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ORIGINAL PAPER

The schizophrenia risk gene ZNF804A influences the antipsychotic response of positive schizophrenia symptoms

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Abstract Genetic factors determining the response to antipsychotic treatment in schizophrenia are poorly understood. A new schizophrenia susceptibility gene, the zinc-finger gene ZNF804A, has recently been identified. To assess the pharmacogenetic importance of this gene, we treated 144 schizophrenia patients and assessed the response of positive and negative symptoms by PANSS. Patients homozygous for the ZNF804A risk allele for schizophrenia (rs1344706 AA) showed poorer improvement of positive symptoms (7.35 ± 0.46) compared to patients with a protective allele (9.41 ± 0.71, $P = 0.022$). This provides further evidence that ZNF804A is of functional relevance to schizophrenia and indicates that ZNF804A may be a novel target for pharmacological interventions.

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Introduction

The unraveling of the genetic causes of schizophrenia is currently progressing rapidly. One of the most compelling new schizophrenia susceptibility genes is ZNF804A, a zinc-finger domain–containing gene [7, 10]. Replication of the observed association of ZNF804A with schizophrenia in further samples has been successful [12, 13, 16]. The intronic variant rs1344706 of ZNF804A has been repeatedly found associated with schizophrenia [10, 12, 13, 16]. This variation is also functional, with significantly increased expression of the A allele of ZNF804A, compared to the C allele [12]. Moreover, the expression of ZNF804A is increased in schizophrenia patients compared to controls, although this change did not reach statistical significance [12]. ZNF804A is likely to exert a role in neurodevelopment, similar to other zinc-finger domain–containing proteins.

To date, the most robust pharmacogenetic findings for the response to antipsychotics have been found for serotonin (5-HT1A, 5-HT2A, and 5-HT2C) and dopamine receptor genes (DRD2 and DRD3) [1, 8]. However, other systems must also be involved to explain the full picture of the genetic contribution of the antipsychotic response. We therefore assessed whether the schizophrenia susceptibility gene ZNF804A has a pharmacogenetic importance. Since previous studies with monoamine system genes have shown that a pharmacogenetic role of a given gene may be primarily restricted to the positive or negative symptom response [8, 11, 14], we queried whether the disease-associated variant of ZNF804A exerts an influence on positive or negative schizophrenia symptoms.

Materials and methods

Patients

A total of 144 schizophrenia patients admitted to the Department of Psychiatry and Psychotherapy of the University of Bonn due to an exacerbation of psychotic symptoms were enrolled in the study. All patients gave written informed consent to participate in the study. Schizophrenia was diagnosed according to the ICD-10 criteria for schizophrenia. All patients were of German Caucasian origin. The schizophrenia patients had an average age of 33.6 years. Eighty-seven patients were men, while 57 patients were women. Patients were treated with atypical antipsychotics prospectively for 4 weeks. PANSS positive and negative schizophrenia symptoms were scored at the start of treatment, and at weeks one and four. The atypical antipsychotics used included olanzapine ($n = 66$), amisulpride ($n = 47$), clozapine ($n = 18$), quetiapine ($n = 19$), risperidone ($n = 13$), ziprasidone ($n = 10$), aripiprazole ($n = 7$), bifeprunox ($n = 3$), and iloperidone ($n = 1$). Some patients were treated with more than one atypical antipsychotic. A major advantage of this study is that it is a study in inpatients. Therefore, compliance was controlled by patients taking the medication under the eyes of the nurse of the ward. Moreover, the patients drank some water directly after taking the medication. Thus, proper taking of the medication was ascertained. Exclusion criteria were pregnancy, contraindication to neuroleptic treatment, mental retardation, organic brain disease, substance abuse or dependence, and suicidal behavior in previous history. The study was approved by the ethics committee of the University of Bonn.

Genotyping

The schizophrenia-associated variant of ZNF804A (rs1344706) was genotyped by allele discrimination using TaqMan technology. 12.5 ng of DNA was used for a TaqMan SNP genotyping assay, employing the Applied Biosystems protocol. The assay consisted of the unlabeled forward and reverse primers and two reporters that are dye-labeled with FAM™ and VIC® and are designed for allelic discrimination of specific SNPs. Both alleles were scored in a single well by measuring the fluorescence at the end of the PCR using a Tecan Ultra 384 reader (Tecan, Crailsheim, Germany). Excitation and emission wavelengths for the FAM-labeled probes were 485 and 535 nm and for the VIC-labeled probes 535 and 590 nm, respectively.

