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ORIGINAL PAPER

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Interaction of tumor necrosis alpha – G308A and epidermal growth factor gene polymorphisms in early-onset schizophrenia

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Abstract The study population comprised 94 Finnish patients with DSM-IV diagnosis of schizophrenia. The patients were placed into two subgroups according to medication response to conventional neuroleptics. The aim of the study was to examine the frequency of tumor necrosis factor *–308* (G > A) polymorphism in these patients and their 98 control subjects who were age- and gender-matched blood donors. Associations between *TNFα –308* polymorphism alone and between the interaction of *TNFα* and epidermal growth factor gene polymorphisms, and medication response and age at onset of schizophrenia were also studied. The frequencies of *TNFα* A-allele were 11.7 % in patients and 12.8 % in controls. The difference was not significant (p = 0.75). *TNFα –308* polymorphism was not associated with medication response. However, patients with EGF AA and TNFα AG/AA genotype had a lower age at onset of schizophrenia compared with the rest of the patients not having this combination (20.0 years, 3.3 vs. 30.2 years, 10.1 mean + SD; p < 0.001). The results support earlier findings according to which *TNFα* polymorphism is not associated with the incidence of schizophrenia. On the other hand, the role of cytokines in schizophrenia may involve genetic interactions predisposing early onset of illness.

Key words biological psychiatry · psychopharmacology · pharmacogenetics · antipsychotic agents · cytokines

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Introduction

Cytokines are a heterogeneous group of proteins functioning as chemical messengers of the immune system. They are actively transported through the blood-brain barrier and also mediate information between the peripheral immune system and the central nervous system (CNS) (Kronfol and Remick 2000; Muller and Ackenheil 1998). A pro-inflammatory cytokine, tumor necrosis factor alpha (TNFα) has a large spectrum of bioactivities and most types of cells show some responsiveness with TNFα. In the CNS, the functions of TNFα have been attributed to neurodegenerative processes, such as demyelinating disease, but also serve a protective function in response to traumatic injury (Wajant et al. 2003). As TNFα is produced by glial cells in the CNS, it has also been classified as one of the glial growth factors (Haydon 2001).

Cytokines have effects on catecholaminergic neurotransmission, which plays an essential role in such psychiatric disorders as schizophrenia. The functions of TNFα have shown similarities with pathophysiological processes in the catecholamine system in schizophrenia

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(Muller and Ackenheil 1998). These functions are also known to be inhibited by conventional antipsychotics (Kronfol and Remick 2000; Pollmacher et al. 1997). In schizophrenia, similar signs of immunological mechanisms of several types have been found in brain as with autoimmune or infectious disorders (Rothermundt et al. 2001).

The glial growth factors, including TNFα, influence on synaptic strength specifically in different parts of the brain. Low glial growth factor signaling and baseline synaptic strength have been proposed as one possible mechanism in genetic vulnerability for developing schizophrenia (Moises et al. 2002). TNFα deficient mice showed increased emotional response to stressful situations in a study by Yamada et al. (Yamada et al. 2000). There is evidence of a connection between TNFα receptor genotype and abnormalities in brain morphology in patients with schizophrenia (Wassink et al. 2000).

The TNFα gene is located in chromosome 6 at 21.1–21.3 region in a major histocompatibility complex. A functional polymorphism at –308 in the *TNFα* promoter (G > A) has shown a 6–9-fold higher transcriptional activation than the common G-allele. The rare A-allele has been associated with a high TNFα production (Wilson et al. 1997). There is some evidence that schizophrenia may be associated with the frequency of *TNFα* A-allele (Boin et al. 2001), but there are also studies reporting no association (Handoko et al. 2003; Pae et al. 2003; Riedel et al. 2002; Tsai et al. 2003) or an inverse association (Schwab et al. 2003). *TNFα* A-allele has also been associated with a higher risk for bipolar disorder (Pae et al. 2004).

TNFα acts synergistically with several other cytokines, and apart from its strong toxic effects it also mediates processes of neuronal development, synaptogenesis, regeneration and plasticity (Muller and Ackenheil 1998). Activation of the TNFα receptor, TNFR1, promotes both cell survival and cell death receptor signaling pathways (Chen and Goeddel 2002). For example, binding of TNFα in TNF receptors mediates inhibition of neurite elongation and branching in hippocampal neurons in mice (Neumann et al. 2002). Activation of TNFR1:s and the receptors of another cytokine, epidermal growth factor (EGF/ErbB:s) have similar effects in cell survival pathways. Furthermore, EGF/ErbB survival signals are inactivated by activation of TNFR1 receptors through caspase cleavage (Danielsen and Maihle 2002). EGF/ErbB receptors have been detected in frontal cortex, hippocampus, striatum and cerebellum in both embryonic and adult mice (Danielsen and Maihle 2002). Futamura et al. have found the EGF protein levels to be decreased in prefrontal cortex and striatum in postmortem samples of patients with schizophrenia (Futamura et al. 2002). EGF has also been shown to induce the expression of dopamine-D2 receptors in rat brain (Missale et al. 1994). The G allele of EGF gene has been associated with higher production of EGF (Shahbazi et al. 2002).

