GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 16, Number 9, 2012
© Mary Ann Liebert, Inc.
Pp. 1135–1137
DOI: 10.1089/gtmb.2012.0066

ZNF804A rs1344706 Variant and Schizophrenia in a Romanian Population from Cluj Napoca

Andreea Zaharie,[1] Emel Ergul,[2] Mavi Deniz Ozel,[2] Ioana Valentina Miclutia,[1] Mihaela Fadgyas Stanculete,[1] and Ali Sazci[2]

The *ZNF804A* rs1344706 variant was the first risk factor to be identified through genome-wide association studies and follow-up studies with meta-analysis for schizophrenia as well as bipolar disorders; we investigated 231 schizophrenia and 222 controls to see whether this particular variant was associated with schizophrenia in a Romanian population from Cluj Napoca. Clearly, there was no association between the *ZNF804A* rs1344706 variant and schizophrenia. Our study provides evidence for those that found no association with schizophrenia. A surprising result of our study was that the T allele frequency is the highest, thus far among the ethnic groups studied. We used a PCR-RFLP method that had been recently developed in our laboratory to the genotype *ZNF804A* rs1344706 variant. In conclusion, the *ZNF804A* rs1344706 variant was not associated with schizophrenia in the Romanian population from Cluj Napoca ($\chi^2 = 0.734$, $p = 0.693$).

Introduction

Schizophrenia (OMIM: 181500) is a complex disorder with a genetic and environmental component, with a lifetime prevalence of 1%. There are strong genetic factors associated with schizophrenia, with an estimated heritability of 80% (Tsuang, 2000).

Although genome-wide association studies (GWASs) are becoming a common place, their use in psychiatric disorders is rather new. A GWAS carried out by O'Donovan *et al.* (2008) identified the variant rs1344706 in the zinc-finger protein *ZNF804A* (2q32.1) gene (OMIM: 612282), which was statistically genome-wide significant for schizophrenia as well as bipolar disorders. Supporting evidence for this finding came from two recent meta-analyses (Williams *et al.*, 2011; Zhang *et al.*, 2011a) as well as three studies in which they have found rs7597593 and rs13423388 variants on chr2, even more profoundly associated with schizophrenia in a gender specific manner (Riley *et al.*, 2010; Zhang *et al.*, 2011a, 2011b; Sazci *et al.*, 2012). Other GWASs have not replicated the evidence of association (Schanze *et al.*, 2011).

In this study, we wanted to show whether the *ZNF804A* rs1344706 variant was associated with schizophrenia in a Romanian population from Cluj Napoca.

Materials and Methods

Patients

The study population was from the Cluj Napoca area of Romania and consisted of 231 schizophrenia patients (43.59 ± 16.665; age range 18–86 years; 35% men, 65% women) and 222 controls (47.08 ± 11.316 years; age range: 18–71 years; 36% men, 64% women). They were selected for the study, having been diagnosed by a clinical examination at the clinic of the Department of Psychiatry, University of Medicine and Pharmacy Iuliu Hatieganu Cluj Napoca, Romania. The diagnosis of schizophrenia was based on DSM-IV criteria. The controls that underwent clinical examination were recruited into the study. The Kocaeli University and the University of Medicine and Pharmacy Iuliu Hatieganu Institutional review boards approved the study. Written informed consent was obtained from all subjects who took part in the study.

Genotyping

Genomic DNA was isolated from whole blood using a conventional method. The genotyping of the subjects was carried out as recently described (Sazci *et al.*, 2012).

Statistics

Allele and genotype frequencies were compared using the χ^2 test. The relative risk as odds ratio (OR) analysis was performed using 2×2 cross-tabulation and a binary logistic regression model for age and sex. Differences between groups were examined using the χ^2 test and the Student's *t*-test. All statistical analyses were carried out using SPSS software package, version 13.0. The Hardy–Weinberg equilibrium was verified for all tested populations. The p-value < 0.05 was considered statistically significant.

[1]Department of Psychiatry, University of Medicine and Pharmacy Iuliu Hatieganu Cluj, Cluj Napoca, Romania.
[2]Department of Medical Biology and Genetics, Faculty of Medicine, University of Kocaeli, Umuttepe, Kocaeli, Turkey.

