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The Gene Polymorphism of *VMAT2* Decrease the Risk of Developing SCZ in Male Han Chinese

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Abstract

SCZ and PD were the common central nervous disorders, both of which the pathophysiology were still elusive. The transmission and metabolism of monoamine including DA was play an important role in the pathogenesis of SCZ and DA. It was reported that both SCZ and PD have genetic background and shared some common gene risk factors such as *MMP9, MAOB*. A recent study shows that two potentially functional SNPs of *VMAT2* were associated with PD. As *VMAT2* protein is mainly expressed in the brain and play an important role in the transportation of monoamine including DA, thus we speculate the gene polymorphism of *VMAT2* may also associate with SC. A total of 430(male 185; female 245; mean age 41.27 ± 14.40, mean onset age 25.05 ± 8.69 yr) patients with SCZ were recruited. Then ligase detection reactions (LDR) method was used to detect the polymorphism of the two SNPs. We did not find any association between the two SNP and SCZ. When we conduct a sex-specific analysis, our data only show that in the recessive model of rs363371, there was an obvious significant difference between the male SCZ group and the male controls (OR, 0.564, 95%CI 0.357-0.892, p=0.014), but not in female SCZ to female controls. Similar sex-specific result was detected in analysis rs363324. We also did not detect a difference between the EOSCZ, LOSCZ and control group for both SNPs. In conclusion, our research indicate the rs363371, rs363324 were not associated with SCZ, while it was seems that the AA genotype of rs363371 conduct a protect effect in male Chinese in developing into SCZ. In the future study more SNPs should be included and more risk factor should be involved in much bigger sample size and different ethnic.

Keywords Schizophrenia; *VMAT2*; Gene polymorphism

Introduction

Schizophrenia (SCZ) is the most common Psychiatric disorders incidence of 1% worldwide and of which the pathophysiology was still elusive. SCZ is a complicate disease which many gene and environmental factors were involved in the pathogenic of it [1]. Among numerous physiologic mechanism of SCZ, DA hypothesis was the most accepted one which means excessive activity or drugs which increase dopamine activity may induce psychiatric symptom. And in the clinic practice, the dopaminergic receptor agonist (eg: chlorpromazine, clozapine, risperidone) were extensively used to treat SCZ.

Many GWAS study and associate studies have detected may gene factors associated with SCZ, which indicate a genetic background of patients with SCZ [2-4]. *VMAT2* gene was located at 10q25, which is a suspected locus of schizophrenia (SCZ) detected by a systematic genome wide linkage study with a LOD score of 3.87 [5]. *VMAT2* protein is mainly expressed in the brain and play an important role in the transmission of monoamine including DA.

Recently, an Italy research reported that two SNP (rs363371, rs36334) in the promoter region of *VMAT2* were detected associated PD. Since DA hypothesis was the common base of both SCZ and PD. For patient with PD, deficiency of DA in substantia nigra account for the main clinic characters of Parkinsonism. Nevertheless, the new revised DA hypothesis of SCZ is hyperactive dopamine transmission in the mesolimbic areas and hypoactive dopamine transmission in the prefrontal cortex in schizophrenia patients [6]. And interestingly, both SCZ and PD have shared some common gene risk factors such as MMP9 [7,8], MAOB, COMT [9-11]. Thus as the shared pathogenic mechanism and genetic factors of SCZ and PD, we conduct a case-control study to detect the association between *VMAT2* gene polymorphism and SCZ in Han Chinese population.

Material and Method

Subject

A total of 430 (male 185; female 245; mean age 41.27 ± 14.40, mean onset age 25.05 ± 8.69 yr) patients with SCZ were recruited from three psychiatric disorder center: The Second Xiangya Hospital of Central South University, located in central China; West China Hospital of Sichuan University, located in southwest China; and the Third Affiliated Hospital of Sun Yat-sen University and Jining Mental Hospital, both located in east China. All patients were diagnosed by two psychiatrists based on the Structured Clinical Interview for DSM Disorders (SCID) and DSM-IV criteria [12]. Patients who were older

than 18 at SCZ onset were subdivided into late-onset SCZ (LOSCZ; n=290 mean age at onset, 27.71 ± 7.96 yr); the others were as early-onset SCZ group (EOSCZ; n=140; mean age at onset, 15.57 ± 2.05 yr) [13]. The control group for SCZ patients comprised 457 Han Chinese (190 males, 280females; mean age, 42.33 ± 12.45 yr). The protocol of the study was approved by the ethics committees of all three hospitals involved in this research, all the written inform consents were got from all the subjects involved in this study (Table 1).

