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Association Between Polymorphisms in GRIK2 Gene and Obsessive-Compulsive Disorder: A Family-Based Study

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Several studies support a genetic influence on obsessive-compulsive disorder (OCD) etiology. The role of glutamate as an important neurotransmitter affecting OCD pathophysiology has been supported by neuroimaging, animal model, medication, and initial candidate gene studies. Genes involved in glutamatergic pathways, such as the glutamate receptor, ionotropic, kainate 2 (GRIK2), have been associated with OCD in previous studies. This study examines *GRIK2* as a candidate gene for OCD susceptibility in a family-based approach. Probands had full DSM-IV diagnostic criteria for OCD. Forty-seven OCD probands and their parents were recruited from tertiary care OCD specialty clinics from France and USA. Genotypes of single nucleotide polymorphism (SNP) markers and related haplotypes were analyzed using Haploview and FBAT software. The polymorphism at rs1556995 ($P = 0.0027$; permuted P-value $= 0.03$) was significantly associated with the presence of OCD. Also, the two marker haplotype rs1556995/rs1417182, was significantly associated with OCD ($P = 0.0019$, permuted P-value $= 0.01$). This study supports previously reported findings of association between proximal *GRIK2* SNPs and OCD in a comprehensive evaluation of the gene. Further study with independent samples and larger sample sizes is required.

Introduction

Obsessive-compulsive disorder (OCD) is characterized by the presence of obsessions and/or compulsions that result in marked anxiety and significant impairment of functioning [1]. This is a common illness with lifetime prevalence rates of 2–3% [2]. Twin, family, and segregation studies have demonstrated that OCD is both familial and genetic [3] and its heritability rate is in the range of 27–65% [4]. Over 60 candidate gene studies have been conducted, most of which focused on serotonergic and dopaminergic pathway genes [3]. Unfortunately, these have not led to conclusive findings. More recently, glutamate has emerged as a focus of interest in the study of OCD pathology.

The role of glutamate as an important neurotransmitter affecting OCD pathophysiology has been supported by neuroimaging, animal model, psychopharmacology and initial candidate gene studies. Altered glutamate levels have also been reported in OCD. Lower levels in anterior cingulate region [5,6], and their projection areas in the striatum [5–7] were found in adults and treated children whereas higher levels of glutamate was found in treatment naïve OCD children [8]. Cerebrospinal fluid glutamate levels were also found to be significantly higher ($P = 0.014$) in OCD cases versus controls [9]. Moreover, glutamate modulating drugs have recently been used in augmentation for pharmacological management of OCD in adults [10,11], adolescents and children [12].

Recent animal models involving glutamatergic pathways have been developed. Knockout mice for the striatum-expressed SAPAP3 gene, which plays a role in the excitatory synapses, developed facial lesions,

repetitive grooming behaviors and anxiety [13]. Other animal studies have shown a significant reduction in fear memory in GRIK2 deficient mice [14]. Shatiel et al. [15] found that *GRIK2* knockout mice demonstrated less anxious behaviors and greater risk-taking and aggressive behaviors compared to wild type mice.

Glutamate related genes have been associated with OCD in human studies. Previous studies found association between OCD and the SLC1A1 glutamate transporter gene [16–19]. Arnold et al. [20] reported that polymorphisms in the 3′ untranslated region of *GRIN2B* (glutamate receptor, ionotropic, *N*-methyl-D-aspartate [NMDA] 2B gene) were associated with OCD in affected families, supporting the hypothesis that NMDA is involved in OCD pathophysiology. Additionally, an association between the previously mentioned SAPAP3 gene and pathologic grooming behaviors in humans has been found [21].

Different glutamate kainate receptor (GRIK) subtypes contribute to the regulation of both excitatory and inhibitory transmission and play an important role in synaptic physiology and plasticity [22]. The GRIK2 gene, which codes for the kainate receptor subunit 2, is split into 17 exons, covering an approximately 670 kb region of chromosome 6 [22]. Abundant GRIK2 mRNA has been found in dentate gyrus, pyramidal neurons of cornu ammonis 3 (CA3 of hippocampus), cerebellar granule cell layer, and neocortical areas [23]. Studies of other mental disorders have reported associations between *GRIK2* polymorphisms and schizophrenia [24], autism [25], early-onset Huntington's disease [26], and autosomal recessive mental retardation [27].

