapped on chromosome 13q14.This study was aimed at evaluating whether 5HTR2A gene polymorphisms are associated with NE. As all children enrolled in this study were assessed in great detail, possible specific associations with phenotypes of NE were also analyzed.

## MATERIALS AND METHODS

### Study Population

This study included 213 Taiwanese children. One hundred sixteen (61 boys and 55 girls) were patients of NE and 97 (50 boys and 47 girls) were healthy control subjects. This study was approved by the Ethics Committee of China Medical University Hospital, Taichung, Taiwan. All children included in this experiment had been above 7 yearsof age and they were studied after their parents had signed informed consent. These children received a thorough physical and neurologic examination, urinalysis, and ultrasonography. Children with secondary NE, neurogenic voiding dysfunction, known structural urinary tract obstruction or complex urinary tract malformations, urinary tract infection or abnormal renal function were excluded from this experiment. The phenotypes of NE were subtyped according to arousal scores, the presences of daytime voiding symptoms (DVS) and constipation. The arousal scores were measured using "scoring system for assessing arousal from sleep" referred to a previous study (12). DVS was defined as more than one of the following voiding problems (urinary urgency, hesitancy, and small bladder functional capacity). Constipation was defined as less than 3 bowel movements per week.

### Genotyping

Genomic DNA was extracted and purified from peripheral whole blood leukocytes using a DNA extractor kit (Genomaker DNA extraction kit; Blossom, Taipei, Taiwan). A total of 50 ng of genomic DNA was mixed with 20 pmol of each polymerase chain reaction (PCR) primer in a total volume of 25 µl containing 10 mM Tris-hydrochloride, pH 8.3; 50 mM potassium chloride; 2.0 mM magenesium chloride; 0.2 mM each deoxyribonucleotide triphosphate; and 1 U of DNA polymerase (Amplitaq; Perkin-Elmer, Foster City, CA). The rs6313 polymorphism in 5HTR2A gene was detected using the oligonucleotide primers 5′-CCAAA-TACCTCGATAGTGCTG-3′ and 5′-CGGCTGTCAG-TAAAGCAGAC-3′ to amplify a 432-bp segment covering the polymorphic site. The PCR conditions were as follows: 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 45 s, then standing at 72°C for 7 min and holding at 4°C. The polymorphism was analyzed by using PCR amplification followed by restriction analysis (restriction enzyme: BspeI). The products were analyzed directly on agarose gel by electrophoresis and each allele was recognized according to its size. Allele frequencies were expressed as a percentage of the total number of alleles. Genotypes and allelic frequencies for rs6313 polymorphism in 5HTR2A gene between different phenotypes were compared.

### Statistical Analysis

Given that the type I error is 0.05, group sample size 34 in group one and 62 in group two achieve 85% power, whereas group sample size 50 in group one and 50 in group two achieve 90% power to detect a difference between the group proportions of 0.3. The observed genotype frequencies were tested for Hardy–Weinberg equilibrium by $\chi^2$analysis. The Woolf logit method was used to determine the odds ratios (OR) and 95% confidence intervals (CI). The software used for the analyses included SPSS for Windows (SPSS Inc., Chicago, IL). A value of $P<0.05$ was defined as statistically significant.

## RESULTS

### Clinical Presentations of Patients with NE and Controls (Table 1)

There were no significant demographic differences between enuresis patients and normal control subjects regarding age, gender, body weight, and height (Table 1).

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## Genotype and Allelic Frequencies of rs6313 Polymorphism in 5HTR2A Gene in Patients with NE and Controls (Table 2)

When we compared the allelic frequencies and genotypes of rs6313 polymorphism in 5HTR2A gene of NE with those of control subjects, no significant differences were found (Table 2).

## Genotypes and Allelic Frequencies of rs6313 Polymorphism in 5HTR2A Gene in NE Children Presented With Different Phenotypes (Table 3)

Table 3 summarized genotypes and allele frequencies of rs6313 polymorphism in 5HTR2A gene between NE children presented with different phenotypes, including arousal scores, DVS, and constipation. The arousal scores were not significantly different among the three genotypes and allelic frequencies. However, we found a significant difference in genotype distribution and allelic frequency of polysymptomatic NE. TT and TC appeared higher risks of NE with DVS compared with CC (OR = 10.71, 95% CI = 2.66–43.12; OR = 2.68, 95% CI = 0.67–10.75, respectively; $P = 0.0002$). TT and TC also appeared to have higher risks of NE with constipation compared with CC (OR = 12.71, 95% CI = 2.59–62.33; OR = 1.83, 95% CI = 0.35–9.6, respectively; $P = 0.00001$). Allele T showed higher frequencies of polysymptomatic NE compared with allele C

**TABLE 1. Demography of the Study Sample**

| | Controls ($n = 97$) | NE ($n = 116$) | $P$-value |
|---|---|---|---|
| Age (years) | 10.7±1.7 | 10.9±1.3 | 0.33 |
| Sex (M/F) | 50/47 | 61/55 | 0.49 |
| BH (cm) | 135.6±12.3 | 132.7±14.3 | 0.12 |
| BW (kg) | 35.3±10.5 | 33.1±13.9 | 0.2 |

**TABLE 2. Genotypes and Allele Frequencies of the T102C Polymorphism in 5HTR2A Gene in Patients with NE and Controls**

| | Controls $n$ (%) ($N = 97$) | NE $n$ (%) ($N = 116$) | $P$-value* |
|---|---|---|---|
| *Genotype* | | | |
| TT | 36 (37.1) | 50 (43.1) | 0.592 |
| TC | 45 (46.4) | 46 (39.7) | |
| CC | 16 (16.5) | 20 (17.2) | |
| *Allelic frequency* | | | |
| Allele T | 117 (57.14) | 146 (59.99) | 0.579 |
| Allele C | 77 (42.86) | 86 (41.01) | |

*Genotype frequencies were compared between control and NE by $\chi^2$ test. A $P$ value of $<0.05$ was considered as statistically significant.