Characteristic	SCZ (n=430)	Controls (n=470)	Comparison*
Age in yr, mean ± SD	41.27 ± 14.40	42.33 ± 12.45	t, p
Gender, n			
Male	185	190	0.80, 0.63-1.02, 3.14, 0.08
Female	245	280	
*Unless otherwise indicated, the values indicate: OR, 95%CI, c^2, p value.			

Table 1: Demographic characteristics of Han Chinese with schizophrenia (SCZ) and healthy controls.

Genotyping

Genomic DNA was obtained from peripheral leukocytes by the classic Phenol-chloroform DNA extract method. All the genotyping experiments were done by the Shanghai Bio-Wing Applied Biotechnology Company using ligase detection reactions (LDR) [14]. The target DNA sequences were amplified using a multiplex PCR method. The primary information for rs363371 and rs363324 (Table S1).

After the completion of the amplification, 1 μl of Proteinase K (20 mg/ml) was added, then heated at 70°C for 10 min and quenched at 94°C for 15 min. The ligation reaction for each subject was carried out in a final volume of 20 μl containing 20 mM Tris-HCl (pH 7.6),25 mM potassium acetate, 10 mM magnesium acetate, 10 mM DTT, 1 mM NAD, 0.1% Triton X-100, 10 μl of MultiPCR product, 1 pmol of each discriminating oligo, 1 pmol of each common probe and 0.5 μl of 40 U/μl Taq DNA ligase (New England Bio labs, USA). The LDR was performed using 40 cycles of 94°C for 30 sec and 63°C for 4 min. The fluorescent products of LDR were differentiated by ABI sequencer 377.

Statistical analysis

Values for age were represented by the mean ± standard deviation (SD), and the gender, allele and genotype were described as percentages. Allele and genotype frequencies were determined by direct counting of HNMT alleles. Concordance of genotype distribution was verified by Hardy-Weinberg Equilibrium (HWE) and then evaluated with the chi-square test. Associations between gender, allele and genotype were also assessed by chi-square test. Age differences between three groups were assessed by the t test. A two-tailed P value<0.05 was considered significant. Statistical SPSS17.0 was used when conduct both chi-square test and t test. Haplotype analysis was conducted by SHEsis 4.0 online (**http://analysis.bio-x.cn/myAnalysis.php**).

Result

From our data, for the SNP rs363371 there were no obvious significant differences of the distribution between the SCZ group and control group (OR=1.607, 95%CI 0.761-1.495, p=0.709) or between the subgroups in dominant model (Table 2). While in the recessive model, there was an obvious significant difference between the male SCZ group and the male controls (OR, 0.564, 95%CI 0.357-0.892, p=0.014). There was not any statistic significant between the SCZ group and the control group, the SCZ female group and controls, the EOSCZ, LOSCZ and control group (Table 2).

Group or subgroup	Genotype, n (%)			Comparison[b]	
	GG	AG	AA	Dominant model	Recessive model
Locus rs363371					
All SCZ	81 (18.8)	227 (52.8)	122 (28.4)	1.607, 0.761-1.495, 0.14, 0.709[c]	0.915, 0.686-1.22, 0.367, 0.545[c]
Male SCZ	32 (17.3)	111 (60)	42 (22.7)	1.013, 0.591-1.736, 0.002, 0.962	0.564, 0.357-0.892, 6.059, 0.014[d]
Male controls	32 (17.1)	91 (48.7)	64 (34.2)		
Female SCZ	49 (20)	116 (47.3)	80 (32.7)	1.239, 0.796-1.930, 0.903, 0.342	1.112,0.769-1.609, 0.32,0.572[d]
Female controls	47 (16.8)	148 (52.8)	85 (30.4)		
EOSCZ	26 (18.7)	80 (57.6)	33 (23.7)	1.057, 0.65-1.721, 0.05, 0.823	0.719, 0.464-1.114, 2.194, 0.139
LOSCZ	55 (18.9)	147 (50.5)	89 (30.6)	1.071, 0.735-1.561, 0.127, 0.721	1.018, 0.741-1.398, 0.012, 0.914
All controls	84 (17.9)	244 (51.9)	142(30.2)		
Locus rs363324					
SCZ	265 (61.6)	147 (34.2)	18 (4.2)	0.968, 0.739-1.268, 0.056, 0.813[c]	1.088, 0.553, 2.138, 0.06, 0.807[c]
Male SCZ	111 (62)	63 (35.2)	5 (2.8)	0.941, 0.615-1.438, 0.08, 0.829	0.735, 0.229-2.359, 0.27, 0.603[d]