In the present study, we examined the frequency of *TNFα* – 308 polymorphism in a Finnish population with schizophrenia. We also investigated the possible effects of the same polymorphism alone and of an interaction with the polymorphism in epidermal growth factor *(EGF 61 G→A)* gene on treatment response with conventional antipsychotic drugs and on age at onset of schizophrenia.

Methods

Subjects

The study sample comprised 94 unrelated patients with schizophrenia who were all of Finnish origin (Northern Caucasians). An experienced psychiatrist interviewed all the patients and checked the diagnoses according to the DSM-IV criteria by evaluating hospital records.

The responder group (group I, n = 43) consisted of patients who had experienced a sufficient and long-lasting response to treatment with conventional neuroleptics. Assessment of response was based on information in hospital and mental health care records and a personal interview with each patient. Before initiation of neuroleptic treatment, the severity of schizophrenic symptoms had to be ≥ 4 according to the Clinical Global Impression scale (CGI). The non-responders group (group II, n = 51) comprised patients on clozapine medication who had failed to respond on at least two different occasions to treatment with two different conventional antipsychotics in a hospital setting. In each treatment period the lowest accepted daily dose was 400 mg chlorpromazine equivalent for a minimum of four weeks. Prior to the initiation of clozapine treatment the severity of schizophrenic symptoms had to be ≥ 4 on the CGI scale, and at least one of the following symptoms had to be present: conceptual disorganization, suspiciousness, hallucinatory behavior, or unusual thought content. Age at onset was determined as the patient's age during first hospitalization at which the diagnosis of schizophrenia was used. These data were obtained from the hospital discharge register. A greater proportion of men (62.2 %) than women (45.8 %) were selected for group II, although the difference was not statistically significant (p = 0.17, chi-square).

The control sample consisted of 98 age- and gender-matched blood donors of Finnish origin (Northern Caucasians). The study was carried out in compliance with the code of ethics of the World Medical Association (Declaration of Helsinki) and the standards established by the local medical ethics committee. The participants gave written informed consent.

TNFα –308 G>A and EGF 61 G>A genotyping

Genomic DNA was extracted from peripheral blood leukocytes using a commercially available kit (Qiagen Inc., Hilden, Germany). TNF alpha genotypes were determined using fluorogenic allele-specific oligonucleotide probes with conjugated minor groove binder (MGB) group (Livak 1999). The nucleotide sequences of the primers (forward CCA AAA GAA ATG GAG GCA ATA GGT T and reverse GGA CCC TGG AGG CTG AAC) and probes used in the PCR were deduced from published sequences deposited in the GenBank database and were chosen and synthesized in conjunction with Applied Biosystems (Foster City, CA, USA) using the Assay-by-Design tool. The reporter dyes chosen were VIC (G-specific probe) and FAM (A-specific probe). DNA samples were genotyped by employing the 5' nuclease assay for allelic discrimination using the ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). PCR reaction containing genomic DNA, 1 × Universal PCR Master Mix, 900 nM of each primer and 200 nM of each probe was performed in 96-well plates using the standard protocol for TaqMan MGB probes in a total volume of 25 µl. Water controls and known control samples previously typed by RFLP-PCR analysis were run in parallel with unknown DNA samples. After cycling, end-point fluorescence was measured and geno-

type calling was carried out by the allelic discrimination analysis module. The genotyping of the *EGF 61 G→A* was carried out as described by Anttila et al. (2004).

Statistical methods

The Pearson chi-square test was used to compare genotype and allele frequencies between different study groups. The interaction analyses were performed with logistic regression model and odds ratios and 95 % confidence intervals were calculated with –2log likelihood test. The association between genotype and the age at onset were analyzed with Kaplan-Meier log rank test and t tests. The limit of statistical significance was set at 0.05. In determining statistical power of the study with regard to the *TNFα* polymorphism in patient and control populations we hypothesized obtaining similar distributions of alleles as in the study of Boin and co-authors (21 % in patients, 11 % in controls) (Boin et al. 2001). According to this assumption, the level of statistical power was equal to 0.80. Data analysis was carried out using SPSS/Win (Version 11.5, SPSS Inc., Chicago, IL) and PS (version 2.1, (Dupont and Plummer 1997)) software.

