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Molecular Association of Inter leukine-1b -511 gene (IL-1b(-511)) with Schizophrenia patients in Babylon province-Iraq

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Abstract : Background : This study includes patient sample consisted of 30 patients (age range19–61 years). They all gavetheir written informed consent for the study after itsnature had been fully explained. The study wasapproved by the ethicscommittees.The control group consisted of 50 healthy blooddonors (age range 18–60 years). It was estimated the molecular association of Inter leukine-1b -511 gene (IL-1b(-511)) with schizophrenia patients and the results were indicated that the B allele is associated with this disease (0.75) and BB genotype has a significant association (p=0.05) and has a risk factor 3.05 more than Bb and bb genotypes.

Introduction

Schizophrenia is a neuro-developmental disorder that affects youth in puberty stage and proved by a confusionin cognition and emotion along with negative and positive signs. According to the neuro-developmental hypothesis, schizophrenia may participatepathologic processes, caused by both environmental and genetic factors, Schizophrenia also has a genetic basis, and a significant number of these genetic risk variants have recently been specified[1,2]. These neurodevelopmental defects, developing in uterus as early as late first or early second trimester for some and then for others, have been suggested to lead to the activation of pathologic neural circuits during pubertyor young adulthood, which leads to the emergence of positive or negative symptoms or both[3,4,5].

Multiple markers of congenital anomalies indicative of neuro-developmental insults have been found in schizophrenia[6,7].

Several studies measuring the levels of proinflammatory cytokines, such as interleukin (IL)-1, -6, -10, -33, andTNF-a in the peripheral blood or cerebrospinal fluid of schizophrenic patients have indicated dysregulation of these cytokines in schizophrenia.It has been recently demonstrated that plasma IL-1b levels were increased in agroup of acute first admission drug-free schizophrenic patients as compared to healthy controls. Others havelinked elevated IL-6 and TNF-a levels to the course, the treatment or progression of schizophrenic illness[8,9, 10]. Although these cytokine abnormalities, as well as the other immunological aberrationsassociated with psychiatric disorders, have been known for some time, the causal relationship has remained enigmatic. Theoretically, the increase in the levels of the pro-inflammatory cytokines can simply be a consequence of mental stress or sleep deprivation associated with the onset or exacerbation of the disease, without having a role in the pathogenesis of the disease[11]. On the other hand, these cytokines can modify the metabolism of neurotransmitters or influence neural development. IL-1 has been described as an astroglial

growth factor and it has been suggested to have a role in the development process of the brain and to be implicated in acute and chronic neurodegeneration[12]. Cytokines are now known to have multiple modulatory effects on cellulargrowth and differentiation. These cytokines could therefore be of primary pathogenic importance, eitherin the acute disease phase or during those stages of brain development which possibly influence the sensitivity of a person to schizophrenia in later life. A geneviral model of schizophrenia suggests that exposure to viruses initiates an immunological process that somehow disorganizes development of the fetal brain[13].

All three genes of the IL-1 gene complex (IL-1a, IL- 1b, IL-1RA) are clustered on the long arm of humanchromosome 2 in a region q13–q21[14]. In the IL-1b gene there are at least two bi-allelic base exchange polymorphisms,one at the promoter region at position -511, and the other at position +3953 in the 5th exon. There is now evidence that geneof IL-1b, ,is polymorphic and the various alleles may have adifferential regulatory effect on cytokine production and, consequently, the allele frequencies are oftenaberrant in various diseases of an autoimmune or inflammatory nature. Results on the IL-1b gene complex, suggest a genetic involvement in the observed dysregulation of thiscytokine in schizophrenia[15,16]. In conclusion, study findings on the allelism of the IL- 1b gene complex suggest that the cytokine aberrations in schizophrenia are, at least partly, genetically determined. It could be speculated that the genetics of infection is somewhat different in schizophrenic patients. This could have etiopathogenic importance bearing in mind the theories of maternal viral infection leaving the foetus at greater risk of developing immunologically mediated brain changes possibly leading to schizophrenia. Future studies on the genetics of immunological changes in schizophrenia should include the mothers of the patients as well.

Methods

Subjects

The patient sample consisted of 30 patients (age range19–61 years). They all gavetheir written informed consent for the study after itsnature had been fully explained. The study wasapproved by the ethicscommittees.The control group consisted of 50 healthy blooddonors (age range 18–60 years).