Male controls	118 (63.4)	61(32.8)	7 (3.8)		
Female SCZ	154 (61.4)	84 (33.5)	13 (5.2)	0.985, 0.695-1.398, 0.007, 0.934	1.486,0.64-3.45,0.858,0.354[d]
Female controls	174 (61.7)	98 (34.8)	10 (3.5)		
EOSCZ	84 (60)	49 (35)	7 (5)	0.904, 0.614-1.331, 0.262, 0.609	1.396, 0.567-3.438, 0.532, 0.466[c]
LOSCZ	181 (62.4)	98 (33.8)	11 (3.8)	1.001, 0.704-1.354, 000, 0.995	1.046, 0.483-2.266, 0.013, 0.909[c]
All controls	292 (64.4)	159 (34)	17 (3.6)		

Abbreviations: SCZ, schizophrenia; EOSCZ, early-onset SCZ; LOSCZ, late-onset SCZ

[a] Frequencies of individual alleles (not shown) did not differ significantly between the entire SCZ group or subgroup and healthy controls.

[b] Values indicate: OR, 95%CI, c2, p value

[c] With respect to all controls

[d] With respect to the corresponding male or female controls

Table 2: Genotype frequencies between the entire group or subgroups of Han Chinese with SCZ and healthy controls [a].

For the other SNP rs363324, there were not obvious differences between the SCZ group and control group and between the subgroups in both dominant and recessive model (Table 2). For the two SNPs the allele frequencies were not different in any compared groups (Table S2).

The linkage disequilibrium D' value between rs363371 and rs363324 was 0.334 for rs373371 and rs363324, suggesting a strong recombination event. Thus we conduct a Haplotype analysis, in which haplotypes with frequencies<0.03 were excluded, identified the three haplotypes A-G, G-A, and G-G. None of these haplotypes showed a significant association with SCZ (Table 3).

SNP	Haplotype*	SCZ, n (%)	Control, n (%)	c2	p	OR [95% CI]
1,2	A-G	470 (54.8)	522 (56.1)	0.323	0.57	0.947 [0.786-1.142]
	G-A	183 (21.3)	195 (20.9)	0.044	0.834	1.025 [0.816-1.286]
	G-G	205 (23.9)	214 (23.0)	0.216	0.642	1.053 [0.846-1.311]

*Only haplotypes with a frequency of p>0.03 were considered in the analysis. A,G, indicates the allele of rs363371; A,G indicates the allele of rs363324

Table 3: Haplotype frequencies at loci rs363371 and rs363324 in Han Chinese with SCZ and healthy controls.

Discussion

Our data did not detect an association between the two SNPs in *VMAT2* gene and PD. Our study was consistence with a previously study which failed to detected an association between *VMAT 2* and SCZ, nonetheless that study did not including the two SNPs of our study [15]. Interesting,we detect the AA genotype of rs363371 as protecting male Han Chinese from developing SCZ (OR,0.564,95%CI 0.357 to 0.892,p=0.014). And this locus, located in the promoter of *VMAT2*, is potential functional. As it in linkage disequilibrium with locus rs2619096, which doubles the activity of the *VMAT2* promoter [16].

Although many research show a relationship between *VMAT2* pathophysiology of neuropsychiatric diseases including schizophrenia, mood disorders, methamphetamine (MAP) neurotoxicity [17-19]. In patients with SCZ, it was reported that the density of *VMAT2* protein increased in an investigation of platelets, which may indicate a schizophrenia-related hyperactivity of the monoaminergic system or an adaptive response to chronic drug treatment [20]. In a 14-3-3 epsilon hetero KO mice model of schizophrenia expression of *VMAT2* was increased significantly in the hippocampal, indicate a possible pathophysiology of schizophrenia includes a monoaminergic transmission abnormality [21]. Recently, a research showed that genetic variation in *VMAT2* may be linked to alterations in cognitive functioning underlying psychotic disorder [22].

Interestingly, our study show a significant difference of AA genotype of rs363371 between the male SCZ group and male controls, while there was not detect such a similar difference between the female SCZ group and female controls. It seems contrary to a GWAS research which supports the idea that no strong sex-specific genetic risk factors exist for schizophrenia [23]. But indeed, there were some support evidence. A case-control study show a gender difference of gene factors, the study show interleukin 1 receptor antagonist protein (IL1Ra) and IGF2BP2 was associated with SCZ in male population but not in female [24,25]. C allele and CC genotype of rs253 was reported confer risk for schizophrenia in men [26]. A male-specific association was also be reported with SNP rs62621676 in a UK case-control cohort [27]. While, there were more female-specific genetic factor. Two case-control study from China show that high-affinity neurotensin receptor 1(NTR1) gene polymorphism and estrogen receptor 1(*ESR1*) gene polymorphism were increase the risk of developing SCZ in female Chinese population [28,29]. A GWAS research found an female-

