GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Association of the *VRK2* gene rs3732136 polymorphism with schizophrenia in a Northwest Chinese Han population

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Genet. Mol. Res. 14 (3): 9404-9411 (2015)
Received October 14, 2014
Accepted May 18, 2015
Published August 14, 2015
DOI http://dx.doi.org/10.4238/2015.August.14.4

ABSTRACT. Previous studies have found that the vaccinia related kinase 2 gene (*VRK2*) polymorphism was associated with schizophrenia (SCZ) in the worldwide population. This association was further supported by *VRK2* mRNA expression patterns and brain structure variations. Here, we analyzed four single nucleotide polymorphisms (SNPs) of the *VRK2* gene in a total population of 893 samples, consisting of 360 patients with SCZ and 533 healthy controls of Han Chinese descent using the SNPscan method. Single SNP, haplotype, and gender-specific association analyses were performed. We found that rs3732136 was significantly associated with SCZ (P = 0.042; odds ratio = 1.25; 95% confidence interval = 1.01-1.55). Further genotype and haplotype association analyses suggested a similar pattern. Our data provide preliminary evidence that the *VRK2* gene might play a

major role in the development of SCZ in the Northwest Chinese Han population.

Key words: *VRK2* gene; SNP; Schizophrenia; Association study

INTRODUCTION

Schizophrenia (SCZ) is a severe and complex mental disorder that shows positive and negative symptoms such as delusions and hallucinations. This disease has a profound impact on the quality of life and social burden (Monti et al., 2013). The cause of SCZ is likely to be a complex interaction between genetic and environmental factors; it has an estimated heritability of 64-80% (Thaker and Carpenter, 2001). Due to lack of clear biological markers, diagnosis of SCZ has long been performed based exclusively on clinical signs and symptoms (American Psychiatric Association, 2000). Recent genome-wide association studies (GWAS) and large-scale meta-analyses have revealed a set of common variations predisposing to SCZ (Shi et al., 2009; Stefansson et al., 2009; Steinberg et al., 2011; Rietschel et al., 2012). One of the GWAS found that a *VRK2* polymorphism was significantly associated with SCZ by analysis of over sixty thousand samples (Ripke, 2011). Researchers have also successfully replicated the association of *VRK2* with SCZ in Asian and European populations (Li et al., 2012). Furthermore, they also found a significant association of *VRK2* with total brain volume and white matter volume (Wirgenes et al., 2012).

Gene expression analyses using DNA micro-arrays have supported the hypothesis that dysfunctional myelination of neurons might be involved in the pathogenesis of SCZ (Yuan et al., 2012). The expression of *VRK2* was shown to be significantly increased in the brains of patients with SCZ. It is possible therefore that genetic variations affecting the expression of the *VRK2* gene might also contribute to the susceptibility of individuals to SCZ.

Therefore, *VRK2* is likely a common risk gene for SCZ in the worldwide populations. In order to test whether *VRK2* is associated with SCZ in the Northwest Chinese Han population, allele, genotype and haplotype frequencies of four single nucleotide polymorphisms (SNPs) of the *VRK2* gene were compared in this study between SCZ and control populations.

MATERIAL AND METHODS

Samples

A total of 893 samples (360 patients with SCZ and 533 normal controls) were enrolled from the Mental Health Center of the First Affiliated Hospital, Xi'an Jiaotong University. All 360 unrelated patients with SCZ (177 men and 183 women, mean age: 37.2 ± 10.4 years) were interviewed independently by two experienced psychiatrists according to DSM-IV diagnostic criteria which included personal history, hospital record, and family-history reports. We also collected 533 normal controls (232 men and 301 women, mean age: 35.6 ± 11.2 years) from the Medical Examination Center of this hospital. Normal controls were confirmed to have a lack of mental illness and were matched with patients in gender,

age, origin and educational level, after undergoing health examination. Informed consent was obtained from all participants.

Genotyping

We identified four SNPs with minor allele frequencies >0.05 in the *VRK2* gene including intron, exon, and 3' UTR regions in the HapMap HCB database. SNPs reported to be significantly associated with SCZ were also examined in this study (Wirgenes et al., 2012). Overall, rs2312147 in the *VRK2* 5' UTR, rs1051061 in exonic sequence, rs2043890 in intronic sequence, and rs3732136 in the 3' UTR were chosen for analysis in the present study.

