Influence of *HTR3* genetic variability on obsessive-compulsive disorder

Hae Won Kim

Department of Medicine

The Graduate School, Yonsei University

Influence of *HTR3* genetic variability on obsessive-compulsive disorder

Directed by Professor Se Joo Kim

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This certifies that the
Doctoral Dissertation of Hae Won Kim
is approved.

Thesis Supervisor: Se Joo Kim

Thesis Committee Member#1: Hyun-Sang Cho

Thesis Committee Member#2: Chul Hoon Kim

Thesis Committee Member#3: Sang-Hyuk Lee

Thesis Committee Member#4: Ji Hyun Lee

The Graduate School
Yonsei University

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I would like to dedicate this dissertation to my mother, to whom my expression of gratitude is never enough.

<TABLE OF CONTENTS>

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

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Purpose: Family, twin, and molecular genetic studies have demonstrated that genetic factors may exert significant influence on the development of OCD and the manifestation of symptoms. Evidence in the extant literature has indicated associations between serotonin-related genetic variants and OCD, but few studies have explored the involvement of serotonin receptor type 3 genes in OCD. The aim of this study was to examine whether *HTR3* genetic variants may affect susceptibility to OCD.

Methods: We performed a case-control study with 596 individuals with OCD and 599 controls. Ten common single nucleotide polymorphisms in the five distinct *HTR3* genes were genotyped. Single-marker association and haplotype-based association analyses were conducted regarding the affected status and different OCD sub-phenotypes, such as age at onset and clinical symptom dimensions. The impact of *HTR3* variants on trait disgust sensitivity was also analyzed.

Results: A significant difference in the genotype distribution of rs1176744 was

detected between individuals with OCD and controls (odds ratio (OR) = 0.74, 95% confidence intervals (CI) = 0.60–0.91, $p = 0.0041$), which was restricted to males when the analyses were stratified by sex (OR = 0.70, CI = 0.54–0.89, $p = 0.0039$). On analyzing clinical characteristics of OCD, significant associations were found for rs3758987 with age at onset in male subjects (OR = 0.49, CI = 0.30–0.78, $p = 0.0025$) and for rs6766410 and rs6443930 with the contamination/cleaning dimension in female subjects (OR = 0.36, CI = 0.18–0.69, $p = 0.0017$ and OR = 0.47, CI = 0.29–0.78, $p = 0.0029$, respectively). In addition, rs6766410 was significantly related to contamination-based disgust scores in the whole OCD sample ($p = 0.0044$). A two-marker composite haplotype in *HTR3B* was associated with OCD in male subjects (OR = 0.75, CI = 0.58–0.97, permutated $p = 0.0339$)

Conclusions: Our results support a role for common variants of *HTR3* in OCD and certain of its clinical phenotypes. These findings may have implications for pharmacogenetic studies of 5-HT_3 antagonists in the treatment of OCD.

Key words : 5-HT_3 receptor, *HTR3*, obsessive-compulsive disorder

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I. INTRODUCTION

Obsessive-compulsive disorder (OCD) is a chronic, debilitating psychiatric disorder characterized by recurrent, intrusive, distressing thoughts (obsessions) and/or repetitive, ritualized behaviors (compulsions) performed to alleviate the anxiety associated with a given obsession,[1] with an estimated prevalence of 1-3% in the general population.[2] OCD is often familial, and results from twin studies and segregation analyses have shown that obsessive-compulsive symptoms are substantially heritable, with a complex pattern of inheritance.[3-6] Given this evidence of a genetic etiology, numerous molecular genetic studies have been conducted to identify chromosomal regions and candidate genes that may confer risk for the development of OCD. The majority of candidate gene association studies have focused on genetic variants relevant to the pathways for serotonin, dopamine, and glutamate, based on

current knowledge of the neurobiology of OCD; dysregulation of neurotransmitter systems may alter the function of the cortico-striato-thalamo-cortical circuits, one of the leading biological models implicated in OCD pathophysiology.[7] Notably, polymorphisms related to serotonergic neurotransmission have been most frequently examined, on the basis of the clinical benefits of serotonin reuptake inhibitors in the treatment of OCD. Indeed, a recent meta-analysis suggested that variations in two serotonin-related genes, *5-HTTLPR* and *HTR2A*, are associated with OCD,[8] while clearer evidence for the impact of other serotonin-related gene variants remains to be found.

Among the several susceptible genes, the involvement of serotonin type 3 (5-HT_3) receptor genes (*HTR3*) in the development of OCD may be supported by several lines of evidence. The 5-HT_3 receptor is a Cys-loop ligand-gated ion channel, which differs structurally and functionally from the other 5-HT receptor subtypes, which are coupled to G-proteins. 5-HT_3 receptor is composed of five subunits surrounding a central non-selective cation channel, and thereby mediates rapid depolarizing responses in neurons when activated.[9,10] The involvement of brainstem 5-HT_3 receptors in the vomiting reflex underlies its well-established role in nausea and emesis. Moreover, activation of 5-HT_3 receptors in the posterior insular cortex has been shown to enhance nausea-induced conditioned disgust reactions in rats.[11] Considering that heightened disgust sensitivity seems to contribute to contamination concerns

and washing rituals in OCD[12,13] and in non-clinical samples,[14] the 5-HT_3 receptor appears to play a pathophysiological role in at least some types of OCD. In addition, these receptors have also been reported to affect cognitive and emotional functions, which may be explained by their influence on the release of various neurotransmitters[15-19] in brain areas such as the hippocampus, amygdala, striatum, and nucleus accumbens. In line with this preclinical evidence, clinical trials of 5-HT_3 antagonists have demonstrated efficacy in reducing symptoms of psychiatric disorders, including OCD.[20-25] Recently, a randomized, double-blind, placebo-controlled study has revealed that 5-HT_3 antagonists may offer additional clinical benefits to conventional OCD treatment with serotonin reuptake inhibitors.[26]