specific association with rs7341475 in reelin (*RELN*) gene [30]. Most interestingly, in a study show in a same gene, different SNPs conduct different sex-spefic effect: eg,SNPs rs3891636, rs7728773, rs2189663,rs3864283, and rs2526303 of *HINT1* gene significant among the female subjects alone, whereas SNP rs7735116 was significant among males alone [31]. These studies indicate there may exit sex-specific genetic risk and protective factors to SCZ.

In terms of us understand, it is the first case-control study try to detect a potential relationship between SCZ and the two potential functional SNPs of *VMAT2* in Chinese population. Nevertheless, Our result have some limitations: Firstly, the relative small sample size may alleviate the power our research(the power for rs363371 was 80.8% while for rs363324 was only 5.1%); Secondly, we only select two SNP in the promoter region and for this low coverage of SNPs the really risky SNP may be neglected; Thirdly, we did not take other risk factors associated SCZ (eg: environmental and social factors) into consideration; Moreover, as a recently research indicated *VMAT2* was associated with Tardive dyskinesia(TD), an involuntary movement disorder that can occur in up to 25% of patients receiving long-term first-generation antipsychotic treatment [32]. Thus, the gene-medical treatment should be take into consideration in the future study.

In conclusion, our research indicate the rs363371, rs363324 were not associated with SCZ, while it was seems that the AA genotype of rs363371 conduct a protect effect in male Chinese in developing into SCZ. In the future study more SNPs should be included and more risk factor should be involved in much bigger sample size and different ethnic.

