Neuropsychiatric Disease and Treatment

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ORIGINAL RESEARCH

Association Between the *SLC1A1* Glutamate Transporter Gene and Obsessive-Compulsive Disorder in the Chinese Han Population

This article was published in the following Dove Press journal:
Neuropsychiatric Disease and Treatment

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Background: Obsessive-compulsive disorder (OCD) is a common, serious and genetically related mental illness; the etiology of OCD has not yet reached a definitive conclusion. Multiple evidence suggests that the glutamatergic system plays a major role in the pathophysiology of OCD. However, subsequent studies on the glutamate transporter gene are not consistent. OCD is a heterogeneous disease. To resolve the complex genetic basis of OCD, division the disorder into different subphenotypes is an effective method for studying the pathogenesis of OCD.

Methods: We recruited 438 OCD patients and 465 age- and sex-matched controls from a Chinese Han population. *rs10491734, rs3780412, rs301434 and rs3087879* SNPs were genotyped by real-time TaqMan polymerase chain reaction, and the chi-squared test was used to compare allele and genotype frequencies of variants between the two groups.

Results: The genotype of *rs301434* was statistically significant in total patients with OCD and the controls. After grouping by age and gender, the genotype of *rs301434* was statistically significant in early-onset OCD, late-onset OCD as well as male OCD, the allele and genotype of *rs3780412* was associated with late-onset OCD. Haplotype analysis showed that four loci haplotypes (G-A-A-G and G-G-A-G) were associated with total OCD, (G-G-A-G) was associated with female OCD, (G-A-G-G) was associated with male OCD, (G-A-A-G and G-G-A-G) were associated with late-onset OCD.

Conclusion: This study provides suggestive evidence that SLC1A1 may be involved in the development of OCD in the Han population. However, these findings require further replication.

Keywords: obsessive-compulsive disorder, glutamate transporter gene, haplotype

Introduction

Obsessive-compulsive disorder (OCD) is a common mental illness with complicated clinical symptoms. The disease is characterized by intrusive unwanted thoughts and repetitive behavior. The lifetime prevalence of patients is between 1% and 3%, and it is listed as one of the ten most disabling diseases in the world by the World Health Organization (WHO).[1,2] OCD is often accompanied by other mental illnesses, such as Tourette's syndrome or eating disorders, which makes treatment more difficult.[3,4]

More and more evidences show that the glutamatergic system plays an important role in the etiology and subsequent treatment of OCD.[5] Imaging and biological studies have shown that glutamate dysregulated neurotransmission in special parts

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DovePress
http://doi.org/10.2147/NDT.S281623

of the brain leads to the appearance of OCD symptoms.[6] According to multiple independent family-based association studies and a case-control analysis, the SLC1A1 gene is closely related to the occurrence of OCD.[7–9] Studies have pointed out that compared with the control group, the concentration of glutamate in the cerebrospinal fluid of OCD patients is higher.[10] And the abnormal glutamatergic transmission in the cortex-striatum-thalamus-cortex (CSTC) circuit plays a certain role in the pathogenesis of OCD.[11] In addition, it has been observed that children with OCD a decreased amount of glutamate in the anterior cingulate cortex. In the brain regions of patients with OCD, the level of glutamate receptors is also modified. It has been reported that serotonin can affect dopaminergic activity indirectly through the glutamatergic and GABAergic systems.[12] It has been reported that serotonin can influence dopaminergic activity indirectly through the glutamatergic and GABA-ergic systems.[13]

SLC1A1 is located on chromosome 9p24, which is expressed in brain regions related to OCD,[14] including the cerebral cortex, striatum, and thalamus.[15] As research into the hereditary pattern of OCD increases, the role of the glutamate transporter gene SLC1A1 in the pathogenesis of the disease has attracted attention.[9,16–18] The glutamate transporter EAAC1 (EAAT3) is a crucial transporter for mammals. Approximately 30–40% of synapses in the mammalian brain are affected by EAAC1, which is encoded by SLC1A1 gene.[19] Bellini et al found that mice lacking EAAC1 showed increased anxiety-like and disrupted grooming behaviors, and then they identify new molecular mechanisms by which EAAC1 can shape glutamatergic and dopaminergic signals and control repeated movement execution.[20] An important function of EAAC1 is to stop the postsynaptic effects of glutamate as well as to regulate extrasynaptic glutamate levels, thus limiting the activation of extra-synaptic neurotransmitter receptors by rapidly removing released glutamate from the synaptic cleft and so alleviating subsequent excitotoxicity. EAAC1 enables excitatory transmission between synapses to function correctly. The SLC1A1 gene polymorphisms may be factors which contribute to glutamate dysfunction in cases of OCD.

