Original article / *Araştırma*

Association between the metabotropic glutamate receptor7 rs3749380 polymorphism and methylphenidate treatment outcome in children with ADHD

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ABSTRACT

Objective: *Attention-deficit/hyperactivity disorder (ADHD) is a heritable neurodevelopmental disorder characterized by inattention, disorganization, and/or hyperactivity-impulsivity. This study investigated the association between the metabotropic glutamate receptors (GRM) 7 rs3749380 polymorphism genotypes and subjective/objective treatment responses to methylphenidate (MPH) in Korean children with ADHD.* ***Methods:*** *This study enrolled 86 medication-naïve children with ADHD in an open-label 8-week trial of MPH. The subjects were genotyped and then evaluated using the ADHD Rating Scale (ARS), the Continuous Performance Test (CPT) and the Clinical Global Impression Scale (CGI) before and after treatment.* ***Results:*** *After 8-week MPH treatment, children with the GRM7 rs3749380 polymorphism T/T genotype had a different response in terms of visual response times and auditory commission errors on the CPT than C/C or C/T genotype groups.* ***Conclusions:*** *These results suggest that the GRM7 rs3749380 polymorphism is associated with the response of MPH in patients with ADHD. Further studies, including replication of our findings using a control or comparison group and a larger sample, are warranted to evaluate the association between the GRM7 genes and treatment responses to MPH in subjects with ADHD.* ***(Anatolian Journal of Psychiatry 2016; 17(6):442-450)***

Keywords: *attention-deficit/hyperactivity disorder, metabotropic glutamate receptor7 rs3749380, T/T polymorphism, methylphenidate, treatment response*

DEHB'li çocuklarda metabotropik glutamat reseptör7 rs3749380 polimorfizmi ile metilfenidat tedavi sonuçları arasındaki ilişki

ÖZ

Amaç: *Dikkat eksikliği hiperaktivite bozukluğu (DEHB) dikkatsizlik, dezorganizasyon ve/veya hiperaktivite-dürtüsellik ile kararkterize kalıtsal bir nörogelişimsel bozukluktur. Bu çalışmada, Koreli DEHB'li çocuklarda metabotropik glutamat reseptör (GRM) 7 rs3749380 polimorfizm genotipleri ile metilfenidata (MPH) öznel/nesnel tedavi yanıtları arasındaki ilişki araştırılmıştır.* ***Yöntem:*** *Çalışmaya sekiz haftalık açık etiketli MPH çalışmasındaki ilaç kullanmayan DEHB'li 86 çocuk alındı. Deneklerin genotipleri çalışıldı ve sonra tedavi öncesinde ve sonrasında DEHB Değerlendirme Ölçeği (ARS), Sürekli Performans Testi (CPT) ve Genel Klinik İzlenim Ölçeği (CGI) ile değerlendirildi.* ***Sonuçlar:*** *Sekiz haftalık MPH kullanılmasından sonra GRM7 rs3749380 polimorfizm T/T genotipindeki çocuklar CPT'de C/C veya C/T genotip grubundakilerden görsel yanıt süreleri ve işitsel görev hatalarıyla ilgili olarak farklı bir yanıta sahipti.* ***Tartışma:*** *Bu sonuçlar GRM7 rs3749380 polimorfizminin DEHB'li hastalarda MPH'ye yanıtla ilişkili olduğunu düşündürür. Bir kontrol veya karşılaştırma grubu ve daha büyük bir örneklem kullanarak bulgularımızın replikasyonunu içeren ileri çalışmalar, GRM7 genleri ile DEHB'li deneklerde MPH'ye tedavi yanıtları arasındaki ilişkiyi*

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Anahtar sözcükler: *Dikkat eksikliği hiperaktivite bozukluğu, metabotropik glutamat reseptör7 rs3749380, T/T polimorfizmi, metilfenidat, tedaviye yanıt*

