Association between obsessive-compulsive disorder and glutamate n-methyl-d-aspartate 2b subunit receptor gene

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The definite cause of obsessive-compulsive disorder (OCD) is still unknown. Family, twin and segregation studies have strongly pointed toward possible involvement of genetic mechanisms in OCD. Recently, neuroimaging and cortico-spinal fluid (CSF) study have provided evidence that glutamate is involved in some OCD patients. However there has been no study in Korea in which a candidate gene in the glutamate system was tested for association with OCD. Therefore, the aims of this study were to investigate the associations between glutamate receptor, ionotropic, n-methyl-d-aspartate (NMDA) subunit 2B gene (GRIN2B) polymorphisms and OCD in Korean.

One hundred and two OCD patients and 130 normal controls participated in this study. Genomic DNA was extracted from their blood then comparison of the genotypes and allele frequencies of the two polymorphisms (5072T/G and 5988T/C) in GRIN2B between the OCD group and the control group was made. Using four symptom factors, which was derived from thirteen main contents of the Yale-Brown Obsessive– compulsive Scale (Y-BOCS) checklist,

we investigated the association between these four factors and the GRIN2B polymorphisms. In addition, student's t-test was used to compare the total Y-BOCS score, the Hamilton Depression Rating Scale (HDRS) score, and the Global Assessment of Functioning (GAF) score with the GRIN2B polymorphisms.

In this case-control study, we could not find any associations between GRIN2B polymorphisms (5072T/G and 5988T/C) and the development of OCD in Korean. There were no significant differences between both groups with regard to gender or age. From statistical analysis of both 5072T/G genes and 5988T/C genes, no significant difference was determined for HDRS score, Y-BOCS score, and GAF score. However in the OCD group, 5072T/G polymorphism was related to obsession with contamination and compulsion of cleaning.

GRIN2B polymorphisms (5072T/G and 5988T/C) does not affect the development of OCD in Korean. But GRIN2B (5072T/G) polymorphisms affect certain factors of OC symptoms, especially obsession with contamination and compulsion of cleaning.

Key words : obsessive-compulsive disorder, n-methyl-d-aspartate (NMDA) receptor 2B gene (GRIN2B), polymorphism

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I. INTRODUCTION

Obsessive-compulsive disorder (OCD) is a severe, chronic neuropsychiatric condition, characterized by recurrent, distressing, unwanted thoughts (obsessions) and repetitive ritualistic behavior (compulsions).[1] OCD is a common disorder, affecting 2-3% of the population worldwide[2] and 1.9% of the Korean population.[3]

Despite its prevalence and severity, very little is known about the disorder's pathogenesis. The concordance rate for OCD symptoms in monozygotic twins ranges from 80% to 87%, whereas the concordance rate in dizygotic twins ranges from 47% to 50%. Overall, family studies report OCD prevalence rates of 6.7%–15% in first-degree relatives of pediatric probands with OCD, and segregation analyses support the presence of a major gene with dominant or codominant transmission.[4-6]

The partial efficacy of selective serotonin reuptake inhibiting agents (SSRIs) has led to the hypothesis that OCD may be associated with dysregulation of serotonergic neurotransmission[7];

however, a substantial proportion (30–40%) of OCD patients do not respond to SSRIs.[8, 9]

It has also been proposed that neurotransmitters other than serotonin play a role in the pathophysiology of OCD. One of the neurotransmitters that have not been investigated until recently with relation to OCD, despite being present in abundance in the neuroanatomical substrate implicated in OCD, has been glutamate. Rosenberg et al. studied 11 psychotropic drug-naive children (8–17years) with OCD, with single-voxel proton magnetic resonance spectroscopy (^{1}H-MRS) examinations and demonstrated that caudate glutamate concentrations were significantly greater in the patients compared to healthy controls.[10] Abnormal glutamate findings were also obtained in cerebrospinal fluid of patients with OCD.[11]

Despite the postulated role for glutamate in the pathogenesis of OCD based on neuroimaging and CSF studies, there has been no study in Korea in which a candidate gene in the glutamate system was tested for association with OCD.

