

ORIGINAL PAPER

Michael Riedel · Holger Krönig · Markus J. Schwarz · Rolf R. Engel · Kai-Uwe Kühn ·
Christopher Sikorski · Safet Sokullu · Manfred Ackenheil · Hans-Jürgen Möller · Norbert Müller

No association between the G308A polymorphism of the tumor necrosis factor- α gene and schizophrenia

Received: 22 April 2002 / Received in revised form: 3 September 2002 / Accepted: 4 September 2002

Abstract Several linkage analyses in schizophrenia research point to a locus on chromosome 6p22, where the gene coding for tumor necrosis factor- α (TNF- α) is located. A marked influence of antipsychotic medication on TNF- α has been described. As the involvement of an immune process in the pathophysiology of schizophrenia has been discussed, a functional TNF- α polymorphism appears to be a candidate in genetic schizophrenia research. The G308A polymorphism of the TNF- α gene was described to be associated with increased TNF- α production.

Boin and colleagues have already described a significant association between the polymorphic allele and schizophrenia, investigating 84 schizophrenic patients (21% polymorphic allele) and 138 healthy volunteers (11% polymorphic allele), recruited in Northern Italy.

We carried out a replication study including 157 schizophrenic patients and 186 healthy persons, who were recruited in Southern Germany. Psychopathology was additionally monitored by PANSS. We were not able to replicate the findings of Boin et al., as we did not find any difference in allele frequency or genotype distribution between our schizophrenic patients (13.7% polymorphic allele) and healthy controls (16.9% polymorphic allele). Moreover, we did not find any association between genotype and psychopathology, as measured by PANSS. The different results between these two studies may be due to ethnic differences.

Key words tumor necrosis factor- α · G308A polymorphism · schizophrenia · ethnic differences · chromosome 6

Introduction

Schizophrenia is a genetically complex disorder with an incidence rate in the range of 0.16–0.42 per 1000 in the general population (Jablensky, 2000). An association between the G308A polymorphism of the tumor necrosis factor- α (TNF- α) gene and schizophrenia has recently been described (Boin et al. 2001). The main finding was a significantly higher frequency of the polymorphic TNF-308A allele (TNF2) in schizophrenic patients ($n = 84$; allele frequency: 0.21) compared to healthy control individuals ($n = 138$; allele frequency: 0.11). Homozygosity for the TNF2 genotype was only found in six of the schizophrenic patients, but in none of the healthy controls (Fisher's Exact test: $p = 0.002$). The functional significance of the TNF G308A polymorphism has been investigated using a reporter gene assay. It was demonstrated that the TNF2 allele is a much more powerful transcriptional activator than the common allele (Wilson et al. 1997). This TNF2 allele has been significantly associated with higher TNF- α production. We were interested in replicating this immunogenetic result for several reasons: 1) the hereditary component of schizophrenia is well established (Lichtermann et al. 2000) and several linkage studies point to a locus on chromosome 6p22, where immunologically relevant genes including the gene coding for TNF- α , are located (Lindholm et al. 1999; Schwab et al. 1995); 2) TNF- α is an important component of immune regulation (Andrews et al. 1990) and an involvement of the immune system in the pathophysiology of schizophrenia has been discussed (Schwarz et al. 2001); 3) the TNF- α system is markedly influenced by antipsychotic medication, indicating a possible role of TNF- α in antipsychotic drug action (Monteleone et al. 1997); 4) as stated by Boin and colleagues, a replication of the genetic study with larger samples would be required.

M. Riedel · H. Krönig · Markus J. Schwarz, M. D. (✉) · R. R. Engel ·
C. Sikorski · S. Sokullu · M. Ackenheil · H.-J. Möller · N. Müller
Psychiatric Hospital
Ludwig-Maximilian University Munich
Nussbaumstr. 7
80336 Munich, Germany
Tel.: + 49-89-5160-2761
Fax: + 49-89-5160-4741
E-Mail: mschwarz@psy.med.uni-muenchen.de

K.-U. Kühn
Psychiatric Hospital
University of Bonn
Bonn, Germany

We analyzed the TNF- α G308A polymorphism of 157 unrelated Caucasian patients suffering from schizophrenia and 186 healthy Caucasian control persons. Age of onset, number of episodes, and psychopathology as measured by PANSS at time of admittance to the hospital were monitored.