INTRODUCTION

Attention-deficit hyperactivity disorder (ADHD) is a common disorder, affecting 3-6% of school-age children.[1] Children with ADHD have manifesttations of continuous traits, including hyperactivity, impulsivity, and dysfunction in executive skills.[2-7] It has been viewed as DNA variants contribute to its complex etiology.[8-10] Extant evidence indicates that dysregulation of the neurotransmitter systems may be involved in its pathophysiology.[11,12] Dysregulated expression of glutamatergic pathway genes has been observed in spontaneously hypertensive rat models.[13,14] Increased concentrations of glutamate were also reported in the neurometabolism of ADHD brains.[15] Glutamatergic neurotransmission is involved in many basic neuronal functions including fast synaptic transmission, neuronal migration, proliferation and excitability.[16] Altered glutamatergic neurotransmission has been implicated in many different CNS processes, physiological and pathological.[17] Genes encoding glutamate receptors represent candidate genes of interest for neuropsychiatric disorders.[18] There are two classes of glutamate receptor. Ionotropic receptors are further classified into N-methyl D-aspartate receptors (NMDA), alpha-amino-3, hydroxy-5-methyl-4-isoxazole propionate receptors (AMPA), kainate (KA) and delta receptors.[16] The metabotropic glutamate receptors (mGluRs) are divided into 3 groups.[14,19] The mGluRs (GRM) in group I are mGluR1 and mGluR5, those in group II are mGluR2 and mGluR3, and those in group III are mGluRs 4, 6, 7, and 8.[20] The mGluR7 is the most highly conserved mGluR subtype across mammalian species.[21] The mGluR7 is thought to be a key player in shaping synaptic responses at glutamatergic synapses as well as in regulating key aspects of inhibitory GABAergic transmission.[22,23] The mGluR7 has putative roles in anxiety, emotional responses, and spatial working memory.[24-27] Neale et al.[28] reported that more than 20 single nucleotide polymorphism (SNP)s of GRM7 showed association with ADHD in the meta-analysis. A genome-wide association study examining the methylphenidate response in children with ADHD found an association with an SNP in GRM7 gene (rs3792452).[29] The above-mentioned evidence suggests that GRM7 represent candidate genes for ADHD or certain phenotypes of ADHD.

Psychostimulants, particularly methylphenidate (MPH), are the most commonly prescribed pharmacological drugs for ADHD. MPH is generally effective not only in reducing symptoms but also in relieving neuropsychological problems such as time variability.[30-32]

We selected this glutamate gene because of its historical prevalence in the literature examining glutamate genes in relation to ADHD. Comparatively fewer studies have investigated the effect of glutamatergic gene polymorphisms on treatment response to MPH. Ohtsuki et al.[20] reported rs 3749380 of GRM7 were associated with schizophrenia. The T allele of GRM7 rs3749380, which is associated with schizophrenia, has lower promoter activity than the C allele.[20] But no published studies have investigated the relationship between the GRM7 rs3749380 polymorphism genotypes and the treatment response to MPH in ADHD subjects. Therefore, the purpose of this study was to evaluate the association between the GRM7 rs3749380 polymorphism genotypes and treatment responses to MPH in Korean children with ADHD.

METHODS

Subjects

Ninety-two drug-naïve ADHD children were enrolled and this study included 86 children with ADHD, as diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text-Revised (DSM-IV-TR) criteria.[33] The children selected for the ADHD group were those who: (1) were 6-12 years old; (2) agreed with informed consent; (3) were diagnosed as having ADHD; (4) had more than 25 points of the pretreatment ADHD Rating Scale (ARS) ; (5) had an IQ score above 80; (6) had no history of exposure to antipsychotic medication. Six children who: (1) had a past history or were currently suffering from medical diseases; (2) had pervasive developmental disorder or mental retardation; (3) had any other psychiatric disorder needed to be treated, were excluded. The study was approved by the institutional review board for human subjects at hospital.

MPH administration and procedures

All of the subjects were drug-naïve at the time of recruitment, and were administered methylphenidate for a total of eight weeks. We started the methylphenidate doses according to their weights. The dosages were increased until sufficient therapeutic effect upon Clinical Global Improvement (CGI) score was reached. Clinical assessment was conducted by certified child and adolescent psychiatrists.