To date, five distinct *HTR3* genes have been cloned for humans: *HTR3A* and *B* are assigned to loci on chromosome 11q23.1-2,[27] and *HTR3C*, *D*, and *E* on 3q27.1.[28] A substantial number of studies have examined the involvement of *HTR3* genes in the pathophysiology and treatment of various psychiatric disorders such as schizophrenia,[29-31] affective disorder,[32-34] eating disorder,[35] and substance dependence.[36-40] In OCD, a large genome-wide linkage study has provided evidence of linkage for markers on chromosome 3q27-28, albeit not reaching the accepted statistical significance.[41] Inasmuch as the markers are 2.5Mb downstream of *HTR3C – E*, these genes may be positional candidates in OCD. In addition, several authors have indicated that a single nucleotide polymorphism (SNP) rs1062613 in *HTR3A* is associated with the

personality trait of harm avoidance[42] and the modulation of amygdala activation[43] in normal human subjects, both of which are suggested to have particular relevance for OCD.[44,45] *HTR3* genes may therefore be considered plausible candidates with regard to involvement in OCD. To the best of our knowledge, however, only two association studies have been conducted with regard to *HTR3* involvement in OCD. In a study with 75 trio samples, no significant association was found between the *HTR3A* variant and individuals with early-onset OCD.[46] The other study was undertaken with case-control samples, which included variants across all *HTR3* genes. The authors suggested that the *HTR3E* variant rs7627615 was related to the washing dimension and to visual organization scores in OCD.[47]

Given this paucity of data and the promising clinical outcomes that follow the use of 5-HT_3 antagonists in the treatment of OCD, we decided that the association between *HTR3* and OCD should be further examined and replicated in an independent sample of individuals with OCD. In the present study, we aimed to perform a case-control association study with common variants of *HTR3* genes in a larger sample of adult OCD probands and controls. Since noticeable heterogeneity of obsessive-compulsive symptoms has been suggested to reflect underlying genetic heterogeneity, separating OCD phenotypes into more homogeneous subgroups would facilitate the identification of susceptibility genes. Thus, clinical characteristics such as age at onset and symptom dimensions have been included in the analyses in terms of

their relationship to genetic variants, as these phenotypes have been proposed as a means of determining more genetically valid subgroups.[48] We also considered gender differences in genetic findings of OCD[49-52] and analyzed gender-specific associations. In addition, we also sought to explore whether genetic variability in *HTR3* contributes to disgust sensitivity, a psychological trait closely associated with OCD,[12,13] as well as with 5-HT_3 receptor function in the brain. We hypothesized that variations within *HTR3* may confer genetic susceptibility to affected status, clinical characteristics, and psychological traits in OCD.

II. MATERIALS AND METHODS

1. Subjects

The study sample consisted of 596 case and 599 control subjects in total. Unrelated individuals with OCD were consecutively recruited from the outpatient department of psychiatry at Severance Hospital, Yonsei University Health System, and diagnosed with the Korean version of the Structured Clinical Interview for DSM-IV Axis I Disorders[53] by a trained psychiatrist. Exclusion criteria were age younger than 19 or older than 65 years, a lifetime history of psychotic symptoms, a history of substance abuse or dependence in the preceding 6 months, or severe organic or neurologic disorders. Subjects with comorbid DSM-IV Axis I disorders were not excluded, provided that obsessive-compulsive symptoms were the primary diagnosis and the main focus

of seeking assistance. Gender-matched, unrelated controls were recruited from the local community through advertisements. Controls with a lifetime history of DSM-IV Axis I disorders or neurological disorders were not included in the study. All subjects identified themselves as ethnically Korean.

Information on age at onset was obtained in clinical evaluation, which was defined as the age of occurrence of obsessive-compulsive symptoms recalled by the subject or family members. We considered 17 years old as a threshold for defining early-onset OCD.[54] The severity of obsessive-compulsive and depressive symptoms was assessed with the clinician-administered version of the Yale-Brown Obsessive-Compulsive Scale (Y-BOCS)[55] and the Montgomery-Åsberg Depression Rating Scale (MADRS),[56] respectively. The Y-BOCS symptom checklist was used to identify the previously ascertained four symptom dimensions[57]: 1) symmetry – symmetry obsessions and repeating, ordering, and counting compulsions, 2) forbidden thoughts – aggressive, sexual, religious, and somatic obsessions and checking compulsions, 3) cleaning – contamination obsessions and cleaning compulsions, and 4) hoarding – hoarding obsessions and compulsions. A dimension was determined to be present with either a current or a lifetime history of at least one symptom in the dimension.

