HLA Associations in Clozapine-Induced Agranulocytosis

By Juan J. Yunis, Deyanira Corzo, Marcela Salazar, Jeffrey A. Lieberman, Alfreda Howard, and Edmond J. Yunis

We previously reported preliminary results of association of clozapine-induced agranulocytosis (CA) with HLA-B38, DR4, DQ3 in five Ashkenazi Jewish patients and with HLA-DR2, DQ1 in four non-Jewish patients. In the present study, 31 additional patients with CA, 10 Ashkenazi Jewish, and 21 of non-Jewish ancestry, were studied. HLA alleles and haplotypes were compared among 52 patients (33 Ashkenazi Jewish, 19 non-Jewish) matched for ethnic background and clinical status. Our results show two associations and define the HLA allele markers for the Ashkenazi Jewish and non-Jewish haplotypes associated with CA. The most important markers for susceptibility for CA in Ashkenazi Jewish patients were DRB1*0402, DQB1*0302, and DQA1*0301, and in non-Jewish patients, HLA-DR*02, DQB1*0502, and DQA1*0102. HLA-DRB1*011 and DQB1*0301 were underrepresented in Ashkenazi Jewish patients when compared with controls. We hypothesize that genes of the major histocompatibility complex, other than class I and class II, are responsible for CA; among them are the variants of the heat-shock proteins 70 or the tumor necrosis factor loci.

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THE ATYPICAL antipsychotic clozapine (clozaril or 8-chloro-11-[4-methyl-1-piperazinyl]-3H-dibenzo-[b,e] [1,4]diazepine) has been shown to have antipsychotic efficacy in 30% to 50% of schizophrenic patients who are refractory to standard pharmacotherapy without the extrapyramidal side effect characteristic of all classical antipsychotic drugs. However, its broader use in the treatment of psychiatric disorders has been limited by clozapine's capacity to produce agranulocytosis in about 1% of patients. It is not clear at present whether the cytotoxic effect is immune mediated or caused by toxicity of the drug or its metabolites.

We have previously reported an association between the haplotype HLA-B38, DR4, DQ3 and CA in five patients with Ashkenazi Jewish ancestry, and with HLA-DR2, DQ2 in four non-Jewish patients. In contrast, although it was reported that a native American who developed agranulocytosis from clozapine treatment carried the B38, DR4 haplotype, others reported no association between agranulocytosis and specific HLA markers. The selective vulnerability of patients to agranulocytosis and its association with HLA haplotypes suggested a genetic basis for this hematologic reaction to clozapine.

We have confirmed the association of CA with HLA-B38, DR4, DQ3 in Ashkenazi Jewish patients and with HLA-DR2, DQ1 in non-Jewish patients. In addition, we characterized molecularly the HLA alleles associated in both groups.

MATERIALS AND METHODS

Patients. An arrangement was established with the Sandoz Clozaril Monitoring System to notify us of the development of agranulocytosis cases nationwide and to identify the attending physician. Patient identities were not disclosed because of confidentiality restrictions. Clinical information on the case was reviewed to determine if the patient met the study's criteria for agranulocytosis, ie, an absolute neutrophil count less than 500/mm³ in the course of the clozapine treatment. For patients who met these criteria, a letter was sent to the treating doctor explaining the purpose and nature of the study and requesting assistance in facilitating access to the patient to request a blood sample. If the patient agreed, additional information was obtained about the natural history of their agranulocytosis, including indication for clozapine, duration of treatment with clozapine, dose, concomitant medication, and past medical history.

For patients who were in the Hillside Hospital system of the New York metropolitan area, an appointment was scheduled for patients to go in for evaluation and blood collection. Patients who lived in other states were grouped by geographic proximity and a team of investigators (J.L., A.H., R.F.) made trips to the patients' locations for blood collections.

Ashkenazi Jewish or non-Jewish ethnicity was determined by historical evidence of the ancestry of the patient's four grandparents. All patients included in this study were white and of European ancestry. Patients were not related and ascertainment of ethnicity was such that the sample of those with or without agranulocytosis were not from an inbred community.

Serologic and DNA typing procedures. For HLA serologic typings, using a sterile technique, 50 mL of whole blood was withdrawn into tubes containing acid citrate dextrose. Tubes were then wrapped in multiple layers of insulating material, placed in styrofoam containers, and maintained at room temperature with heat packs added in cold weather and cold packs in hot weather. Containers were shipped using next-day air delivery service to assure arrival before noon at the Immunogenetics Laboratory of the Dana-Farber Cancer Institute. All specimens arrived within 24 hours of being drawn. Only one specimen arrived in an unusable condition because of reaching an excessively high temperature. Peripheral blood leukocytes were isolated by differential centrifugation in a Ficoll-Hypaque (Pharmacia, Piscataway, NJ) density gradient. Cells were frozen in RPMI 1640 containing 50% heat-inactivated fetal calf serum and 10% dimethylsulfoxide until further use. HLA-A, -B, -C, -DR, and -DQ generic and alleles were typed using the microcytotoxicity assay.