Clinical assessments

Kiddie-Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (K-SADS-PL):[34] K-SADS-PL was performed for the diagnosis of ADHD. It is a semi-structured interview tool, designed to evaluate the severities of ADHD symptoms and to evaluate the 32 different psychiatric disorders included in DSM-IV. The Korean version of K-SADS-PL (K-SADS-PL-K) was translated, and its validity and reliability of ADHD, tic disorder and oppositional defiant disorder were proven by Kim et al. [34]

The Attention-Deficit Hyperactivity Disorder Rating Scale (ARS): The Korean version of the ADHD Rating Scale (ARS) was used for the evaluation of symptoms of ADHD, consisting of a total of 18 items. Each item is scored on a 4-point scale. The scale includes nine items for inattention and nine items related to hyperactivity and impulsivity.

Clinical Global Impression (CGI): CGI is rated by the clinician and uses measure of symptom severity, treatment response and the efficacy of treatments in most clinical studies with mental disorders. The scale was composed of two subscales, both of which are seven-point scales, that rate symptom severity (CGI-S) and symptom improvement (CGI-I). Scoring on the CGI-I is as follow: (1) very much improved; (2) much improved; (3) minimally improved; (4) no change; (5) minimally worse; (6) much worse; (7) very much worse. CGI-S is as follow: (1) normal; not at all ill; (2) borderline mentally ill; (3) mildly ill; (4) moderately ill; (5) markedly ill; (6) severely ill; or (7) extremely ill.

Neuropsychological assessments

The Continuous Performance Test (CPT): Attention and response inhibition were assessed using a standardized visual and auditory form of the Korean version of the computerized Continuous Performance Test (CPT).

The four variables that were recorded were (1) omission errors, which are commonly interpreted as a measure of inattention; (2) commission errors, which are interpreted as a measure of impulsivity; (3) response times for correct responses to the target, which are interpreted as a measure of information processing and motor response speed; and (4) response time variability, which is interpreted as a measure of variability or consistency of attention.

Treatment response

A response criterion was defined based on the CGI-I, CGI-S, and the ARS score at baseline and three weeks after MPH treatment.[35] The dichotomous response criterion, 'good' responder vs. 'poor' responder, was applied. First criterion, based on a change of ARS total score, 'good' responder had an improvement of ≥50% compared with the baseline ARS score. Second criterion was defined by CGI-I score, 'good' responder who had 1 or 2 points after MPH treatment, whereas a 'poor' responder had a CGI-I score in the range of 3-7 points.[36] Third criterion defined by CGI-S score after MPH treatment, a 'good' responder had a 1 or 2 score and 3 to 7 score as a 'poor' responder. Fourth criterion, a 'good' responder had satisfied of all aforementioned criteria.

Genotyping

The genotyping was screened using single base primer extension assay using ABI PRISM SNaPshot Multiplex kit (ABI, Foster City, CA, USA) according to manufacturer's recommenddation.

The genomic DNA flanking the interested SNP was amplified with PCR reaction with 5'- CGT CCT GAC TTT GAT GAA G and 5'- AAA GTA AGC GAC TGT TCG AG for the GRM7 polymorphism (rs3749380) and standard PCR reagents in 10 microliter reaction volume, containing 10ng of genomic DNA, 0.5pM of each oligonucleotide primer, 1microliter of 10X PCR buffer, 250mM dNTP (2.5mM each) and 0.25 unit i-StarTaq DNA Polymerase (5unit/μl) (iNtRON Biotechnology, Sungnam, Kyungki-Do, Korea).

Statistical analyses

The allele frequencies were estimated by counting, and the Hardy-Weinberg equilibrium was calculated based on these allele frequencies, using χ^2 (chi-square) test. Group differences in the clinical variables involving continuous data were summarized using median (minimum, maximum) and tested by a Kruskall-Wallis test rather than parametric method because of

small size of data. Between-group comparisons involving categorical data were assessed using a Fisher's exact test. For assessing treatment outcome, Kruskall-Wallis test was used for analyzing effects of MPH treatment and polymorphism. The Dunn's method was applied for pair-wise multiple comparisons for significant outcomes from Kruskall-Wallis tests. It controls inflation of type I error due to multiple comparisons. Dependant variables included ARS and CPT score and the fixed factors were genes. All tests were two-tailed and significance was defined as an alpha <0.05.