Table 1. Allele and Genotype Distribution and Hardy–Weinberg Equilibrium Exact Test of the *ZNF804* rs1344706 Variant of Schizophrenia and Controls

Genotype	*Schizophrenia*	*Controls*	χ^2	p	*OR, 95%CI*
ZNF804A	231 (100.0)	222 (100.0)	0.734	0.693	
TT	95 (41.1)	92 (41.4)	0.005	0.946	0.987 (0.679–1.435)
TG	104 (45.0)	105 (47.3)	0.236	0.627	0.912 (0.631–1.321)
GG	32 (13.9)	25 (11.3)	0.691	0.406	1.267 (0.725–2.216)
Allele frequency					
T allele	63.64	65.09	0.691	0.406	0.789 (0.451–1.380)
G allele	36.36	34.91	0.005	0.946	1.013 (0.697–1.473)
HWE exact test (p)	0.66	0.67			

HWE, Hardy–Weinberg equilibrium; OR, odds ratio.

Results and Discussion

The T allele frequency of the *ZNF804A* rs1344706 variant was 65.09% in controls and 63.64% in cases (Table 1). Overall, the *ZNF804A* rs1344706 variant was not associated with schizophrenia ($\chi^2 = 0.734$; $p = 0.693$). Neither the TT genotype nor T allele were associated with schizophrenia (OR = 0.987, 95% CI = 0.679, 1.435, $p = 0.946$; OR = 0.789, 95% CI = 0.451, 1.380, $p = 0.406$, respectively). The controls and patients with schizophrenia were in Hardy–Weinberg equilibrium (0.67 and 0.66, respectively) (Table1).

In the present study, the *ZNF804A* rs1344706 variant was not associated with schizophrenia in a Romanian population from Cluj Napoca. Likewise, there was no allele or genotype association (Table 1). Our study was also supported by the results of two independent studies (Schanze *et al.*, 2011; Cousij *et al.*, 2012).

The *ZNF804A* gene has four exons and three introns with a gene size of 341 kb located on chr 2q32.1, and encodes a zinc-finger protein of 1210 amino acids with an unknown function. The *ZNF804A* gene is expressed in the brain and contains a C2H2-type domain associated with the zinc-finger protein family. Further analysis of the region with rs1344706 revealed that there is a short 3-kb conserved mammalian region located downstream of rs1344706. Consequently, this conserved mammalian region may act as a *cis*-acting element of the *ZNF804A* gene and may play a critical role in the etiology of schizophrenia (Zhang *et al.*, 2011b). Haplotype analysis of rs4666998-rs13423388-rs56280129 showed association with schizophrenia. Similarly haplotype analysis of rs1344706-rs4666998-rs13423388-rs56280129 revealed association with schizophrenia (Zhang *et al.*, 2011b). Usually proteins with zinc-finger domains tend to have a modulatory function and behave as transcription factors, but have diverse interactions with many molecules, including RNA and proteins. How many genes are modulated by this protein is not clear. Most recently, it has been shown that the *ZNF804A* gene regulates expression of the schizophrenia-associated genes *PRSS16*, *COMT*, *PDE4B*, and *DRD* (Girgenti *et al.*, 2012). It has been reported that risk allele carriers revealed increased gene expression levels (Riley *et al.*, 2010). The presence of transcription factor-binding sites in the conserved mammalian DNA sequence around rs1344706 revealed interactions with Myt1L zinc-finger protein and the POU3F1/Oct-6 POU domain transcription factor, both of which participate in oligodendrocyte differentiation and proliferation. Evidence from mouse studies suggested that the mouse homolog of the *ZNF804A* gene is a target gene for the *HOXC8* gene, indicating that the gene also is involved in the regulation of early neurodevelopment (Chung *et al.*, 2012). There appear to be differences in the allelic distribution of rs1344706 among ethnic groups, such as T allele frequencies in a descending order in Romania: 65.09, Germany: 61, United States: 61, Ireland: 59, United Kingdom: 59, Turkey: 58.39, Hungary: 58, China: 52, Japan: 42% (Sz Gene database: www.szgene.org).

