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Association between –G308A tumor necrosis factor alpha gene polymorphism and schizophrenia

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Dysregulation of the inflammatory response system has been linked to pathophysiology of schizophrenia.[1,2] Evidence of immune activation has derived from the detection of abnormal levels of proinflammatory cytokines and their receptors in peripheral blood and cerebrospinal fluid from schizophrenic patients.[3–7] Cytokines are involved in normal CNS development as well as in the pathogenesis of many neuro-psychiatric disorders, acting directly on neural cells or modulating neurotransmitter and neuropeptide systems.[8,9] In particular tumor necrosis factor α (TNFα), depending on its concentration, can exert both neurotrophic and neurotoxic effects and influence neural cell growth and proliferation.[10,11] Moreover, TNFα gene is located on the small arm of chromosome 6 (6p21.1–21.3), a locus associated with genetic susceptibility to schizophrenia.[12,13] We studied the distribution of –G308A TNFα gene polymorphism in 84 schizophrenic patients and in 138 healthy volunteers. This biallelic base exchange polymorphism directly affects TNFα plasma levels.[14–18] Frequency of the *TNF2(A)* allele is significantly increased in schizophrenic patients as compared to controls ($P = 0.0042$). Genotype distribution is also significantly different ($P = 0.0024$). *TNF2* homozygotes are represented only in the patient group ($P = 0.002$). These data suggest a potential role of TNFα as a candidate gene for susceptibility to schizophrenia and suggest that immune dysregulation in schizophrenic patients could also have a genetic component. *Molecular Psychiatry* (2001) **6,** 79–82.

Activation of monocytes/macrophages and T lymphocytes might play a role in the pathogenesis of schizophrenia.[1,2] Significant increases in plasma concentrations of interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-6 receptor (IL-6R), tumor necrosis factor α (TNFα) and interleukin-1 receptor antagonist (IL-1RA), as well as in cerebrospinal fluid levels of interleukin-2 (IL-2) have been reported in schizophrenic patients.[3–7] Cytokines are actively transported through the blood–brain barrier, but they are also released by activated astrocytes and microglial cells.[20] It is now well established that cytokines can interact with neural cell functions, directly acting as messengers or influencing neurotransmitters and neuropeptide balance.[8,9] Moreover, there is some evidence suggesting that cytokine production during chronic activation of the immune system observed in schizophrenia could modulate prodromal, active and residual phases of this disease, and influence the response to treatment.[5,6] The origin, role and nature of the inflammatory process in schizophrenia still remain to be defined. It is still unclear if immune abnormalities detected in schizophrenic patients are expressions of the pathogenesis of the disease or are a consequence of different underlying confounding factors, such as mental stress, sleep deprivation, smoking habits, ongoing infectious diseases and prior or current medications.[21] Recently, it has been shown that typical and atypical antipsychotic drugs could modulate cytokine secretion and exert immunoregulatory effects. Some studies reported that short-term treatment with clozapine may induce the production of pro-inflammatory cytokines such as IL-6, INFγ and TNFα, an effect that tends to disappear upon prolonged treatment.[22,23] Moreover, dysregulation in cytokines production (IL-1, IL-6 and TNF) has also been described in patients with major depressive disorders, Alzheimer's disease, panic disorder, obsessive-compulsive disorder, autism and stress-induced anxiety.[24–29] This lack of specificity and the possible biasing influence of the above-mentioned factors weaken the relevance of serum/plasma-based investigations on immune system abnormalities in schizophrenic patients. Together with the evidence that genetic factors play a major role in the etiology and pathogenesis of schizophrenia as confirmed by family, twin and adoption studies, the evaluation of genetic polymorphisms involving cytokines and other immune-response mediators properly addresses the need for a more rigorous and objective approach.[30,31]