RESULTS

Our sample consisted of 86 children with ADHD including 74 boys and 12 girls. Of the DSM-IV subtypes of ADHD, the hyperactive impulsive subtype was the most common in our subjects, followed by combined and the inattentive subtype.

Table 1. Demographic and clinical characteristics of children with ADHD according to GRM7 rs3749380 polymorphism genotype

	Total (n=86)		CC genotype (n=55)		CT genotype (n=28)		TT genotype (n=3)		p value
Age, median (min-max) years	8.5 (6-14)		9.0 (6-14)		8.5 (6-12)		7.0 (6-7)		0.116*
Weight, median (min-max) kg	31.6 (19.8-60.3)		34.7 (19.8-60.3)		29.8 (19.9-59)		26.2 (25.4-27)		0.231*
Subtype	n	%	n	%	n	%	n	%	
Combined	24	27.91	14	25.45	10	35.71	0	0	0.499**
Inattentive	2	2.33	1	1.82	1	3.57	0	0	
Hyperactive/Impulsive	60	69.77	40	72.73	17	60.71	3	100	
Gender	n	%	n	%	n	%	n	%	
Male	74	86.05	48	87.27	23	82.14	3	100	0.701**
Female	12	13.95	7	12.73	5	17.86	3	100	

*: *Kruskall-Wallis test,* **: *Fisher's exact test*

Table 2. Association between GRM7 rs3749380 polymorphism and clinical and neuropsychological measures baseline and after MPH treatment

Time	Baseline				Eight week			
Subtype	CC genotype (n=55)	CT genotype (n=28)	TT genotype (n=3)	p value*	CC genotype (n=55)	CT genotype (n=28)	TT genotype (n=3)	p value*
ARS, median (min-max)								
Total	33 (22-50)	30.5 (25-50)	34 (28-34)	0.989	18 (5-41)	13 (4,36)	13 (11,26)	0.363
Inattentive	19 (9-29)	19.5 (11-26)	15 (15-18)	0.359	10 (3-23)	9 (2,19)	7 (5,14)	0.437
Hyperactive/impul.	14 (2-24)	15.5 (6-24)	18 (13-19)	0.648	8 (0-18)	6 (0,17)	6 (6,12)	0.273
CPT visual, median (min-max)								
OE	2 (0-25)	2 (0-30)	16 (4-32)	0.065	1 (0-50)	0 (0-15)	17 (7-38)	0.012
CE	6 (0,46)	8.5 (0-42)	26 (3-30)	0.635	3 (0-61)	2.5 (0-45)	26 (7-31)	0.094
RT	558(325-836)	508(354-835)	868(765-960)	0.017	536(312-1026)	483(322-912)	612(561-722)	0.226
RVT	188(60-632)	189(101-357)	437(312-596)	0.029	181(53-400)	150(39-286)	404(160-438)	0.099
CPT auditory, median (min-max)								
OE	1 (0-75)	1 (0-18)	3 (2-27)	0.291	0 (0.25)	0 (0,9)	7 (2,23)	0.032
CE	4 (0,41)	2.5 (0,55)	7 (5,23)	0.325	2 (0,27)	2 (0,9)	9 (9,27)	0.034
RT	630(0-1230)	697(462-875)	859(822-873)	0.007	596(377-1005)	621(287-918)	793(454-890)	0.618
RVT	207(71-784)	198(73-597)	332(273-580)	0.073	181(53-400)	166(51-288)	273(188-331)	0.140
CGI-S	5 (3,7)	5 (3,6)	5 (4,6)	0.638	3 (1,5)	3 (1,5)	3 (2,4)	0.406

*: *Kruskall-Wallis test; ARS: Attention-Deficit/Hyperactivity Disorder Rating Scale; CPT: Continuous Performance Test; OE: Omission Errors; CE: Commission Errors; RT: Response Time; RTV: Response Time Variability; CGI-S: Clinical Global Impression-Severity; MPH: Methylphenidate*

Genotype analysis of the GRM7 rs3749380 polymorphism identified the C/C genotype in 55 subjects, the C/T genotype in 28 subjects, and the T/T genotype in three subjects. The C allele of the GRM7 rs3749380 polymorphism was identified in 138/172 chromosomes and the T allele was identified in 34/192 chromosomes.