Delorme et al. [28] in a case-control and trio approach, evaluated the association of three *GRIK2* single nucleotide polymorphism (SNPs) with OCD. The Delorme et al. study [23] found that the *GRIK2* SNP I867 (rs2227283), previously found to be associated with autism, was undertransmitted ($P < 0.03$) in OCD trios, supporting an etiological role for this variant. However, the GRIK2 gene and OCD association has not been evaluated by other studies to date and a broad evaluation of the gene was still needed.

In the present study, an extensive exploration of GRIK2 gene was performed: twenty-one polymorphisms on the GRIK2 gene, comprehensively selected, were evaluated in 47 trios of OCD probands.

Material and Methods

Subjects

Forty-seven probands meeting DSM-IV criteria for OCD and their parents (47 trios) were enrolled in this study. Trios were recruited from specialized OCD clinics in New Haven, CT, U.S.; Boston, MA, U.S.; and Paris, France. In order to establish OCD diagnosis, OCD probands and their parents underwent structured clinical interviews including the Structured Clinical Interview for DSM-IV-Non-Patient edition (SCID-NP) [29] for adults, and the Kiddie Schedule for Affective Disorders and Schizophrenia-Present and Lifetime Version (KSADS-PL) [30] for children younger than 18 years of age. Interviewers from the Paris site were child psychiatrists with expertise in OCD and tic disorders. Interviewers from the Yale and Harvard sites had at least a Bachelor's degree and went through a rigorous training program for the conduct of structured interviews. At all sites, interviews were reviewed by two expert clinicians, a best estimate diagnosis was assigned [31], and then diagnoses were confirmed by an expert clinician interview. Twenty-one of the subjects from France, included in the current study, were also examined in a previous OCD and GRIK2 association study by Delorme et al. [28]. Written informed consent was obtained from all adult participants and written assent was obtained from children under 18 years of age in the presence of their parents, who provided the written consent on their behalf.

Genotyping

DNA was extracted from peripheral blood and buccal cell samples using Gentra protocols [32]. Samples with low yields underwent whole genome amplification via the REPLI-g protocol (QIAGEN). SNPs thought to be highly polymorphic were selected using the program Tagger [33], and by downloading all available SNP information from dbSNP, Celera, and HapMap databases. Nineteen SNPs were chosen across 684 kb to create a Linkage Disequilibrium map in 93 CEPH samples (12 families, 24 trios) for the region surrounding GRIK2. These SNPs, in addition to 2 of the 3 SNPs evaluated in the Delorme and others [28] study, were then used to select an informative pairwise set of 21 SNPs evaluated on this study [33]. Genotyping was performed on the Sequenom hME platform [34]. Markers with genotyping success rates <85% in our sample, with minor allele frequency <1%, with greater than 2 Mendel errors, or with a Hardy–Weinberg *P*-value of <0.05 were excluded. The 21 SNPs covered GRIK2, spanning 610.9 kb, with a density of 38.9 kb/SNP. The average r^2 value between tested (e.g., TAG) SNPs and other known SNPs examined in our CEPH samples was 0.929. After the 21 TAG SNPs were genotyped, both single marker and haplotype associations were examined.

Statistical Analyses

Analyses were completed with Family Based Association Test (FBAT) version 1.5.5 [35] and Haploview [36]. The bi-allelic mode of FBAT under an additive model, with a minimum of 1 informative family as a default threshold and minor alelle frequancy equal to 0.001, was used. FBAT was used to conduct single-marker and haplotype analyses by calculating *P*-values for individual SNPs and their associated haplotypes. Haploview was used to define linkage disequilibrium in the region. To minimize the odds of false-positive association, permutation analysis with 100,000 permutations was performed. Using the FBAT program, haplotype-specific and global permuted *P*-values were calculated for the haplotypes selected by Haploview.