The study was approved by the Institutional Review Board of Severance Hospital. Written informed consent was obtained from each subject at the beginning of the study.

2. Disgust scale-revised

Information on individual differences for trait sensitivity to disgust was obtained with the Korean version of the disgust scale-revised (DS-R)[58] in a subset of cases (n = 256) and controls (n = 478). The DS-R is a five-point Likert-type scale (0 – 4), comprised of 25 disgust-eliciting items and two catch questions. The latent factor structure of the DS-R constitutes three subscales, including the 12-item core disgust scale, the 8-item animal reminder disgust scale, and the 5-item contamination-based disgust scale. Core disgust is based on the avoidance or rejection response to disgusting stimuli such as bodily waste products, rotting foods, and small animals. Animal reminder disgust reflects the aversion to reminders of the animal origins of humans, including body envelope violations and death. Finally, contamination-based disgust is related to the perceived threat of disease contagion.[13]

3. SNP selection

We selected 10 SNPs according to the either of the following criteria: 1) functional variants annotated in dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/) that reside within the regulatory region or alter the amino acid sequence of a protein, or 2) variants previously reported to be associated with OCD or other psychiatric disorders. All selected variants had a verified minor allele frequency higher than 0.05 in Asians, as ascertained by means of the HapMap project database

(http://hapmap.ncbi.nlm.nih.gov/; Data Release 28, phase II+III August10, on NCBI B36 assembly, dbSNP b126). Information about the selected SNPs is provided in Table 1.

Table 1. Characteristics of the *HTR3* variants

Gene	rs number	Position[1]	cDNA	Amino acid exchange
HTR3A	rs1062613	chr11:113975284	c.-24T>C	-
HTR3A	rs1176713	chr11:113989703	c.1491A>G	p.Leu497=
HTR3B	rs3758987	chr11:113904553	c.-381T>C	-
HTR3B	rs1176744	chr11:113932306	c.386A>C	p.Tyr129Ser
HTR3B	rs3782025	chr11:113936885	c.696+3792G>A	-
HTR3C	rs6766410	chr3:184056974	c.489C>A	p.Asn163Lys
HTR3C	rs6807362	chr3:184060222	c.1214G>C	p.Gly405Ala
HTR3D	rs6443930	chr3:184036506	c.107G>C	p.Gly36Ala
HTR3D	rs1000952	chr3:184038034	c.155G>A	p.Arg52His
HTR3E	rs7627615	chr3:184100628	c.256G>A	p.Ala86Thr

[1]Information on chromosome position is based on NCBI genome build 38.p2.

4. Genotyping

Genomic DNA was prepared from peripheral blood samples using a nucleic acid isolation device, QuickGene-mini80 (FUJIFILM, Tokyo, Japan). In a subset of controls (n = 160), DNA was extracted from saliva using the Oragene DNA collection kit (DNA Genotek, Kanata, Ontario, Canada).

Genotyping of SNP rs3782025 was screened using a single base primer extension assay using an ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to the manufacturer's recommendations. Briefly, the

genomic DNA flanking the interested SNP was amplified with polymerase chain reaction (PCR) with forward and reverse primer pairs and standard PCR reagents in a 10 microliter reaction volume containing 10ng of genomic DNA, 0.5pM of each oligonucleotide primer, 1 microliter of 10X PCR buffer, 250M dNTP (2.5mM each), and 0.25 units of DiaStar Taq DNA Polymerase (5unit/μl) (SolGent co. Ltd., Daejeon, South Korea). The PCR was carried out as follows: 10 min at 95°C for 1 cycle, and 35 cycles at 95°C for 30 seconds, 60°C for 1 minute, 72°C for 1 minute followed by 1 cycle of 72°C for 10 minutes. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (USB Corporation, Cleveland, OH, USA) and exonuclease I (USB Corporation, Cleveland, OH, USA) at 37°C for 75 minutes and at 72°C for 15 minutes to purify the amplified products. One microliter of the purified amplification products was added to a SNaPshot Multiplex Ready reaction mixture containing 0.15 pmols of genotyping primer for a primer extension reaction. The primer extension reaction was carried out for 25 cycles at 96°C for 10 seconds, at 50°C for 5 seconds, and at 60°C for 30 seconds. The reaction products were treated with 1 unit of SAP at 37°C for 1 hour and at 72°C for 15 minutes to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products was added to 9 microliters of Hi-Di formamide (ABI, Foster City, CA). The mixture was incubated at 95°C for 5 minutes, placed on ice for 5 minutes, and then analyzed by electrophoresis in an ABI Prism 3730xl DNA analyzer. Analysis was carried

out using Genemapper software (version 4.0; Applied Biosystems).

Genotyping of the remaining nine SNPs – rs1062613, rs1176713, rs3758987, rs1176744, rs6766410, rs6807362, rs6443930, rs1000952, and rs7627615 – was screened using the TaqMan fluorogenic 5' nuclease assay (ABI, Foster City, CA, USA). The final volume of the PCR was 5ul, containing 10ng of genomic DNA and 2.5ul of TaqMan Universal PCR Master Mix, together with 0.13ul of 20X Assay Mix. Thermal cycle conditions were as follows: 50°C for 2 minutes to activate the uracil N-glycosylase and to prevent carry-over contamination, 95°C for 10 minutes to activate the DNA polymerase, followed by 45 cycles of 95°C for 15 seconds and of 60°C for 1 minute. All PCR were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (ABI, Foster City, CA, USA), and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (ABI, Foster City, CA, USA). Duplicate samples and negative controls were included to ensure accuracy of genotyping. Table 2 shows the primer sets and assay IDs used in the experiment.