Human genomic DNA was extracted using a standard method according to manufacturer recommendations (Omega Bio-tek, Norcross, GA, USA). Sample DNA (10 ng) was amplified by polymerase chain reaction (PCR). A 48-Plex SNPscan™kit (Genesky Biotechnologies Inc., Shanghai, China) was designed and used to determine genotypes of the four SNPs. The SNPscan technique was developed according to patented SNP genotyping technology by Genesky Biotechnologies Inc., which provides a high-throughput and cost-saving SNP genotyping method based on double ligation and multiplex fluorescence PCR as previously described (Du et al., 2014).

The genotyping method is accurate and has been used by many studies (Ren et al., 2014; Wang et al., 2014; Yin et al., 2014). Repeated analyses were performed to guarantee the genotyping quality by randomly choosing 5% samples with high DNA quality. The average genotype call rate for all markers was 98.2%.

Statistical analysis

Using the G*Power program (Franz Faul, University Kiel, Germany), the statistical power of the sample size was calculated according to Cohen's method (Faul et al., 2007). This demonstrated >85% power to detect a significant ($P < 0.05$) association for genotypes, alleles, and haplotypes when an effect size index of 0.1 (corresponding to a "weak" gene effect) was used in our samples.

The genotype, allele, and haplotype frequency differences between patients and controls were calculated by chi-square analysis. The deviation of the genotype counts from Hardy-Weinberg equilibrium (HWE) was tested by SHEsis (http://analysis.box-x.cn). The odds ratio (ORs) and 95% confidence interval (CIs) were calculated by unconditional logistic regression of the association between the *VRK2* gene and the presence of SCZ. Rare haplotypes (less than 1% of both patient and control subjects) were excluded from further analysis. Furthermore, stratified analyses were conducted to examine whether differences in gene and gender influenced identified associations. All statistical analyses were performed using the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS

No significant deviation from HWE was found in patients or controls for the four SNPs. The frequencies of genotypes and alleles of the four *VRK2* SNPs are shown in Table 1. All SNPs

were highly polymorphic in patients and controls. We first conducted a single SNP association analysis. When all of the samples were considered, we observed a significant association for rs3732136 (P = 0.042; OR = 1.25; 95%CI, 1.01-1.55). Genotype association analysis for rs3732136 suggested a similar pattern with a significant P value (P = 0.039). When divided according to gender, there was no significant difference in allele and genotype frequencies (Table 2).

Linkage disequilibrium analyses of the patients and controls revealed that the four SNPs were located in one haplotype block (Figure 1). Although some haplotypes (HAP1: P = 0.039; HAP4, P = 0.049) and a gender-specific haplotype (HAP4, P = 0.006 in women) showed significant differences between patient and control populations, they did not meet standard significance criteria for haplotype frequencies based on the global P values (Table 3).

Table 1. Genotype and allele frequencies of four SNPs of the *VRK2* gene.

SNP	Genotypes/Alleles	SCZ (N = 360)		CTR (N = 533)		OR	95%CI	P value
		N	%	N	%			
rs2312147[a]	CC	202	56.11	284	51.36	1		0.111
	CT	134	37.22	222	40.14	1.20	0.90-1.58	0.211
	TT	24	6.67	47	8.50	1.39	0.83-2.35	0.214
	CC *vs* CT+TT					1.23	0.94-1.60	0.134
	CC+TC *vs* CC					0.77	0.46-1.29	0.324
	Allele C	538	74.72	790	71.43	1		
	Allele T	182	25.28	316	28.57	1.19	0.96-1.47	0.108
rs1051061[b]	AA	88	24.51	165	29.84	1		0.857
	AG	182	50.70	265	47.92	1.14	0.73-1.76	0.567
	GG	89	24.79	123	22.24	1.43	0.84-2.43	0.187
	AA *vs* AG+GG					1.22	0.80-1.84	0.356
	AA+AG *vs* GG					0.76	0.49-1.19	0.230
	Allele A	358	49.86	595	53.80	1		
	Allele G	360	50.14	511	46.20	1.19	0.91-1.54	0.201
rs2043890[c]	AA	211	58.77	323	58.51	1		0.837
	AG	128	35.65	195	35.33	1.00	0.75-1.32	0.973
	GG	20	5.58	34	6.16	1.11	0.62-1.98	0.938
	AA *vs* AG+GG					1.01	0.77-1.32	0.938
	AA+AG *vs* GG					0.90	0.51-1.59	0.713
	Allele A	550	76.60	841	76.18	1		
	Allele G	168	23.40	263	23.82	1.02	0.82-1.28	0.835
rs3732136[d]	CC	193	54.21	260	48.87	1		***0.039***
	CT	142	39.89	221	41.54	1.16	0.87-1.53	0.317
	TT	21	5.90	51	9.59	1.80	1.05-3.10	***0.038***
	CC *vs* CT+TT					1.24	0.95-1.62	0.132
	CC+CT *vs* TT					1.69	1.00-2.87	0.059
	Allele C	528	74.16	741	69.64	1		
	Allele T	184	25.84	323	30.36	1.25	1.01-1.55	***0.042***

[a]One individual failed to be genotyped for rs2312147; [b]two individuals failed to be genotyped for rs1051061; [c]two individuals failed to be genotyped for rs2043890; [d]five individuals failed to be genotyped for rs3732136; SNP = single nucleotide polymorphism; SCZ = schizophrenia; CTR = control; OR = odds ratio; CI = confidence interval. Significant P values are in italic bold text.