For allele typings, DNA was isolated with a DNA isolation kit (Stratagene, La Jolla, CA) or by the salting-out method. DNA samples were amplified by polymerase chain reaction (PCR) for DRB generic, DQ1, and DQB1 loci in 100 μL reaction mixture containing 50 pmol of each primer, 50 mmol/L KCl, 10 mmol/L...
TRIS-HCl (pH 8.3), deoxynucleotide triphosphates (200 μmol/L each), 2.5 U of AmpliTaq DNA polymerase (Perkin Elmer-Cetus, Emerville, CA) and 1.5 to 2 mMol/L MgCl₂. The primers and conditions used in this study have been published elsewhere. In addition, allele-specific amplifications for DRB1*04, DRB1*15/16, and DRB1*08 were performed when required. Several negative controls (no DNA) were always included to detect any contamination. All reactions were performed in a thermal cycler (model 9600; Perkin Elmer-Cetus, Norwalk, CT). Amplification was confirmed by electrophoretic analysis of 5 μL of PCR-amplified product in a 2% agarose gel on TBE buffer containing 0.5 μg/mL ethidium bromide. Resulting patterns were analyzed by comparing band migration to size markers and recording those that were present in each digest.

Allele assignments. The DQA1 and DQB1 alleles were determined in the locus-specific PCR-amplified products by sequence-specific oligonucleotide (SSO) probe hybridization as described elsewhere. For DRB and allele-specific typing (DRB1*15/16 and DRB1*08), a panel of 36 SSO probes were used. The majority of these SSO probes have been described in the reference protocol of the 11th International Histocompatibility Workshop and are listed in Table 1. The sequence, orientation, codons, allele specificity, and melting profile of those SSO probes, used in this study but not described on the 11th International Histocompatibility Workshop, are also given in Table 1. For DRB1*04 allele typing, a PCR-RFLP method was used. Instead of using the enzyme MnlI, we hybridized with a sequence-specific oligonucleotide probe to differentiate alleles DRB1*0401/0402, DRB1*0405/0409, and DRB1*0404/0413. For HLA-DP assignments we used the patterns described.

Data analysis. HLA generic type, allele, and haplotype frequencies were determined by direct counting of alleles or haplotypes from unrelated individuals and, in some cases, by segregation analysis of families. An unrelated individual was presumed homozygous when typing results showed the presence of only one allele, or by segregation analysis in families. In the majority of the individuals of non-Jewish ethnic background and the majority of individuals that were treated with clozapine and did not develop agranulocytosis, the family studies were not sufficient to assign segregation of HLA-A, C, B, or HLA-DPB1 and, therefore, generic types of alleles of those loci were listed as phenotypes.

Statistical significance of the differences in frequency of individual major histocompatibility complex (MHC) generic types, alleles, or haplotypes in patients with agranulocytosis and patients without agranulocytosis after treatment with clozapine was estimated by chi-square analyses or Fisher’s exact test, as appropriate, with the aid of the Instat software (Graphpad, San Diego, CA).

RESULTS

Samples were obtained from 31 patients who had agranulocytosis. Five patients were ascertained at the Long Island Jewish Medical Center (LIJMC), six patients from outside our institution but within the New York metropolitan area and 19 patients within the United States, but outside the metropolitan area. Fifty-two patients treated with clozapine for at least 1 year (past the period of peak risk), who did not develop agranulocytosis were ascertained from LIJMC as a control group.

The agranulocytosis sample had a mean age of 41.6 years (SD, 12.7 years); 52.5% were males and 47.5% were females. Five patients were ascertained at the Long Island Jewish Medical Center (LIJMC), six patients from outside our institution but within the New York metropolitan area and 19 patients within the United States, but outside the metropolitan area. Fifty-two patients treated with clozapine for at least 1 year (past the period of peak risk), who did not develop agranulocytosis were ascertained from LIJMC as a control group.