Table 2. Primer sets and assay IDs used in the experiment

rs number	Primer sequence
rs3782025	Forward: CAGGAAACAGCTATGACCgtggcacatccctgtaaa
	Reverse: TGTAAAACGACGGCCAGTAAAACTGCCCCTACCTACA
	SNP primer: AGGCAAYTACAGGCTAACCTTGG
rs number	Assay ID
rs1062613	C___7488465_10
rs1176713	C___1372122_10
rs3758987	C__27512451_10
rs1176744	C___7488596_1_
rs6766410	C__26004660_10
rs6807362	C__28948667_10
rs6443930	C__28960641_10
rs1000952	C____109464_10
rs7627615	C__28960643_10

SNP, single nucleotide polymorphism

5. Sample power calculation

Statistical power was evaluated under a dominant genetic model, using Quanto software version 1.2.4 (http://biostats.usc.edu/software) with statistical significance set at $p < 0.05$. Given the available sample size, the statistical power for detecting a risk allele with an arbitrarily established effect size of 1.5 ranged from 0.77 to 0.88, depending on the minor allele frequencies.

6. Statistical analysis

Single-marker association analyses were performed on affected status

and each sub-phenotype (age at onset, symmetry, forbidden thoughts, cleaning, and hoarding) with multivariate logistic regression under codominant, dominant, recessive, overdominant, and additive models of inheritance. The impact of genetic variants on disgust sensitivity for each DS-R subscale was also evaluated under the five genetic models, with linear regression. The analyses were adjusted for age, and the model with the least Akaike information criteria (AIC) was selected as the best fitting model. Analyses were conducted using the R software version 3.2.1 (http://www.r-project.org/) and the R package SNPassoc.[59] The overall statistical significance was set at $p < 0.005$ after Bonferroni correction for 10 independent SNPs tested.

Haploview software version 4.2 (http://www.broad.mit.edu/mpg/haploview) was used to estimate pairwise linkage disequilibrium (LD) patterns of the tested SNPs. Haplotype blocks were identified using the solid spine of LD method with a D' threshold of 0.8.[60] Haplotype-based associations were analyzed using the R package haplo.stats,[61] which estimates haplotype frequencies with an expectation-maximization algorithm. Haplotype-specific score statistics were computed to test associations with various traits. Only haplotypes with frequencies above 0.01 were considered in the analyses. A permutation procedure ($n = 10000$) was performed in order to estimate the corrected significance of the best results.

III. RESULTS

1. Data quality control

The threshold for genotyping call rate was set at 0.95 for each SNP, with an average call rate of 0.992 (0.983 – 0.997). All SNPs were in Hardy-Weinberg equilibrium in both OCD and control samples ($p > 0.05$), except for rs1176713 ($p = 0.023$ in OCD) and rs3782025 ($p = 0.033$ in control). Minor allele frequencies were higher than 0.05 in all SNPs.

2. Subjects

The demographic and clinical characteristics of subjects are summarized in Table 3. No significant differences were found in sex distribution ($\chi^2 = 0.004$, $df = 1$, $p = 0.950$) and years of education ($t = -1.464$, $df = 1150$, $p = 0.1435$) between the two groups, but individuals with OCD were significantly older than controls ($t = -13.88$, $df = 759.47$, $p < 0.001$). A total of 103 individuals with OCD (17.3%) were drug-naïve at the enrollment. For trait disgust sensitivity, both groups obtained similar scores for core disgust ($t = -1.610$, $df = 471.47$, $p = 0.108$), whereas individuals with OCD scored significantly higher on animal reminder disgust ($t = -7.045$, $df = 732$, $p < 0.001$) and contamination-based disgust ($t = -6.888$, $df = 455.28$, $p < 0.001$).

Table 3. Sociodemographic and clinical characteristics of study sample

Variable	OCD (*n* = 596)	Controls (*n* = 599)
Age, years (range)	29.77 ± 10.41 (19–63)	23.44 ± 3.92 (19–48)
Male/Female, *n*	390/206	393/206
Education, years	13.57 ± 2.36	13.39 ± 1.95
Age at onset of OCD, years	18.42 ± 8.90	
Early-onset (< 18years), *n* (%)	328 (55.0)	
Late-onset (≥ 18years), *n* (%)	268 (45.0)	
Illness duration, years	11.36 ± 8.28	
Current medications, *n* (%)		
SSRIs	493 (82.7)	
Atypical antipsychotics	85 (14.3)	
Benzodiazepines	160 (26.8)	
Mood stabilizers	11 (1.8)	
Basal Y-BOCS score		
Total	24.92 ± 5.94	
Obsessions	12.67 ± 3.03	
Compulsions	12.24 ± 3.43	
Basal MADRS score	18.59 ± 8.71	
Comorbid diagnosis, *n* (%)		
Any comorbid diagnosis	138 (23.2)	
Affective disorders	77 (12.9)	
Major depressive disorder (*n* = 46)		
Depressive disorder, NOS (*n* = 21)		
Bipolar I disorder (*n* = 5)		
Bipolar II disorder (*n* = 5)		
Anxiety disorders	35 (5.9)	
Panic disorder (*n* = 22)		
Social phobia (*n* = 9)		
Posttraumatic stress disorder (*n* = 3)		
Generalized anxiety disorder (*n* = 1)		
Eating disorders	2 (0.3)	
Tic disorder or Tourette's disorder	24 (4.0)	
Others	8 (1.3)	
Symptom dimensions, present, *n* (%)		
Symmetry	441 (74.0)	