The expression of the T allele of the rs1344706 variant is considerably higher in the prefrontal cortex as compared to that of the G allele (Riley *et al.*, 2010). However, how the *ZNF804A* variant causes schizophrenia is unclear, and this variant has been associated with cognitive impairments (Walters *et al.*, 2010; Esslinger *et al.*, 2011; Chen *et al.*, 2012; Hargreaves *et al.*, 2012; Wei *et al.*, 2012). rs1344706 is located on intron 2 of the gene, which contains the zinc-finger DNA-binding domain.

However, several clues have emerged through cognitive studies by which this variant is associated with functional connectivity of the dorsolateral prefrontal cortex, both across hemispheres and with the hippocampus, can increase functional connectivity of the amygdala with the regulatory limbic and prefrontal areas and neuropsychological performance, such as visual memory, episodic and working memory, and attention. Consequently, these functions are impaired in patients with schizophrenia as well as bipolar disorders (Esslinger *et al.*, 2009, 2011; Donohoe *et al.*, 2010; Hashimoto *et al.*, 2010; Walters *et al.*, 2010; Balog *et al.*, 2011; Steinberg *et al.*, 2011; Walter *et al.*, 2011; Kuswanto *et al.*, 2012; Mössner *et al.*, 2012). It has been recently shown that the *ZNF804A* gene alters expression of genes involved in cell adhesion (Hill *et al.*, 2012).

In conclusion, the *ZNF804A* rs1344706 variant was not associated with schizophrenia in a Romanian population from Cluj Napoca. Perhaps, other SNPs in the region that we did not study may be responsible for an association. It is also likely that other genetic and environmental factors are at work in Romanian schizophrenia.

Acknowledgments

We are grateful for the subjects who participated in the study. We also thank the Kocaeli University Research Fund for supporting this study (2008/009).

Author Disclosure Statement

All authors declare that they have no conflicts of interest.

References

Balog Z, Kiss I, Keri S (2011) *ZNF804A* may be associated with executive control of attention. Genes Brain Behav 10:223–227.

Chen M, Xu Z, Zhai J, *et al.* (2012) Evidence of IQ-modulated association between *ZNF804A* gene polymorphism and cognitive function in Schizophrenia patients. Neuropsychopharmacology [Epub ahead of print]; DOI: 10.1038/npp.2012.1.

Chung HJ, Lee JY, Deocaris CC, *et al.* (2012) Mouse homologue of the Schizophrenia susceptibility gene *ZNF804A* as a target of Hoxc8. J Biomed Biotechnol 2010:231708.

Cousijn H, Rijpkema M, Harteveld A, *et al.* (2012) Schizophrenia risk gene *ZNF804A* does not influence macroscopic brain structure: an MRI study in 892 volunteers. Mol Psychiatry [Epub ahead of print]; DOI: 10.1038/mp.2011.181.

Donohoe G, Morris DW, Corvin A (2010) The psychosis susceptibility gene *ZNF804A:* associations, functions, and phenotypes. Schizophr Bull 36:904–909.

Esslinger C, Kirsch P, Haddad L, *et al.* (2011) Cognitive state and connectivity effects of the genome-wide significant psychosis variant in *ZNF804A.* Neuroimage 54:2514–2523.

Esslinger C, Walter H, Kirsch P, *et al.* (2009) Neural mechanisms of a genome-wide supported psychosis variant. Science 324:605.

Girgenti MJ, Loturco JJ, Maher BJ (2012) *ZNF804A* regulates expression of the schizophrenia-associated genes PRSS16, COMT, PDE4B, and DRD2. PLoS One 7:e32404.

Hargreaves A, Morris DW, Rose E, *et al.* (2012) ZNF804A and social cognition in patients with schizophrenia and healthy controls. Mol Psychiatry 17:118–119.

Hashimoto R, Ohi K, Yasuda Y, *et al.* (2010) The impact of a genome-wide supported psychosis variant in the *ZNF804A* gene on memory function in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 153B:1459–1464.

Hill MJ, Jeffries AR, Dobson RJ, *et al.* (2012) Knockdown of the psychosis susceptibility gene *ZNF804A* alters expression of genes involved in cell adhesion. Hum Mol Genet 21:1018–1024.