Table 2. Gender specific allele and genotype association analysis.

Marks and gender		Allele frequency (%)		P value	Genotype frequency (%)			P value
		C	T		CC	CT	TT	
rs2312147								
M								
	SCZ	74.72	25.28	0.693	57.96	33.52	8.52	0.707
	CTR	73.49	26.51		56.04	34.91	9.05	
F								
	SCZ	75.00	25.00	0.064	54.95	40.11	4.94	0.056
	CTR	69.44	30.56		47.18	44.52	8.30	
rs1051061								
M								
	SCZ	48.58	51.42	0.283	23.30	50.57	26.13	0.299
	CTR	52.37	47.63		30.60	43.54	25.86	
F								
	SCZ	51.09	48.91	0.201	25.68	50.82	23.50	0.194
	CTR	55.32	44.68		29.57	51.50	18.93	
rs2043890								
M								
	SCZ	77.27	22.73	0.746	60.23	34.09	5.68	0.753
	CTR	76.30	23.70		59.57	33.48	6.95	
F								
	SCZ	75.96	24.04	0.895	57.38	37.16	5.46	0.894
	CTR	75.58	24.42		56.81	37.54	5.65	
rs3732136								
M								
	SCZ	75.00	25.00	0.219	55.17	39.66	5.17	0.213
	CTR	71.12	28.88		50.43	41.38	8.19	
F								
	SCZ	73.35	26.65	0.105	53.30	40.11	6.59	0.108

SCZ = schizophrenia; CTR = control; M = male; F = female.

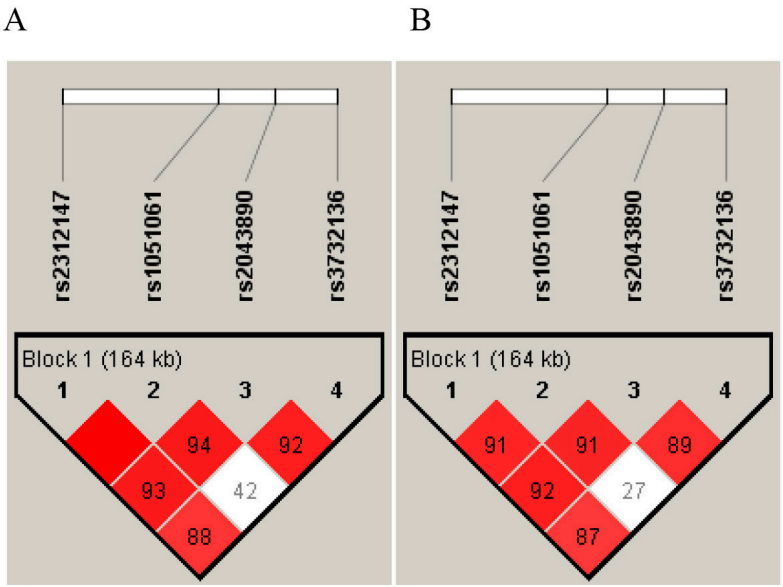

Figure 1. Linkage disequilibrium (LD) plot of the four SNPs in the *VRK2* gene in patients and controls (below). Values in squares are the pair-wise calculation of D'. Empty squares indicate D' = 1 (i.e. complete LD between a pair of SNPs). LD, linkage disequilibrium; SNP, single nucleotide polymorphism. **A.** Patients; **B.** controls.

Table 3. Haplotype frequencies and association analysis.

