Int J Clin Exp Med 2015;8(3):4606-4610
www.ijcem.com /ISSN:1940-5901/IJCEM1401005

Original Article

Association study of dopamine transporter gene (DAT1) variable tandem repeat sequence (VNTR) with obsessive-compulsive disorder in Chinese Han Population

Shaoyan Zhang[1*], Weihua Jiang[2*], Xiuming Tang[3], Quanchen Xu[4], Jingli wang[5], Rui Gui[6], Xinhua Zhang[2], Shiguo Liu[5,7]

[1]Clinical Laboratory, The Affiliated Hospital of Qingdao University, Qingdao 266003, China; [2]Department of Psychiatry, Medical College, Qingdao University, Qingdao, 266021, China; [3]Reproductive Medicine Centre, The Affiliated Hospital o Qingdao University, Qingdao 266003, China; [4]The Affiliated Hospital of Qingdao University, Qingdao 266003, China; [5]Prenatal Diagnosis Center, The Affiliated Hospital of Qingdao University, Qingdao, 266003, China; [6]Clinical Laboratory, Pingyi Hospital Chinese Medicine, Shandong, China; [7]Genetic Laboratory, The Affiliated Hospital of Qingdao University, Qingdao 266003, China. [*]Equal contributors.

Received December 7, 2013; Accepted January 31, 2015; Epub March 15, 2015; Published March 30, 2015

Abstract: Objective: Multiple evidence suggests an involvement of the dopamine neurotransmitter system in Obsessive-compulsive disorder (OCD). Therefore, we explore the association of 3'UTR region of 40 bp variable tandem repeat (VNTR) polymorphism in Dopamine Transporter Gene (DAT1) in Chinese Han population. Methods: A total of 305 OCD patients and 435 healthy individuals were recruited for the study. OCD was diagnosed with the Forth Edition (DSM-IV) diagnostic criteria. After polymerase chain reaction of VNTR was used to evaluate the 40 bp VNTR polymorphism in *DAT1*, a case-control association analysis was performed by the χ^2 test. Results: The results showed that no association was found between OCD patients and controls for the genotype distribution (X^2 =0.743, P=0.690, df=2) as well as allelic (X^2=0.172, P=0.678, OR=0.928, 95% CI=0.885-1.224) distribution. Conclusions: Our data suggest that the 40 bp VNTR polymorphism in *DAT1* may not be associated with susceptibility to OCD in the Chinese Han population studied. However, this result needed to be replicated from different populations.

Keywords: OCD, DAT1, 40 bp VNTR, susceptibility, association analysis

Introduction

Obsessive-compulsive disorder (OCD), which has an estimated incidence of approximately 1.1%~3.3% [1], is the common revalent psychiatric disorder, characterized by force concept, forced impulse or compulsive behavior, etc. OCD usually onsets from 20 to 24 years old [2], which has serious affected the normal living, work and sociality badly and brought heavy burden to the society. Many studies showed that OCD is a much more complex factors disease and genetic factors may play a significant role [3-7]. But until now, no common vulnerability genetic variants which clearly associated with the disorder have been reported.

Previous studies showed several lines of evidence illustrated that dopamine system may be related to OCD. Vandenbergh *et al.* reported that reduced serotonin function and increased norepinephrine activity in the brain lead to aggressive and impulsive behavior in animal and human studies [8]. In addition, some antipsychotic drugs such as selective serotonin reuptake inhibitors (SSRIs), which adjust dopamine function activities in brain, can be used as a kind of aggrandizement lonely auxiliary instrument for OCD treatment [9]. As one of important dopamine transporters, dopamine transporter gene (DAT1, SLC6A3), located on 5p15.3, is responsible for the bulk of dopamine degradation in the striatum and the prefrontal cortex and the transfer of dopamine in the synaptic cleft, thus affecting the signaling transmission mediated by postsynaptic membrane. Some unit of length 40 bp variable tandem repeat (VNTR) repeat sequence in the 3'end of the

Table 1. Demographic Characteristics of OCD Patients and Controls

	OCD patients	Controls	*P*-value
Age, mean [SD]	31.4 [12.4]	31.6 [10.8]	0.15
Gender			
female	154	232	0.45
male	151	203	

non-translated region (3'UTR) of *DAT1* [8] may affect its expression. Mill et al. observed *DAT1* expression of the 10-repeat allele of VNTR polymorphism was increased in the cerebellum, frontal lobe, and lymphocytes [10]. This polymorphism has been associated with susceptibility to Tourette syndrome (TS) and Attention Deficit and Hyperactivity Disorder (ADHD) [11, 12].