It has been speculated that genetic variation within or near the SLC1A1 gene is associated with OCD in the Chinese Han population. OCD is a complex disease, in which patients with early-onset OCD and patients with late-onset OCD have different genetic foundations and clinical symptoms, which makes their treatment results often different. We classified the onset-age <18 as early-onset and the onset-age≥18 as late-onset in the present study. We aimed to provide basic evidence for SLC1A1 as a candidate gene for the etiology of OCD in this population. A total of 438 OCD patients and 465 healthy controls were genotyped, and four SNPs (*rs10491734, rs3780412, rs301434 and rs3087879*) were selected to validate our hypothesis.

Materials and Methods

Case Control Sample

A total of 438 OCD patients (mean age, 29.27±13.96 years) and 465 controls (mean age, 28.77±9.25 years) from the Affiliated Hospital of Medical College Qingdao University participated in this study. All subjects provided written informed consent, children in the present study were written and provided by their guardians. The OCD patient group included 260 male patients and 178 female patients, while the control group comprised 276 male subjects and 189 female subjects. We diagnosed patients according to the criteria of the Diagnostic and Statistical Manual of mental disorders (DSM-IV) and Obsessive compulsive symptoms of participants were assessed through the Yale–Brown Obsessive Compulsive Scale Checklist (YBOCS-CL),[21] indicating that all patients were severely affected (26.72 ±4.43). YBOCS severity scale scores range from 0 to 40, with a score ≥16, indicating clinically significant symptoms. A score of≥16 on the YBOCS severity scale was required for inclusion in this study. Subjects with a diagnosis, according to DSM-IV, of schizophrenia, recurrent major depression, bipolar disorder, mental retardation, alcohol or other substance abuse within the last 6 months or a history of psychosurgery, encephalitis, or significant head trauma were excluded. Subjects showing slight OCD symptoms with serious comorbidity symptoms, such as anxiety, depression and tic, were also excluded. Subjects refusing to participate or permit the extraction of venous blood were excluded.

Healthy control (n=465) subjects were recruited from the Center of Health Examination of the Affiliated Hospital of Qingdao University Medical College. All controls were included after being interviewed using the Diagnostic Interview for Genetic Studies[22] and Family Interview for Genetic Studies (assessing first-degree relatives of control families according to the

reports of controls) to confirm the absence of both personal and familial history of OCD and other psychiatric disorders. Two experienced psychiatrists conducted a MINI for each member of the control samples to ensure that none of the controls suffered from any psychiatric disorders before beginning the current study.

The protocol of this study conformed to the ethical guidelines of the 1975 Declaration of Helsinki. This study was approved through the Ethics Committees of Affiliated Hospital of Qingdao University Medical College. All subjects provided written informed consent. In particular, the informed consent for children in the present study was written and provided by their guardians.

