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Tryptophan metabolism and immunogenetics in major depression: A role for interferon-γ gene

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ABSTRACT

The tryptophan metabolism and immune activation play a role in pathophysiology of major depressive disorders. The pro-inflammatory cytokine interferon-γ transcriptionally induces the indoleamine 2,3-dioxygenase enzyme that degrades the tryptophan and thus induces serotonin depletion. The polymorphism of certain cytokine genes was reported to be associated with major depression. We investigated the association between interferon-γ (IFNγ) gene CA repeat polymorphism, the profile of serotonin and tryptophan pathway metabolites and clinical parameters in 125 depressed patients and 93 healthy controls. Compared to controls, serum tryptophan and 5-hydroxyindoleacetic acid (5HIAA) concentrations in the patients were significantly lower and serum kynurenine concentrations were significantly higher at baseline ($p < 0.0001$). The presence of IFNγ CA repeat allele 2 homozygous has significant association with higher kynurenine concentrations in controls ($F = 4.47$, $p = 0.038$) as well as in patients ($F = 3.79$, $p = 0.045$). The existence of interferon-γ CA repeat allele 2 (homo- or heterozygous) showed significant association with increase of tryptophan breakdown over time during the study period ($F = 6.0$, $p = 0.019$). The results indicated the association between IFNγ CA repeat allele 2, tryptophan metabolism and the effect of medication.

1. Introduction

The importance of tryptophan metabolism in the pathophysiology of major depressive disorders has been well documented. Several studies in depressed patients who responded to the selective serotonin reuptake inhibitors (SSRIs) and other antidepressants showed that acute tryptophan depletion could reverse the antidepressant effects (Aberg-Wistedt et al., 1998; Bremner et al., 1997; Delgado et al., 1990, 1991, 1999; Smith et al., 1999; Spillmann et al., 2001).

Tryptophan, instead of being synthesized into 5-hydroxytophan and serotonin, could be degraded into kynurenine and could lead to tryptophan depletion and then to serotonin depletion. In addition, the kynurenines which are neuroactive are proposed to be involved in the pathophysiological mechanism in depression (Lapin and Oxenkrug, 1969; Lapin, 1973). This depletion is the result of enhanced tryptophan catabolism by the enzymes tryptophan 2,3-dioxygenase (TDO) in the liver (Hayaishi, 1980) and the indoleamine 2,3-dioxygenase (IDO) in the lungs, placenta, blood and brain (Heyes et al., 1995; Mellor and Munn, 1999). TDO2 immunoreactivity was demonstrated in the frontal cortex of the schizophrenic brain (Miller et al., 2004).

The TDO specifically metabolises tryptophan only (Hayaishi, 1980) whereas IDO also metabolises serotonin and melatonin (Hayaishi, 1976). The activity of TDO is enhanced by the tryptophan concentration (Saito et al., 1990; Satyanarayana and Rao, 1980; Smith et al., 1980) and by high cortisol (Salter and Pogson, 1985) whereas IDO activity is transcriptionally induced by the pro-inflammatory cytokines such as interferon-γ (Carlin et al., 1987, 1989; Hu et al., 1995; Taylor and Feng, 1991; Yasui et al., 1986) and inhibited by anti-inflammatory cytokine, interleukin-4 (IL4) (Musso et al., 1994).

Both increased pro-inflammatory cytokines (Anisman et al., 1999; Connor and Leonard, 1998; Kaestner et al., 2005; Kim et al., 2002; Lanquillon et al., 2000; Mikova et al., 2001; Myint et al., 2005; Thomas et al., 2005) and hypercortisolaemia (Asnis et al., 1981a,b; Cohen et al., 1984; Lin et al., 1986; Sher et al., 2005) have been well reported in patients with major depression. An increase in interferon-γ/IL-4 ratio was observed in medication naive depressed patients (Myint et al., 2005). Enhanced IDO activity which was indirectly indicated by an increased neopterin level

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was observed in cancer patients treated with interferon-α, a pro-inflammatory cytokine and subsequently developed depression (Capouron et al., 2002). Enhanced formation of kynurenine from tryptophan, indicated by kynurenine/tryptophan ratio which indirectly indicates the IDO activity, was also observed in interferon-α treated patients who developed depression (Wichers et al., 2005) and in medication naïve patients with major depression compared to normal controls (Myint et al., 2007). However, another study reported that tryptophan depletion in depressed patients occurs without enhanced an kynurenine pathway (Hughes et al., 2012), although that conclusion was drawn without measuring concentration of quinolinic acid which is one of the crucial metabolites from the downstream kynurenine metabolism. Taken all together, the high pro-inflammatory cytokines could induce serotonin depletion by (1) depletion of tryptophan, which is the precursor of serotonin through increased tryptophan breakdown due to enhanced IDO activity; and (2) direct breakdown of serotonin by IDO into anthraniloylalkylamine fragment (Sun, 1989) apart from the catabolic pathway through monoamineoxidase (MAO) activity into the end metabolite 5-hydroxyindoleacetic acid (5HIAA), which might deplete serotonin and shunt the serotonin away from degradation into 5HIAA and result in a reduction of 5HIAA.