Variable	OCD (n = 596)	Controls (n = 599)
Forbidden thoughts	505 (84.7)	
Cleaning	437 (73.3)	
Hoarding	200 (33.6)	
DS-R score		
Total score	60.30 ± 15.45	53.76 ± 14.27
Core disgust	29.55 ± 8.08	28.58 ± 7.18
Animal reminder disgust	21.63 ± 6.34	18.11 ± 6.52
Contamination-based disgust	9.12 ± 4.03	7.07 ± 3.44

OCD, obsessive-compulsive disorder; SSRI, selective serotonin reuptake inhibitor;
Y-BOCS, Yale-Brown obsessive-compulsive scale;
MADRS, Montgomery-Åsberg depression rating scale; DS-R, disgust scale-revised

3. Single SNP association analysis

Four SNPs showed nominal significance ($p < 0.05$) in genotype distributions between individuals with OCD and controls: rs1062613, rs3758987, rs1176744, and rs3782025. After Bonferroni correction, only rs1176744 remained significant under an additive model (Table 4). The association between this SNP and affected status was restricted to males when the analyses were stratified by sex (OR = 0.70, CI = 0.54–0.89, $p = 0.0039$).

The analyses based on clinical characteristics, regarding age at onset and symptom dimensions, yielded no significant results for the whole OCD sample. However, significant associations were observed in further analysis stratified by sex. Considering age at onset, the genotype distribution of rs3758987 differed significantly between early-onset OCD and late-onset OCD

in males under a dominant model (Table 5). No other variants were related to age at onset. For symptom dimensions, two SNPs, rs6766410 and rs6443930, were significantly associated with the cleaning dimension in females under an overdominant model and an additive model, respectively (Table 6).

Concerning the relationship between disgust sensitivity and *HTR3* variants, we found a significant effect of rs6766410 on contamination-based disgust scores under an overdominant model in OCD (mean score difference = -1.430, $p = 0.0044$). Analyzing by sex stratification, only nominal significance was shown in females ($p = 0.030$). No significant associations were found between rs6766410 and scores on other DS-R subscales or between other *HTR3* variants and DS-R subscale scores in OCD.

Table 4. Association analysis of *HTR3* SNPs in individuals with OCD and controls

	Controls, *n* (%)	OCD, *n* (%)	OR (95% CI)	*p* value
rs1062613[1]				
CC-CT	592 (99.3)	584 (98.0)	1.00	0.0269
TT	4 (0.7)	12 (2.0)	3.56 (1.08–11.69)	
rs1176713[1]				
AA-AG	550 (93.2)	544 (91.7)	1.00	0.6317
GG	40 (6.8)	49 (8.3)	1.12 (0.70–1.81)	
rs3758987[2]				
TT, TC, CC	586 (49.9)	589 (50.1)	0.80 (0.65–0.98)	0.0317
rs1176744[2]				
AA, AC, CC	593 (49.9)	596 (50.1)	0.74 (0.60–0.91)	0.0041
rs3782025[3]				
AA	290 (49.8)	253 (43.0)	1.00	0.0104
AG-GG	292 (50.2)	335 (57.0)	1.39 (1.08-1.78)	
rs6766410[1]				
AA-AC	501 (84.2)	510 (85.7)	1.00	0.2198
CC	94 (15.8)	85 (14.3)	0.81 (0.57–1.14)	
rs6807362[1]				
CC-CG	548 (92.6)	557 (93.6)	1.00	0.3393
GG	44 (7.4)	38 (6.4)	0.79 (0.48–1.29)	
rs6443930[1]				
CC-CG	476 (79.9)	483 (81.3)	1.00	0.5946
GG	120 (20.1)	111 (18.7)	0.92 (0.67–1.25)	
rs1000952[4]				
AA	494 (82.9)	509 (85.4)	1.00	0.0688
AG	100 (16.8)	81 (13.6)	0.73 (0.51–1.03)	
GG	2 (0.3)	6 (1.0)	3.00 (0.58–15.57)	
rs7627615[2]				
AA, AG, GG	597 (50.1)	595 (49.9)	0.92 (0.75–1.14)	0.4574

[1]recessive model, [2]additive model, [3]dominant model, [4]codominant model

OCD, obsessive-compulsive disorder; OR, odds ratio

Table 5. Effect of rs3758987 on age at onset

	OCD subjects, total				Male subjects				Female subjects			
	Early-onset OCD, *n* (%)				Early-onset OCD, n (%)				Early-onset OCD, n (%)			
Genotype	No	Yes	OR (95% CI)	*p* value	No	Yes	OR (95% CI)	*p* value	No	Yes	OR (95% CI)	*p* value
TT	142 (53.6)	203 (62.7)	1.00		71 (49.3)	156 (65.0)	1.00		71 (58.7)	47 (56.0)	1.00	
TC-CC	123 (46.4)	121 (37.3)	0.66 (0.45–0.96)	0.0327	73 (50.7)	84 (35.0)	0.49 (0.30–0.78)	0.0025	50 (41.3)	37 (44.0)	1.19 (0.61–2.31)	0.6090