Glutamate is the major excitatory neurotransmitter in the CNS and acts via two different groups of receptors, metabotropic (mGluR) and ionotropic (iGluR) receptors.[12] The mGluRs are G-protein coupled and modify neural and glial excitability. The iGluRs are ionic channels, permeable to cations. There are three different families within the iGluR group: NMDA, AMPA and kainate receptors. The NMDA receptor is a heteromeric ligand-gated ion channel that interacts with multiple intracellular proteins by way of different subunits.[13] NMDA receptors are concentrated at postsynaptic sites, although some appear to be presynaptic.[14] The glutamate receptor, ionotropic, N-methyl-d-aspartate 2B (GRIN2B) subunit is a critical structural and functional component of the NMDA receptor .[15, 16] The gene encoding the GRIN2B subunit is located on chromosome 12p12.[17, 18] In both rats and humans, the GRIN2B subunit is primarily expressed in forebrain structures such as the prefrontal cortex, hippocampus, striatum, thalamus, and olfactory bulb.[19] Prefrontal cortex and striatum are regions of metabolic abnormality in

OCD.[20] Therefore, there is a possibility of a positive association between OCD and GRIN2B.

No uniformly replicated case-control association studies with OCD probands have yet emerged, although positive association have reported significant findings for genes in brain neurotransmitter pathways including serotonin, and catecholamine. These results suggest OCD is not a homogenous condition, and various underlying etiological mechanisms may exist. The clinical presentation of OCD is remarkably diverse, and can vary both within and across patients over time. This variability in the phenotypic expression has led to the hypothesis that OCD is a heterogeneous disorder possibly based on etiologic heterogeneity. This hypothesis indicates the need to study the relationship between the symptom and genetic etiology.

Based on the data above, we hypothesized that there would be significant genetic association between GRIN2B and OCD. To test this hypothesis, we examined two GRIN2B SNPs in both healthy control group and OCD patients group and also evaluated the association of four symptom factors from Yale-Brown Obsessive– compulsive Scale (Y-BOCS checklist)[21] with the GRIN2B.

II. MATEREALS AND METHODS

1. Subjects

One hundred and two unrelated patients with OCD were recruited from Hallym University Sacred Heart Hospital and Youngdong Severance Hospital OCD clinic over a period of 24 months. All OCD patients met the DSM-IV criteria for OCD. Subjects with major depression (without psychotic features) were included in the study only if the OC symptoms were the most prominent and only if the onset of OCD antedated the onset of depression. A total of 12 subjects

(11.8%) were diagnosed with major depressive disorder according to DSM-IV. Subjects were excluded if they presented with a movement disorder other than a tic, any psychotic symptoms, mental retardation, alcohol or other substance abuse within the last 6 months, or a history of psychosurgery, encephalitis, or significant head trauma. Of the 102 subjects, 44 were taking medications (mainly SSRIs and low-dose of benzodiazepines) and/or exposure/response-prevention behavioral therapy. None of the remaining 58 patients have received any treatment for their OCD symptoms at least within the previous 6 months.

One hundred and thirty unrelated control individuals were recruited from the nurses, students and volunteers at Hallym University Sacred Heart Hospital and Anyang community mental health center. Control individuals had no history and family history of OCD and other psychiatric disorders. All subjects were of Korean descent based on their home language and reported descent. The protocol was approved by the ethic committees of Youngdong Severance Hospital and Hallym Sacred Heart Hospital, and all subjects provided written informed consent prior to study participation.

The OC symptoms and their severity were evaluated and scored by the Y-BOCS.[22] Obsessions and compulsions were recorded according to the Y-BOCS checklist,[22] including more than 60 items organized in eight categories of obsessions and seven categories of compulsions. Y-BOCS checklist was performed following the methodology of Leckman et al.[1] Briefly, a score of 0, 1, or 2 was assigned to each of the seven major obsessive symptom categories and to each of the six major compulsion categories of the Y-BOCS checklist as follows: if the patient did not endorse any of the specific symptoms under the heading, then that category was assigned a score of 0; if the patient endorsed at least one of the specific symptoms but did not consider it a principal problem, that category was assigned a score of 1; or if the patient identified at least one of the specific symptoms as a principal or major problem, that

category was assigned a score of 2. Note that miscellaneous obsessions and compulsions were excluded because each contained many heterogeneous symptoms. OC symptoms from the Y-BOCS checklist were reclassified into four factors - hoarding/repeating, contamination/cleaning, aggressive/sexual, and religious/ somatic - based on Kim's report.[23] The severity of depressive symptoms in OCD patients was rated using the Hamilton Depression Rating Scale(HDRS, 21-item version).[24] The Global Assessment of Functioning (GAF) Score was also rated according to psychological, social, and occupational functioning.