Statistical analysis

The difference of PANSS negative or positive symptom scores between admission and week four, stratified by genotype, were investigated by analysis of variance. A Bonferroni correction for multiple testing was performed. The level of significance was set at 0.05 (two-tailed). Since the seminal study of O'Donovan and colleagues [10] only assessed allele frequencies of this very common variant, we chose to focus on the homozygous risk allele carriers to enable a meaningful analysis. This approach is borne out by subsequent structural neuroimaging studies [3, 5]. The PANSS positive or negative symptom scores at admission were compared between the ZNF804A genotypes by analysis of variance. Pearson chi-squared test was conducted to test for the distribution of gender and genotype.

Results

A total of 144 schizophrenia patients admitted to hospital due to an exacerbation of schizophrenia symptoms gave written informed consent to participate in the study. A description of the clinical and demographic characteristics of the patients as well as the genotype frequencies are given in Table 1. The distribution of the ZNF804A genotypes was in Hardy–Weinberg equilibrium ($P = 0.535$). The genotype distribution did not differ with regard to gender ($P = 0.51$; $df = 2$) or age ($P = 0.92$; $df = 2$). Moreover, positive ($P = 0.20$; $df = 2$) and negative ($P = 0.53$; $df = 2$) PANSS symptom scores at baseline did not differ between the genotypes.

The response of the positive and negative symptoms to treatment with atypical antipsychotics is shown in Fig. 1. We calculated the difference of the positive or negative symptoms score at admission versus week 4 for each individual patient. When assessing the response of positive symptoms, those patients with the disease-associated genotype AA of ZNF804A improved less than those with the other genotypes. Thus, patients with the AA genotype showed a mean improvement of 7.35 ± 0.46 (mean ± s.e.m.) of positive symptoms, while patients with at least one C allele improved by 9.41 ± 0.71 points (mean ± s.e.m.) ($P = 0.022$) (Fig. 1). This difference remained significant after Bonferroni correction ($P = 0.044$).

For negative symptoms, patients with the AA genotype improved by 4.25 ± 0.47 points (mean ± s.e.m.), while patients with at least one C allele improved by 5.08 ± 0.5 points (mean ± s.e.m.). The poorer response of the negative symptoms of the patients with the disease-associated genotype AA was not significant ($P = 0.236$).

In this study, female patients showed superior improvement of symptoms, compared to male patients. This held both for positive symptoms ($P = 0.028$) and for negative symptoms ($P = 0.019$). Interestingly, there was no gender * ZNF804A genotype interaction for positive

Table 1 Clinical characteristics of the 144 schizophrenia patients

Genotype	AA	AC	CC
Number (%)	65 (45%)	61 (42%)	18 (13%)
Age (years) ± SD	33.8 ± 9.6	33.1 ± 10	33.9 ± 11.5
Gender (m;f)	37; 28	37; 24	13; 5
Positive symptoms (PANSS) at baseline ± SD	21.6 ± 3.4	23.2 ± 6.4	22.6 ± 3.4
Positive symptoms (PANNS) at week 4 ± SD	14.3 ± 3	13.6 ± 2.8	13.7 ± 2.2
Negative symptoms (PANSS) at baseline ± SD	21.4 ± 3.5	21.9 ± 4.3	20.9 ± 3.6
Negative symptoms (PANNS) at week 4 ± SD	17.1 ± 3.3	16.8 ± 3.3	16.2 ± 3.4

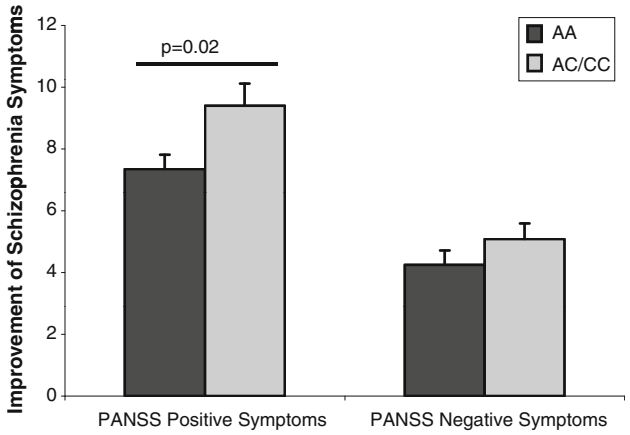

Fig. 1 Improvement of positive and negative symptoms. The improvement of positive and negative schizophrenia symptoms after 4 weeks of treatment with atypical antipsychotics is shown. Patients homozygous for the ZNF804A risk allele for schizophrenia (AA) show poorer improvement of positive symptoms compared to patients with a protective allele. Means ± s.e.m. are shown

