



Genetic variants of IL-6 and its receptor are not associated with schizophrenia in Taiwan

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ABSTRACT

The pathophysiological process of schizophrenia is still unclear. The levels of interleukine-6 (IL-6) and its receptor, soluble IL-6R, have been reported to be elevated in the plasma and cerebrospinal fluid of schizophrenic patients. In this study, we tested the association of genetic variants of IL-6 and IL-6R with schizophrenia. Genotyping of three single nucleotide polymorphisms (SNP) for each IL-6 (IL-6-1, IL-6-2, and IL-6-3) and IL-6R (rs4845617 = IL-6R1, rs4553185 = IL-6R2, and rs4379670 = IL-6R3) gene was performed in 100 patients with schizophrenia and 113 normal controls. The polymorphisms of IL-6R2 were genotyped using Tetra-primer ARMS PCR. IL-6R3 polymorphisms were genotyped using restriction fragment length polymorphism (RFLP) with Apo I enzyme as the restriction enzyme. All other polymorphisms were genotyped using the direct sequencing method. We found a di-nucleotide haplotype block and a tri-nucleotide haplotype block in the genes of IL-6 and IL-6R, respectively. All six SNPs and their haplotypes failed to show a significant association with schizophrenia. The IL-6-2 SNP showed a nominally significant association with the positive symptoms of schizophrenia ($p = 0.0472$). We conclude that the genetic variants of IL-6 and IL-6R are not associated with schizophrenia. In order to verify this result, further study using a larger sample size and exploring the association between the genotype of IL-6-2 and plasma level of IL-6 is recommended.

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Schizophrenia is a complex mental disorder affecting 1% of all populations. The disease primarily affects the central nervous system, but immune alterations have been actively proposed to play a role in the pathogenesis of schizophrenia [10,16,18]. An autoimmune involvement in schizophrenia has been suggested by several cellular and humoral cytokine changes in patients [22]. Among these altered cytokines, interleukine-6 (IL-6; online Mendelian Inheritance in Man (MIM) number *147620) has been most consistently found to be related to schizophrenia [6,9,14–15,26].

IL-6 is a pleiotropic cytokine released both from peripheral immune cells and from neurons and microglia of the central

nervous system (CNS) [24,27]. In the CNS, soluble IL-6 receptor (sIL-6R; MIM number *147880) levels in the CSF have increased in schizophrenic patients with a marked paranoid-hallucinatory syndrome [17]. In the periphery, IL-6 has been found to be persistently elevated in the plasma of patients in different ethnic groups [1,11,19,26]. High IL-6 levels have been found to be related to duration and treatment resistance in schizophrenia [11,19]. These results suggested that an elevated plasma level of IL-6 was associated with an unfavorable course of schizophrenia with a longer duration of illness, greater treatment resistance, and more marked paranoid-hallucinatory symptoms.

We selected single nucleotide polymorphisms (SNPs) located within these two genes and genotyped them in 113 normal controls and 100 schizophrenic patients to decipher the potential genetic association of IL-6 and IL-6 receptor in schizophrenia. Endophenotypes are recommended to define the role of the gene in the complex traits of schizophrenia [5]. In this study, we used the sus-

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tained attention endophenotype of schizophrenia as measured by the continuous performance test (CPT) and the severity of the positive and negative symptom dimensions to assess the potential association of these endophenotype indicators with IL-6 and its receptor in the disease.

We collected the DNA of schizophrenic patients, fulfilling criteria defined by the Diagnostic and Statistical Manual 4th edition (DSM-VI) [2] from the Department of Psychiatry of the National Taiwan University Hospital and normal controls without a history of any psychotic symptoms or family history of psychotic disorder from hospital staff and community subjects. All individuals were screened for the presence of acute infectious disease. The average age was 33.3 ± 10.6 years for normal controls and 34.5 ± 11.3 years for patients. The male to female ratios were 50:62 in normal controls and 48:52 in patients. Clinical symptoms were rated using the schedule for assessment of negative symptoms (SANS) [4] and the schedule for assessment of positive symptoms (SAPS) [3]. The sum score of negative symptoms (SUMN) was the sum of the global scores of four negative symptom dimensions, including affective blunting, alogia, avolition-apathy, and anhedonia-asociality. The sum score of positive symptoms (SUMP) was the sum of the global scores of hallucinations and delusions. The sum score of disorganizing symptoms (SUMDIS) was the sum of the global scores of Bizarre behavior and positive formal thought disorder. The patients were recruited from the outpatient clinics and their psychiatric status was stable as they received maintenance neuroleptic treatment due to the potential fluctuations in positive symptoms. Their mean positive symptom score was 1.44 ± 1.18 , mean disorganizing symptom score 1.27 ± 0.97 , and mean negative symptom score 1.80 ± 0.96 using the SAPS and SANS scales (rating range 0–5).

After signing informed consent, the study subjects took the continuous performance task (CPT) in the morning and 10 cc of blood was drawn from the antecubital vein in the morning after overnight fasting.