2. Genotyping

Peripheral blood was collected and genomic DNA was extracted by a standard procedure.[25] Two single nucleotide polymorphisms (SNPs), located in the 3′-untranslated region (3′-UTR) of GRIN2B were examined: 5072T/G (rs890 in dbSNP data base[26]), and 5988T/C.[27]

5072T/G (rs890)

Genotyping was carried out using the TaqMan fluorogenic 5' nuclease assay (Applied Biosystems). The final volume of polymerase chain reaction (PCR) was 5ul, containing 2 ng of genomic DNA and 2.5ul TaqMan Universal PCR Master Mix, with 0.125ul of 40X Assay Mix or 0.25ul of 20X Assay Mix. Thermal cycle conditions were as follows: 50℃ for 2 min to activate the uracil N-glycosylase and to prevent carry-over contamination, 95℃ for 10 min to activate the DNA polymerase, followed by 40 cycles of 92℃ for 15 s and 60℃ for 1 min. All PCR were performed using 384-well plates by a Dual 384-Well GeneAmp PCR System 9700 (Applied Biosystems) and the endpoint fluorescent readings were performed on an ABI PRISM 7900 HT Sequence Detection System (Applied Biosystems). Duplicate samples and negative

controls were included to ensure accuracy of genotyping.

5988T/C (rs1805502)

The genomic DNA were prepared from peripheral blood samples using PUREGENE blood DNA kit (Gentra Inc. Minneapolis, MN, USA) following manufacturer's protocol. The genotypes of the patients and control samples were assayed by single base primer extension assay using ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's recommendation. Briefly, the genomic DNA flanking the SNP (P2RY12 52G/T rs6809699) was amplified with PCR reaction with 5'- CTCTTCAGCAGAGATGCAA (Forward) and 5'- AGTGGTCCTGTTCCCAGT (Reverse) primer pairs and standard PCR reagents in 10 microliter reaction volume, containing 10ng of genomic DNA, 0.5pM of each oligonucleotide primer, 1 microliter of 10X PCR Gold buffer, 250μM dNTP, 3mM MgCl2 and 0.25 unit i-StarTaq DNA Polymerase (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea). The PCR reactions were carried out as follows : 10 min at 95℃ for 1 cycle, and 30 cycles on 95℃ for 30s, 55℃ for 1min, 72℃ for 1min followed by 1 cycle of 72℃ for 7mins. After amplification, the PCR products were treated with 1 unit each of shrimp alkaline phosphatase (SAP) (Roche) and exonuclease I (USB Corporation) at 37℃ for 60 minutes and 72℃ for 15 minutes to purify the amplified products. One microliter of the purified amplification products were added to a SNaPshot Multiplex Ready reaction mixture containing 0.15pmols of genotyping primer (5'- ACAAYCTCACCTCTGCGCCTGG) for primer extension reaction. The primer extension reaction was carried out for 25cycles of 96℃ for 10 seconds, 50℃ for 5 seconds, and 60℃ for 30 seconds. The reaction products were treated with 1 unit of SAP at 37℃ for 1 hour and 72℃ 15 minutes to remove excess fluorescent dye terminators. One microliter of the final reaction samples containing the extension products were added to 9

microliter of Hi-Di formamide (ABI, Foster City, CA). The mixture was incubated at 95℃ for 5 min, followed by 5min on ice and then analyzed by electrophoresis in ABI Prism 3730xl DNA analyzer. Results were analyzed using GeneScan analysis software (ABI).