OCD, obsessive-compulsive disorder; OR, odds ratio; CI, confidence intervals

Table 6. Effect of rs6766410 and rs6443930 on cleaning dimension

	OCD subjects, total				Male subjects				Female subjects			
	Symptoms, *n* (%)				Symptoms, *n* (%)				Symptoms, *n* (%)			
Genotype	Absent	Present	OR (95% CI)	*p* value	Absent	Present	OR (95% CI)	*p* value	Absent	Present	OR (95% CI)	*p* value
rs6766410												
AA-CC	67 (42.4)	218 (49.9)	1.00		51 (49.5)	137 (47.9)	1.00		16 (29.1)	81 (53.6)	1.00	
AC	91 (57.6)	219 (50.1)	0.75 (0.52–1.08)	0.1158	52 (50.5)	149 (52.1)	1.07 (0.68–1.69)	0.7541	39 (70.9)	70 (46.4)	0.36 (0.18–0.69)	0.0017
rs6443930												
CC, CG, GG	157 (26.4)	437 (73.6)	0.91 (0.70– 1.19)	0.4942	104 (26.7)	286 (73.3)	1.21 (0.88–1.66)	0.2388	53 (26.0)	151 (74.0)	0.47 (0.29– 0.78)	0.0029

OCD, obsessive-compulsive disorder; OR, odds ratio; CI, confidence intervals

4. Haplotype association analysis

We identified four LD blocks, three of which contained two markers from each gene, and one of which contained three markers from *HTR3C* and *HTR3E* (Figure 1). For haplotypes estimated in *HTR3B*, we found a significant difference in the distribution of haplotypes between individuals with OCD and controls. As shown in Table 7, a composite C-C was significantly associated with a lower risk of being affected by OCD. On stratifying by sex, the association remained significant only in males (OR = 0.75, CI = 0.58 – 0.97, permutated p = 0.0339). For haplotypes in other *HTR3* genes, we could not find evidence of a relationship with OCD.

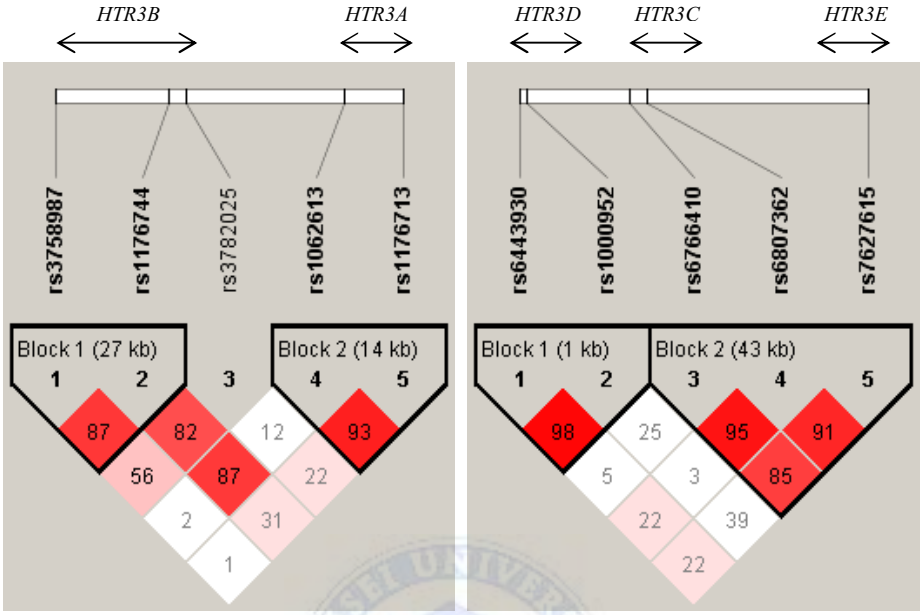

Figure 1. Linkage disequilibrium patterns and haplotype blocks estimated with markers that were examined in this study. All numbers in the square represent the D' value as a percentile.

Table 7. Estimated haplotype frequencies in individuals with OCD and controls

Haplotype (*HTR3B*)						
rs3758987	rs1176744	Controls %	OCD %	OR (95% CI)	Crude *p* value	Permutated *p* value
T	A	69.1	73.6	1.00 (reference)		
C	C	22.0	18.1	0.77 (0.63–0.95)	0.0173	0.0185
C	A	3.8	4.9	1.17 (0.79–1.72)	0.2398	0.2395
T	C	2.0	2.2	1.04 (0.59–1.82)	0.7751	0.7709

OCD, obsessive-compulsive disorder; OR, odds ratio; CI, confidence intervals

IV. Discussion

In the present study, we aimed to explore whether *HTR3* genetic variants confer risk for OCD and/or for certain clinical characteristics of the disorder. The main findings of this study provide further evidence of involvement of *HTR3* in OCD affected status, as well as in age at onset and in the manifestation of specific symptom dimensions.