In the present study, we hypothesized the association between OCD and the 40 bp VNTR polymorphism in *DAT1* and performed a case-control association analysis to assess whether the polymorphism in this gene could be implicated in susceptibility to OCD in a Chinese Han population.

Materials and methods

Study population

A total of 305 OCD patients [mean age (SD)=31.4 (12.4) years] and 435 control subjects [mean age (SD)=31.6 (10.8) years] were included in this study from the Affiliated Hospital of Medical College, Qingdao University (**Table 1**). The affected individuals included 154 male and 151 female patients and control included 232 male and 203 female. All patients were diagnosed according to Diagnostic and Statistical Manual of mental disorders (DSM-IV) diagnostic criteria, and the severity of the disorder was evaluated with the Yale-Brown Obsessive Compulsive Scale [13]. The control subjects were selected among healthy volunteers and all had no history of any psychiatric disorder. The patients with OCD and healthy controls were Chinese people from the same geographic region and of the same ethnic origin. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Ethics Committee of Affiliated Hospital of Medical College, Qingdao University.

Genetic analysis

Genomic DNA was extracted from peripheral blood leukocytes using standard methods. The segments of polymorphism were replicated using the primers as follows: 5-TCTGCTACA-AGTTCTGGC-3 and 5-CTGCAGCTTTTTCTCTAG-3. PCR reactions were carried out in a final volume of 10 μl containing 2 × PCR MasterMix, 0.5 μl primer and 50 ng of DNA. The reaction of DAT1 involved a starter step at high temperature of 94°C for 5 min followed by 35 cycles with the following cycle profile: denaturation at 94°C for 1 minute, annealing temperature 66°C for 1 min and elongation at 72°C for 30 s and a final elongation at 72°C for 10 min. The PCR products were directly detected by 3.5% agarose gel electrophoresis. A 50 bp DNA ladder was used to mark the relative molecular mass of PCR product. The allele produces present five fragments of 520, 480, 440, 400 and 360 bp, which were visualized under ultraviolet (UV) light.

Statistical analysis

For all data of patients and controls, the Hardy-Weinberg equilibrium of the genotype distribution was tested using the homogeneity chi-squared test. A case-control study was performed using the homogeneity chi-squared test, which was carried out using the Statistical Package for Social Sciences (Version 12.0 for Windows; SPSS, Inc., Chicago, IL, USA) and Microsoft Excel 2000.

Results

We found that this VNTR polymorphism consisted of the 11, 10, 9, 8 and 7 repeats units, respectively. Each allele consisted of the 520, 480, 440, 400, and 360 bp nucleotide sequences through sequencing, respectively. Nine genotypes which were 10-10, 11-10, 11-9, 9-10, 8-10, 7-10, 7-7, 7-9, 9-9 were found in all the participants. Major allele is 10 repeat and minor alleles are 11, 9, 8 and 7 repeat. X represents allele of 11, 9, 8, 7 repeat. In the VNTR loci, the subjects were categorized into three genotypes: major allele homozygote (10-10), heterozygote with major and minor alleles (10-X), and minor allele homozygote (X-X), respectively.

VNTR polymorphism followed the HardyWeinberg equilibrium (HWE) in the control group

Table 2. The associations in the distributions of genotypes and allele frequency for VNTR polymorphism in *DAT1* in cases and controls

	(1)	(2)	(1) VS (2)
	Case	Control	X^2, df, *P*-value, AND (95% CI)
	N=305	N=435	
Genotypes			
10-10	249	358	X^2=0.743, df=2, P=0.690, P=0.690
10-X	52	74	
X-X	4	3	
Alleles			X^2=0.172, df=1, P=0.678, OR=0.928, 95% CI=0.653-1.320
10	550	790	
X	60	80	