A CPT machine from Sunrise System, v. 2.20 (Pembroke, MA, USA), was used to assess sustained attention. The procedure has been described in detail elsewhere [7]. Briefly, numbers from 0 to 9 were randomly presented for 50 ms each, at a rate of one number per second. Each subject undertook two CPT sessions: the undegraded 1–9 task and the 25% degraded 1–9 task. Subjects were asked to respond whenever the number “9” preceded by the number “1” appeared on the screen. A total of 331 trials, 34 (10%) of which were target stimuli, were presented over five minutes for each session. During the 25% degraded session, a pattern of snow was used to toggle the background and foreground so that the image was visually distorted. Each test session began with 2 min of practice (repeated if subjects required). One signal-detection index of performance on the test, sensitivity (d'), was derived from the hit rate (probability of response to target trials) and false-alarm rate (probability of response to nontarget trials) [20]. Sensitivity is an individual's ability to discriminate target stimuli from nontarget stimuli. In a 1-week test–retest reliability study [7] of the CPT versions used in this study, the intraclass correlation coefficients or reliability of d' were 0.83 and 0.82 for the undegraded and the 25% degraded 1–9 task, respectively.

The SNP markers were selected according to the potential proteomic function alterations which include the exon and the promoter regions of the loci with average distance of 31 kb for IL-6R (IL-6R1, IL-6R2, and IL-6R3) and 6 kb for IL-6. We initially selected 3 SNPs (rs1800797, rs3087236, and rs3087236) of IL-6 and 3 SNPs (rs4845617, rs4553185, and rs4379670) of IL-6R from the NCBI SNP database (dbSNP). However, no polymorphisms were found at the originally selected SNP.ID in IL-6 and we found three novel SNPs (IL-6-1, IL-6-2, IL-6-3) in the region near the originally selected three SNPs, respectively, using direct sequencing. The chromosome position of each SNP marker on each gene was genotyped with the

primer pairs as shown in Table 1. The SNP of IL-6R2 was genotyped by Tetra-primer ARMS PCR [25], the IL-6R3 was genotyped by RFLP with Apo I enzyme, and the others were genotyped by direct sequencing. All polymerase chain reactions (PCRs) were carried out according to the protocol of the Pro Taq (Protech Technology, Taiwan) on a DNA Thermal Cycler ABI 9700. The PCR for IL-6R2 Tetra-primer was performed with an initial denaturation step at 95°C for 2 min, followed by two rounds; 16 cycles of denaturing at 95°C for 1 min, annealing at 71°C for 1 min and extension at 72°C for 1 min, and 20 cycles with initial 95°C for 1 min, annealing at 57°C for 1 min and extension at 72°C for 2 min. The PCR products of direct sequencing were purified to remove reaction buffer and remaining primers with PCR DNA Fragments Extraction Kit (Geneaid, Taiwan). The sequences of PCR products were directly determined by BigDye Terminator Cycle Sequencing kit (Applied Biosystems, CA, USA).

The Hardy–Weinberg equilibrium was assessed using the ALLELE procedure in SAS/GENETICS release 8.2 [8] for each SNP. We used Haploview software to construct haplotype blocks constituted by “strong LD” markers [12]. The genotype and allele-type association analyses were performed by using the CASECONTROL procedure in SAS/GENETICS release 8.2 with 10000 permutation resamples between the normal controls and the schizophrenic patients. The phenotype and genotype association analyses were performed by the Kruskal–Wallis non-parametric one-way analysis of variance.

Three SNPs of both IL-6 receptor (at chromosome 1q21) and IL-6 (at chromosome 7p21) were designed with specific primer pairs for genotyping and were validated in this study (Table 1). The SNPs at the IL-6 region (IL-6-1, IL-6-2 and IL-6-3) are novel compared to the IL-6R. The polymorphism of IL-6-1 is 25 bps at the 3'-end on the rs1800797 locus, IL-6-2 is 38 bps at the 3'-end on the rs3087236 and IL-6-3 is 63 bps at the 3'-end on the rs3087236.

No polymorphisms were found at the rs1800797 and the rs3087236 in these subjects.

The genotype frequency and minor allele frequency of each SNP in both control and schizophrenia groups are presented in Table 2. All SNPs were compatible with Hardy–Weinberg's equilibrium distribution, except IL-6R2 ($p = 0.022$ in control, and $p = 0.0171$ in schizophrenia). However, considering multiple testing, the SNP IL-6R2 was still compatible with Hardy–Weinberg's equilibrium. We found no significant associations of these SNP genotypes with schizophrenia.

The intermarker linkage disequilibrium analyses for haplotype block revealed a three-SNP block of IL-6 with D' of 0.81 and a two-SNP block of IL-6R with D' of 0.95 (Fig. 1). The haplotype frequencies

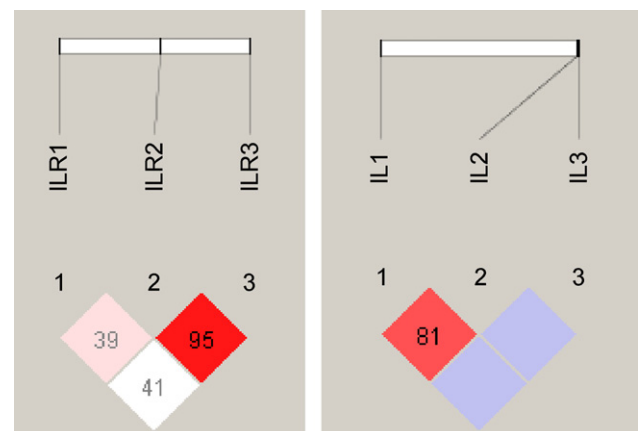


Fig. 1. Haploview linkage disequilibrium (D') displays the haplotype structures of both IL-6R and IL-6 genes. The number in each square is $D' \times 100$ between two SNPs.

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