Results

The frequencies of *TNFα* A-allele were 22/188 (11.7 %) in patients and 25/196 (12.8 %) in controls. The difference was not statistically significant (p = 0.75) (Table 1). The patient groups with different medication response did not differ in their *TNFα* genotypes (p = 0.96). The TNFα polymorphism was not associated with the age at onset of schizophrenia (p = 0.35).

As reported earlier, the frequencies of *EGF 61 G→A* polymorphism within the same sample were 108/188 (57.4 %) in patients and 119/196 (60.7 %) in controls (Table 1). *EGF* G allele was associated with later age of onset in male subjects (Anttila et al. 2004).

The model of interaction of *EGF* and *TNFα* polymorphisms was not associated with the incidence of schizophrenia (p = 0.39, –2log likelihood test). However, the interaction between *EGF* and *TNF* polymorphisms was associated with the age at onset of schizophrenia (p = 0.0015, log rank test) (Fig. 1). The mean difference in age at onset between subgroups according to *EGF/TNFα* combination (subgroup I: combination of *EGF* AA and *TNFα* AG/AA genotypes, n = 6; subgroup II: any other combination of *EGF/TNFα* genotypes, n = 88) was 9.2 years (subgroup I, mean 20.0 years, SD 3.3 years; subgroup II, mean 30.2 years, SD 10.1 years; p < 0.001, t-test). The difference of means is presented in Fig. 2.

Discussion

This study represents one of the few works reporting on the relationship between gene polymorphisms of cy-

Table 1 Clinical and genetic characteristics of patients with schizophrenia and control subjects

	Sex M/F	Age years	Age at onset years	TNFα allele[2]		TNFα genotype[3]			EGF allele		EGF genotype		
				G	A	GG	AG	AA	G	A	GG	AG	AA
Responders	17/26	48.1±11.4	32.2±9.5	76	10	35	6	2	36	50	7	22	14
Non-responders	29/22	44.1±10.5	27.5±10.2[1]	90	12	41	8	2	44	58	8	28	15
Controls	47/51	45.9±11.2		171	25	74	23	1	77	119	18	41	39

[1] P = 0.02 compared to responders (chi-square test); [2] P = 0.75 between all patients and controls; [3] P = 0.14 between all patients and controls

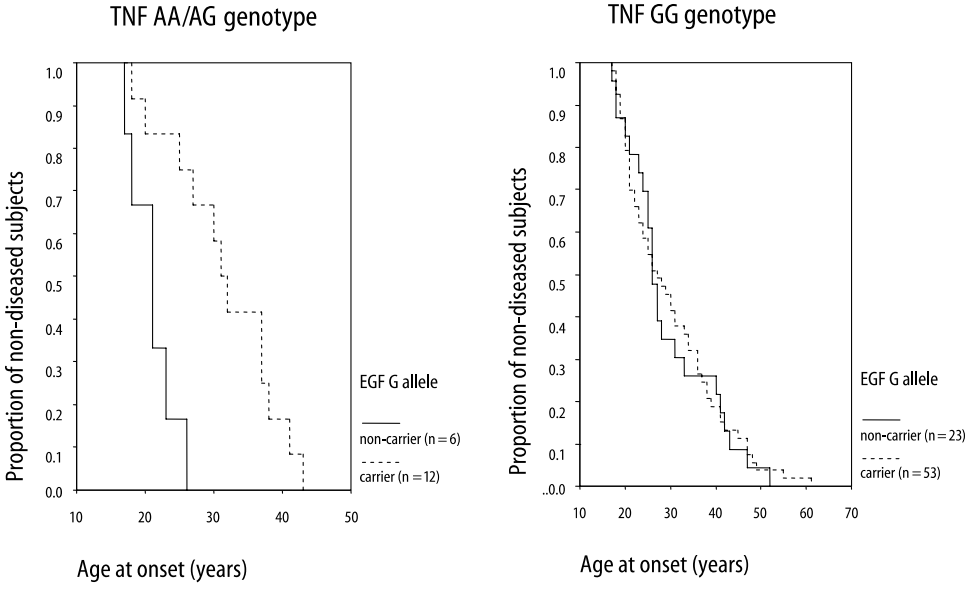

Fig. 1 Survival plots showing age at onset with schizophrenia in patients with tumor necrosis factor AA/AG genotype (left plot) and GG genotype (right plot). The patients not carrying epidermal growth factor G allele have lower age at onset in the TNF AA/AG group compared with patients carrying EGF G allele (p = 0.0015, log rank test). Corresponding difference is not found in the TNF GG group (p = 0.81)