(cutoff *p*-value =0.697). The genotype frequencies and allelic frequency distributions in cases and controls are listed in **Table 2**. Evaluation of VNTR polymorphism in DAT1 showed that the prevalence of the 10/10 genotype was 249 (81.64%) in patients and 358 (82.30%) in controls, the frequency of the 10/X genotype was 52 (17.05%) and 74 (17.01%) in patients and controls, respectively, and the values for the X/X genotype in the patient group was 4 (1.31%) and in controls was 3 (0.69%) (**Table 2**). Statistical analysis showed no difference between groups regarding these genotypes (X^2=0.743, P=0.690, DF=2). The frequency of the 10 allele was 550 (90.16%) and 790 (90.80%) in patients and controls, respectively. 60 (9.84%) the X alleles were seen in patients, but the frequency of this allele was 80 (9.20%) in controls. No significant difference were found in these alleles (X^2=0.172, P=0.678, OR=0.928, 95% CI=0.653-1.320, DF=1).

Discussion

Due to its high morbidity and a chronic course lead to serious consequences [14, 15], OCD is paid more attention by the National Institute of Mental Health. In the past few years, many researches were performed for OCD etiology such as family survey and candidate genes [16, 17], and there are many evidence of genetic contribution to its etiology, but environmental risk factors also are likely to be involved. Animal experiments and pharmacological studies have indicated that serotonin and dopamine play an important role in OCD [18]. As a core factor in dopamine transmission, *DAT1* plays a key role in the transfer of dopamine in the synaptic cleft [19]. The VNTR polymorphism 40 bp core sequence in the 3'-UTR of *DAT1* may occur 3 to 11 times, repeating the common is 9 (440 bp allele 9), 10 repeats (480 bp, allele 10). Fuke et al. indicated this VNTR polymorphism affected its expression, when the 3'UTR contains 10 repetitions of the sequence, the gene expression levels rise [20].

Previous studies were performed for DAT1 40 bp VNTR polymorphism with susceptibility to TS and ADHD. Tarnok Z and his colleague found that the DAT1 40 bp VNTR showed an association with the peak tic-severity by means of transmission disequilibrium test (TDT) design in 103 TS trios in Hungary population and patients with at least one copy of the 9-repeat allele had significantly more severe symptoms than individuals with the homozygous 10/10 genotype [21], while no significant association were found in mixed European descent [22]. Hawi et al. indicated a significantly association between ADHD and 10-repeat allele of *DAT1* in 187 trios in Omani population by HHRR studies [23]. Chen et al. reported evidence of increased transmission of the 10-repeat allele using TRANSMIT in a sample of 110 Taiwanese probands with a DSM-IV diagnosis of ADHD [24]. However, Shang et al. examined the linkage disequilibrium structure of the *DAT1* and investigated whether the *DAT1* was associated with ADHD in Chinese family-based association sample in 273 probands and found although a haplotype rs27048 (C)/rs429699 (T) was significantly associated with the inattentive subtype, no association between DAT1 40 bp VNTR and ADHD [18]. There are also several negative association studies [25, 26].

In this present study, we explored the association of VNTR polymorphism of *DAT1* and OCD in the Chinese Han population. We found that this tandem repeat polymorphism consisted of the 11, 10, 9, 8 and 7 repeats units. Major allele is

10 repeat while minor alleles are 11, 9, 8 and 7 repeat, which represented by X allele. So the VNTR loci were categorized into three genotypes: major allele homozygote (10-10), heterozygote with major and minor alleles (10-X), and minor allele homozygote (X-X), respectively. However, our results showed that no association was found between OCD patients and controls for the genotype and alleles distribution. Our results did not confirm an involvement of the VNTR polymorphism of *DAT1* in conferring susceptibility to OCD in Chinese Han population. Walitza S et al. investigated the association between this VNTR polymorphism and OCD by 69 OCD trios in German population and found similar result with us [27]. Miguita et al. also found lack of association analysis between a VNTR intron 8 polymorphism of DAT1 and OCD in a Brazilian sample [28]. However, two disadvantages can be pointed in our study: on the one hand, this study was limited by the small size of the available samples. On the other hand, ethnic difference in genetic polymorphisms may determine their functions in different populations. Therefore, our finding remains to be confirmed by further studies that should consider larger specimens in more area and update method adopting.

