Gene Expression to Genetical Genomics

ORIGINAL RESEARCH

Interleukin 1 Beta Gene Polymorphism in Schizophrenia and Psychotic Depression

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Abstract: The proinflammatory interleukin 1 beta serum level is elevated in schizophrenic patients. Like other proinflammatory cytokine, it is in part dependant on genetic expression, so it may represent a candidate susceptibility gene for schizophrenia. It plays a crucial role in development of the Central Nervous System (CNS) and may, hence, influence brain morphology in schizophrenia.
The theory that psychotic depression is a distinct syndrome that has to be differentiated from non psychotic depression, is supported by literature showing substantial difference between them in clinical manifestations, biological laboratory measures, family transmission, course, response to treatment and prognosis. Our work attempts to assess Interleukin 1 beta gene polymorphism in psychotic depression and schizophrenia, which may potentially represent a genetic differentiating biological marker, taking in consideration the diagnostic difficulty that rises when evaluating those two disorders, though of different management and outcome, but largely similar in terms of presenting symptoms.

Keywords: schizophrenia, depression, psychogenetics, interleukin 1 beta, genetics

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Introduction

Schizophrenia and affective psychoses are severe and prevalent psychiatric disorders described in all cultures and populations. Whether these functional psychoses are two distinct disorders, or are closely related in etiology, has been debated in the literature during the last century. Several studies have suggested that schizophrenia and bipolar disorder are on a continuum of liability.[1–4]

Psychopathological dimensions and psychiatric symptoms shared by both groups of patients would be compatible with this overlap. Likewise, other risk factors, such as cerebral ventricle enlargement, markers of prenatal suffering, or life events, have been described in both mental disorders. Recent molecular linkage studies have suggested the possible existence of shared disease loci for both disorders.[1–4]

Genes coding for some of these cytokines are located on the Inter Leukin IL-1 cluster within chromosome 2q13. This cluster contains nine genes of the IL-1 family of cytokines (IL-1A, IL-1B, IL-1RN, and IL-1F5–F10). Several polymorphic variants of these genes have been associated with human diseases. The IL-1b gene (IL-1B) consists of seven exons with an extension of 7 kb and codes for a precursor form (proIL-1b) which is cleaved by a protease (ICE) to give the active IL-1b form.[5]

This pro-inflammatory cytokine is involved in acute and chronic neurodegeneration and in embryonic development of the CNS. During CNS neurodevelopment, IL-1b promotes proliferation and production of cytokines and trophic factors, such as nerve growth factor (NGF), in astrocytes and inhibits normal expression of brain-derived neurotrophic factor (BDNF). In addition, IL-1 (α and β) participates in differentiation of mesencephalic progenitor cells into dopaminergic neurons in cell culture.[5,6]

On the other hand, the IL-1Ra gene (IL-1RN) consists of seven exons in a region of 16 kb, and several splice variants can be obtained from the coding sequence. Its product, IL-1 receptor antagonist (IL-1Ra), is an endogenous antagonist at IL-1 receptors and modulates the action of IL-1 agonists. Altered levels of IL-1Ra have been described in the pathogenesis of several diseases where inflammatory or autoimmune processes are involved.[5]

Recent studies have shown that drug-naive schizophrenic patients exhibit a significant increase in both IL-1b and IL-1Ra plasma levels when compared with healthy controls. It is generally agreed that regulatory processes affecting IL-1 function are, at least in part, determined by genetic variation.[26] Thus, production of IL-1b and IL-1Ra may be modulated by the effect of polymorphisms in the IL-1 cluster.[6]

Since higher plasma levels of interleukin-1β have been found in schizophrenic patients and interleukin-1β levels are partly genetically determined, interleukin-1β has been regarded as a candidate gene in schizophrenia. Interleukin-1β is a cytokine that is not only involved in inflammatory responses but which also plays a crucial role in the development of the central nervous system. Interleukin-1β has previously been shown to stimulate astrocytes to proliferate and produce a variety of cytokines and trophic factors, including nerve growth factor. Moreover, it is involved in acute and chronic neurodegeneration.[7]

Two biallelic base-exchange polymorphisms, which are in nearly complete linkage disequilibrium, have been reported at positions −511 and −31 in the promoter region of the interleukin-1β gene. There is growing evidence that allele 2 at position −511 is associated with enhanced interleukin-1β production. The interleukin-1β polymorphisms and a repeat polymorphism in the neighboring interleukin-1Ra gene are in much weaker linkage disequilibrium. Nevertheless, there is an interaction: interleukin-1β −511 allele 2 is associated with higher levels of interleukin-1Ra, while allele 2 (IL1RN*2) in the interleukin-1Ra gene is associated with higher levels of interleukin-1β.[8]

Depression has been associated with increases in circulating cytokines in younger adults, and there is evidence for prefrontal inflammation in late-life depression. Thomas et al tested the hypothesis that levels of cytokine interleukin-1β (IL-1β) would be higher in subjects with late-life major depression.[9]

Our work aims at assessing polymorphism of Interleukin 1 beta gene in both psychotic depression and schizophrenia in relation to non psychotic normal control group.