Methods

Subjects

A total of 157 unrelated Caucasian patients suffering from schizophrenia (93 male, 64 female; mean age 33.34 ± 11.71 years, ranging from 17 to 65 years) were recruited at the Psychiatric Hospital of the University of Munich. Diagnoses were established according to the criteria of the DSM-IV (Diagnostic and Statistical Manual) by two independent experienced psychiatrists. In 111 of the patients, paranoid schizophrenia was diagnosed (DSM-IV: 295.3x), 24 carried a diagnosis of disorganized schizophrenia (DSM-IV: 295.1x), 3 of residual schizophrenia (DSM-IV: 295.6x), 2 were diagnosed as catatonic schizophrenics (DSM-IV: 295.2x), 9 as undifferentiated schizophrenics (DSM-IV: 295.9x), and 7 had a schizophreniform disorder (DSM-IV: 295.4x). The mean age of onset was 27.6 ± 9.5 years. Sixty-five of the patients were suffering from first episode schizophrenia. Psychopathology at time of admittance to the hospital was monitored by the structured interview of the positive and negative symptoms scale (PANSS) (Kay et al. 1987). PANSS was administered by rater trained investigators.

From the general population of Munich and surrounding areas, 186 healthy Caucasians (88 male, 98 female; mean age 46.9 ± 13.6 years, ranging from 22 to 76 years), representing different social groups, were recruited as the control group. All controls were screened for past or present psychiatric illness and were medically examined including standard laboratory tests. History of a psychosis in a first degree relative was estimated by interviewing the control persons and was considered an exclusion criterion.

The study was approved by the local Ethics Committee, and all patients and controls gave their written informed consent after the aim of the study had been fully explained.

Genotyping

Genomic DNA was isolated from whole blood according to standard procedures. All genotypings were performed by the fluorescence resonance energy transfer method (FRET) using the Light Cycler System (Roche Diagnostics). A detailed description of the theoretical background and methodology is given by Toyota et al. (Toyota et al. 2000). For the single base polymorphism at position -G308A in the TNF- α promoter region, the following conditions were applied: forward primer: 5'- TCC TgCATC CTG TCT ggA Ag -3'; reverse primer: 5'- gTC TTC Tgg gCC ACT cAC Tg - 3'; donor hybridization probe: 5'- TTg Agg ggC ATg ggg ACg ggg T- fluorescein-3'; acceptor hybridization probe: 5'- LCRed640-AgC CTC CAG ggT CCT ACA CACAAA TCA gTC - 3'. The PCR was performed with 50 ng DNA in a total volume of 10 μ l containing 1 μ l reaction mix, 0.4 μ M MgCl₂, 0.25 μ M of each primer and 0.1 μ M of each hybridization probe according to the manufac-

turer's instructions for 45 cycles of denaturation (95 °C, 0 s, ramp rate 20 °C/s), annealing (58 °C, 10 s, ramp rate 20 °C/s) and extension (72 °C, 10 s, ramp rate 20 °C/s). After amplification, a melting curve was generated by holding the reaction at 40 °C for 30 s and subsequent careful heating to 95 °C with a ramp rate of 0.1 °C/s. The fluorescence signal was plotted against temperature to give melting curves for each sample. Peaks were obtained at 63°C for the A-allele and at 68 °C for the G-allele.