Results

Among the 47 probands, 26 were from United States and 21 were from France. All of them were non-Hispanic white individuals. Among the U.S. probands, 17 were male and 9 were female; 24 were children; and the mean age was 12–13 years. Among French probands, 15 were male and 6 were female; only 3 of them were adults; and the mean age was 20–25 years. Data regarding age was lacking for 2 individuals in the U.S. subsample and for 8 probands in the French subsample.

In Table 1, the SNPs examined are described. Among the 21 single markers, the SNP # 16, rs1556995 ($P = 0.0027$) was significantly associated with the presence of OCD (Table 1). This SNP has a minor allele frequency of 23.8%, with the major and minor alleles being A and G, respectively. The A allele was over-transmitted in 19 informative trios (Table 1). A two marker haplotype (#16/#17, rs1556995/rs1417182, G/C), that included the associated locus, was significantly under-transmitted for OCD probands ($P = 0.0012$). The single marker #16, rs1556995 (permuted $P = 0.03$) and G/C haplotype (frequency: 25%; permuted *P*-value = 0.01) remained significantly associated after 100,000 permutations.

Table 2 compares the significant findings of this study and of the previous study by Delorme [28].

As noted in the methods section, some overlap exists between the present sample and the French study sample [28]. In an effort to provide stringent and conservative analyses, a secondary analysis excluding all French samples was performed. In this secondary analysis, data from 26 U.S. trios were analyzed. The secondary analysis found significant association with the 2 SNPs #16, rs1556995 ($P = 0.0067$) and #17, rs1417182

Table 1 Association with single marker GRIK2 SNPs in entire sample and U.S. subsample

Marker #	SNP name	Position	Allele	Total sample						U.S. subsample					
				OT	MAF	T:U	Chi- square	Z score	*P*-value	OT	MAF	T:U	Chi-square	Z score	*P*-value
1	rs2787554	101999105	G:C	C	0.398	18:14	0.5	0.707	0.48	C	0.451	8:07	0.067	0.258	0.79
2	rs1856310	102004252	G:T	T	0.383	16:12	0.571	0.756	0.45	=	0.439	7:07	0	0	1
3	rs10485268	102010156	G:A	–	0.05	4:03	0.143	–	1	=	0.073	3:02	0.2	0	1
4	rs2518224	102013370	A:C	A	0.102	8:04	1.333	1.155	0.25	A	0.122	4:01	1.8	1.342	0.18
5	rs2518166	102020415	C:T	T	0.375	16:15	0.032	0.18	0.86	T	0.425	9:07	0.25	0.5	0.62
6	rs990386	102035896	C:T	T	0.464	22:16	0.947	1.121	0.26	T	0.476	11:07	0.889	1.147	0.25
7	rs989467	102039780	C:T	T	0.373	17:14	0.29	0.707	0.48	T	0.415	8:07	0.067	0.5	0.62
8	rs2579945	102041883	G:C	–	0.105	7:07	0	0	1	G	0.049	2:01	0.333	0.577	0.56
9	rs1856133	102207593	T:C	T	0.34	16:14	0.133	0.365	0.72	T	0.375	8:06	0.286	0.535	0.59
10	rs12193068	102208930	A:C	A	0.069	4:03	0.143	0.378	0.71	–	0.075	1:01	0	0	1
11	rs9498659	102210130	A:G	A	0.354	18:15	0.273	0.522	0.60	A	0.378	8:06	0.286	0.535	0.59
12	rs9485526	102217877	A:G	A	0.331	18:15	0.273	0.522	0.60	A	0.366	10:06	1	1	0.32
13	rs1337415	102219218	G:A	G	0.31	17:14	0.29	0.539	0.59	G	0.346	8:06	0.286	0.535	0.59
14	rs1415483	102219857	C:T	C	0.114	9:06	0.6	0.775	0.44	C	0.134	6:03	1	1	0.32
15	rs6926170	102418194	C:T	T	0.494	24:15	2.077	1.441	0.15	T	0.5	11:05	2.25	1.5	0.13
16	rs1556995	102424038	A:G	A	0.238	20:05	9	3	**0.0027**	A	0.269	10:01	7.364	2.714	**0.0067**
17	rs1417182	102440034	T:C	T	0.494	25:16	1.976	1.406	0.16	T	0.476	11:03	4.571	2.138	0.03
18	rs1475919	102465548	G:A	G	0.222	14:08	1.636	1.279	0.20	G	0.25	6:04	0.4	0.632	0.53
19	rs3213607	102590049	C:A	–	0.102	7:07	0	0	1	–	0.11	3:03	0	0	1
20	rs2227283	102610010	G:A	G	0.423	17:15	0.125	0.354	0.72	G	0.408	7:05	0.333	0.577	0.56
21	rs2235076	102622953	G:A	G	0.026	2:00	2	1.41	0.16	–	0.026	0:00	N/A	–	NN