Subjects and Methods

Patients were recruited from the psychiatric outpatient service of Alexandria University Hospital, patients aged between 18 and 50 years, scoring 4 or above

on the Clinical Global Impression for Severity Scale CGI-S.[10] Diagnosis was done in conformity with the Diagnostic and Statistical Manual of Mental Illness in it's 4th edition DSM-IV.[11]

Patients were categorized in 3 groups.

Group I Psychotic depression patients (n = 17)

Group II schizophrenic patients (n = 19)

Group III control group, healthy non psychotic group (n = 17)

All subjects were subjected to physical and psychiatric assessment by competent physicians and laboratory investigations for complete blood count, renal functions, hepatic function, electrolytes and Thyroid gland profile.

Genetic analysis

DNA extraction

PCR amplification

The region containing the C/T transition at position –511 in the IL1B gene promoter was amplified by PCR (Polymerase Chain Reaction) using the primers:

5′-TGGC ATTGATCTGGTTC ATC-3′ and 5′-GTTTAGGAATCTTCCCACTT-3′

PCR was performed in a Thermo-Hybaid PCR Express thermocycler, in a final volume of 50 µl containing the following:

- ~300 ng of genomic DNA
- 1X PCR buffer (750 mM Tris-HCl, pH 8.8 at 25 °C, 200 mM NH_4SO_4, 1% Tween, MBI Fermentas)
- 3 mM $MgCl_2$
- 400 µM dNTPs (MBI Fermentas)
- 30 picoM of each primer (ILBF and ILBR, Metabion). The sequence of the primers in reference …
- 1 unit Taq DNA polymerase.

The reaction was denatured initially at 94 °C for 4 minutes followed by 35 cycles of denaturation at 94 °C for 30 seconds, annealing at 48 °C for 30 seconds and extension at 72 °C for 30 seconds. This was followed by a final extension step at 72 °C for 10 minutes.

Ten µl of the PCR product were mixed with 4 µl of 6X loading buffer (0.09% bromophenol blue, 0.09% xylene and 60 mM EDTA in 60% glycerol), then loaded on 2% agarose gel (Gibco) containing ethidium bromide 20 ng/µl to check the PCR product.

Restriction

For detection of the polymorphism, the PCR products were digested overnight at 37 °C with the restriction enzyme AvaI (MBI Fermentas). Digestion was done in a total volume of 30 µl containing 15 µl of the PCR product, 2 U of the enzyme, 3 µl buffer and milliQ water for the remaining volume. The restriction digests were electrophoresed on 3% agarose gels and visualized with ethidium bromide staining and ultraviolet illumination.

Interpretation of results

The digested products Allele 1 (CC), Allele 2 (TT) and Allele ½ (CT).

Statistical methods

Data were analysed using PC with Statistical Package for Social Sciences version 13, the 0.05 was used as cut off value for statistical significance. Due to small numbers we opted a rather for student's t test and non parametric chi square (X^2) test for independent groups.

Results

Group I patients with psychotic depression

Group II patients with schizophrenia

Group III control group

In group I the duration of illness ranged between 2 and 12 with a mean of 5.10 ± 2.83 years while in group II it ranged between 2 and 11 with a mean of 3.85 ± 2.01 years. Both groups I an II are matched as regards duration of illness (Fig. 1).