symptoms ($P = 0.293$), while a gender * ZNF804A genotype interaction was seen for negative symptoms ($P = 0.011$). Thus, female patients carrying a C allele experienced an improvement of negative symptoms by 7.21 points, while females with the AA genotype improved their negative symptoms by 4.14 points during the study.

Age of the patients correlated neither with improvement of positive symptoms ($P = 0.394$) nor with improvement of negative symptoms ($P = 0.084$). While PANSS symptom scores at baseline did not differ between the genotypes, as shown above, improvement of positive symptom scores stratified by ZNF804A genotype was reduced to a trend ($P = 0.125$) when correcting for positive symptom score at baseline as a potential confounding factor.

Discussion

We have shown that the schizophrenia susceptibility gene ZNF804A influences the response of positive symptoms to antipsychotics. The disease-associated genotype AA of rs1344706 led to a poorer response of the positive symptoms. This indicates that ZNF804A does not only influence the development of schizophrenia but also has a role in the response of symptoms. In addition to the effect of ZNF804A on disturbed connectivity in the brain of schizophrenia patients [4], this also shows that ZNF804A has a functional role in schizophrenia. The present data suggest that the ZNF804A protein may be an important target for the development of novel pharmacological treatment strategies of schizophrenia.

In a large meta-analysis of ZNF804A, which encompassed 18,945 schizophrenia patients and 38,675 controls, a detailed search for new ZNF804A polymorphisms and a detailed association analysis showed that rs1344706 remained the most strongly associated variant in ZNF804A [16]. As the authors state, the allelic association at the ZNF804A locus is now one of the most compelling in schizophrenia to date [16]. The pharmacogenetic findings regarding ZNF804A reported in the present study thus show a pharmacogenetic effect of the most robust disease-associated variant of ZNF804A.

As a caveat, it should be considered that ZNF804A is one out of potentially hundreds or even thousands of schizophrenia risk genes. The role of ZNF804A in explaining the pathogenesis of schizophrenia should therefore not be overestimated, and the risk of developing schizophrenia will probably be determined by ZNF804A in concert with variants in many other risk genes.

Regarding the mechanism by which ZNF804A influences the response to atypical antipsychotics, two points should be considered. ZNF804A may exert its pharmacogenetic effects by a direct involvement in the mechanism of action of the drugs. Alternatively, the ZNF804A genotype AA may represent a biomarker which defines a distinct subtype of schizophrenia that is more resistant to treatment with atypical antipsychotics. Elucidation of a possible direct involvement of ZNF804A in the mechanism of action of antipsychotic drugs will require knowledge on the function of ZNF804A, which is currently not known. Regarding a possible distinct subtype of schizophrenia defined by the ZNF804A genotype AA, this is an attractive hypothesis that could be assessed in future large studies to see whether any psychopathological characteristics stand

out in these patients, compared to carriers of the ZNF804A C allele. We anticipate that it will be possible in several years' time to differentiate between these possible mechanisms.

The ZNF804A variant influences the response of positive symptoms to antipsychotics. The 5-HT1A receptor variant C-1019G, on the other hand, has been shown to primarily influence the improvement of negative symptoms to antipsychotics [8, 11, 14]. Therefore, a picture is emerging where variants in some genes appear to moderate the response of positive symptoms, while other genes may influence the improvement of negative symptoms. It is anticipated that with an increasing number of such genes, a prediction of the differential response of schizophrenia symptoms may eventually become possible.

ZNF804A has been shown to be a risk gene not only for schizophrenia but also for bipolar disorder [10, 15]. ZNF804A is among a growing group of genes affecting the risk for both disorders [2, 6, 9, 15]. It will therefore be interesting to assess whether ZNF804A also influences the drug response in affective disorders. An example in point is the 5-HT1A receptor gene, which has a pharmacogenetic role in both schizophrenia and depression [8, 11].

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Conflict of interest None.

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