MAF: minor allele frequency; T:U: transmitted: untransmitted alleles; N/A: not applicable due to MAF; OT: Over-Transmitted Alelle.

Table 2 Comparison between significant SNPs in the current study and in the study of Delorme et al. [23]

			Current study		Delorme and others (2004) study	
Marker #	SNP name	Position	MAF	Single-marker *P*-value	MAF	Single marker *P*-value
15	rs6926170	102418194	0.494	0.150	N/A	N/A
16	rs1556995	102424038	0.238	0.0027	N/A	N/A
17	rs1417182	102440034	0.494	0.160	N/A	N/A
20	rs2227283	102610010	0.423	0.724	0.45	0.60
21	rs2235076	102622953	0.026	0.157	0.02	0.03
N/A	rs2227281	102609889	N/A	N/A	0.37	0.45

MAF: minor allele frequency; N/A: not applicable.

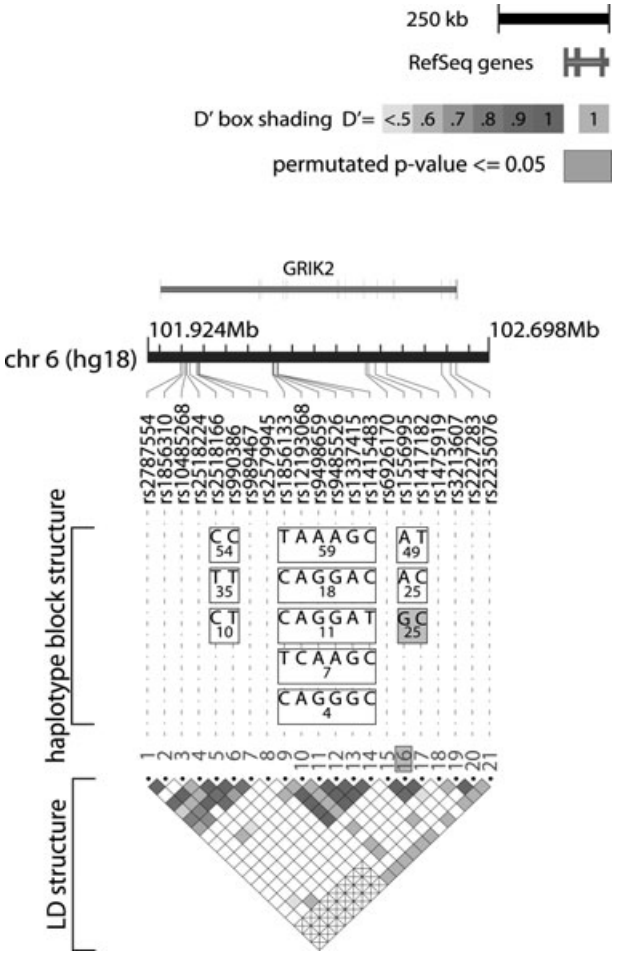

Figure 1 LocusView [37] diagram of GRIK2 association with OCD.

($P = 0.0325$) (Table 1) which comprise the significant haplotype found in the primary analysis (Fig. 1). The A allele of SNP #16, rs1556995, was over-transmitted in 14 informative trios. SNP #17, rs1417182 has a minor allele frequency of 47.6%, with the major and minor alleles being T and C, respectively. The T allele was over-transmitted in 15 informative trios. These two SNPs (#16, rs1556995; #17, rs1417182) and the SNP #15, rs6929170 comprise the three SNP haplotype (Fig. 2) found to be significantly undertransmitted to OCD probands (transmitted:untransmitted = 1.2:10.9) in the secondary analysis. The C/G/C haplotype had a frequency of 30.3% and a *P*-value = 0.0057, and also remained significant after 100,000 permutations ($P = 0.05$). The secondary analysis found the same results as the primary analysis.