3. Analyses

First, differences in the genotype and allele frequencies between patients and controls were tested using the Chi-square(χ^2) association test. Second, a Kruskal-Wallis test was used in association study between OCD and the GRIN2B polymorphism using factor score, representing the correlation of the contents profile of the subject with each factor, from the Y-BOCS checklist as quantitative phenotypes. One-way anova was used to compare the total Y-BOCS score, the HDRS score, and the GAF score with the GRIN2B polymorphisms. The criterion for significance was set at $p<0.05$ for all of the tests. Data are presented as mean ± standard deviation.

III. RESULTS

We included 102 OCD subjects who were diagnosed as having OCD in the study and 130 control subjects. The ethnic background of the subjects was 100% Korean.

There were no differences in the age and gender distributions between the OCD and control groups (table 1), and the genotype distribution did not deviate from Hardy-Weinberg equilibrium in either OCD patients or controls for both 5072T/G polymorphism and 5988T/C polymorphism. The onset age was not significantly different comparing the three genotypic groups of both 5072T/G polymorphism ($p=0.580$) and 5988T/C polymorphism ($p=0.985$).

Table 1. Demographic characteristics of OCD patients and controls

	OCD patients (n= 102)	Controls (n=130)	p
Age, mean±SD, years	31.9±10.9	29.9±6.8	0.13 [a]
Sex			
Male	70 (68.6%)	78 (60.0%)	0.18 [b]
Female	32 (31.4%)	52 (40.0%)	

[a] t test, [b] χ^2 test (χ^2= 1.842, d.f.=1)

Despite remarkable differences in heterozygote prevalence in polymorphisms, it showed no statistical difference in the genotype distributions and allele frequencies between OCD patients and control group (table 2).

Table 2. Genotype frequency of GRIN2B gene polymorphism in obsessive-compulsive disorder patients and controls (χ2 test)

	5072T/G					5988T/C				
	Genotype (n=102)			Allele (n=204)		Genotype (n=102)			Allele (n=204)	
	T/T	T/G	G/G	T	G	T/T	T/C	C/C	T	C
OCD patients	63 (62%)	32 (31%)	7 (7%)	158 (77%)	46 (23%)	68 (67%)	32 (31%)	2 (2%)	168 (82%)	36 (18%)
Controls	76 (58%)	47 (36%)	7 (5%)	199 (77%)	61 (23%)	91 (70%)	35 (27%)	4 (3%)	217 (83%)	43 (17%)
χ^2	0.70			0.05		0.76			0.10	
d.f.	2			1		2			1	
p value	0.71			0.82		0.68			0.75	

We also studied association between OCD and GRIN2B polymorphism using factor scores from the Y-BOCS checklist by Kruskal-Wallis test (table 3). For 5072T/G polymorphism, comparison of factor scores among OCD patients with TT, TG and GG each other detected significant difference in factor 2: obsession with contamination and compulsion of cleaning. For 5988T/C polymorphism, no difference was found in four factors.

Table 3. Comparisons of factor scores between obsessive-compulsive disorder patients with GRIN2B polymorphism

	5072T/G				5988T/C			
Factor score	T/T (n=63)	T/G (n=32)	G/G (n=7)	p-value	T/T (n=68)	T/C (n=32)	C/C (n=2)	p-value
Factor1	0.02±1.05	0.02±0.95	0.48±0.70	0.57	-0.14±1.12	0.27±0.67	-0.9±0.23	0.08
Factor2	0.16±0.90	0.52±1.10	0.57±0.58	0.022 [a]	0.05±1.02	-0.56±1.01	0.13±0.66	0.93
Factor3	0.07±0.84	0.05±1.13	0.23±1.46	0.70	-0.15±0.92	0.15±1.05	0.09±1.01	0.52
Factor4	0.18±0.91	0.09±0.87	0.15±1.19	0.24	-0.03±0.95	-0.19±0.89	0.16±0.48	0.66

[a] Kruskal-Wallis test

From statistical analysis of both three 5072T/G genes and three 5988T/C genes, no significant difference was determined for HDRS score, Y-BOCS score, and GAF score (table 4).

