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 $\chi^2$ 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 $\chi^2$ 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.

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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) (Table 1).

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, 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 rs4669998-rs13423388-rs56280129 showed association with schizophrenia. Similarly haplotype analysis of rs1344706-rs4669998-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.

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Author Disclosure Statement

All authors declare that they have no conflicts of interest.
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