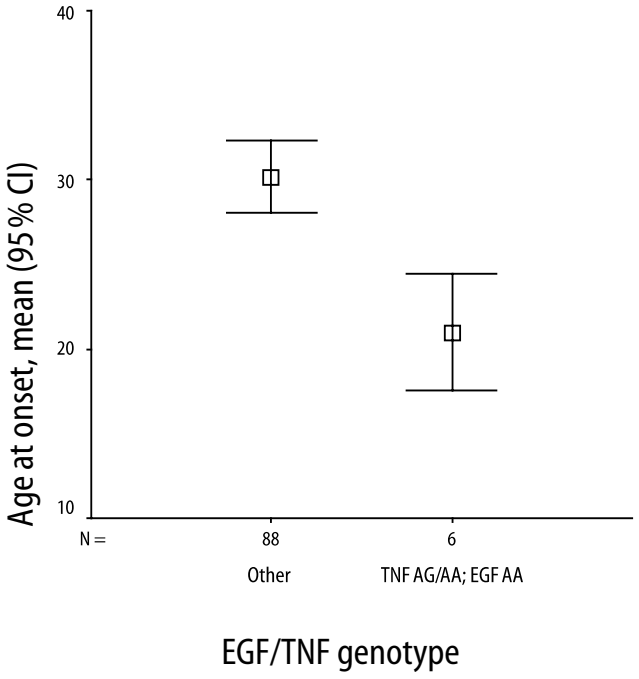

Fig. 2 Difference in the age at onset of schizophrenia between subgroups according to combination of epidermal growth factor and tumor necrosis factor genotypes. In the subgroup with a combination of TNFα AG/AA and EGF AA genotypes the mean age at onset is 20.0 years and SD 3.3 years. In the other subgroup with any other combination of EGF and TNF genotypes the mean age at onset is 30.2 years and SD 10.1 years ($p < 0.001$, t-test)

tokines and schizophrenia. Due to the wide spectrum of functions with cytokines in the CNS, and the limitations of the present sample size, the results should be regarded as preliminary evidence possibly leading to more precise hypotheses on the role of cytokines in pathogenesis of schizophrenia.

Most of the studies so far have not found any association between *TNFα-308A* polymorphism and the incidence of schizophrenia (Handoko et al. 2003; Pae et al. 2003; Riedel et al. 2002; Tsai et al. 2003), and the present finding gives further support to this hypothesis without any association. Neither did the *TNFα* polymorphism alone have any significant effect on medication response with conventional antipsychotics nor on the time at onset of schizophrenia.

Schizophrenia is a neurodevelopmental disorder, and gene-gene interactions with cytokine genes could explain early abnormalities in synaptic plasticity and functional selection during the maturation process of the CNS (Moises et al. 2002). However, the study did not show any association between the interaction of *TNFα* and *EGF* polymorphisms and the incidence of schizophrenia, or medication response.

The interaction of *EGF* and *TNF* genotypes was related to the age at onset of schizophrenia. Although the number of patients with *EGF* AA and *TNFα* AG/AA genotype is small, the analysis according to combination of *TNFα* and *EGF* genotypes revealed two clearly different groups in relation to the age at onset of schizophrenia. The finding could refer to marked developmental vulnerability resulting in early onset of the clinical disorder in the group with *TNFα* AG/AA and *EGF* AA genotype. EGF enhances cell survival in dopaminergic neurons (Yamada et al. 1997), and the G allele of *EGF* polymorphism has been associated with high EGF production in peripheral blood (Shahbazi et al. 2002). On the other hand, TNFα A-allele is associated with a high level of TNF production (Wilson et al. 1997). It is known that TNFα stimulates EGF receptor expression in airway epithelium (Nadel 2001). TNFα converting enzyme that converts membrane-anchored TNFα into its soluble form markedly impairs EGF receptor availability in mice (Sunnarborg et al. 2002). In post mortem brain tissues of patients with schizophrenia, EGF receptor expression has been found to be elevated and EGF levels decreased in prefrontal cortex (Futamura et al. 2002). The combination of the *TNF* AG/AA and *EGF* AA genotype may result in high TNFα expression with low EGF levels and low EGFR availability in brain tissue, too. This genotype combination may further predispose to enhanced cell receptor death signaling via TNFR1, inhibition in survival signaling via EGF/ErbB receptor and abnormal functional development in dopaminergic regulatory pathways in prefrontal cortex.

The study gives further evidence to support the earlier finding that a functional polymorphism in *TNFα* does not have any association with the incidence of schizophrenia. The medication response with conventional anti-psychotic medication was likewise not associated with this polymorphism. The interaction of *TNFα* and *EGF* may have an effect on greater vulnerability resulting in early onset of schizophrenia. It is, however, important to note that the background of this finding has not been clearly verified, but is partly hypothetical and thus needs further support from complementary studies.

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