Table 4. Comparisons of obsessive-compulsive, depressive symptom and global function between obsessive-compulsive disorder patients with GRIN2B gene (one-way anova)

	5072T/G				5988T/C			
Rating scale	T/T (n=63)	T/G (n=32)	G/G (n=7)	p-value	T/T (n=68)	T/C (n=32)	C/C (n=2)	p-value
Y-BOCS	27.10±6.06	27.71±6.09	27.75±4.35	0.92	26.17±6.20	29.48±5.05	27.50±0.71	0.07
HDRS	13.44±8.74	16.33±11.77	13.67±8.96	0.52	14.74±10.36	13.08±8.32	18.50±12.02	0.66
GAF	53.11±9.65	52.15±9.84	53.75±16.00	0.92	53.91±10.08	50.75±9.83	55.00±7.07	0.44

IV. DISCUSSION

The aim of our work was to determine the genetic association between GRIN2B and OCD. In this study, we found no evidence of association between OCD and both 5072T/G and 5988T/C polymorphism.

In the previous associated study for the three polymorphisms (5072T/G, 5806A/C, 5988T/C) in the GRIN2B gene using the Family Based Association Test (FBAT) with 389 individuals in

130 families, Arnold et al. reported a significant positive association between both 5072T/G polymorphism and 5072T/G-5988T/C haplotype and OCD in the 97% caucasian population.[28] The frequencies of the T and G alleles of 5072T/G in our OCD subjects were different from Arnold's (77% & 23% vs 49% & 51%, respectively), in contrast to the T and C alleles of 5988T/C (82% & 18% vs 80% &20%, respectively).

Possible explanations for our findings are as follow. First, variations in the GRIN2B gene play no significant role in the development of OCD in Korean, in contrast to the Caucasian. The different ethnicity of the sample could be invoked to explain the discrepancies between our study and Arnold's. Second, the effects of GRIN2B variation on OCD are too minimal to be detected given our relatively small sample size.

In addition to GRIN2B, regarding glutamate receptor ionotrpic kainate 2 (GRIK2) and 3 (GRIK3), Delorme et al. performed a case-control study in 156 patients and 141 controls, and also a transmission disequilibrium test in 124 parent-offspring trios.[29] Although there were no associations of GRIK3 S301A or GRIK2 rs2227281 (intron14) and rs2227283 (exon15) with OCD in the case-control of family-base analyses, the GRIK2 SNP 1867 allele (rs2238076) in exon 16 was transmitted less than expected in OCD.[29] However, it has also been reported the positive association between glutamate transporter gene solute carrier family 1, member 1 gene (SLC1A1) and OCD.[30, 31] These results suggest the possibility of positive association of glutamate associated gene with OCD; however association between GRIN2B and OCD was not found in the present study.

We estimated the association between GRIN2B gene polymorphism and phenotypic characteristics of OCD from the principal component analysis. There was significant difference with 5072T/G gene polymorphism in obsession with contamination and compulsion of cleaning. This suggests that the GRIN2B gene is associated with a certain dimension of OC symptoms,

especially obsession with contamination and compulsion of cleaning. It is also interesting to note that the L-genotype of dopamine receptor D4 may have negative effects on the development of OCD and religious/somatic factor of the obsessive-compulsive symptoms, in our subject.[32] It has been suggested that clinical heterogeneity is due to genetic heterogeneity since some clinical features of neuropsychiatric disorders aggregate in families and twins.[33] Considering our result and previous studies, we suggest that OCD may be a clinically heterogenous and multidimensional condition mediated by genetic heterogeneity.

In addition, from analysis of the HDRS score, the Y-BOCS score and the GAF score, no significant difference was determined comparing the genotype groups, suggesting that this GRIN2B genetic polymorphism does not affect the HDRS score, the Y-BOCS score and the GAF score for our OCD population. However, in contrast to the Y-BOCS checklist, which represents both current and past symptoms, Y-BOCS and HDRS reflected only current states. Therefore, our results must be treated with caution since these variables are state-dependent; thus would fluctuate over time.