The TNFα gene is located within the class III region of the major histocompatibility complex (MHC) on the small arm of chromosome 6 (6p21.1–21.3), a locus associated with genetic susceptibility for schizophrenia.[12,13] TNFα is a pleiotropic pro-inflammatory cytokine, whose biological effects include induction of apoptosis and activation in target cells of NF-κB-inducing kinase (NIK), a MAP3K-related kinase critical for the amplification of immunological cellular processes.[32] Human monocytes, the main source of TNFα have large and stable differences in TNFα production levels. Several polymorphisms have been identified in the TNFα gene promoter region. Among these variants,

the polymorphism located at nucleotide position –308 has been shown to directly affect TNFα expression.[14] This is a well-defined biallelic base exchange polymorphism, which includes a common variant with a guanine (G) at position –308 (*TNF1*), and an uncommon variant with an adenine (*A*) at –308 (*TNF2*). The *TNF2* allele has been significantly associated with higher TNFα production and in some cases with increased morbidity and mortality in many different infectious (sepsis, malaria, leishmaniasis, COPD), autoimmune (type 1 autoimmune hepatitis, SLE) and other immune-mediated disorders (asthma, contact dermatitis).[15–18,33] To date no study to determine an association between this polymorphism and schizophrenia, major depression or other psychiatric illnesses has been performed.

We analyzed the distribution of TNFα –308 polymorphism in 84 unrelated schizophrenic patients and in 138 unrelated healthy volunteers. Data reported in Table 1 show allelic and genotype distributions of TNFα –308 gene polymorphism. Genotype distributions have no deviation from Hardy–Weinberg equilibrium. There is a significant increase in frequency of TNF –308A (*TNF2*) allele in schizophrenic patients (0.21) as compared to healthy individuals (0.11; P = 0.0042). Genotype distribution is also significantly different between patients and the control population (P = 0.0024) and homozygosity for -A substitution (*TNF2*) is only represented in the patient group (P = 0.002, Fisher exact test). No significant differences in allele and genotype frequencies are detected when the four subtypes of disease, the different classes of treatment, and gender are considered. Allelic distribution in Italian controls did not differ significantly from that of control individuals recruited from a UK population.[34]

TNFα is a critical cytokine for the onset and amplification of the inflammatory response and plays a pivotal role on host immunoreactivity to infectious agents or to self-antigens in autoimmune disorders. The polymorphism described in the present study could result in higher constitutive and inducible levels of TNFα and partly explain the increased concentration of this cytokine measured during acute and chronic phases of the disease in the sera of schizophrenic patients bearing the *TNF2* allele. Epidemiological studies have linked structural abnormalities in the brains of schizophrenics (enlarged ventricles, lower brain weight, reduced volume of temporal lobe structure) with maternal influenza during pregnancy, prenatal or delivery obstetric complications and seasonality of birth, supporting the neurodevelopmental hypothesis of schizophrenia.[35,36] Cytokines such as TNFα are implicated in acute and chronic neurodegeneration and interact with neurotransmitters' balance, particularly in the catecolaminergic system.[8–11] Thus, an abnormal TNFα production, supported by an infection during critical stages of brain development, could determine in subjects with –308 *TNF2* allele an increased risk to develop schizophrenia later in life. This is an example of a possible gene–environment interaction, where a genetic polymorphism may have relevant implications for the risk of developing the illness when associated with different environmental stressors. Our findings represent the first observation on the potential role of TNFα for susceptibility to schizophrenia and are in accordance with a previous study on allelism of the IL-1 gene complex suggesting that cytokine abnormalities in schizophrenia have a strong genetic basis.[19] Further studies with larger samples are required to better understand the influence of TNFα regulation on different symptoms of the complex phenotype of schizophrenia. Finally, since increases in proinflammatory cytokines have also been detected in major depression, Alzheimer's disease and other psychiatric disorders, extension of the study of TNFα polymorphism to these conditions will help to better define the specificity of our findings.

