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A promoter variant in the *chitinase 3-like 1* gene is associated with serum YKL-40 level and personality trait

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ABSTRACT

The *chitinase 3-like 1* (*CHI3L1*) gene, a cellular survival factor against several environmental and psychosocial stresses, has been sown to be more highly expressed in the hippocampus and prefrontal cortex of patients with schizophrenia than unaffected individuals. We recently reported a significant association between schizophrenia and SNP rs4950928, which is located in the promoter region of the *CHI3L1* gene, in a Japanese population. The G-allele at this SNP in the gene has been associated with higher transcriptional activity in a luciferase reporter assay and with higher mRNA levels in the peripheral blood cells of patients with schizophrenia. We investigated the impact of the *CHI3L1* polymorphism rs4950928 on serum YKL-40 levels, the protein product of *CHI3L1*. We found that individuals with the G-allele, who were more prevalent among patients with schizophrenia, had significantly higher serum YKL-40 levels ($p = 0.043$). Personality traits are considered to be an important aspect of schizophrenia primarily because they may influence symptoms and social functioning. Personality trait analyses using the temperament and character inventory (TCI) indicated that schizophrenic patients have a unique personality profile that appears to be present across cultures. We hypothesized that higher serum YKL-40 levels are associated with personality trait in patients with schizophrenia. Thus, we next examined the impact of the risk *CHI3L1* polymorphism on personality traits using the TCI. We found that individuals with the G-allele had significantly higher self-transcendence scores ($p = 0.0054$). These findings suggest possible associations between the SNP in the *CHI3L1* gene, the risk for schizophrenia, and higher serum YKL-40 levels and personality traits in a Japanese population.

Schizophrenia is a common and complex psychiatric disease. Many genes have been implicated in the pathogenesis of schizophrenia [8,9,19,30,33], and the *chitinase 3-like 1* gene (*CHI3L1*) gene has been reported to be associated with the disease [34,35]. We have recently reported a significant association between schizophrenia and a SNP rs4950928 ($p = 0.009$) located in the promoter region of the *CHI3L1* gene (the most significant $p < 0.001$) in a Japanese population using the largest sample size to date (1463 cases and 1795 controls) [25]. Elevated expression of the *CHI3L1* gene has been indicated in the schizophrenic hippocampus and prefrontal cortex in independent postmortem studies [1,5]. The G-allele of the gene at rs4950928, which was found to be more prevalent in patients with schizophrenia, has been associated with higher transcriptional activity in a luciferase reporter assay and higher mRNA levels in peripheral blood cells in patients with schizophrenia [35]. *CHI3L1* gene acts as a cellular survival factor in responses to a variety of adverse environments, including various types of physiologic stress such as inflammation, hypoxia and nutrient deprivation. These stressors may induce high expression of *CHI3L1* [15,26]. The protein product of the *CHI3L1* gene was named YKL-40 [14]. YKL-40 is a secreted protein, produced by activated macrophages and neutrophils in different tissues characterized by inflammation and increased remodeling of the extracellular matrix [16,28,32]. YKL-40 initiates phosphoinositide-3 kinase (PI-3K) signaling cascades

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in fibroblasts [27]. The PI-3K pathway, in particular the phosphorylation of protein kinase B (AKT), is strongly associated with cell survival [2], which suggests a role for YKL-40 as an anti-apoptotic protein. The genetic variants of the *CHI3L1* gene and the higher serum YKL-40 levels are associated with several inflammatory diseases, such as sarcoidosis, asthma and inflammatory bowel diseases [13,17,18,24]. It has been hypothesized that YKL-40 plays a protective role in inflammatory processes in patients with schizophrenia and is highly expressed in patients with schizophrenia. In this study, we investigated whether the G-allele has an effect on YKL-40 levels in schizophrenic patients. To achieve this goal, we measured the serum YKL-40 levels of patients with schizophrenia and control subjects.

Personality traits are considered to be an important aspect of schizophrenia primarily because they may influence symptoms and social functioning [20,21]. The temperament and character inventory (TCI) is a well-established self-report questionnaire. It measures four temperament dimensions [novelty seeking (NS), harm avoidance (HA), reward dependence (RD) and persistence (PS)] and three character dimensions [self-directedness (SD), cooperativeness (CO) and self-transcendence (ST)] [6]. Personality trait analyses using the TCI have indicated that schizophrenic patients have a unique personality profile that appears to be present across cultures [higher scores of ST and HA and lower scores of NS, RD, SD and CO in schizophrenia] [3,4,7,11,29]. We hypothesized that higher serum YKL-40 levels would be associated with personality traits in patients with schizophrenia. Thus, we secondly examined the impact of the risk *CHI3L1* polymorphism on personality traits using the TCI.