Haplotype	SNP1	SNP2	SNP3	SNP4	Gene counting (frequency %)			
ID					SCZ	CTR	P value	Global
HAP1	C	G	A	C	48.5	43.5	***0.039***	0.241
HAP2	C	A	A	T	21.1	22.9	0.362	
HAP3	T	A	G	C	20.5	21.1	0.775	
HAP4	T	A	A	T	3.8	5.9	***0.049***	
HAP5	C	A	A	C	2.42	1.64	0.245	
HAP6	C	A	G	C	1.94	1.8	0.890	
Male								
HAP1	C	G	A	C	50.2	45.4	0.168	0.766
HAP2	C	A	A	T	19.9	23.5	0.221	
HAP3	T	A	G	C	19.3	19.9	0.855	
HAP4	T	A	A	T	4.6	4.3	0.832	
HAP5	C	A	A	C	2.3	1.6	0.489	
HAP6	C	A	G	C	2.4	2.2	0.856	
Female								
HAP1	C	G	A	C	46.8	42.1	0.149	0.264
HAP2	C	A	A	T	22.2	22.5	0.938	
HAP3	T	A	G	C	21.6	22.0	0.890	
HAP4	T	A	A	T	3.0	7.2	***0.006***	
HAP5	C	A	A	C	2.5	1.7	0.391	
HAP6	C	A	G	C	1.5	1.6	0.870	

SNP = single nucleotide polymorphism; SCZ = schizophrenia; CTR = control. Significant P values are in italic bold text.

DISCUSSION

In this research, we present a case-control study of four polymorphisms (rs2312147, rs1051061, rs2043890, and rs3732136) of the *VRK2* gene including 533 controls and 360 patients with SCZ from the Northwest Chinese Han population. In this association study, we found that the C allele and the CC genotype of the *VRK2* rs3732136 SNP were associated with a decreased risk of SCZ. This significant association was not reflected by other SNPs (rs1051061, rs2043890, etc.) that showed high linkage disequilibrium with rs3732136 (Figure 1). To the best of our knowledge, this is the first report of a significant association of the rs3732136 polymorphism with SCZ. Although the significance is uncertain, we note that SNP rs3732136 is located in intron 1 of the *FANCL* gene as well as in the alternative exon16 of the *VRK2* gene.

FANCL is a member of the *FANC* gene family, and disruption of the function of *FANC* genes causes Fanconi anemia (FA) (Alpi and Patel, 2009). Studies have reported that *FANC* genes might have a role in the recognition or repair of DNA damage due to the increased risk of cancer seen in patients with FA (Juko-Pecirep et al., 2011). Compared with the study performed in the *FANCL* gene rs3732136 locus, the frequency of rs3732136 C allele was shown to be 70.1% in healthy Swedish subjects (Juko-Pecirep et al., 2011), 69.8% in the French population (St-Laurent Pedneault, 2013), *vs* 69.6% in the control population in our study.

The serine/threonine-protein kinase VRK2 is an enzyme encoded by the *VRK2* gene, which is located on human chromosome 2p16.1. There have been eight VRK2 isoforms described which arise because of alternatively spliced transcripts of the *VRK2* gene (Blanco et al., 2006). VRK2 is widely expressed in human tissues and has increased expression in highly proliferative cells (Nezu et al., 1997).

Although previous studies revealed that the *VRK2* rs2312147 polymorphism was associated with SCZ in Asian and European samples, our results did not support these findings. Compared with the study performed in the Chinese Yunnan population, the frequency of the

rs2312147 C allele in our study (71.4% in controls and 74.7% in patients) was comparable (Wirgenes et al., 2012) (68% in controls and 74% in patients with SCZ), whereas the C allele frequency was much lower in European Americans (61% in controls and 63% in patients with SCZ) (Steinberg et al., 2011).

It should be noted that our study participants were all recruited from Shaanxi Province, while the study samples from Li et al. (2012) were drawn from Yunnan Province, and the Steinberg et al. (2011) samples were collected from European Americans. It is therefore possible that differences in the *VRK2* polymorphism profiles could reflect regional differences not generalizable to all people. Multiple additional factors might contribute to the variable results as well, including cohort homogeneity, sample size, ethnic background-specific effects, epistasis, and notably the selection pattern of genetic loci.

Taken together, we investigated whether four SNPs (rs2312147, rs1051061, rs2043890, and rs3732136) of the *VRK2* were associated with SCZ in the Northwest Chinese Han population. We found that the rs3732136 polymorphism of the *VRK2* gene was associated with SCZ ($P = 0.042$). These results suggested that rs3732136 might be associated with the susceptibility to SCZ. However, positive associations might sometimes be due to genetic heterogeneity, and protective alleles might vary according to ethnic differences. Therefore, further studies using a larger number of subjects in different ethnic groups should be performed to determine whether the *VRK2* gene polymorphism might be truly involved in the development of SCZ.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (Grant #31301949), the National Science Foundation for Post-doctoral Scientists of China (Grant #2013M532056), and the National Science Foundation for Post-doctoral Scientists of Shaanxi.

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