Statistical analysis

Statistical analyses were performed using SPSS for windows (Version 10.0.7). Results are reported as means \pm SD. Genotype and allele frequencies for comparison of the following groups were calculated using Chi² test: 1) patients vs. controls, 2) the herein investigated control group vs. the control population published by Boin et al., (2001), 3) first vs. multiple episode patients. The relationship between genotype and age of onset or PANSS ratings was established by ANOVA test. A two-tailed p value of at least 0.05 was considered significant. Together with genotype distribution, significancies for allele frequencies are reported. For estimation of the power of our sample to replicate the original finding, we used SOLO Power Analysis (J.L. Hinze, 1992, BMDP Statistical Software, Los Angeles).

Results

We could not replicate the finding reported by Boin and colleagues: there was no difference between schizophrenic patients and healthy controls, neither regarding genotype distribution, nor regarding allele frequency. Homozygosity for the TNF2 allele was present in only two patients (1.27%) and five control persons (2.69%). Homozygosity for the wildtype allele was found in 73.89% of the schizophrenic patients and 68.82% of the healthy controls, in contrast to the findings of Boin and colleagues, who reported allele frequencies of 66% and 79%, respectively. The A allele (TNF2) was present in 43 of our schizophrenic patients (13.7%; Boin et al.: 21%) and in 63 of the healthy control persons (16.9%; Boin et al.: 11%).

The power to replicate the described allele frequencies was 0.82 at a p value of 0.05, indicating a sufficient number of cases in our replication study.

Table 1 shows the lack of relationship between TNF- α genotype and psychopathology as measured by the PANSS scores. Additionally, we found no association between age of onset and genotype distribution among patients (age of onset - AA: 38.0 ± 1.4 years; AG: 29.0 ± 11.8 years; GG: 26.9 ± 8.6 years; ANOVA: $F = 1.45$; $p = 0.24$). Comparison of the two ethnically different control populations, as investigated in the present study and in the

Table 1 The relationship between psychopathology as measured by the PANSS 'positive', 'negative', 'global', and 'total' scores and TNF- α A308G genotype, calculated by ANOVA

PANSS scores	Genotype distribution			ANOVA	
	AA	AG	GG	F	p
Positive	15.5 \pm 12.0 (7–24)	22.2 \pm 5.9 (10–36)	21.2 \pm 6.0 (8–35)	1.44	0.24
Negative	18.5 \pm 12.0 (10–27)	23.0 \pm 7.6 (7–44)	23.5 \pm 8.1 (4–45)	0.45	0.64
Global	38.5 \pm 27.6 (19–58)	45.8 \pm 11.0 (27–72)	43.3 \pm 10.9 (10–75)	0.98	0.38
Total	72.5 \pm 51.6 (36–109)	91.0 \pm 20.9 (53–129)	88.0 \pm 19.9 (48–145)	0.93	0.40

Table 2 Comparison of the two control groups, investigated in the present study and in the study published by Boin et al. (2001) regarding TNF- α G308A allele frequency. Statistical calculation unraveled a significant difference: $\chi^2 = 4.860$, $df = 1$, $p = 0.0275$

Population	G allele	A allele
Southern German	309	63
Northern Italian	247	29

study published by Boin et al. (2001), showed a significantly higher frequency of the A allele in the Southern German population (Table 2).

Discussion

We have investigated the TNF- α G308A polymorphism in schizophrenia using a case-control study design. Our study does not replicate the positive association of the TNF2 allele (308A) with schizophrenia as published by Boin et al. (2001). The divergent findings of the two studies may be due to ethnic differences between the patient and control groups. Boin et al. investigated persons recruited in Northern Italy, whereas the herein investigated groups have been recruited in Southern Germany. Statistical comparison of these two control groups unraveled a significantly higher frequency of the A allele in the German group. Former studies have confirmed the differences in the TNF2 allele frequency between ethnic groups: Milicic et al. (Milicic et al. 2000) described a significant reduction in TNF2 alleles only in patients suffering from ankylosing spondylitis recruited in Southern Germany, but not in those recruited in the UK. Another study showed that the TNF2 allele was significantly correlated with self-reported childhood asthma in the UK, but not in a South Asian population (Winchester et al. 2000). Additionally, Gallagher et al. (Gallagher et al. 1997) argued that important ethnic differences in the TNF2 allele distribution may exist. Nevertheless, the differences in the sample sizes have to be considered. Therefore, a statistical artifact based on a small number of cases has to be considered as possible cause for the different results.