**TABLE 3. Genotypes and Allele Frequencies of T102C Polymorphism in 5HTR2A Gene in NE Children Presented With Different Phenotypes**

| | DVS | | | High arousal score | | | Constipation | | |
|---|---|---|---|---|---|---|---|---|---|
| No. patients | Yes ($n = 48$) | No ($n = 57$) | Odds ratio (95%CI) | Yes ($n = 54$) | No ($n = 62$) | Odds ratio (95%CI) | Yes ($n = 37$) | No ($n = 68$) | Odds ratio (95%CI) |
| *Genotype* | | | | | | | | | |
| TT | 30 | 14 | 10.71 (2.66,43.12) | 25 | 25 | 1.5 (0.52,4.30) | 27 | 17 | 12.71 (2.59,62.33) |
| TC | 15 | 28 | 2.68 (0.67,10.75) | 21 | 25 | 1.26 (0.43,3.66) | 8 | 35 | 1.83 (0.35,9.6) |
| CC | 3 | 15 | 1 | 8 | 12 | 1 | 2 | 16 | 1 |
| | $P = 0.0002$ | | | $P = 0.74$ | | | $P = 0.00001$ | | |
| *Allelic frequency* | | | | | | | | | |
| T | 75 | 56 | 3.7 (2.01,6.79) | 71 | 75 | 1.25 (0.73,2.14) | 62 | 69 | 5.02 (2.48,10.14) |
| C | 21 | 58 | 1 | 37 | 49 | 1 | 12 | 67 | 1 |
| | $P = 0.000015$ | | | $P = 0.41$ | | | $P = 0.000002$ | | |

The Pearson $\chi^2$ test was performed to obtain the $P$ value. A $P$ value of $<0.05$ was considered as statistically significant. Patient numbers may not add up to 116 because of some clinical data are missing.

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(OR = 3.7, 95% CI = 2.01–6.79, $P = 0.000015$). Allele T also showed higher frequencies of NE with constipation compared with allele C (OR = 5.02, 95% CI = 2.48–10.14, $P = 0.00002$).

## DISCUSSION

Imipramine, a tricyclic antidepressant, was introduced to treat NE in 1960 and since then several studies revealed Imipramine had effects on a reduction in wet nights (13,14). Although TCA had been primarily used to treat the affective disorders, clinical investigations in the last decade suggested that non-affective disorders, such as enuresis, also have therapeutic effects (15). It was also suggested that associations between a variety of psychiatric disorders and incontinence, such as depression and attention deficit hyperactivity disorder (16,17). The "shotgun" hypothesis explains the multiple therapeutic effects of TCA based on multiple actions of TCA (e.g., on adrenergic receptors vs. muscarinic receptors vs. serotonin receptors) (15). TCA would increase neurotransmitters in the synaptic cleft and promote serotonin or noradrenaline transmission (18) Animal studies also showed that TCA exerted an inhibitory action on the maturation reflex by a central cholinergic mechanism resulting in detrusor muscle relaxation. Another studies showed imipramine had acute modulatory effect on increasing threshold of the spinal reflex via inhibition of serotonin reuptake in nerve terminals (19). On the other hand, lowering monoamines, such as serotonin and noradrenaline, in the central nervous system leads to depression, urinary frequency, and a hyperactive bladder in experimental animals (16). Recently, some case studies presented the efficacy of SSRI, such as sertraline and fluoxetine, in the treatment of enuresis (20–22). These findings also have the same implications that serotonin might play an important role in NE.

Serotonin is an important neurotransmitter, which participates in many physiologic processes such as sleep, appetite, thermoregulation, pain perception, and hormone secretion. Lack of arousal responses to distension-induced maturation reflex has been accepted as the main dysfunctions of NE and both of them are mediated by nuclei in the brainstem (23–25). The 5HTR2A gene mapped on chromosome 13q14, which contains three exons and two introns, encodes the 5-hydroxytryptamine receptor 2A, which is a postsynaptic G protein-linked receptor. Genetic variation in 5HTR2A gene may contribute to these discrepancies in 5HTR2A receptor expression and subsequently influence serotonin transmission.

This study is the first one to search the associations between 5HTR2A gene polymorphisms and NE. It was determined that rs6313 polymorphism in 5-hydroxytryptamine receptor 2A gene may not be associated with NE in Taiwanese children. These associations were statistically significant only when analyses were performed in polysymptomatic NE and NE with constipation. These results further support the previous observations that NE is heterogeneous disorder. The heterogeneity of NE has been manifested in the distinction between mono- and polysymptomatic forms, based on the absence or presence of bladder overactivity. The causes of monosymptomatic NE and polysymptomatic NE might be different and serotonin might have influence on polysymptomatic NE. Further studies with larger samples together may help to determine the exact role of 5HTR2A gene in enuresis. Children with the polysymptomatic form had a number of associated bladder and bowel problems. Clinically, it is important to distinguish the two types of NE to find the most appropriate treatment. This may open a new door in NE molecular genetics research and elucidate the complex interplay among the neurotransmitter systems in the etiology of NE.

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