DNA Analysis and Statistical Analysis

Genomic DNA was extracted from leukocytes in the peripheral blood using standard methods. DNA amplification was conducted using polymerase chain reaction (PCR). The following PCR primer sequences were used: *rs301434* forward, 5-ACGTTGGATGGCCCTGAAAAATCCCTTGAC-3, and *rs301434* reverse, 5-ACGTTGGATGCAAGGGCAAG GACTTGTCTC-3; *rs3780412* forward, 5-ACGTTGGA TGAGCCCCCACAAAATACTCTG-3, and *rs3780412* reverse, 5-ACGTTGGATGGAAGAGGTTTTATGTTTG TC-3; *rs10491734* forward, 5-ACGTTGGATGGGAGA CTTTGACTTTGCCAC-3, and *rs10491734* reverse, 5-ACGTTGGATGCTTTTTGTTTCTGAATGCCC-3; *rs3087879* forward, 5-ACGTTGGATGTGCAGAGT AAATCCCACGAC-3, and *rs3087879* reverse, 5-ACGTTGGATGGGAGGAGACAAGAGTCATAG-3. PCR was performed in a final volume of 5 μL containing 1 μL of genomic DNA, 0.95 μL of H_2O, 0.625 μL of PCR Buffer (10×), 0.325 μL of Mg Cl_2 (25 mM), 1 μL of dNTP (2.5 mM each), 1 μL of primer, and 0.1 μL of HotStarTaq DNA Polymerase (5 U/μL). The following cycling conditions were used: initial denaturation at 94°C for 15 min, followed by 45 cycles at 94°C for 20 s, 56°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 3 min and storage at 4°C. Subsequently, we added the SAP reaction mix (final volume of 2 μL), containing 1.53 μL of H_2O, 0.17 μL of SAP Buffer (10×), and 0.3 μL of SAP enzyme (1 U/μL), to the PCR product. The reaction was initiated at 37°C for 40 min, followed by incubation at 85°C for 5 min. The reaction was maintained at 4°C. iPlex reagent (Sequenom, San Diego, CA) (final volume of 2 μL, containing 0.755 μL of H_2O, 0.2 μL of iPlex Buffer (10×), 0.2 μL of iPlex Termination mix, and 0.041 μL of iPlex enzyme) was then added to the reaction product. The following cycling conditions were used: 94°C for 30 s, followed by 40 cycles at 94°C for 5 s, five cycles at 52°C for 5 s, and 80°C for 5 s, with a final extension at 72°C for 3 min and storage at 4°C.

Following purification, the reaction product was analyzed using MassARRAY SpectroCHIP (Sequenom, San Diego, CA). SNPs were detected with a MassARRAY Compact Analyzer (Sequenom, San Diego, CA). Results were analyzed using TYPER software (Sequenom, San Diego, CA) and genotyping data were obtained. Detection accuracy was 99.6%. SNP genotyping was performed at Shanghai Benegene Biotechnologies Co. Ltd., and data analysis was conducted using SPSS software (version 17.0 for Windows; SPSS, Inc., Chicago, IL, USA). Age comparisons between the OCD and control groups were made with a *t*-test. Allelic, genotypic and haplotype frequencies between OCD participants and controls, as well as to estimate the Hardy-Weinberg equilibrium were established by SHEsis software (http://analysis.bio-x.cn).[23]

Results

No deviation of the Hardy-Weinberg equilibrium was found in the distribution of the four SNPs among OCD and control groups (P>0.05). We found significant differences in genotype frequencies of *rs301434* between all OCD and control groups, while there were significant differences in genotype frequency of *rs301434* between early-onset OCD and control groups, late-onset OCD and control groups as well as male OCD and control groups (total $\chi2$=9.948, P=0.007; male $\chi2$=8.766, P=0.013; female $\chi2$=2.331, P=0.311; early-onset $\chi2$=8.982, P=0.011; late-onset $\chi2$=8.839, P=0.012).

We also found that genotype and allele frequencies of *rs3780412* were statistically significant for the late-onset OCD and control groups (genotype $\chi2$=7.196, P=0.027; allele $\chi2$=5.575, P=0.018). However, we found that genotype and allele frequencies of *rs10491734* and *rs3087879* were not statistically significant for OCD or control groups.

Four loci haplotypes (*rs10491734-rs3780412-rs301434-rs3087879*) were found to be associated with OCD. Haplotypes G-A-A-G and G-G-A-G were statistically significant for all OCD and control groups (P=0.033 and 0.030, respectively). Haplotype

G-A-G-G was associated with the male OCD group (P=0.010), while G-G-A-G was associated with the female OCD group (P=0.039). Finally, we found that G-A-A-G, G-G-A-G were associated with the late-onset OCD groups (P=0.019 and 0.050, respectively). In addition, our use of haplotype analysis showed that G-A-G-G is a risk factor for male OCD (OR = 1.737, 95% CI: 1.134–2.660), while G-G-A-G is a risk factor for total (OR = 1.412, 95% CI: 1.033–1.930), female (OR = 1.670, 95% CI: 1.021–2.730) and late-onset OCD (OR = 1.469, 95% CI: 0.997–2.166).