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The presence of the DFU31*0402, DRB4*0101, DQBI*0501 haplotype is displayed. It is clear that 9 of 10 patients carry that haplotype, confirming our previous results. Also listed are four (nos. 11 to 14) patients reported before as DR4, DQ3 that have been confirmed by DNA typing. It is also clear from the results that the haplotype

Table 1. DRB SSO Probes Used to Characterize DRB* Alleles

<table>
<thead>
<tr>
<th>SSO Probe</th>
<th>Sequence 5′-3′</th>
<th>Tm</th>
<th>Codon</th>
<th>Allele Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DR1200</td>
<td>GAGGAGCTCTCCTGCCCCT</td>
<td>56</td>
<td>34-GEELL</td>
<td>DRB1*1201/1202</td>
</tr>
<tr>
<td>DRB55I</td>
<td>CGGCTCACCGCCGAGTAC</td>
<td>62</td>
<td>55-RPSAEY</td>
<td>DRB1*0405/0409/0412, 1303, 1304, 0801, 0803, 0805, 0806</td>
</tr>
<tr>
<td>DRBE14I</td>
<td>GATGCTCAATCTTCTGAAT</td>
<td>48</td>
<td>14-ECOFFN</td>
<td>DRB1*1405</td>
</tr>
<tr>
<td>DRBE69I</td>
<td>GAAGACAGCCGCCGCTCCTG</td>
<td>62</td>
<td>69-EDRRA1</td>
<td>DRB1*0412, 1403, 0801-0804, 0806</td>
</tr>
<tr>
<td>DRB1303</td>
<td>ACATCTCGGAGACAGACGGC</td>
<td>54</td>
<td>68-DILEDK</td>
<td>DRB1*1303</td>
</tr>
<tr>
<td>DRB1305</td>
<td>ACTCTCTAGCAGACAGGCGG</td>
<td>56</td>
<td>68-DFTEDR</td>
<td>DRB1<em>0415, 1305, 1101, 1104-1105, 1202, 1601, 0801, 0802, 0804-0806, DRB5</em>0101, 0102</td>
</tr>
<tr>
<td>DRB0302</td>
<td>TCCCTGGAGAGATACTCC</td>
<td>54</td>
<td>28-FLERYF</td>
<td>DRB1<em>0302, 0303, 1402, 1403, 1406, DRB3</em>0301</td>
</tr>
<tr>
<td>DRB1402</td>
<td>ACCTCTGGAGACAGGCGCGG</td>
<td>60</td>
<td>68-DLEGR</td>
<td>DRB1<em>04 (except 0401, 0402, 0409, 0412), DRB1</em>0101, 0102, 1402, 1406, 1409</td>
</tr>
<tr>
<td>DRB-91</td>
<td>TGGAAACGGACGACAGAAGGC</td>
<td>56</td>
<td>61-WNSQKD</td>
<td>DRB1, DRB3, DRB4, DRB5</td>
</tr>
</tbody>
</table>

11th International Workshop SSO Probes: DRB1001, 1006, 1012, 1208, 1207, 1200, 1202, 12007, 12009, 123712, 123717, 123704, 123708, 123703, 123703, 123701, 123602, 123601, 123605, 123601, 123603.

Abbreviation: Tm, melting temperature. 
† Tm based on 4°C for each G and C, and 2°C for each A and T. 
‡ S.Y. Yang, Memorial Sloan-Kettering Cancer Institute, personal communication, July 1992.
DRB1*0402, DRB4*0101, DQB1*0302, DQA1*0301 was present in patients who were HLA-B38. Table 3 shows the HLA haplotypes in non-Jewish patients with agranulocytosis, displayed for examination of the presence of haplotype HLA-DR2, DQ1, which has been defined molecularly as HLA-DRB1*1601, DRB5*02, DQB1*0502, DQA1*0102. It is of interest that 13 of 21 patients had the haplotype DR2, DQ1, but the HLA-B allele present in patients with the haplotype HLA-DRB1*1601, DRB1*0101, DQB1*0502, DQA1*0102 was not HLA-B7, a specificity generally associated with HLA-DRB1*1501.

Frequencies of certain HLA generic types, alleles, and haplotypes in clozapine-induced agranulocytosis. Tables 4 and 5 show the comparison of the frequency of HLA generic types, alleles, or haplotypes in patients with CA compared with the corresponding frequencies in patients treated with clozapine who did not develop agranulocytosis.

In Ashkenazi Jewish agranulocytosis patients, the frequency of HLA-B38, DR4, or DQ3 was 90% compared with the respective generic types of 21.8%, 35.7%, and 71.8% in controls. There was a statistically significant association between agranulocytosis and HLA-B38 (P < .0001), DR4 (P = .009) and no significant association of HLA-DQ3 in the Ashkenazi Jewish sample. The haplotype HLA-B38, DR4, DQ3 had a frequency of 90% compared with 18.7% in the controls (P < .0001), with an odds ratio of 48 (95% confidence interval [CI], 4.1 to 369.7). High-resolution typing of class II alleles showed that the frequency of HLA-DRB1*0402 (43.7%), DQB1*0302 (43.7%), and DQA1*0301 (75%) was increased when compared with the frequency of these alleles in the control group (11.1%, 34%, 14.8%, and 24% respectively). There was a statistically significant association between agranulocytosis and HLA-DRB1*0402 (P = .007), DQB1*0302 (P = .03), DQA1*
Table 4. HLA Associations in Ashkenazi Jewish Patients With Clozapine-induced Agranulocytosis