Table 1 Allele and genotype frequencies of TNFα gene polymorphism at position –308

	Schizophrenic patients (n = 84)	*Controls (n = 138)*
TNFα (–308) allele frequency		
Allele 1 (*TNF1*)	133 (0.79)	247 (0.89)
Allele 2 (*TNF2*)	35 (0.21)	29 (0.11)
χ^2 = 8.2 (df = 1); P = 0.0042; OR = 2.24 (95% CI 1.3–4.0)		
TNFα (–308) genotype		
1.1 (*TNF1/TNF1*)	55 (0.66)	109 (0.79)
1.2 (*TNF1/TNF2*)	23 (0.27)	29 (0.21)
2.2 (*TNF2/TNF2*)	6 (0.7)	0* (0.0)
χ^2 = 12.1 (df = 2); P = 0.0024; *P = 0.002 Fisher exact test		

Methods

Subjects

Eighty-four schizophrenic patients (56 males, 28 females), all recruited from inpatient facilities of the Psychiatric Rehabilitation Center IRCSS S Giovanni di Dio, Fatebenefratelli (Brescia, Italy) and 138 unrelated healthy volunteers (79 males, 59 females) gave, after detailed information, written informed consent to participate in the study, as indicated on the approval note by our Institutional Ethics Committee. All patients met DSM-IV criteria for schizophrenia as diagnosed by two staff-psychiatrists (RP and CAA) and assessed using the *Structured Clinical Interview for DSM-IV Patient Edition* (SCID-P).[37] Exclusionary criteria included other axis I (substance abuse, organic mental disorders, affective disorders) and axis II diagnosis, as well as neurological illness and epilepsy. A general physical exam and laboratory blood tests were performed on patients at enrollment time to rule out acute and chronic inflammatory or infectious diseases. The Brief Psychiatric Rating Scale (BPRS) was used to measure symptoms. Normal controls were free from present, past and family history (first-degree relatives) of psychiatric illness or substance abuse diagnoses

(excluding nicotine and caffeine dependence). All the subjects enrolled in this study, patients and controls, were Caucasoid living in Northern Italy. Table 2 shows the demographic and clinical characteristics for the patient study group. They include: age (mean = 43.4, SD = 9.5); age at onset (mean = 25.6; SD = 5.9); length of illness (mean = 17.9, SD = 8.2); study admission BPRS score (mean = 54.8, SD = 9.7). Forty-two patients met criteria for paranoid, 23 for undifferentiated, seven for disorganized subtypes of schizophrenia and 12 for schizoaffective disorder. At the time of enrollment (January 1999–June 2000) 35 patients were treated with typical, 21 with atypical antipsychotic and 28 were neuroleptic free (washing-out period).

DNA analysis

A venous blood sample of 7 ml was obtained from all subjects enrolled in the study and frozen at –20°C for later DNA isolation. Genomic DNA was extracted from frozen samples using standard methods.[38] The single base polymorphism at position –308 in the TNFα promoter region was screened by polymerase chain reaction (PCR) using the same methods described by Wilson *et al*.[34] Flanking oligonucleotides primers were (forward) 5′ AGGCAATAGGTTTTGAGGGCCAT 3′ and (reverse) 5′ TCCTCCCTGCTCCGATTCCG 3′. Conditions for PCR were denaturation at 94°C for 5 min followed by 30 cycles at 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s, with a subsequent final extension step at 72°C for 7 min. PCR products were digested at 37°C overnight with 10 units of *NcoI* restriction enzyme (Promega Corp, Madison, WI, USA) and electrophoresed on a 3.5% MetaPhor agarose gel (FMC, Rockland, ME, USA), stained with ethidium bromide (product size after digestion: *TNF1* allele = 87 and 20 bp, *TNF2* allele = 107 bp).

Table 2 Demographic and clinical characteristics of the patient study group

Characteristics	*Mean*	*SD*
Age (years)	43.4	9.5
Age at illness onset (years)	25.6	5.9
Duration of illness (years)	17.9	8.2
BPRS score at study	54.8	9.7
	n	*%*
Gender		
Male	56	66.6
Female	28	33.4
DSM-IV diagnosis		
Schizophrenia	72	85.7
Paranoid	42	50.0
Undifferentiated	23	27.4
Disorganized	7	8.3
Schizoaffective disorder	12	14.3

Statistical analysis

Different allele and genotype frequencies in each group were compared using standard Chi-square (χ^2) analysis and Fisher exact test. Statistical calculations were performed using the Sigmastat software (Jandel Scientific, UK).

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