Discussion

This is the second study reporting an association between SNPs in the GRIK2 gene and the OCD phenotype. Two separate analyses were performed: a primary analysis with 47 U.S. and French trios and a conservative secondary analysis with only the subsample of 26 U.S. trios. In the primary analysis, the SNP rs1556995 ($P = 0.0027$; permuted $P = 0.03$) and a two marker haplotype (#16/#17; rs1556995/rs1417182) ($P = 0.0012$; permuted $P = 0.01$) were significantly associated with OCD, and withstood permutation testing.

Glutamate has been shown to play important roles in many brain processes including neurodevelopment, learning, acute and chronic neurodegeneration, stress response and anxiety. Exposure to severe stress has been associated with glutamate excitotoxicity, which can cause neuronal damage and/or death [38]. Glutamate exerts its actions through ligand-gated ion channel (ionotropic) receptors, including the NMDA, kainate (GRIK), and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtypes, and G protein-coupled metabotropic receptors (mGluR1-8) [23].

Ionotropic kainate glutamate receptor-2 (GRIK2) mRNA has been found to be prominent in striatal neurons and the anterior caudate areas [23]. GRIK receptors play a role in synaptic transmission, synaptic plasticity, and glutamate-induced neuronal degeneration in basal ganglia.

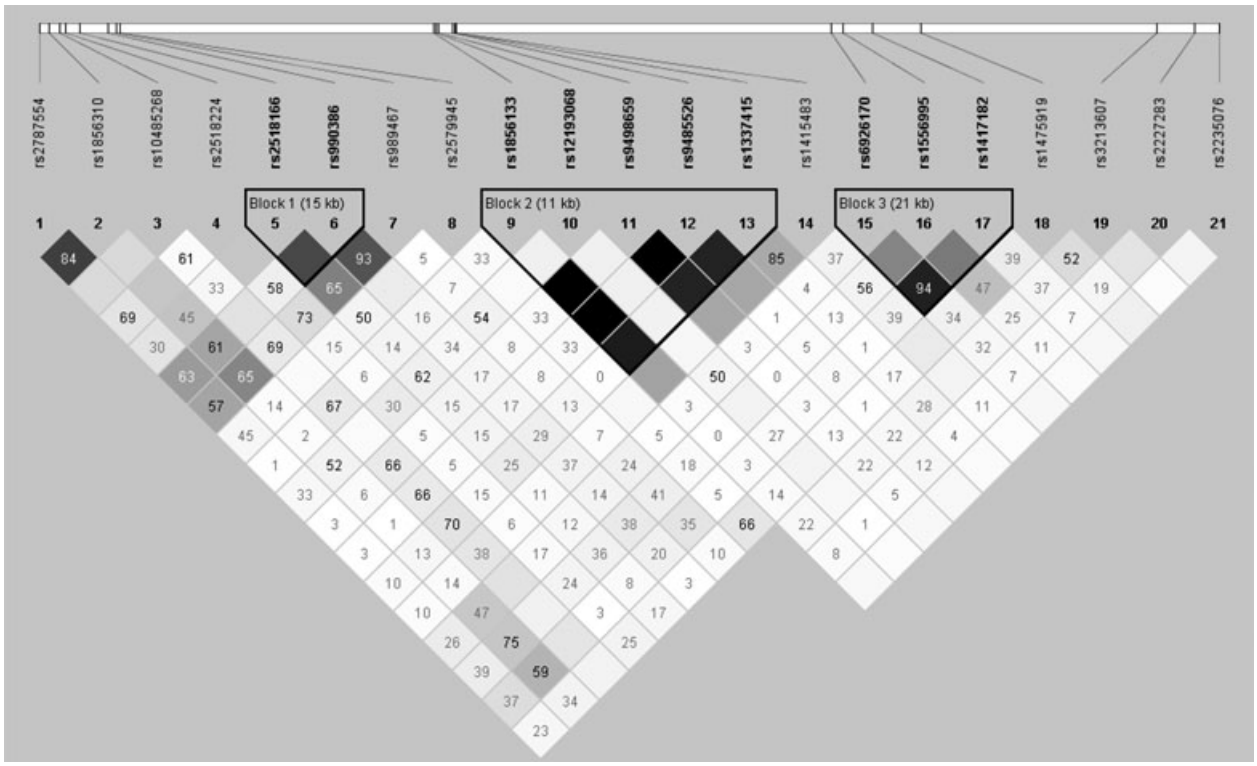

Figure 2 GRIK2 haplotype blocks in the U.S. subsample.

As previously mentioned, genes involved in serotonergic, dopaminergic, and other neurotransmitters pathways have been examined in OCD. Glutamate interacts in a complex manner with monoaminergic systems impacting the release of other neurotransmitters including monoamines and GABA. Glutamate agonists also facilitate presynaptic synthesis and release of dopamine in the prefrontal cortex [7]. Similarly, there is a mutual influence between glutamate and serotonin transmission. Glutamate receptor antagonism leads to an enhancement of 5HT2A receptor-mediated transmission; 5HT2A agonist leads to reduced glutamatergic transmission [7] and 5-HT receptor activation reduces the excitatory effect of glutamate on cellular activity [38]. This interaction is believed to contribute with some mechanisms of psychiatric symptoms [39]. Consistent with this, the efficacy of antiglutamatergic agents, such as riluzole and memantine, in treating OCD has also been reported in early studies [11]. Based on the above, it is possible that the genetic vulnerability for OCD includes genetic variations in glutamate related pathways. The most accepted etiological model for OCD is that for complex disorders, which involves the interaction of several genes, each with small effects, and the environment. As such, the GRIK2 gene could have a role, in combination with other genes, in OCD etiology.