Some human and experimental studies had been performed on the genes related to proinflammatory cytokines or markers and their relationship to depression (Jun et al., 2002, 2003a,b; Simen et al., 2006; Yu et al., 2003). The human study on IL1β (C-511T) genetic polymorphism showed that −511T allele of the IL1β gene had a trend of less severity of depressive symptoms and more favourable fluoxetine therapeutic response than −511C carriers (Yu et al., 2003). An experimental study also showed that the deletion of tumour necrosis factor receptor (TNFR) 1 and 2 leads to an antidepressant-like response in the forced swim test (Simen et al., 2006). Regarding the polymorphism of cytokine genes and IDO activity, one study recently demonstrated that enhanced blood IDO activity of Helicobactor pylori seropositive individuals in terms of kynurenine/tryptophan ratio is related to TGFβ1-509 allele T (Raitala et al., 2007). These findings indicated the possible relationship between certain cytokine genes, depression and IDO activity which will affect the tryptophan breakdown.

Based on the above findings, we hypothesised that the tryptophan metabolism, with emphasis on the balance between tryptophan breakdown into kynurenine and serotonin synthesis from tryptophan, may be related to genetic polymorphisms of certain cytokine genes. Since interferon-γ (IFN-γ) is the key cytokine that enhances IDO activity, we focused on this cytokine. The CA repeat microsatellite box in the first intron of the IFN-γ gene is well documented to be associated with altered in vitro production of interferon-γ by peripheral blood mononuclear cells of healthy subjects (Pravica et al., 1999). In addition, it was reported that the single nucleotide polymorphism of IFN-γ +874(T/A) is associated with kynurenine/tryptophan ratio in women (Raitala et al., 2005). We therefore placed emphasis on the association between biochemical parameters and the IFN-γ CA repeat polymorphism. In particular, we explored the relationship between tryptophan metabolism in terms of tryptophan breakdown, indicated by kynurenine/tryptophan ratio, and the balance between tryptophan breakdown and serotonin conversion from tryptophan, indicated by serotonin metabolite 5HIAA/kynurenine ratio and interferon-γ gene CA repeat polymorphism, in patients with major depression.

2. Materials and methods

2.1. Patients and controls

We investigated 125 unrelated in-patients (46 men, 79 women; age 49.75 ± 13.5 years) suffering from major depression (93.0%) or depressed states of bipolar disorder (7.0%) according to DSM-IV criteria. 30.3% of the patients had a first episode, 69.7% a recurrent episode. Prior to the inclusion of the patient in the study, blood sampling for a routine laboratory screening and a medical examination were carried out to exclude severe medical disorders. Patients with any psychiatric comorbidity or any systemic chronic diseases or acute diseases that can affect the immune system were excluded from the study. All patients and controls were Caucasians and recruited in the Southern Germany/Bavaria region; 88% of them were of German descent. The mean sum score of the Hamilton Depression Rating Scale (17-item version) at admission was 26.6 ± 6 and 6.7 ± 2.1 before discharge from hospital. The patients were drug-free at baseline for at least 5 days prior to inclusion. Major medical disorders, addiction or other comorbid psychiatric diagnoses, pregnancy, use of oral contraceptives or hormone replacement therapy led to exclusion from the study. None of the patients had been pre-treated with fluoxetine or depot neuroleptics. Blood for neurochemical and genetic analyses was drawn exactly at 8:00 AM after overnight fasting to prevent an influence of circadian fluctuations, and psychopathology was assessed. Neurochemical analyses and ratings were consecutively carried out after 2 and 4 weeks and before discharge from hospital. During their time of admission patients received various pharmacological and non-pharmacological treatments, among them treatment with mirtazapine (18%), sertraline (12%) or reboxetine (13%). Forty five percent of the patients received a combination of one of those medications with benzodiazepine or lithium or valproate or lamotrigine. Five percent of the patients were treated with electroconvulsive therapy and 7% were treated with other non-pharmacological therapies.

A total of 93 healthy controls (35 men, 58 women) were recruited. All controls were asked and investigated for past or present psychiatric and rheumatic illness through questionnaires including MMPI-2 testing and were medically examined for the presence of infectious illness through standard laboratory tests including CRP and differential blood count. This was done with the intention that an infectious status according to the actual laboratory parameters would be considered an exclusion criterion. Additionally, control individuals were asked for any history of psychosis in a first-degree relative, which was also considered an exclusion criterion.

The study was carried out according to the Declaration of Helsinki and local ethics committee approval. Patients were included in the study after they gave written informed consent. A written informed consent was taken from each patient and control.

2.2. Analyses of genetic polymorphism

Genomic analysis was done on the blood samples taken at the time of recruitment for both patients and controls. Genomic DNA was isolated from whole blood according to standard procedures.

The CA-repeat polymorphism in intron 1 of the interferon-γ gene was genotyped by fluorescence detection using the PISM 310 automated sequencer (Applied Biosystems, Foster City, CA, USA). A DNA fragment containing the repeat sequence was amplified with the following conditions: Forward primer: 5′-6-FAM-gCT gTC ATA ATA ATA TTC AgA C-3′; reverse primer: 3′-CgA gCT TTA AAA gAT AgT TCC-5′. PCR was performed with 50 ng of genomic DNA in a total volume of 25 μl containing 0.5 μM each primer, 200 μM dNTPs, 2.5 μl 10 × PCR buffer and 0.5 Units Taq (Ampli Taq Gold; Perkin Elmer). PCR conditions were as follows: 95 °C for 5 min; 30 cycles of denaturation 95 °C/60 s; annealing 57 °C/45 s; elongation 72 °C/45 s; and final 72 °C/7 min. Prior to loading onto the PRISM 310 10 μl formamide was added to 1 μl of the reaction mixture and samples were denaturated at 95 °C for 5 min. Reactions were electrophoresed on a 41 cm capillary array at