Collection of the blood samples:

Blood were collected in EDTA tubes, stored in - 40˚C (deep freeze) in order to be used later in DNA extraction.

PCR Amplification of Inter leukine-1b-511 gene (IL-1b -511)

The region that contains the *Ava*I polymorphic site at the position -511 of the IL-1b genewas amplified by PCR. The oligonucleotides 5TGGCAT TGA TCT GGT TCA TC3 and 5GTT TAG GAATCT TCC CAC TT3flanking this region were used as primers. PCR conditions were as follows: 95°C for2 min, 55°C for 1 min, 74°C for 1 min, then 38 cycles of 95°C for 1 min, 55°C for 1 min, 74°C for 1 min, and finally 74°C for 4 min. The products were digested with 6 units of *AvaI* at 37°C for 3 h. Fragments were analyzed by electrophoresis on 2.5% agarose gel electrophoresis, stained with ethidium bromide. This gave products of BB (304bp), Bb (190 bp, 114 bp and 304 bp), and bb (190 bp, 114 bp)[15].

Statistical Analysis:

Genetic analysis was performed using Chi-square($\chi 2$) test. P values less than (0.05) is considered significant and less than (0.01) is considered highly significant.

Results and Discussion

The PCR product of IL-1b (-511)gene amplification was 304bp figure (1).

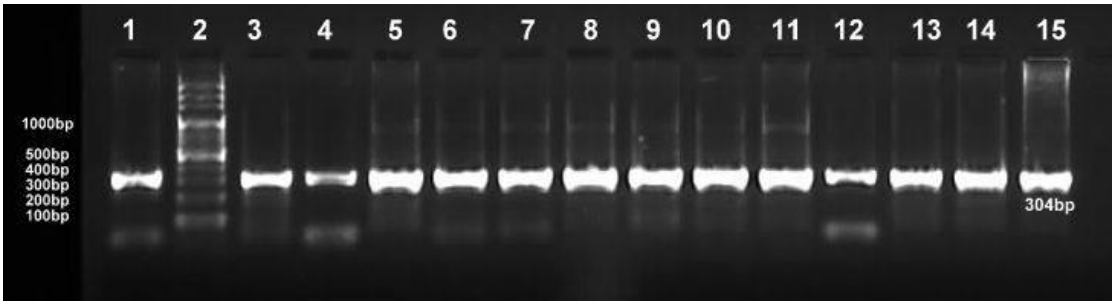

Figure (1)electrophoresis pattern of PCR product of IL-1b (-511) gene, the optimum annealing temperature was 55.0°C

The Genotype of IL-1b (-511)gene polymorphism between the two group control and patient group were detected using PCR-RFLP technique. Results from figure (2) show the genotype of IL-1b (-511)gene in the two study groups control and patients (the control were 50 samples while the patients were 30 samples), BBhomogenotype represented (304bp), Bb heterogenotype represented (304bp, 190bp,and114bp) and bb homogenotype represented (190bp,and114bp)

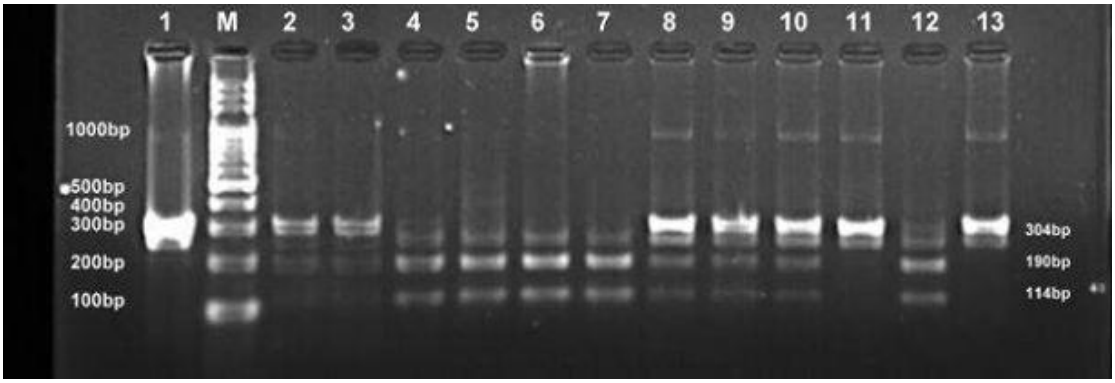

Figure (2) Electrophoresis pattern of PCR-RFLP by 2.5% agarose gel for PCR product (304bp) with restriction enzyme *AvaI*. Lane M DNA ladder.Lane (1-4) control andLane: (5-14) patients. Lane (4) heterozygote (Bb) genotype, Lane (1-3, 11,13) mutant homozygote (BB) genotype and Lane (12) homozygote (bb) genotype.