In conclusion, our study did not confirm the hypothesis of a possible gene-environment interaction based on a higher frequency of the polymorphic TNF2 allele in schizophrenia.

Acknowledgments We thank the Theodore and Vada Stanley Foundation for financial support.

References

- Andrews JS, Berger AE, Ware CF (1990) Characterization of the receptor for tumor necrosis factor (TNF) and lymphotoxin (LT) on human T lymphocytes. TNF and LT differ in their receptor binding properties and the induction of MHC class I proteins on a human CD4+ T cell hybridoma (Published Erratum Appears in J Immunol 1990 Jul 15;144(12):4906). J Immunol 144:2582-2591
- Boin F, Zanardini R, Pioli R, Altamura CA, Maes M, Gennarelli M (2001) Association between -G308A tumor necrosis factor alpha gene polymorphism and schizophrenia. Mol Psychiatry 6:79-82
- Gallagher G, Eskdale J, Oh HH, Richards SD, Campbell DA, Field M (1997) Polymorphisms in the TNF gene cluster and MHC serotypes in the West of Scotland. Immunogenetics 45:188-194
- Jablensky A (2000) Epidemiology of schizophrenia: the global burden of disease and disability. Eur Arch Psychiatry Clin Neurosci 250:274-285
- Kay SR, Fiszbein A, Opler LA (1987) The Positive and Negative Syndrome Scale (PANSS) for schizophrenia. Schizophr Bull 13: 261-276
- Lichtermann D, Karbe E, Maier W (2000) The genetic epidemiology of schizophrenia and of schizophrenia spectrum disorders. Eur Arch Psychiatry Clin Neurosci 250:304-310
- Lindholm E, Ekholm B, Balciuniene J, Johansson G, Castensson A, Koisti M, Nylander PO, Pettersson U, Adolfsson R, Jazin E (1999) Linkage analysis of a large Swedish kindred provides further support for a susceptibility locus for schizophrenia on chromosome 6p23. Am J Med Genet 88:369-377
- Milicic A, Lindheimer F, Laval S, Rudwaleit M, Ackerman H, Wordsworth P, Hohler T, Brown MA (2000) Interethnic studies of TNF polymorphisms confirm the likely presence of a second MHC susceptibility locus in ankylosing spondylitis. Genes Immun 1:418-422
- Monteleone P, Fabrazzo M, Tortorella A, Maj M (1997) Plasma levels of interleukin-6 and tumor necrosis factor alpha in chronic schizophrenia: effects of clozapine treatment. Psychiatry Res 71: 11-17
- Schwab SG, Albus M, Hallmayer J, Honig S, Borrmann M, Lichtermann D, Ebstein RP, Ackenheil M, Lerer B, Risch N, et al. (1995) Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. Nat Genet 11:325-327
- Schwarz MJ, Chiang S, Müller N, Ackenheil M (2001) T-helper-1 and T-helper-2 responses in psychiatric disorders. Brain Behav Immun 15:340-370
- Toyota T, Watanabe A, Shibuya H, Nankai M, Hattori E, Yamada K, Kurumaji A, Karkera JD, Detera-Wadleigh SD, Yoshikawa T (2000) Association study on the DUSP6 gene, an affective disorder candidate gene on 12q23, performed by using fluorescence resonance energy transfer-based melting curve analysis on the light-cycler. Mol Psychiatry 5:489-494
- Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW (1997) Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. Proc Natl Acad Sci USA 94:3195-3199
- Winchester EC, Millwood IY, Rand L, Penny MA, Kessling AM (2000) Association of the TNF-Alpha-308 (G→A) polymorphism with self-reported history of childhood asthma. Hum Genet 107: 591-596