Discussion

Obsessive-compulsive disorder is a complex multifactorial disease, which seems to be affected by environmental and genetic factors. Recent reports indicate that glutamate transporter gene mutations play a role in the etiology of OCD. Our study found significant differences in genotype frequencies of *rs301434* between total OCD group and control group. After grouping by gender and age, the results indicate a significant difference in the genotype frequency of *rs301434* between early-onset OCD and control groups, late-onset OCD and control groups as well as male OCD and control groups (See Table 2). Arnold et al found that two variants located within a single haplotype block, *rs301434* and *rs301435*, were associated with the transmission of OCD,[7] while de Salles Andrade and colleagues found that the A-A-G (*rs301434-rs3780412-rs301443*) haplotype was twice as common for people with OCD than for controls. Regarding clinical characteristics, the G-A (*rs301434-rs3780412*) haplotype in patients with OCD seems to be related to the symptoms of hoarding.[24] This finding implicated that *rs301434* may be an important SNP to OCD in the Han Chinese population. *Rs301434* may influence the development of OCD through expression of the neuronal glutamate transporter.

Dickel et al found a positive relationship between *rs3780412, rs301430* and OCD, where the association is limited to males in *rs3780412*.[8] Wendland et al found three highly significant synthetic markers in haplotype analysis, where *rs3087879, rs301430* and *rs3858819* were significant in the OCD haplotype test.[9] When we tested *rs3780412* and *rs3087879* in the Han population, we found a significant difference in allele and genotype frequency of *rs3780412* between late-onset OCD and control groups. However, genotypes and alleles of *rs3087879* were not statistically significant between OCD patients and control groups, nor between the stratified groups (See Tables 1 and 2). The further research significance of dividing OCD into early and late subtypes lies in exploring the genetic and neurobiological determinants of OCD and predicting the best treatment plan. The symptoms of OCD are heterogeneous and are thought to emerge from complex genetic, environmental, and epigenetic interactions. Compared with late-onset OCD, early-onset OCD are more likely to be associated with Tourette syndrome, have greater heritability, and have more difficult treatment. We speculate that late-onset OCD may be related to *rs3780412*, which may have connection with the influence of psychosocial factors on patients. Certain social factors may have an impact on this SNP, which is expressed in patients with late-onset OCD. We will make further analysis of related influencing factors in future research.

Some authors have found that 3-SNP haplotype *rs4740788-rs10491734-rs10491735* was associated with a total sample of OCD patients as well as with male OCD.[16] Wu and colleagues found that *rs10491734* was significantly associated with early-onset OCD.[25] However, we did not reach this conclusion for the Han population (See Tables 1 and 2). This may be due to the polymorphism of the locus.

Although we only found positive results in the two SNPs *rs301434* and *rs3780412*, haplotypes studies found more positive results, which may be due to polymorphisms at multiple loci alleles affect different subtypes of OCD and clinical symptoms (See Table 1). We found that haplotypes of the four SNPs (*rs10491734-rs3780412-rs301434-rs3087879*) showed significant differences between the OCD and control groups as a whole. After classifying OCD participants on the basis of sex and age, we found significant differences between male OCD and controls, female OCD and controls as well as late-onset OCD and controls. The haplotype G-A-A-G was associated with both total OCD and late-onset OCD. Haplotype G-A-G-G was associated with male OCD and is a risk factor for male OCD. Haplotype G-G-A-G was associated with total OCD, female OCD and late-onset OCD, and is a risk factor for these groups (See Table 3).

