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Brief Research Communication

Genes for Interleukin-2 Receptor β Chain, Interleukin-1 β, and Schizophrenia: No Evidence for the Association or Linkage

Masahiko Tatsumi,[1] Tsukasa Sasaki,[2] Toshiyuki Sakai,[1] Kunitoshi Kamijima,[1] Rimmei Fukuda,[2] Hiroshi Kunugi,[2] Mineko Hattori,[2] and Shinichiro Nanko[2]*

[1]*Department of Psychiatry, Showa University School of Medicine, Tokyo, Japan*
[2]*Department of Psychiatry, Teikyo University School of Medicine, Tokyo, Japan*

We studied a CA repeat polymorphism of the interleukin-2 receptor β chain (IL-2RB) gene and a C/-514/T variation of the interleukin-1 β (IL-1B) gene in Japanese schizophrenia patients. Both a case-control association study (54 patients and 54 controls) and a linkage study using six multiplex families (the number of the affected ⩾4 in each family) were employed. No evidence for the association or the linkage was obtained either for the IL-2RB or IL-1B gene. Am. J. Med. Genet. 74:338–341, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: schizophrenia; interleukin-1 β; interleukin-2 β receptor; microsatellite marker; linkage; association

INTRODUCTION

Cytokines, including interleukin-1 (IL-1) and interleukin-2 (IL-2), play several roles in brain functions and may be related to the development of schizophrenia. Several studies have found increased serum soluble IL-2 receptors in schizophrenia patients [Ganguli and Rabin, 1989; Rapaport et al., 1989, 1993; Rapaport and Lohr, 1994]. The finding was confirmed in neuroleptic-naive as well as neuroleptic-treated patients [Rapaport and Lohr, 1994]. Sasaki et al. [1994] found a significant increase of IL-2 receptors on lymphocytes in schizophrenia patients after acute exacerbation, compared to the controls. Production of IL-2 after mitogenic stimulation was decreased in the lymphocytes of neuroleptic-naive as well as -treated patients with schizophrenia [Ganguli and Robin, 1989; Villemain et al., 1989; Ganguli et al., 1995].

In addition to these immunological findings, recent genome scan studies suggested a linkage of schizophrenia to the chromosome 22q12–q13 region, where the IL-2 β receptor (IL-2RB) gene is mapped. Pulver et al. [1994a] observed suggestive evidence for IL-2RB (a lod score of 1.54 at $\theta = 0.2$) under dominant model. Coon et al. [1994] obtained a lod score of 2.09 for D22S276 (4 cM proximal to IL-2RB [Coon et al., 1995], at $\theta = 0.1$) under recessive model. Vallada et al. [1995] and Polymeropolous et al. [1994] obtained lod scores of 1.51 (at $\theta = 0.1$) and 0.37 (at $\theta = 0.2$) for D22S278 (11 cM telomeric to IL-2RB [Coon et al., 1994]). Schwab et al. [1995] reported a lod score of 0.61 for D22S304 (3.5 cM centromeric to D22S278, at $\theta = 0.2$). Finally, a multicenter study of these research groups showed suggestive evidence for the linkage of schizophrenia with this region, by using a non-parametric analysis [Gill et al., 1996], although some follow-up studies failed to observe evidence for the linkage [Pulver et al., 1994b]. Thus, the IL-2RB gene is considered a good candidate gene for schizophrenia.

IL-1 may also be involved in the pathogenesis of schizophrenia. IL-1 affects several brain functions, including deep sleep and appetite [Krueger et al., 1984], which are often interrupted in acute patients with schizophrenia. In addition, recent studies have shown that IL-1 β (IL-1B) augments release or metabolism of monoamines, including dopamine, norepinephrine and serotonin [Palazzolo and Quadri, 1990; Carmelina et al., 1991; Mohankumar et al., 1991; Shintani et al., 1993]. In major psychosis, including schizophrenia, functions of the monomania system are disturbed. The IL-1B gene may therefore, be considered a candidate gene for psychosis including schizophrenia.

In the present study, we investigated a "C" to "T" substitution in the 5′ region of the IL-1B gene (2q13–q21) [di Giovine et al., 1992] and a dinucleotide (CA) repeat polymorphism of the IL-2RB gene (22q12–13.1) [Brewster et al., 1991] in Japanese patients with schizophrenia. A linkage analysis using multiplex families and a case-control association study were both conducted.