In male subjects with OCD, we found a global relation between *HTR3B* variant rs1176744 and OCD under an additive model, which suggested that the odds of being affected by OCD was reduced by 0.7 times with a one copy increase of the variant C allele. In line with this, a major *HTR3B* haplotype carrying the C allele was also associated with OCD male subjects in a protective manner. Therefore, these results support a plausible role for the variant C allele of rs1176744 in decreasing OCD susceptibility. This functional Tyr129Ser variant, located in exon 5, results in slow deactivation and desensitization kinetics for variant (p.129S) 5-HT_3AB receptors compared to wild-type ones, thereby allowing substantially increased maximal response to serotonin.[62,63] For receptors composed of both wild-type (p.Y129) and variant (p.129S) 5-HT_3B subunits, an intermediate maximal response to serotonin has been shown;[62] this finding may correlate with our result, which found varying degrees of OCD susceptibility according to variant allele dose. Considering that 5-HT_3B subunits are expressed in the hippocampus and in the amygdala,[64-66] one possible explanation for our result is that alteration in receptor responsiveness might play

a role in fear conditioning and extinction, which could in turn contribute to OCD susceptibility.[67]

Similar to our findings, previous studies in major depressive disorder,[34] bipolar disorder,[32,33] and eating disorders[35] have shown that the A allele of rs1176744 was largely overrepresented in these patients compared to controls. By contrast, the C allele was related to an increased risk of alcohol dependence,[36] a seemingly counterintuitive result given the frequent co-occurrence of OCD and alcohol use disorder.[2] The explanation may lie in a different LD structure, where these alternative alleles might be linked to different unidentified causative loci that may be population or disease specific. In addition to the abovementioned disease susceptibilities, it has been suggested that the AA genotype of this SNP might increase the risk of developing nausea during paroxetine treatment,[68] a commonly prescribed drug in OCD. As a common side effect of selective serotonin reuptake inhibitors (SSRIs) is nausea, which can be severe enough to cause discontinuation of treatment, identifying individuals prone to developing nausea may facilitate more tailored treatment with SSRIs. Taken together, this functional variant rs1176744 may underlie the genetic etiology of OCD and might serve as a potential predictor of individual drug response.

Regarding the onset age of obsessive-compulsive symptoms, we found a significant association of *HTR3B* variant rs3758987 with a lower risk of developing OCD at an early age in males. The extant literature has suggested

that individuals with early-onset disorder may represent a genetically more valid subgroup, which is associated with more familial loadings of OCD, higher comorbidity with tics, and poorer response to treatment.[69,70] Although this 5' upstream variant does not exert a direct effect on the amino acid sequence of the encoded protein, this variant may be in LD with a nearby functionally important, but not yet explored, polymorphism. On the other hand, this SNP might influence regulatory processes related to *HTR3B* expression, such as gene transcription. Further research on the physiological relevance of the variant and replication of this finding in different populations is needed to verify the role of this common variant in influencing the onset age of OCD.

With regard to the symptom dimensions of OCD, the cleaning dimension was significantly associated in females with two non-synonymous SNPs, rs6766410 and rs6443930. Previous studies have reported that the *HTR3C* and *HTR3D* genes are almost ubiquitously expressed in the human body, including in the brain,[71] where these variants might contribute to the development of specific symptom dimensions by modulating channel function. Since there are no data concerning the function of these SNPs on the protein level, we investigated their putative effects with a PolyPhen-2 prediction.[72,73] The analysis revealed that neither of the variants was predicted to be damaging; however, on analysis of rs6807362 and rs1000952, which were in high LD with rs6766410 and rs6443930, respectively, these variants were predicted to be possibly damaging or probably damaging (PolyPhen-2 scores (HumDiv) 0.647

and 0.998, respectively). Thus, the observed association with rs6766410 and rs6443930 may be attributed to other tightly linked functional variants, such as the ones examined in this study or to other unexplored variants. Notably, *HTR3C* variant rs6766410 was also related to contamination-based disgust sensitivity as measured by the DS-R scale, which may possibly affect susceptibility to the cleaning dimension in OCD.[13] Interestingly, these relationships between rs6766410, the cleaning dimension, and disgust sensitivity were most robust under an overdominant model in the same direction, in which the heterozygote genotype AC was significantly associated with reduced risk of the cleaning dimension, as well as with lower scores on contamination-based disgust. These results suggest that molecular heterosis[74] may be a mechanism by which this *HTR3C* variant is relevant to the cleaning dimension and to disgust sensitivity. Molecular heterosis refers to a situation in which heterozygote genotypes are related with a greater (positive heterosis) or lesser (negative heterosis) effect on a trait than homozygote genotypes, which has been frequently observed in genes involved in the dopaminergic[75-77] or serotonergic[35,40,78,79] pathways. According to Comings and MacMurray,[74] our results may be most likely attributed to interaction effects between 5-HT_3 receptor subunits; receptor function might be modified depending on how these wild-type and variant subunits are assembled and interact in the pentameric structure. Further research on functional significance of this variant may provide more valid explanations. Additionally, as there were relatively few

female subjects in the study sample, a larger sample of female OCD probands would be needed to draw a more definite conclusion.