Kuswanto CN, Woon PS, Zheng XB, *et al.* (2012).Genome-wide supported psychosis risk variant in *ZNF804A* gene and impact on cortico-limbic WM integrity in schizophrenia. Am J Med Genet B Neuropsychiatr Genet 159B:255–262.

Mössner R, Schuhmacher A, Wagner M, *et al.* (2012) The schizophrenia risk gene *ZNF804A* influences the antipsychotic response of positive schizophrenia symptoms. Eur Arch Psychiatry Clin Neurosci [Epub ahead of print]; DOI: 10.1007/s00406-011-0235-1.

O'Donovan MC, Craddock N, Norton N, *et al.* (2008) Molecular genetics of schizophrenia collaboration. Identification of loci associated with schizophrenia by genome-wide association and follow-up. Nat Genet 40:1053–1055.

Riley B, Thiselton D, Maher BS, *et al.* (2010) Replication of association between schizophrenia and *ZNF804A* in the Irish case-control study of schizophrenia sample. Mol Psychiatry 15:29–37.

Sazci A, Ozel MD, Ergul E, Yildiz MA (2012) Polymerase chain reaction-restriction fragment length polymorphism method for screening *ZNF804A* gene polymorphism (rs1344706) in patients with schizophrenia: a significant association. Genet Test Mol Biomarkers 16:157–161.

Schanze D, Ekici AB, Gawlik M, *et al.* (2011) Evaluation of risk loci for schizophrenia derived from genome-wide association studies in a German population. Am J Med Genet B Neuropsychiatr Genet 156:198–203.

Steinberg S, Mors O, Børglum AD, *et al.* (2011) Expanding the range of *ZNF804A* variants conferring risk of psychosis. Mol Psychiatry 16:59–66.

Tsuang M. (2000) Schizophrenia: genes and environment. Biol psychiatry 47:219–220.

Walter H, Schnell K, Erk S, *et al.* (2011) Effects of a genome-wide supported psychosis risk variant on neural activation during a theory-of-mind task. Mol Psychiatry 16:462–470.

Walters JT, Corvin A, Owen MJ, *et al.* (2010) Psychosis susceptibility gene *ZNF804A* and cognitive performance in schizophrenia. Arch Gen Psychiatry 67:692–700.

Wei Q, Kang Z, Diao F, *et al.* (2012) Association of the ZNF804A gene polymorphism rs1344706 with white matter density changes in Chinese schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 36:122–127.

Williams HJ, Norton N, Dwyer S, *et al.* (2011) Fine mapping of *ZNF804A* and genome-wide significant evidence for its involvement in schizophrenia and bipolar disorder. Mol Psychiatry 16:429–441.

Zhang F, Chen Q, Ye T, *et al.* (2011a) Evidence of sex-modulated association of *ZNF804A* with schizophrenia. Biol Psychiatry 69:914–917.

Zhang R, Valenzuela RK, Lu S, *et al.*, (2011b) Is the conserved mammalian region of *ZNF804A* locus associated with schizophrenia? A population-based genetics analysis. Schizophr Res 133:159–164.

Address correspondence to:
Ali Sazci, Ph.D.
Department of Medical Biology and Genetics
Faculty of Medicine
University of Kocaeli
Umuttepe 41380
Kocaeli
Turkey

E-mail: alisazci@gmail.com

This article has been cited by:

1. Sibylle G. Schwab, Agung A.A.A. Kusumawardhani, Nan Dai, WenWen Qin, Mutiara D.B. Wildenauer, Feranindhya Agiananda, Nurmiati Amir, Ronald Antoni, Tiana Arsianti, Asmarahadi Asmarahadi, Hervita Diatri, Prianto Djatmiko, Irmansyah Irmansyah, Siti Khalimah, Irmia Kusumadewi, Profitasari Kusumaningrum, Petrin R. Lukman, Lukman Mustar, Martina W. Nasrun, Safyuni Naswati, Prasetiyawan Prasetiyawan, Gerald M. Semen, Kristiana Siste, Heriani Tobing, Natalia Widiasih, Tjhin Wiguna, Widayanti Dewi Wulandari, Beben Benyamin, Dieter B. Wildenauer. 2013. Association of rs1344706 in the ZNF804A gene with schizophrenia in a case/control sample from Indonesia. *Schizophrenia Research* . [CrossRef]