*Correspondence to: Shinichiro Nanko, MD, Department of Psychiatry, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi, Tokyo 173, Japan.

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TABLE I. Allelic Distributions of the C/-514/T Variation of the IL-1B Gene and the Dinucleotide Repeat Polymorphism of the IL-2RB Gene in Unrelated Subjects

	IL-1B		IL-2RB												
Alleles	1	2	<2	3	4	5	6	7	8	9	10	11	12	13	14
Patients (n = 54)	64	44	1	4	5	4	20	26	28	4	2	7	1	5	1
Controls (n = 54)	64	44	0	1	4	2	21	29	32	8	6	1	1	3	0

SUBJECTS AND METHODS

Subjects

Association study. In the case-control association study, the subject consisted of 54 unrelated Japanese patients with schizophrenia. The patients were recruited from the Outpatient Clinic, Department of Psychiatry, Teikyo University Hospital (TUH), Tokyo. The consensus diagnosis was made by two psychiatrists according to the DSM-III-R criteria. The controls comprised the same number of unrelated healthy Japanese volunteers, without a history of major psychosis, recruited from the hospital staff and students of the TUH. The age of the patients and the controls ranged from 19 to 63 (mean = 35, SD = 11) and from 19 to 53 (mean = 28, SD = 7), respectively.

Linkage study. The subjects were 53 family members from six Japanese multiplex schizophrenia pedigrees. The family members were interviewed using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) [Spitzer and Endicott, 1979]. Consensus diagnoses were made by at least two psychiatrists independently according to the Research Diagnostic Criteria (RDC) [Spitzer et al., 1978]. Information from relatives and medical and social records were also used when available. Each family contained four or more patients with psychiatric disorders, including two or more schizophrenia patients. Diagrams of two families were demonstrated in Gill et al. [1993]. DNAs were available in 53 members from the six families. Out of the 53 subjects, 20 were diagnosed with schizophrenia, two with delusional disorder, one with unspecified functional psychosis, one with bipolar I disorder (with psychotic symptoms) and one with unipolar disorder (major depression).

Genotyping

For the IL-1B gene, a 405 bp fragment including the polymorphism site in the 5′ region was amplified using PCR [di Giovine et al., 1992]. The PCR products were digested with *Ava* I. Electrophoresis was performed on 5% polyacrylamide gel, followed by ethidium bromide staining. For the IL-2RB gene, a fragment including the dinucleotide repeat was amplified using PCR [Brewster et al., 1991]. Silver staining was performed to detect the allele after electrophoresis on 12% polyacrylamide gel.

Statistical Analysis

For the linkage analysis, lod scores were calculated using the MLINK program (the LINKAGE package). Because the exact mode of inheritance is unknown in schizophrenia, we employed several different models (Model 1–3), which were used in a previous study [Gill et al., 1993]. These models encompass the range between dominant and recessive transmission and are compatible with the known distribution of schizophrenia in the population and the relative of the proband. The parameters were as follows; $f_{AA} = 0.999$, $f_{Aa} = 0.95$, $f_{aa} = 0.001$, and the gene frequency = 0.02 in the Model 1; $f_{AA} = 0.999$, $f_{Aa} = 0.60$, $f_{aa} = 0.001$ and the gene frequency = 0.02 in the Model 2; and $f_{AA} = 1.0$, $f_{Aa} = 0.10$, $f_{aa} = 0.001$ and the gene frequency = 0.10 in the Model 3. Two definitions of the affected status (narrow and broad definitions) were tested for each model. Narrow definition included schizophrenia, schizoaffective disorder and unspecified functional psychosis (RDC). Broad definition comprised the narrow definition plus bipolar affective disorder and unipolar major depression (RDC).

RESULTS

No significant difference in the allelic distribution was observed between the unrelated patients and controls, either in the "C/T" variation of the IL-1B gene or the dinucleotide polymorphism of the IL-2RB gene (Table I). Also, no significant difference was observed in the genotype distribution between the patients and controls, in either of the genes.