In group I the score of CGI-S ranged between 4 and 5 with a mean of 4.6 ± 0.5, while in group II it ranged between 4 and 6 with a mean of 5.1 ± 0.55 with no

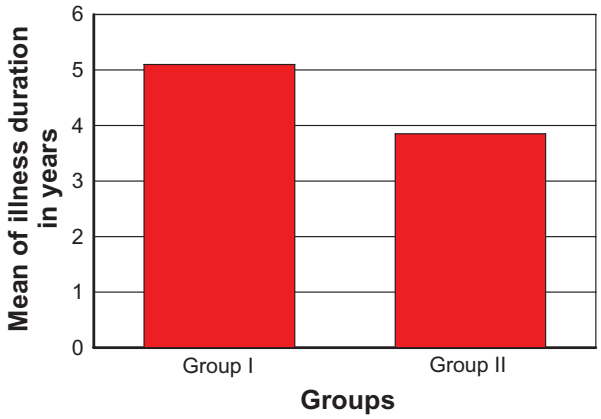

Figure 1. Comparison between groups I and II as regards duration of illness.

Table 1. Comparison between Group I and Group II as regards duration of illness.

Duration of illness in years	Group I (n = 17) Psychotic depression	Group II (n = 19) Schizophrenia
Range	2–12	2–11
Mean	5.10	3.85
SD	2.83	2.01
t	1.61	

Table 2. Comparison between both groups I and II as regards CGI-S scores.

CGI-S score	Group I (n = 17) Psychotic depression	Group II (n = 19) Schizophrenia
Range	4–5	4–6
Mean	4.6	5.1
SD	0.5	0.55
t	0.8962	

significant statistical difference. Both groups I and II are matched as regards illness severity as measured by CGI-S (Fig. 2).

In group I, the percentage of different genotypes for interleukin 1 beta gene was 23.5%, 35.3% and 41.2% for genotypes CC, TT and CT respectively. In group II, the percentage of different genotypes for interleukin 1 beta gene was 31.6%, 26.3% and 41.1% for genotypes CC, TT and CT respectively. Finally, in group III, the percentage of different genotypes for interleukin 1 beta gene was 29.4%, 35.3% and 35.3% for genotypes CC, TT and CT respectively (Fig. 3).

In group I, the percentage of different genotypes for interleukin 1 beta gene was 58.8% and 41.2% for genotypes (CC or TT) and CT respectively. In group II, the percentage of different genotypes for interleukin 1 beta gene was 57.9% and 42.1% for genotypes (CC or TT) and CT respectively. Finally, In group III, the percentage of different genotypes for interleukin 1 beta gene was 64.7% and 35.3% for genotypes (CC or TT) and CT respectively. Chi = 0.53, so there's no significant statistical difference as regards the distribution of different genotypes for each group (Fig. 4).

Discussion

In the present research work no significant statistical difference as regards genotypes and polymorphism of the interleukin 1 beta gene among the studied groups, schizophrenic patients and psychotically depressed patients in relation to healthy control subjects.

Such finding support results from previous works. Meizeinzehl EM et al conducted a study investigating the effect on brain morphology of an interleukin-1β genetic polymorphism (C→T transition at position –511) in patients with schizophrenia. Genotype analysis were used in the examination of 44 male schizophrenic patients and 48 healthy male comparison subjects. No association between the interleukin-1β polymorphism and schizophrenia was detected.[7]

In contrast, Previous studies have suggested that these polymorphisms of the IL-1b and IL-1RN genes may influence susceptibility to schizophrenia.

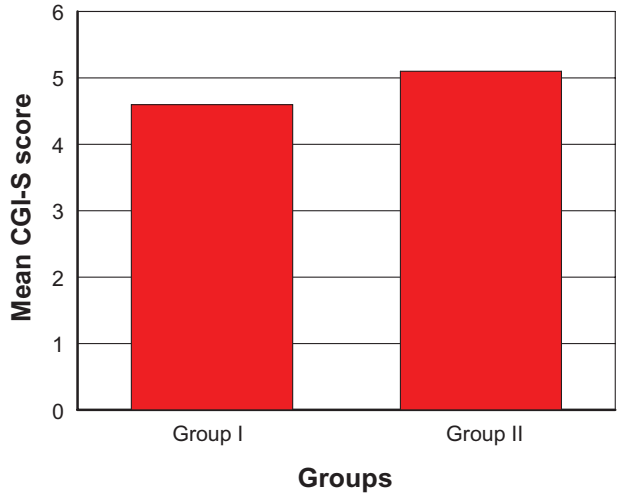

Figure 2. Comparison between both groups I and II as regards CGI-S.