The present study did not replicate the findings of the previous GRIK2 and OCD association study, performed by Delorme et al. [28]. The SNP rs2235076, associated with OCD in the Delorme et al. [28] study, was negative in our study. Additionally, Delorme et al. [28] did not analyze rs1556995, the SNP significantly associated with OCD in our study, or rs1417182, which, with rs1556995, makes up the 2-marker haplotype significantly associated with OCD in our study. It is possible that our findings are false negative, particularly in light ofthe low minor allele frequency (0.026) of rs1556995 and our smaller sample size (47 trios). However, GRIK2 is a large gene that spans 185.851bp and the SNPs found to be associated with OCD, in the present study, are relatively close to the GRIK2 SNP previously reported as associated with OCD by Delorme et al. [28]. A possible explanation for the different results of the current study and the Delorme et al. study is that the association between the OCD phenotype and significant SNPs/haplotypes may be not directly related to the SNPs found to be associated with OCD in the two studies, but to linked genes or variants.

The SNP rs1556995, found to be associated with OCD in this study, is in an intronic region of the GRIK2 gene. Although introns had previously been assumed to be "junk DNA," the regulatory role of RNA coded by intronic regions (noncoding RNAs) has recently been studied in complex psychiatric disorders [40]. Although there is no known noncoding RNA on this region, 2 expressed sequence tags (ESTs) were identified in the area of rs6926170: CV376356 and CV376382. This is important to note as ESTs represent portions of expressed genes and may become tools to refine prediction of gene transcripts, their protein products, and eventually of their function [41].

Limitations of this study must be acknowledged. With the small sample size of 47 families, the association power is about 17.5% (MAF = 0.238; $r^2 = 1$, alpha = 5%, disease frequency = 2%) [42]. In addition 21 SNPs were examined, raising the possibility of Type I error. Despite this, a significant association was found and remained significant when the risk of false positive was controlled by permutation analysis. Furthermore, different assessment methods between sites as well as the OCD clinical heterogeneity may have been sources of confounding bias.

As such, these preliminary findings provide support for the GRIK2 gene as a possible candidate for OCD etiology. Studies with independent samples and large sample sizes are required for confirmation.

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Conflict of Interest

The authors have no conflict of interest.

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