As summarized in Table II, no positive lod scores were observed either for the IL-1B gene or the IL-2RB gene, under any tested model (the three models of transmission; narrow and broad definitions of the affected status). Significant negative (<−2) lod scores were achieved for both of the IL-1B and IL-2RB genes (at $\theta = 0$), under all the models. Evidence for genetic heterogeneity among six families was not demonstrated by the HOMOG program [Ott, 1991].

DISCUSSION

Our results did not detect differences in allelic or genotype distribution between the patients and controls in the IL-2RB or IL-1B gene. Nimgaonkar et al. [1995] studied the same polymorphism of the IL-2RB gene and failed to detect differences in allele frequencies between schizophrenia patients and controls, consistent with our result with this gene. This suggests that the IL-2RB gene may not play a major role in the etiology of schizophrenia. However, the following limitations remain. First, because the polymorphism is located at the non-coding region of the gene, functional differences between the alleles are unclear. Second, the sample size was relatively small in both studies [54 patients and the same number of controls (df = 12) in our study, and 42 patients and 47 controls (df = 7) in Nimgaonkar et al. [1995]. The data of the two studies

TABLE II. Lod Scores for the IL-1B Gene and the IL-2RB Gene in Six Multiplex Families

(IL-1B)	θ	0.00	0.01	0.05	0.1	0.2	0.3	0.4
Narrow	Model 1	−3.9	−3.1	−2.2	−1.5	−0.7	−0.3	−0.1
	Model 2	−2.6	−2.3	−1.6	−1.1	−0.5	−0.2	−0.0
	Model 3	−2.4	−2.1	−1.5	−1.0	−0.4	−0.1	−0.1
Broad	Model 1	−3.9	−3.1	−2.1	−1.5	−0.7	−0.3	−0.1
	Model 2	−2.6	−2.3	−1.6	−1.0	−0.4	−0.2	−0.0
	Model 3	−2.0	−1.7	−1.1	−0.7	−0.2	−0.0	0.0
(IL-2RB)	θ	0.00	0.01	0.05	0.1	0.2	0.3	0.4
Narrow	Model 1	−2.7	−1.9	−1.3	−0.9	−0.5	−0.2	−0.1
	Model 2	−2.0	−1.8	−1.2	−0.8	−0.5	−0.2	−0.0
	Model 3	−2.4	−2.2	−1.6	−1.1	−0.4	−0.2	−0.0
Broad	Model 1	−4.1	−2.7	−1.5	−0.9	−0.4	−0.1	0.1
	Model 2	−2.8	−2.4	−1.5	−1.0	−0.4	−0.1	0.0
	Model 3	−2.6	−2.4	−1.7	−1.1	−0.4	−0.2	−0.0

may not be combined due to the ethnic difference. Therefore, it might be that the studies were not capable of detecting the association, if only a small portion of the cases were due to the IL-2RB gene. Also, because a previous study [Ganguli et al., 1995] suggested that abnormality of IL-2 production in schizophrenia patients might be associated with early age of onset and negative symptoms, studies of patients with those features may help detect the possible association.

For the C/−514/T variation of the IL-1B gene, our sample size is large enough to detect a relative risk of 2.5 with statistical power of 80% and significance of 5% (two-sided test), assuming that the frequency of the allele 1 in the population ranges from 35% to 40% (the present data). A larger sample size might be required to detect a smaller effect of the gene; however, exactly the same allele frequencies between our patients and controls suggest that this variation of the IL-1B gene may not play a major role in the etiology of schizophrenia. This variation is located at the 5′ region of the gene and may play a role in the control of the expression of the gene. However, studies of variations in the coding region of the gene may be more helpful to detect the possible association.

In the linkage analysis, we employed three models of inheritance ranging from dominant to recessive mode, and two criteria of the affected status. No models provided any suggestive lod scores and all the models achieved lod scores of −2 or less at $\theta = 0$, for either of the genes. Thus, we may conclude that no evidence is obtained for the linkage of the chromosomal regions, including IL-2RB or IL-1B in our six Japanese multiplex families. The previous multicenter studies in a large number of pedigrees have suggested a linkage of schizophrenia with the 22q region including IL-2RB. However, the lod scores or *P* values were relatively low and did not reach the statistically significant level [Lander and Kruglyak, 1995] in those studies, suggesting that only a fraction of the pedigrees were linked to this region. Our result also suggests that a portion of the multiplex schizophrenia families linked to the 22q region may be small, even if they exist at all.

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