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Pharmacogenomic Study of Clozapine-Induced Agranulocytosis/Granulocytopenia in a Japanese Population

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ABSTRACT

BACKGROUND: Clozapine-induced agranulocytosis (CIA)/clozapine-induced granulocytopenia (CIG) (CIAG) is a life-threatening event for schizophrenic subjects treated with clozapine.

METHODS: To examine the genetic factor for CIAG, a genome-wide pharmacogenomic analysis was conducted using 50 subjects with CIAG and 2905 control subjects.

RESULTS: We identified a significant association in the human leukocyte antigen (HLA) region (rs1800625, $p = 3.46 \times 10^{-9}$, odds ratio [OR] = 3.8); therefore, subsequent HLA typing was performed. We detected a significant association of HLA-B*59:01 with CIAG ($p = 3.81 \times 10^{-8}$, OR = 10.7) and confirmed this association by comparing with an independent clozapine-tolerant control group ($n = 380$, $p = 2.97 \times 10^{-5}$, OR = 6.3). As we observed that the OR of CIA (OR: 9.3∼15.8) was approximately double that in CIG (OR: 4.4∼7.4), we hypothesized that the CIG subjects were a mixed population of those who potentially would develop CIA and those who would not develop CIA (non-CIA). This hypothesis allowed the proportion of the CIG who were non-CIA to be calculated, enabling us to estimate the positive predictive value of the nonrisk allele on non-CIA in CIG subjects. Assuming this model, we estimated that 1) ∼50% of CIG subjects would be non-CIA; and 2) ∼60% of the CIG subjects without the risk allele would be non-CIA and therefore not expected to develop CIA.

CONCLUSIONS: Our results suggest that HLA-B*59:01 is a risk factor for CIAG in the Japanese population. Furthermore, if our model is true, the results suggest that rechallenging certain CIG subjects with clozapine may not be always contraindicated.

Keywords: Genome-wide association study, Human leukocyte antigen, Pharmacogenomics, Schizophrenia, Side effect, Single nucleotide polymorphism

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Schizophrenia is a chronic, serious, and disabling mental disorder with a lifetime prevalence of approximately 1% of the world population (1). Antipsychotics are the most useful therapeutic option for schizophrenia; however, approximately one third of patients do not respond adequately to first-line antipsychotics, resulting in treatment-resistant schizophrenia (TRS) (2,3).

Clozapine (CLZ) is a gold standard drug for managing TRS, with a lower incidence of movement abnormality and efficacy superior to that of other antipsychotics in the management of TRS (2-5). Despite its efficacy with TRS, the use of CLZ is significantly restricted by severe side effects such as CLZ-induced agranulocytosis (CIA)/CLZ-induced granulocytopenia (CIG) (CIAG), which is rare but potentially life threatening. CIAG events are observed in approximately 1% (for CIA) and 3% (for CIG) of CLZ-treatment patients, although the prevalence of CIA differs between populations (Caucasian: .3%∼.9%; Asian: greater than 1%; Japanese: 1.1%) (6-11).

In the clinical setting, patients treated with CLZ require frequent blood monitoring for reduced neutrophil count, an indication of CIG and CIA. One of the main reasons for this is that, in general, CIG and CIA are considered to be a continuous phenotype; psychiatrists expect to control the development of CIA by detecting patients at the less serious CIG stage (12).

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However, as psychiatrists recognize based on experience, and as Hummer *et al.* (13) reported, some patients with CIG may not go on to experience a serious outcome such as immediate development of CIA (i.e., benign or transient neutropenia) (12,13). For example, a routine blood examination showing an absolute neutrophil count (ANC) of 1400 cells/mm^3 may result in discontinuation of CLZ (as this is below the Japanese cutoff level of 1500 cells/mm^3 at which discontinuing CLZ treatment is mandatory), but the ANC may then recover quickly to an acceptable value. This may be because an ANC of around 1500 cells/mm^3 can fluctuate; for example, it may increase with exercise (14,15) or decrease with viral infection (16).

In such clinical situations, a perfect genetic test to screen for the risk of CIAG before treatment by CLZ is not always mandatory. As described, frequent blood monitoring can be used to detect CIA before CIG develops. Also, CLZ is one of the last treatment options for TRS; the psychiatrist may have little choice other than CLZ treatment. In this regard, a screening test may be useful for reducing the frequency of blood monitoring, thus relieving the burden on quality of life and reducing cost. More importantly, however, psychiatrists need more information on whether or not rechallenging is possible or need a screening test to select CIAG patients who can be treated again with CLZ as a rechallenge.