After eight weeks of treatment, the total ARS score decreased, from 33 at baseline to 18 in C/C genotype, from 30.5 at baseline to 13 in C/T genotype and from 34 at baseline to 13 in T/T genotype, indicating an improvement in symptoms.

No statistically significant differences in age, weight, ADHD subtype, and sex were observed among genotypic groups.

There was no significant effect of the GRM7 rs3749380 polymorphisms and on ARS scores. However, we observed statistically significant differences in visual response times (p=0.017), response time variability (p=0.029) and auditory response times (p=0.007) on CPT depending on genotypes (Table 2).

Dunn's nonparametric comparison for post-hoc test revealed that with respect to visual response times, TT genotype was significant different from CT (p=0.014) and CC (p=0.02). With respect to visual response time variability, TT genotype was statistically different from CT (p=0.032) and CC (p=0.025). And with respect to auditory response time, TT genotype was significant different from CC (p=0.02) (Table 3).

In comparing distributions of ARS, CPT between GRM7 rs3749380 polymorphism after MPH treatment, Kruskall-Wallis test was performed. There was no significant effect of the GRM7 rs3749380 polymorphisms on ARS scores. However, we observed statistically significant differences in visual omission errors (p=0.0123), auditory omission errors (p=0.0325) and commission errors (p=0.0336) on CPT (Table 2).

The post-hoc test using Dunn's method revealed that with respect to visual omission errors, TT genotype was significant different from CT (p=0.01) and CC (p=0.034). With respect to auditory omission errors, TT genotype was significant different from CT (p=0.028) and CC (p=0.036). And with respect to auditory commission errors, TT genotype was significant different from CT (p=0.029) and CC (p=0.038) (Table 3).

Table 3. Dunn's nonparametric comparison for post-hoc test

			CC-CT	CT-TT	CC-TT
Baseline	Visual	RT	1	0.014	0.020
		RTV	1	0.032	0.025
	Auditory	RT	0.139	0.180	0.020
8 Week	Visual	OE	0.733	0.010	0.034
	Auditory	OE	1	0.028	0.036
		CE	1	0.029	0.038

RT: Response Time; RTV: Response Time Variability; OE: Omission Errors; CE: Commission Errors

After eight weeks of treatment with MPH, the MPH treatment response according to the GRM7 rs3749380 polymorphism genotype was examined. No significant differences were found between the treatment improvement and ARS scores according to the GRM7 rs3749380 polymorphism. However, we found significant difference between the T/T genotype and response in terms of visual response times and auditory commission errors on the CPT compared with the other genotypes (Table 4).

Dunn's test revealed that with respect to visual response times, TT genotype was marginally significantly different from CT (p=0.052) and significantly different from CC (p=0.046). And with respect to auditory commission errors, TT genotype was significantly different from CT (p=0.034) (Table 5).

DISCUSSION

To the best of our knowledge, this is the first study to examine the associations between the GRM7 rs3749380 polymorphism genotypes and

Table 4. Post-treatment changes in clinical and neuropsychological measures according to GRM7 rs3749380 polymorphism genotype

	CC genotype (n=55)	CT genotype (n=28)	TT genotype (n=3)	p*
ARS, median (min, max)				
Total	-16 (-36,4)	-20 (-39,3)	-15 (-23,-10)	0.319
Inattentive	-9 (-19,-1)	-10 (-21,0)	-8 (-10,-4)	0.288
Hyperactive/impulsive	-6 (-17,6)	-9 (-18,8)	-7 (-13,-6)	0.374
CPT visual, median (min, max)				
OE	-1 (-23,48)	-1 (-25,12)	6 (-9,13)	0.503
CE	-3.5 (-41,20)	-4.5 (-29,5)	-4 (-19,28)	0.991
RT	-35.6 (-271.8,305.7)	-39.1 (-180.3,245.2)	-152.4 (-397.8,-146.2)	0.049
RTV	-25.1 (-373.8,193.6)	-34.3 (-217.7,148.7)	-157.4 (-277.3,91.1)	0.586
CPT auditory, median (min, max)				
OE	-1 (-75,5)	-0.5 (-15,1)	0 (-20,20)	0.744
CE	-1 (-39,17)	-2 (-46,6)	4 (2,4)	0.037
RT	-28.45 (-838.5,598.3)	-73.3 (-320.9,131.4)	-79.4 (-405,67.7)	0.198
RTV	-29.05 (-383.8,177.5)	-44.25 (-450.4,143.2)	-1.2 (-392.1,-0.4)	0.653
CGI-S, Median (min, max)	-2 (-4,0)	-2 (-4,0)	-1 (-4,-1)	0.905