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References

1. Ayhan Y (2009) Animal models of gene-environment interactions in schizophrenia. Behav Brain Res 204: 274-81.
2. Ripke S, O'Dushlaine C, Chambert K, Kähler AK, Akterin S, et al. (2013) Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 45: 1150-1159.
3. Ripke S, Neale BM, Corvin A, Walters JT, Farh KH, et al. (2014) Schizophrenia working group of the psychiatric genomics consortium biological insights from 108 schizophrenia-associated genomic loci. Nature 511: 421-427.
4. Harrison PJ (2014) Recent genetic findings in schizophrenia and their therapeutic relevance. J Psychopharmacol 29: 85-96.
5. Williams NM, Norton N, Williams H, Ekholm B, Hamshere ML, et al. (2003) A systematic genome wide linkage study in 353 sib pairs with schizophrenia. Am J Hum Genet 73: 1355-67.
6. Silva AF, Figee M, Amelsvoort T, Veltman D, Haan L (2008) The revised dopamine hypothesis of schizophrenia: Evidence from pharmacological MRI studies with atypical antipsychotic medication. Psychopharmacol Bull 41:121-132.
7. He X, Zhang L, Yao X, Hu J, Yu L, et al. (2013) Association studies of MMP-9 in Parkinson's disease and amyotrophic lateral sclerosis. PLoS One 8: 73-77.
8. Han H, He X, Tang J, Liu W, Liu K, et al. (2011) The C(-1562)T polymorphism of matrix metalloproteinase-9 gene is associated with schizophrenia in China. Psychiatry Res 190: 163-164.
9. Kiyohara C, Miyake Y, Koyanagi M, Fujimoto T, Shirasawa S, et al (2011) Fukuoka kinki Parkinson's disease study group. genetic polymorphisms involved in dopaminergic neurotransmission and risk for parkinson's disease in a Japanese population. BMC Neurol 25: 11-89.
10. Ira E, Zanoni M, Ruggeri M, Dazzan P, Tosato S (2013) COMT, neuropsychological function and brain structure in schizophrenia: A systematic review and neurobiological interpretation. J Psychiatry Neurosci 38: 366-380.
11. Carrera N, Sanjuán J, Moltó MD, Carracedo A, Costas J (2009) Recent adaptive selection at MAOB and ancestral susceptibility to schizophrenia. Am J Med Genet B Neuropsychiatr Genet 150: 369-374.
12. First MB, Spitzer RL, Gibbon M, Williams J (1996) Structured clinical interview for dsm-IV axis I disorders, clinician version (SCID-CV). American Psychiatric Press Inc Washington, USA.
13. Rapoport JL, Addington AM, Frangou S, Psych MR (2005) The neurodevelopmental model of schizophrenia: Update 2005. Mol Psychiatry 10: 434-449.
14. Thomas G, Sinville R, Sutton S, Farquar H, Hammer RP, et al. (2005) Capillary and micro electrophoretic separations of ligase detection reaction products produced from low-abundant point mutations in genomic DNA. Electrophoresis 25: 1668-1677.
15. Talkowski ME, Kirov G, Bamne M, Georgieva L, Torres G, et al. (2008) A network of dopaminergic gene variations implicated as risk factors for schizophrenia. Hum Mol Genet 17: 747-758.
16. Lin Z, Walther D, Yu XY, Li S, Drgon T, et al. (2005) SLC18A2 promotet haplotypes and identification of a novel protective factor against alcoholism. Hum Mol Genet 14: 1393- 1404.
17. Taylor SF, Koeppe RA, Tandon R, Zubieta JK, Frey KA (2000). In vivo measurement of the vesicular monoamine transporter in schizophrenia. Neuropsychopharmacology 23: 667-675.
18. Zubieta JK, Taylor SF, Huguelet P, Koeppe RA, Kilbourn MR, et al. (2001). Vesicular monoamine transporter concentrations in bipolar disorder type I, schizophrenia, and healthy subjects. Biol Psychiatry 49:110-116.
19. Volz TJ, Farnsworth SJ, Rowley SD, Hanson GR, Fleckenstein AE (2009) Age-dependent differences in dopamine transporter and vesicular monoamine transporter-2 function and their implications for methamphetamine neurotoxicity. Synapse 63:147-151.
20. Zucker M, Valevski A, Weizman A, Rehavi M (2002). Increased platelet vesicular monoamine transporter density in adult schizophreniapatients. Eur Neuropsychopharmacol 12:343-347.
21. Iritani S, Sekiguchi H, Habuchi C, Hikita T, Taya S, et al. (2010) Immunohistochemical study of vesicle monoamine transporter 2 in the hippocampal region of genetic animal model of schizophrenia. Synapse 64: 948-953.
22. Simons CJ, Winkel R (2013) Intermediate phenotype analysis of patients, unaffected siblings, and healthy controls identifies VMAT2 as a candidate gene for psychotic disorder and neurocognition. Schizophr Bull. 39: 848-856.
23. Bergen SE, O'Dushlaine CT, Lee PH, Fanous AH, Ruderfer DM, et al. (2014) Genetic modifiers and subtypes in schizophrenia: Investigations of age at onset, severity, sex and family history. Schizophr Res 154: 48-53.
24. Nejma M, Zaabar I, Zaafrane F, Thabet S, Mechri A,et al. (2013) A gender-specific association of interleukin 1 receptor antagonist polymorphism with schizophrenia susceptibility. Acta Neuropsychiatr 25: 349-55.
25. Zhang X, Hui L, Liu Y, Wang ZQ, You Y, et al. (2013) The type 2 diabetes mellitus susceptibility gene IGF2BP2 is associated with schizophrenia in a Han Chinese population. J Clin Psychiatry 74: 287-92.
26. Xie C, Wang ZC, Liu XF, Wang L, Yang MS (2011) Association between schizophrenia and single nucleotide polymorphisms in lipoprotein lipase gene in a Han Chinese population. Psychiatr Genet 21: 307-314.
27. Carroll L, Williams N, Moskvina V, Russell E, Norton N, et al. (2010) Evidence for rare and common genetic risk variants for schizophrenia at protein kinase C, alpha. Mol Psychiatry 15: 1101-1111.
28. Wang LW, He SP, Zhang JL, Tong DX, He Y, et al. (2013) Association between ESR1 gene polymorphisms and haplotypes with schizophrenia. Europe PMC 30: 21-25.

29. Ma H, Huang Y, Zhang B, Li J, Wang Y, et al. (2013) Association between neurotensin receptor 1 (NTR1) gene polymorphisms and schizophrenia in a Han Chinese population. J Mol Neurosci 50: 345-352.

30. Shifman S, Johannesson M, Bronstein M, Chen SX, Collier DA, et al. (2008) Genome-wide association identifies a common variant in the reelin gene that increases the risk of schizophrenia only in women. PLoS Genet 4: 28.

31. Chen Q, Wang X, O'Neill FA, Walsh D, Kendler KS, et al. (2008) The histidine triad nucleotide-binding protein 1 (HINT1) gene a candidate for schizophrenia?. Schizophr Res 106: 200-207.

32. Zai CC, Tiwari AK, Mazzoco M, de Luca V, Müller DJ, et al. (2013) Association study of the vesicular monoamine transporter gene SLC18A2 with tardive dyskinesia. J Psychiatr Res 47: 1760-1765.