To date, several pharmacogenetic/pharmacogenomic (PGx) studies have been carried out, mainly in Caucasian populations; however, there is no report to clarify the difference between CIA and CIG. For example, classical candidate gene-based pharmacogenetic studies suggested that a specific sequence variant in human leukocyte antigen (HLA) (HLA-DQB1, 6672G > C) was associated with CIA, but the significance was modest ($p \sim .001$) (17,18). A recent PGx study using a genome-wide association approach and Exome sequencing analysis reported a large impact on CIAG genetics: it showed that CIAG was associated with two independent amino acid changes in HLA-B (158T: $p = 6.4 \times 10^{-10}$) and HLA-DQB1 (126Q: $p = 4.7 \times 10^{-14}$) (19). It is of note that the p values surpassed the genome-wide significance threshold (5×10^{-8}), although these associations were detected by imputation of nonsynonymous single nucleotide polymorphisms (SNPs) of HLA genes and not by the genotyping of classical HLA alleles.

In this study, we aimed to explore the genetic risk for CIAG in the Japanese population by a genome-wide SNP survey. Although the sample size of CIAG subjects was small, we detected significant SNPs in the HLA region compared with healthy control subjects drawn from the general population. To detect the responsible risk allele, we conducted classical HLA typing for the CIAG subjects and for CLZ-tolerant control subjects, which showed that a specific allele in HLA-B was significantly associated with CIAG. We investigated the clinical utility of this risk allele and whether it could increase posterior probability in the screening test for CIAG or could provide information regarding the possibility of CLZ rechallenging for CIG subjects.

METHODS AND MATERIALS

Ethical Statement

After providing a complete description of the study to the subjects, written informed consent was obtained. The ethics committees of each university, institute, and hospital participating in this project approved this study.

Participants

CIAG Subjects. Fifty-two patients with CIAG were included (28 male and 24 female patients, age 44.0 ± 15.6 years old). Of these, 23 were diagnosed with CIA, defined as a decrease in ANC to less than 500 cells/mm^3, and 29 were diagnosed with CIG, defined as an ANC between 500 cells/mm^3 and 1500 cells/mm^3 or for three of these subjects as leukopenia (white blood cell count less than 3000 cells/mm^3). These criteria were followed by the Clozaril Patient Monitoring Service in Japan and many other countries, which can reflect a real-world clinical setting. All subjects experienced CIAG within 180 days of first being prescribed CLZ, in accordance with the definition of CIAG in a previous paper (20). All of the subjects were diagnosed as TRS, and all identified themselves as Japanese.

Healthy Comparison Subjects. A total of 2948 subjects (1120 male and 1828 female subjects; age 37.0 ± 15.2 years old) were genotyped as healthy control subjects; they had no personal history of mental disorders and were Japanese descent by self-report. Among them, 1108 subjects were nurses at Fujita Health University Hospital (21), while the others were recruited from the general population.

CLZ-Tolerant Control Subjects. For the classical HLA association analysis, we recruited 380 CLZ-tolerant individuals (210 male and 170 female individuals, age 42.7 ± 11.8 years old) who had been treated with CLZ for more than 180 days without suffering from CIAG. Again, all of the subjects were diagnosed as having TRS and self-reported as Japanese.

Genotyping and Quality Control

In the PGx analysis, we genotyped the CIAG cases and the healthy comparison subjects using the Illumina HumanOmniExpressExome v1.0 (for 825 healthy comparison subjects) or v1.2 (for the rest of the subjects) (Illumina, San Diego, California).

The following quality control (QC) procedure was applied: 1) we extracted overlapping SNPs between the v1.0 and v1.2 chips; 2) we ensured gender consistency by investigating the SNPs on chromosome X (one CIAG subject); 3) we removed the subjects with a low call rate < .99 (null subjects); and 4) we removed subjects with two degrees or less of relatedness using an identity-by-state analysis (22 healthy comparison subjects). After this first QC filtering, 51 CIAG and 2926 healthy comparison subjects were still eligible, with 643,234 SNPs with a minor allele frequency of more than 1%. To investigate the population structures as following QC, we carried out principal component analysis (22). Using HapMap datasets for four populations (Japanese in Tokyo; Han Chinese in Beijing; Yoruba in Ibadan, Nigeria; and Utah residents with ancestry from northern and western Europe), we confirmed that samples in the East Asian population were grouped in one cluster (Supplemental Figure S1). Then, using only the East Asian HapMap populations (Japanese in Tokyo and Han Chinese in Beijing), we classified the population clusters,