ARS: Attention-Deficit/Hyperactivity Disorder Rating Scale; CPT: Continuous Performance Test; OE: Omission Errors; CE: Commission Errors; RT: Response Time; RTV: Response Time Variability; CGI-S: Clinical Global Impression-Severity
**: Kruskall-Wallis test*

Table 5. Dunn's nonparametric comparison for post-hoc test

		CC-CT	CT-TT	CC-TT
Visual	RT	1	0.052	0.046
Auditory	CE	0.789	0.034	0.102

RT: Response Time; CE: Commission Errors

treatment response to MPH in ADHD subjects.

In this study, total ARS scores were also indifferent between the groups at baseline as showed in Table 2. No significant association was found between the GRM7 rs3749380 genotype and treatment response measured by total ARS and this result suggested that the GRM7 rs3749380 interaction may have a limited impact on the effect of MPH on ADHD symptoms in particular. Our small sample size may have reduced the likelihood of finding statistical significance.

But there was a significant effect on several variables in CPT performance. At baseline, we found that homozygous subjects for the T allele (T/T genotype) were significantly different on the CPT visual and auditory response times and visual response time variability as compared with those with the C/C or C/T genotypes. After 8-week MPH treatment, subjects with the T/T genotype were different in visual and auditory omission errors and commission errors on CPT.

With regard to the assessment of treatment response to MPH using objective neuropsychological measures, the ADHD subjects with more copies of the T allele of GRM7 gene (rs3749380) polymorphism showed more reduction in visual response times, but more increase in auditory commission errors on the CPT. These results showed somewhat differences with other previous studies,[20,37,38] which identified the T allele as a putative risk allele of the GRM7 gene (rs3749380) polymorphism. Those studies reported an association between the T allele of the

of the GRM7 rs3749380 polymorphism and schizophrenia symptoms.[20,37,38] We can infer that possessing the risk (T) allele of this polymorphism may contribute to a greater improvement in some profiles in neuropsychological performance after MPH treatment in subjects with ADHD, but not in others. These result suggested that MPH may have a limited impact on some ADHD symptoms in particular. Our short treatment time may cause poor treatment effects. In this study, it also might be noted that the association between the GRM 7 genotypes and baseline clinical characteristics such as symptom severity may influence these pharmacogenetic findings because higher baseline severity has been reported to predict a greater treatment response in ADHD subjects.[39,40] Buitelaar et al.[39] suggested that there may be a greater potential for improvement in more severe compared with less severe patients.

In summary, results of the present study were T/T genotype group showed significant difference on the CPT measure of visual response times and auditory commission errors compared with C/T or C/C genotype group. These have been proposed as a putative endophenotype with the potential to index genetic variability in ADHD.[41] Our data provide further support for the candidacy of response times and commission errors as an endophenotype for ADHD and suggest that variations in this phenotype are related to genetic variations in the GRM 7 gene.

Several limitations of this study should be noted. First, this study has no placebo controlled group and open-label design. Second, we did not compare the GRM7 rs3749380 polymorphisms in subjects with ADHD with those in healthy controls. Third, our study assessed short-term outcomes, which are incomplete assessments of treatment response. Long-term outcomes might prove to be much more clinically relevant for investigation. Fourth, we did not stratify our results according to DSM-IV subtypes and thus cannot comment upon whether our effects are driven by a particular dimension. And another possible limitation is that candidate genes in our study are not a major gene on ADHD genetic study. Finally, due to small sample size of our study, it must be required for replication with large sample size. Thus, the results should be interpreted cautiously.

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