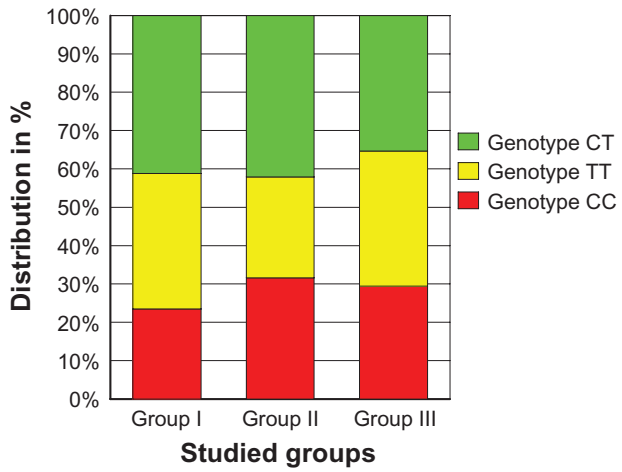

Figure 3. Distribution of different genotypes for each group.

Table 3. Distribution of different Alleles at position –511 promoter region of IL 1 beta gene for each of the studied groups.

	Group I (%) Psychotic depression n = 17	Group II (%) Schizophrenia n = 19	Group III (%) Control n = 17
Allele 1 (CC)	23.5	31.6	29.4
Allele 2 (TT)	35.3	26.3	35.3
Allele 1/2 (CT)	41.2	41.1	35.3
Total	100	100	100

Katila et al reported a positive association with schizophrenia for the combination –511 (allele 1)-VNTR (allele 1), authors described an excess of allele 2 in schizophrenic patients showing decreases in bifrontal-temporal gray matter volume and generalised white matter tissue deficits. Rosa and colleagues found the same allele 2 associated with depressive dimension in schizophrenia.[8]

It should be noted that none of these studies used the haplotypic approach in fact, owing to the strong linkage disequilibrium between loci located on the IL-1 cluster, analysis of haplotypes would be the most appropriate method to study the involvement of this genomic region in psychiatric disorders.[7]

Authors hypothesise that an imbalance in the ratio between pro-inflammatory and anti-inflammatory cytokines may affect embryonic neurodevelopment and promote neurodegeneration in adulthood.

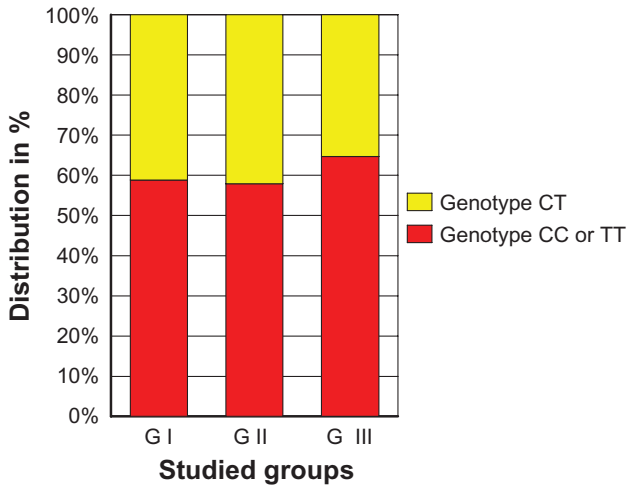

Figure 4. Distribution of different genotypes for each group.

Table 4. Distribution of different genotypes for each group (both homogenous groups added together for statistical purposes to apply Chi square).

	Group I (%) Psychotic depression n = 17	Group II (%) Schizophrenia n = 19	Group III (%) Control n = 17
Genotype CC or TT	58.8 (n = 10)	57.9 (n = 11)	64.7 (n = 11)
Genotype CT	41.2 (n = 7)	42.1 (n = 8)	35.3 (n = 6)
Total	100	100	100

Additionally, IL-1b plays a key role in the dopaminergic differentiation of neural progenitors. It should be mentioned that alterations in the dopaminergic system have been classically related to the origin of functional psychoses and, particularly, to the origin of positive symptoms. According to evidence mentioned above, the polymorphic regions analysed in this study, which are thought to confer subtle changes in the expression pattern of the IL-1B and IL-1RN genes, could contribute, with a moderate effect, to destabilising the pro-inflammatory/anti inflammatory equilibrium during neuro development.[12,13]

Our study is limited by small sample which render extrapolation and generalization of results to schizophrenic and psychotically depressed populations unreliable, further studies with larger samples are highly recommended in order to have a solid answer for our research hypothesis.

Disclosure

This manuscript has been read and approved by all authors. This paper is unique and is not under consideration by any other publication and has not been published elsewhere. The authors and peer reviewers of this paper report no conflicts of interest. The authors confirm that they have permission to reproduce any copyrighted material.

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