There were a number of limitations to this study. First, it is still possible that we missed areas of the gene that could be associated with OCD, particularly the 5′-UTR containing the functional promoter polymorphism.[34] Second, more than half of the control subjects in our study were nurses and medical students; hence, stratification bias may have been present in our sample. Third, we use the case-control design rather than the more robust tests, e.g., HRR or TDT. Case control studies are more vulnerable to genetic diversity within populations, whereas the family-based transmission tests are less vulnerable. Last, our failure to find other significant associations might reflect a relatively small size.

Nonetheless, this study represents the first published report of an association between a glutamate system gene and OCD in Korean. Therefore, it merits further investigations to

determine the association between GRIN2B polymorphisms and OCD by perhaps using a larger sample size, various phenotypic characters, another population, and other SNPs.

V. CONCLUSION

In this study, we examined two GRIN2B SNPs in both health subjects group and OCD patients group to search for genetic association between GRIN2B polymorphism and OCD. We did not find any differences in the GRIN2B polymorphisms between two groups. However, there were significant association between 5072T/C polymorphism and both obsessions in OCD patients: contamination and compulsion of cleaning. No significant difference was found in the HDRS score, the Y-BOCS score and the GAF score.

In conclusion, there were no genotype frequencies of the two SNPs (5072T/G, and 5988T/C) in GRIN2B between OCD patients and controls in Korean. However, these results indicate that 5072T/G polymorphisms affect certain factors of OC symptoms.

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ABSTRACT (IN KOREAN)

강박장애와 글루타메이트 수용체 유전자 다형성과의 관련성

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황성식

강박장애의 원인에 대해서는 아직 명확히 밝혀진 바가 없다. 지금까지 보고된 강박장애에 대한 가족연구, 쌍생아 연구, 분리 연구(segregation study)등을 살펴보면 유전적인 요소들이 강박장애의 원인에 어떠한 역할을 할 것이라는 가능성을 강력히 시사하고 있다. 강박장애의 병태생리와 글루타메이트의 관련성이 의심되는 연구결과에도 불구하고 한국에서는 아직 강박장애와 관련된 글루타메이트 체계의 후보 유전자 연구는 보고되지 않고 있다. 본 연구의 목적은 한국인에서, 글루타메이트 수용체 n-methyl-d-aspartate (NMDA) subunit 2B gene (GRIN2B) 유전자형과 강박장애와의 연관성을 밝히는 것이다.

한림대학교 의과대학 성심병원과 연세대학교 의과대학 영동세브란스 병원 정신과에 내원한 강박장애 환자 102명과 정상대조군 130명을 모집하여 혈액에서 유전자를 추출하여 GRIN2B의 5072T/G와 5988T/C의 유전자형 빈도와 대립형질 빈도를 비교하였다. 강박장애 환자군에서는 유전자형에 따른 Yale-Brown Obsessive- compulsive Scale (YBOCS) checklist로부터 추출된 4가지 요인들의 점수와 YBOCS 총점수, Hamilton Depression Rating Scale (HDRS), Global Assessment of Functioning (GAF) 점수를 t test를 사용하여 분석하였다.

본 연구에서 GRIN2B 유전자형(5072T/G와 5988T/C)과 강박장애의 발병에

있어 유전자형에 따른 의미 있는 상관관계를 발견할 수 없었다. 환자군과 대조군 사이에 연령과 성별분포에 있어 차이는 없었으며 환자군에서는 GRIN2B 유전자형(5072T/G와 5988T/C)과 비교한 YBOCS, HDRS와 GAF점수에서 유전자형에 따른 의미 있는 차이는 보이지 않았다. 그러나 강박장애 환자군 내에서 YBOCS checklist로부터 추출된 4가지 요인들의 점수의 비교에서 5072T/G의 유전자형에 따른 오염/청결 강박요인의 점수는 유의한 차이가 있었다.

이번 연구결과는 한국인에서 GRIN2B 유전자형(5072T/G와 5988T/C)은 강박장애의 발병에 영향을 미치지 않는다는 것을 보여준다. 그러나 GRIN2B (5072T/G) 유전자형은 특이적인 강박장애의 증상, 특히 오염/청결 증상요인의 심각도에 영향을 미칠 것으로 생각되었다.

핵심되는 말 : 강박장애, 글루타메이트 수용체, 유전자 다형성