The genotype frequencies of BB, Bb, and bb of IL-1b (-511) gene polymorphism were 17(56.67%), 11(36.67%)and 2(6.66%) in the patient group, while 15(30%), 31(62%) and 4(8%) in the control group , table (1).

Table(1): Genotype of IL-1b (-511)gene polymorphism with Allele frequency associated with schizophrenia .

Genotype IL 1B -511	**Control**	**Patient**	χ^2 **(N=80)**	***P* value**	**O.R.**
BB	15(30%)	17(56.67%)	5.670	0.05	3.05
Bb	31(62%)	11(36.67%)	5.602	0.018	0.35
bb	4(8%)	2(6.66%)	0.136	0.713	0.82
Total number	50 (100%)	30 (100%)			
Allele frequency					
Allele	**Control**		**Patient**		
B	0.6		0.75		
b	0.4		0.25		

Results from table(1) show that the *P-value* of the genotype frequency of IL-1b (-511)gene in the two study groups control and patients has mean differences (0.000) which is less than 0.05. So there were significant differences of IL-1b (-511)gene ($p \leq 0.05$).

The data of allele frequencies of point mutations on IL-1b (-511)gene in two study group control(50) and patients(30) are presented in Table (3). For patient groups the allele frequency of(B) variant allele was, but(b) allele variant frequency was according to Hardy-Wienberg equation. While for control groups the allele frequency of (B) variant allele was, but (b) allele variant frequency was according to Hardy- Wienbergequation.

PCR product of IL-1b (-511)gene amplification was 304bp[15]. The Genotype of IL-1b (-511)gene polymorphism between the two group control and patient group were detected using PCR-RFLP technique. Results show the genotype of IL-1b (-511)gene in the two study groups control and patients (the control were 50 samples while the patients were 30 samples). The genotype frequencies ofBB, Bb , and bb of IL-1b (-511)gene polymorphism were 17(56.67%), 11 (36.67%)and 2 (6.66%) in the patient group, while 15 (30%), 31 (62%) and 4 (8%) in the control group. Results also show that the *P-value* of the genotype frequency of IL-1b (-511)gene in the two study groups control and patients has mean differences (0.05, 0.018, 0.713)for BB, Bb , and bbrespectively, and from these results BB, BbIL-1b (-511)gene polymorphism have significant differences which is less than 0.05, while bbhas no significant differences. Results from table (1) indicate that the patients with genotype BB has a risk factor (3.05) times than the genotype of Bb which has a risk factor (0.35) and bbwhich has a risk factor (0.82).

So from the above results it has been demonstrated that there is a significant relationship between IL-1b (-511)gene and schizophrenia, and the (B) was the risk allele [16, 17, 18].

IL-1 has been described as an astroglial growth factor and it has been suggested to have a role in the development process of the brain and to be implicated in acute and chronic neurodegeneration. Cytokines are now known to have multiple modulatory effects on cellular growth and differentiation. These cytokines could therefore be of primary pathogenic importance, either in the acute disease phase or during those stages of brain development which possibly influence the sensitivity of a person to schizophrenia in later life [17,19, 20].

Schizophrenia has a high heritability, and evidence suggests a polygenicinheritance, with an established role of both rare variants with large effects, as well as commonSingle Nucleotide Polymorphisms (SNPs) with small effects. Given this complexity, earlygenetic studies failed to replicate previous associations, leading to a pessimistic outlook onschizophrenia genetics[21]. By studying the interface of peripheral cytokines and CNScellular processes contributing to depression, we may be able todevelop a new class of therapeutics to treat mood disorders by sequestering and preventing these peripherally-derived inflammatory cytokines from acting on mood circuits in the brain[22].

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