In a previous study with a German sample,[47] it was suggested that the *HTR3E* variant rs7627615 might be associated with the cleaning dimension, a finding that we could not replicate in this study despite the larger sample size. Considering that this failure of replication might have arisen from the different operational criteria in defining the symptom dimensions, we reanalyzed the data in accordance with the earlier study's analysis, a process whereby the symptom dimensions were derived from factor scores. No significant association was found between this SNP and the cleaning dimension, however (data not shown). Given the benign effect of rs7627615, as predicted by PolyPhen-2 analysis, the association might reflect unidentified causal variants linked to this SNP. Fine mapping of this region in different populations may uncover the novel genetic variants related to OCD and its particular symptom dimensions.

The gender-specific associations of *HTR3* polymorphisms are consistent with previous studies which revealed sexually dimorphic effects of genetic variants on OCD.[49-52] Interestingly, these associations were in line with previously reported differences in clinical findings of OCD, such as an earlier onset in men[80,81] and more contamination-related symptoms in women.[82,83] Different clinical manifestations between males and females might thus reflect underlying genetic heterogeneity.

Previous studies have shown that 5-HT_3 antagonists, such as

ondansetron and granisetron, may yield tangible benefits for OCD treatment, either as a primary or an augmentative agent.[20-26] The results of this study may thus have implications in pharmacogenetic studies with 5-HT_3 antagonists in OCD. It is plausible that genetic variations and subsequent alterations in receptor function might elicit different responses to 5-HT_3 antagonists and that clarification of genetic variations affecting individual treatment responses could be beneficial in selecting treatment options.

To the best of our knowledge, this is the second largest candidate gene association study on OCD, and the largest study for investigating *HTR3* involvement in OCD. Nonetheless, our study has several limitations. The major limitation of this study is its potential proneness to population stratification, which may serve as a confounding factor. Although the possibility of false-positive associations stemming from population stratification could not be completely excluded, a high degree of genetic homogeneity in the Korean population[84] might make bias less likely. Second, since controls were significantly younger than individuals with OCD, subjects in the control group could potentially develop obsessive-compulsive symptoms later in life. However, this inevitable factor may exert only a trivial effect on power given that controls had largely passed the mean age of OCD onset[1] and that lifetime prevalence of OCD is approximately 1-2% in the general population. Third, DS-R scores were obtained from a subset of subjects, which could have led to reduced power for detecting associations between genetic variants and disgust

sensitivity. Fourth, as the information on age at onset was collected retrospectively, recall bias could not be disregarded. Finally, we investigated only 10 polymorphisms in *HTR3* genes, introducing the possibility of missing other variants that might also be associated with OCD.

V. CONCLUSION

We have conducted a large candidate gene association study, exploring the relationship between *HTR3* genetic variability and OCD. The present study identified an influence of *HTR3* variants on affected status and certain phenotypes of OCD. These findings may support the role of 5-HT_3 receptors in OCD pathophysiology and clinical manifestations. Future studies focusing on the relationship between these *HTR3* variants and treatment response to 5-HT_3 antagonists may elucidate whether genetic variations of the 5-HT_3 receptor also influence medication response in OCD.

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ABSTRACT(IN KOREAN)

강박장애와 세로토닌 제3형 수용체 유전적 변이의 상관성

<지도교수 김 세 주>

연세대학교 대학원 의학과

김 혜 원

목적: 가족연구, 쌍생아연구 등에서 강박장애의 원인에 유전적 요소들이 관여할 것이라는 가능성이 제시되었으며 현재까지 세로토닌 관련 유전자와의 연관성이 가장 주목 받고 있다. 본 연구에서는 세로토닌 제3형 수용체의 유전적 변이와 강박장애의 연관성을 살펴보고자 하였다.

방법: 강박장애 환자 596명과 정상 대조군 599명을 대상으로 5개의 세로토닌 제3형 수용체 유전자에서 10개의 단일 염기 다형성(single nucleotide polymorphism, SNP)을 분석하였다. 단일 염기 다형성과 일배체 분석을 강박장애의 이환 여부 및 발병 나이, 강박장애의 증상차원, 혐오민감성(disgust sensitivity)에 대해 시행하였다.

결과: 남자에서 rs1176744 유전자형의 빈도가 환자군과 대조군 간에 유의한 차이가 있었으며 이 단일 염기 다형성을 포함한 일배체형의 빈도 또한 두 군간에 유의한 차이가 있었다. rs3758987은 남자에서

강박장애의 발병 나이와 유의한 연관성을 보였으며, rs7677410과 rs6443930은 여자에서 오염 및 청결에 대한 증상차원과 유의한 연관성을 보였다. 또한 rs6766410은 오염과 관련된 혐오민감성과 유의한 연관성을 보였다.

결론: 본 연구의 결과는 세로토닌 제3형 수용체의 유전적 변이가 강박장애의 이환 상태 및 특정 임상 양상과 관련이 있을 가능성을 시사한다. 향후 강박장애에서 이러한 유전적 변이가 세로토닌 제3형 수용체 길항제(5-HT_3 antagonist)에 대한 치료반응에 미치는 영향을 연구한다면 유전적 변이에 따른 개별화된 치료 전략을 수립하는데 도움이 될 것이다.

핵심되는 말 : 세로토닌 제3형 수용체, 단일 염기 다형성, 강박장애