Acknowledgements

We thank all probands for their participation. This work was supported by the National Natural Science Foundation of China (81371499 and 30971586).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Shiguo Liu, Genetic Laboratory, The Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266003, China. E-mail: liushiguo2002@126.com; Dr. Xinhua Zhang, Department of Psychiatry, Medical College, Qingdao University, 16 Jiangsu Road, Qingdao 266003, China. E-mail: xhzhang@126.com

References

[1] Fontenelle LF, Mendlowicz MV, Versiani M. The descriptive epidemiology of obsessive-compulsive disorder. Prog Neuropsychopharmacol Biol Psychiatry 2006; 30: 327-337.

[2] Cavallini MC, Albertazzi M, Bianchi L, Bellodi L. Anticipation of age at onset of obsessive-compulsive spectrum disorders inpatients with obsessive-compulsive disorder. Psychiatry Res 2002; 111: 1-9.

[3] Dickel DE, Veenstra-VanderWeele J, Cox NJ, Wu X, Fischer DJ, Van Etten-Lee M, Himle JA, Leventhal BL, Cook EH Jr, Hanna GL. Association testing of the positional and functional candidate gene SLC1A1/EAAC1 in early-onset obsessive-compulsive disorder. Arch Gen Psychiatry 2006; 63: 778-785.

[4] Szekely A, Ronai Z, Nemoda Z, Kolmann G, Gervai J, Sasvari-Szekely M. Human personality dimensions of persistence and harm avoidance associated withDRD4 and 5-HTTLPR polymorphisms. Am J Med Genet B Neuropsychiatr Genet 2004; 126B: 106-110.

[5] Chabane N, Millet B, Delorme R, Lichtermann D, Mathieu F, Laplanche JL, Roy I, Mouren MC, Hankard R, Maier W, Launay JM, Leboyer M. Lack of evidence for association between serotonin transporter gene (5-HTTLPR)and obsessive-compulsive disorder by case control and family association study inhumans. Neurosci Lett 2004; 363: 154-156.

[6] Nordstrom EJ, Burton FH. A transgenic model of comorbid Tourette's syndrome and obsessive-compulsivedisorder circuitry. Mol Psychiatry 2002; 7: 617-25, 524.

[7] Hemmings SM, Kinnear CJ, Niehaus DJ, Moolman-Smook JC, Lochner C, Knowles JA, Corfield VA, Stein DJ. Investigating the role of dopaminergic and serotonergic candidate genes inobsessive-compulsive disorder. Eur Neuropsychopharmacol 2003; 13: 93-98.

[8] Vandenbergh DJ, Persico AM, Hawkins AL, Griffin CA, Li X, Jabs EW, Uhl GR. Human dopamine transporter gene (DAT1) maps to chromosome 5p15.3 and displays aVNTR. Genomics 1992; 14: 1104-1106.

[9] Vermeire S, Audenaert K, De Meester R, Vandermeulen E, Waelbers T, De Spiegeleer B, Eersels J, Dobbeleir A, Peremans K. Serotonin 2A receptor, serotonin transporter and dopamine transporter alterationsin dogs with compulsive behaviour as a promising model for humanobsessive-compulsive disorder. Psychiatry Res 2012; 201: 78-87.

[10] Mill J, Asherson P, Browes C, D'Souza U, Craig I. Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR:Evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet 2002; 114: 975-979.

[11] Chen CK, Chen SL, Mill J, Huang YS, Lin SK, Curran S, Purcell S, Sham P, Asherson P. The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample. Mol Psychiatry 2003; 8: 393-396.

[12] Tarnok Z, Ronai Z, Gervai J, Kereszturi E, Gadoros J, Sasvari-Szekely M, Nemoda Z. Do-

paminergic candidate genes in Tourette syndrome: association between ticseverity and 3'UTR polymorphism of the dopamine transporter gene. Am J Med Genet B Neuropsychiatr Genet 2007; 144B: 900-905.