For serum YKL-40 measurements, 20 patients with schizophrenia and 19 controls were enrolled. The subjects for personality trait analysis consisted of 99 patients with schizophrenia and 179 controls. All controls and 18 of 20 patients with schizophrenia enrolled for serum YKL-40 measurements were also enrolled for personality trait analysis. Cases were recruited at Osaka University hospitals. Each schizophrenic research subject had been diagnosed and assessed by at least two trained psychiatrists according to the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria based on unstructured clinical interview. Symptoms of schizophrenia were assessed using the positive and negative syndrome scale (PANSS). Three of 20 patients with schizophrenia enrolled for serum YKL-40 measurements were not treated with anti psychotic drugs and 7 of 99 patients with schizophrenia enrolled for personality trait analysis were not treated with anti psychotic drugs. Cases of schizophrenia with the comorbidities of substance-related disorders or mental retardation were excluded. Controls were recruited through local advertisements. Psychiatrically, medically and neurologically healthy controls were evaluated using the DSM-IV structured clinical interview, non-patient version. Subjects were excluded if they had neurological or medical conditions that could potentially affect the central nervous system, such as atypical headache, head trauma with loss of consciousness, chronic lung disease, kidney disease, chronic hepatic disease, thyroid disease, active stage cancer, cerebrovascular disease, epilepsy or seizures. Written informed consent was obtained for all subjects after the procedures had been fully explained. This study was carried out in accordance with the World Medical Association's Declaration of Helsinki and approved by the Research Ethical Committee of Osaka University.

Serum YKL-40 was measured using an enzyme-linked immunosorbent assay kit (Metra YKL-40, Quidel Corporation, San Diego, CA, USA), in accordance with the manufacturer's instructions. All samples were run in duplicate and mean values were used for analysis. The average intra-assay coefficient of variation determined by triplicate of 10 CSF samples was 3.2%. To examine reproducibility, four CSF samples were analyzed in each of two experiments. After normalization, the average inter-assay coefficient of variation was 5.6%.

The TCI is administered through a self-report questionnaire based on 240 items requiring a true or false item response [6]. We only examined the main scores of the four temperaments (HA, NS, RD and PS) and three characters (SD, CO and ST) dimensions of the scale. The concepts of each dimension are as follows: NS is the activation of behavior in response to novelty and signals of reward or relief of punishment; HA is the inhibition of behavior in response to signals of punishment or non-reward; RD is the maintenance of behavior that was previously rewarded; PS is the perseveration with behavior despite frustration and fatigue; SD is the concept of the self as an autonomous individual; CO is the concept of the self as an integral part of humanity or society; and ST is the concept of the self as an integral part of the universe and its source [6].

Venous blood was collected from the subjects and genomic DNA was extracted from whole blood according to standard procedures. The timing of blood collection was not consistent among the samples. Genotyping of the SNP was carried out via TaqMan assays (Applied Biosystems, Foster City, CA, USA) as previously described [10,23]. The TaqMan probe and Universal PCR Master Mix were obtained from Applied Biosystems. The TaqMan probe ID for the SNP rs4950928 was C__27832042_10. Allelic-specific fluorescence was measured using an ABI PRISM 7900 Sequence Detector System (Applied Biosystems).

Statistical analyses were performed using SNPAlyze V5.1.1 Pro software (DYNACOM, Yokohama, Japan) and SPSS 16.0J software (SPSS Japan Inc., Tokyo, Japan). Differences in clinical characteristics between patients and controls or between genotype groups were analyzed using χ^2 tests for categorical variables and the Mann–Whitney *U*-test for continuous variables. Deviation from Hardy–Weinberg equilibrium (HWE) was tested separately in cases and controls. The analysis revealed age and gender differences in some dimensions. Therefore, the effect of the *CHI3L1* genotype and the effect of diagnosis on the serum YKL-40 levels were analyzed by a two-way analysis of covariance (ANCOVA), with age and gender as covariates. In previous personality traits analyses using the TCI, it has been suggested that possible confounding factors affect personality traits [22,31]. The number of years of education was lower in patients with schizophrenia than in healthy controls in a Japanese population [11]. Therefore, with age, gender and education years as covariates, the effect of *CHI3L1* genotype and the effect of diagnosis on personality traits were analyzed by a two-way ANCOVA. The significant level for statistical tests of genetic and personality association was set at $p < 0.05$.

We examined possible associations between the *CHI3L1* genotype at rs4950928 and serum YKL-40 levels in patients with schizophrenia and controls, because this variant was indicated to have a significant association with schizophrenia in a previous study. Supplementary Table 1 shows the characteristics of the subjects and the distribution of genotypes. There was no difference in demographic variables, age, gender, years of education, chlorpromazine equivalents of total antipsychotics (CPZeq) and positive and negative symptom scale (PANSS) scores between *CHI3L1* genotype groups. Given that there was only one CC homozygous individual in subjects for serum YKL-40 analysis, we removed the data of the subject with CC genotype and compared GG genotype with GC genotype. The effects of *CHI3L1* genotype and diagnosis on serum YKL-40 levels were shown in Table 1. Two-way ANCOVA revealed significant effects of genotype ($F = 4.46$, $p = 0.043$, $\eta^2 = 0.122$). No effect of diagnosis ($p > 0.70$) or genotype–diagnosis interaction was found ($p > 0.80$). Individuals homozygous for the G-allele, which was more common in the patient group, showed higher serum YKL-40 levels than the C-carriers (Fig. 1). There was no genotype effect when we separately analyzed the effect of genotype on YKL-40 in patients ($p > 0.30$) and controls ($p > 0.10$).