Table 1 The Results of Single-Site Allele Association Analysis for the Overall, Male, Female, Early-Onset, and Late-Onset OCD Samples

SNP	Group	No	Allele		OR (95% CI)	X^2	P
rs10491734	overall OCD	438	A(0.212)	G(0.788)	0.89 (0.71–1.12)	0.994	0.319
	control	465	0.231	0.769			
	male OCD	260	A(0.214)	G(0.786)	0.82 (0.62–1.09)	1.909	0.167
	male control	276	0.250	0.750			
	female OCD	178	A(0.208)	G(0.792)	1.04 (0.72–1.49)	0.052	0.819
	female control	189	0.201	0.799			
	early onset OCD	252	A(0.221)	G(0.779)	0.95 (0.73–1.23)	0.150	0.698
	control	465	0.230	0.770			
	late onset OCD	186	A(0.199)	G(0.801)	0.83 (0.62–1.12)	1.499	0.221
	control	465	0.230	0.770			
rs3780412	overall OCD	438	A(0.751)	G(0.249)	0.89 (0.72–1.11)	0.976	0.323
	control	465	0.771	0.229			
	male OCD	260	A(0.754)	G(0.246)	0.96 (0.73–1.27)	0.071	0.789
	male control	276	0.761	0.239			
	female OCD	178	A(0.747)	G(0.253)	0.79 (0.56–1.12)	1.746	0.186
	female control	189	0.788	0.212			
	early onset OCD	252	A(0.782)	G(0.218)	1.06 (0.81–1.37)	0.177	0.674
	control	465	0.772	0.228			
	late onset OCD	186	A(0.710)	G(0.290)	0.72 (0.55–0.95)	5.575	0.018*
	control	465	0.772	0.228			
rs301434	overall OCD	438	A(0.852)	G(0.148)	0.79 (0.61–1.04)	2.802	0.094
	control	465	0.878	0.122			
	male OCD	260	A(0.850)	G(0.150)	0.74 (0.52–1.06)	2.702	0.100
	male control	276	0.884	0.116			
	female OCD	178	A(0.854)	G(0.146)	0.87 (0.57–1.32)	0.417	0.518
	female control	189	0.870	0.130			
	early onset OCD	252	A(0.857)	G(0.143)	0.83 (0.60–1.14)	1.326	0.249
	control	465	0.878	0.122			
	late onset OCD	186	A(0.844)	G(0.156)	0.75 (0.53–1.05)	2.757	0.097
	Control	465	0.878	0.122			
rs3087879	overall OCD	438	C(0.113)	G(0.887)	0.94 (0.70–1.25)	0.177	0.674
	control	465	0.119	0.881			
	male OCD	260	C(0.121)	G(0.879)	1.15 (0.79–1.68)	0.541	0.462
	male control	276	0.107	0.893			
	female OCD	178	C(0.101)	G(0.899)	0.72 (0.46–1.14)	2.004	0.157
	female control	189	0.135	0.865			
	early onset OCD	252	C(0.099)	G(0.901)	0.82 (0.58–1.17)	1.199	0.273
	control	465	0.118	0.882			
	late onset OCD	186	C(0.132)	G(0.868)	1.13 (0.78–1.62)	0.448	0.503
	Control	465	0.118	0.882			

Note: *p-value < 0.05 indicated significant statistical differences.

In conclusion, we found that the genotype of SLC1A1 *rs301434* is significantly associated with all OCD and control groups, early-onset OCD and control groups, late-onset OCD and control groups as well as male OCD and control groups. The genotype and allele of *rs3780412* is significantly associated with late-onset

Table 2 The Results of Single-Site Genotype Association Analysis for the Overall, Male, Female, Early-Onset, and Late-Onset OCD Samples