[13] Arnold PD, Rosenberg DR, Mundo E, Tharmalingam S, Kennedy JL, Richter MA. Association of a glutamate (NMDA) subunit receptor gene (GRIN2B) withobsessive-compulsive disorder: a preliminary study. Psychopharmacology (Berl) 2004; 174: 530-538.

[14] Tibrewal P, Kumar HB, Shubha GN, Subhashree D, Purushottam M, Thennarasu K, Reddy YC, Jain S. Association of serotonin transporter gene polymorphisms with obsessive-compulsivedisorder (OCD) in a south Indian population. Indian J Med Res 2010; 132: 690-695.

[15] Scharf JM, Miller LL, Mathews CA, Ben-Shlomo Y. Prevalence of Tourette syndrome and chronic tics in the population-based Avonlongitudinal study of parents and children cohort. J Am Acad Child Adolesc Psychiatry 2012; 51: 192-201. e5.

[16] O'Rourke JA, Scharf JM, Platko J, Stewart SE, Illmann C, Geller DA, King RA, Leckman JF, Pauls DL. The familial association of tourette's disorder and ADHD: the impact of OCD-symptoms. Am J Med Genet B Neuropsychiatr Genet 2011; 156B: 553-560.

[17] Nestadt G, Grados M, Samuels JF. Genetics of obsessive-compulsive disorder. Psychiatr Clin North Am 2010; 33: 141-158.

[18] Hoffman KL. Animal models of obsessive compulsive disorder: recent findings and futuredirections. Expert Opin Drug Discov 2011; 6: 725-737.

[19] Lind PA, Eriksson CJ, Wilhelmsen KC. Association between harmful alcohol consumption behavior and dopamine transporter (DAT1) gene polymorphisms in a male Finnish population. Psychiatr Genet 2009; 19: 117-125.

[20] Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S. The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. Pharmacogenomics J 2001; 1: 152-156.

[21] Tarnok Z, Ronai Z, Gervai J, Kereszturi E, Gadoros J, Sasvari-Szekely M, Nemoda Z. Dopaminergic candidate genes in Tourette syndrome: association between ticseverity and 3' UTR polymorphism of the dopamine transporter gene. Am J Med Genet B Neuropsychiatr Genet 2007; 144B: 900-905.

[22] Yoon DY, Rippel CA, Kobets AJ, Morris CM, Lee JE, Williams PN, Bridges DD, Vandenbergh DJ, Shugart YY, Singer HS. Dopaminergic polymorphisms in Tourette syndrome: association with the DAT gene(SLC6A3). Am J Med Genet B Neuropsychiatr Genet 2007; 144B: 605-610.

[23] Hawi Z, Lowe N, Kirley A, Gruenhage F, Nöthen M, Greenwood T, Kelsoe J, Fitzgerald M, Gill M. Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. Mol Psychiatry 2003; 8: 299-308.

[24] Chen CK, Chen SL, Mill J, Huang YS, Lin SK, Curran S, Purcell S, Sham P, Asherson P. The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample. Mol Psychiatry 2003; 8: 393-396.

[25] Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA, Hutz MH. Attention-deficit hyperactivity disorder: a study of association with both thedopamine transporter gene and the dopamine D4 receptor gene. Am J Med Genet 2001; 105: 471-478.

[26] Simsek M, Al-Sharbati M, Al-Adawi S, Ganguly SS, Lawatia K. Association of the risk allele of dopamine transporter gene (DAT1*10) in Omanimale children with attention-deficit hyperactivity disorder. Clin Biochem 2005; 38: 739-742.

[27] Walitza S, Scherag A, Renner TJ, Hinney A, Remschmidt H, Herpertz-Dahlmann B, Schulz E, Schafer H, Lange KW, Wewetzer C, Gerlach M. Transmission disequilibrium studies in early onset of obsessive-compulsive disorder for polymorphisms in genes of the dopaminergic system. J Neural Transm 2008; 115: 1071-1088.

[28] Miguita K, Cordeiro Q, Siqueira-Roberto J, Shavitt RG, Castillo JC, Castillo AR, Miguel EC, Vallada H. Association analysis between a VNTR intron 8 polymorphism of the dopamine-transporter gene (SLC6A3) and obsessive-compulsive disorder in a Braziliansample. Arq Neuropsiquiatr 2007; 65: 936-941.