SNP	Group	No	Genotype			X^2	P
rs10491734	overall OCD	438	AA(0.046)	AG(0.332)	GG(0.622)	1.057	0.589
	control	465	0.058	0.346	0.596		
	male OCD	260	AA(0.050)	AG(0.328)	GG(0.622)	1.898	0.387
	male control	276	0.072	0.355	0.572		
	female OCD	178	AA(0.039)	AG(0.337)	GG(0.624)	0.053	0.973
	female control	189	0.037	0.328	0.635		
	early onset OCD	252	AA(0.048)	AG(0.347)	GG(0.606)	0.334	0.846
	control	465	0.058	0.344	0.598		
	late onset OCD	186	AA(0.043)	AG(0.312)	GG(0.645)	1.459	0.482
	control	465	0.058	0.344	0.598		
rs3780412	overall OCD	438	AA(0.555)	AG(0.393)	GG(0.053)	4.128	0.127
	control	465	0.606	0.329	0.065		
	male OCD	260	AA(0.565)	AC(0.377)	CC(0.058)	1.903	0.386
	male control	276	0.598	0.326	0.076		
	female OCD	178	AA(0.539)	AG(0.416)	GG(0.045)	3.052	0.217
	female control	189	0.624	0.328	0.048		
	early onset OCD	252	AA(0.599)	AG(0.365)	GG(0.036)	3.217	0.200
	control	465	0.609	0.327	0.065		
	late onset OCD	186	AA(0.495)	AC(0.430)	GG(0.075)	7.196	0.027*
	control	465	0.609	0.327	0.065		
rs301434	overall OCD	438	AA(0.735)	AG(0.233)	GG(0.032)	9.948	0.007*
	control	465	0.761	0.234	0.004		
	male OCD	260	AA(0.731)	AG(0.238)	GG(0.031)	8.766	0.013*
	male control	276	0.768	0.232	0.000		
	female OCD	178	AA(0.742)	AG(0.225)	GG(0.034)	2.331	0.312
	female control	189	0.751	0.238	0.011		
	early onset OCD	252	AA(0.746)	AG(0.222)	GG(0.032)	8.982	0.011*
	control	465	0.761	0.234	0.004		
	late onset OCD	186	AA(0.720)	AG(0.247)	GG(0.032)	8.839	0.012*
	Control	465	0.761	0.234	0.004		
rs3087879	overall OCD	438	CC(0.007)	CG(0.212)	GG(0.781)	1.391	0.499
	control	465	0.015	0.209	0.776		
	male OCD	260	CC(0.012)	CG(0.219)	GG(0.796)	0.621	0.733
	male control	276	0.011	0.192	0.797		
	female OCD	178	CC(0.000)	CG(0.202)	GG(0.798)	4.294	0.117
	female control	189	0.021	0.228	0.751		
	early onset OCD	252	CC(0.004)	CG(0.190)	GG(0.806)	2.159	0.339
	control	465	0.015	0.206	0.778		
	late onset OCD	186	CC(0.011)	CG(0.242)	GG(0.747)	1.118	0.572
	Control	465	0.015	0.206	0.778		

Note: *p-value < 0.05 indicated significant statistical differences.

OCD and control groups. The haplotypes (G-A-A-G, G-G-A-G) are associated with total OCD and control groups. Haplotype (G-G-A-G) is associated with female OCD, haplotype (G-A-G-G) is associated with male OCD, and haplotype (G-A-A-G, G-G-A-G) is associated with late-onset OCD. Our findings support the idea that SLC1A1 is a susceptibility gene for OCD, but this study also has limitations, due to the limited sample size, our results need to further increase the sample data for verification.

Table 3 The Results of the Haplotype Analysis for SNP rs10491734-rs3780412-rs301434-rs3087879 in OCD Patients and Controls

Haplotype[a]	Group	Case (Frequency, %)	Control (Frenquency, %)	X^2	P	OR (95%CI)
GGAG	All OCD	100.59(0.115)	77.22(0.083)	4.707	0.030	1.412 [1.033~1.930]
	Late-onset OCD	46.64(0.125)	77.03(0.083)	5.040	0.024	1.552 [1.055~2.283]
	Female OCD	44.69(0.126)	29.39(0.078)	4.243	0.039	1.670 [1.021~2.730]
GAGG	Male OCD	59.52(0.115)	38.21(0.069)	6.578	0.010	1.737 [1.134~2.660]
GAAG	All OCD	403.99(0.462)	468.24(0.503)	4.536	0.033	0.815 [0.675~0.984]
	Late-onset OCD	165.67(0.445)	469.92(0.505)	5.439	0.019	0.748 [0.585~0.955]

Note: Haplotypes with frequency<0.03 are ignored in analysis.

Acknowledgments

The authors wish to thank the patients, healthy volunteers, and their family members.

Disclosure

All authors declare no conflicts of interest for this work.

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