<table>
<thead>
<tr>
<th>Allele or Haplotype</th>
<th>New Cases</th>
<th>All Cases Included</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenotype Frequency</td>
<td>Gene Frequency</td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>HLA-B38*</td>
<td>9:10</td>
<td>7:32</td>
</tr>
<tr>
<td>HLA-DR4*</td>
<td>9:10</td>
<td>12:32</td>
</tr>
<tr>
<td>HLA-DRw3*</td>
<td>9:10</td>
<td>23:32</td>
</tr>
<tr>
<td>[HLA-B38, DR4, DQw3]</td>
<td>9:10</td>
<td>6:32</td>
</tr>
<tr>
<td>HLA-DRB1*0402</td>
<td>7:16</td>
<td>6:54</td>
</tr>
<tr>
<td>HLA-DRB4*0101</td>
<td>9:16</td>
<td>18:54</td>
</tr>
<tr>
<td>HLA-DQB1*0301</td>
<td>12:16</td>
<td>13:54</td>
</tr>
<tr>
<td>HLA-DQA1*0301</td>
<td>0:16</td>
<td>18:54</td>
</tr>
<tr>
<td>HLA-DRB1*11</td>
<td>0:16</td>
<td>13:54</td>
</tr>
<tr>
<td>[DRB1<em>0402, DRB4</em>0101, DQB1*0301]</td>
<td>7:16</td>
<td>6:54</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

† 10 new Ashkenazi cases, 8 DNA typed.
‡ 33 Ashkenazi controls, 27 DNA typed.
§ 15 total Ashkenazi cases, 12 DNA typed.
∥ Allele was not assigned in one Ashkenazi control.

The frequency of HLA-B38, DR4, DQw3 was increased when compared with that of controls (5.2% and 26.3%, respectively). There was a statistically significant association between agranulocytosis and HLA-B7 (P = .04) and DR2 (P = .03). Haplotypes HLA-B7, DR2, DQ1 (23.8%) and DR2, DQ1 (61.9%) were increased when compared with the frequency of 6.2% and 21% in the controls (P = .05 and .01, respectively). High-resolution typing of class I alleles 0301 (P = .0006). The haplotype HLA-DRB1*0402, DRB4*0101, DQB1*0302, DQA1*0301 had a frequency of 43.7% patients with agranulocytosis compared with the frequency in controls of 11.1% (P = .007), with an odds ratio of 6.2 (95% CI, 1.7 to 22.9). It is of interest that the allele DQB1*0301 was absent in patients and found in a frequency of 33.3% in controls (P = .007). HLA-DR11 was also absent in patients and found in 24% of controls (P = .03).

Table 5. HLA Associations in Non-Jewish Patients With Clozapine-induced Agranulocytosis

<table>
<thead>
<tr>
<th>Allele or Haplotype</th>
<th>New Cases</th>
<th>All Cases Included</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenotype Frequency</td>
<td>Gene Frequency</td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>HLA-B7*</td>
<td>7:20</td>
<td>1:19</td>
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<tr>
<td>HLA-DR2</td>
<td>13:21</td>
<td>5:19</td>
</tr>
<tr>
<td>HLA-DQw1</td>
<td>13:21</td>
<td>14:19</td>
</tr>
<tr>
<td>[HLA-DR2, DQw1]</td>
<td>13:21</td>
<td>4:19</td>
</tr>
<tr>
<td>[HLA-B7, DR2, DQw1]</td>
<td>5:21</td>
<td>0:19</td>
</tr>
<tr>
<td>HLA-DRB1*1601</td>
<td>10:42</td>
<td>2:32</td>
</tr>
<tr>
<td>HLA-DRB5*02</td>
<td>10:42</td>
<td>2:32</td>
</tr>
<tr>
<td>HLA-DQB1*0502</td>
<td>10:42</td>
<td>1:32</td>
</tr>
<tr>
<td>HLA-DQA1*0102</td>
<td>15:42</td>
<td>3:32</td>
</tr>
<tr>
<td>[DRB1<em>1601, DRB5</em>02, DQB1<em>0502, DQA1</em>0102]</td>
<td>10:42</td>
<td>0:32</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

† 21 new non-Jewish cases, all DNA typed.
‡ 19 non-Jewish controls, 16 DNA typed.
§ 25 total non-Jewish cases, 21 DNA typed.
∥ Allele was not assigned in one non-Jewish case.
shown that the alleles DQB1*0502 (23.8%) and DQA1*0102 (23.8%) but not DRB1*1601 (23.8%) or DRB5*02 (23.8%) were more frequent when compared with their frequencies in controls: 3.1% and 9.3%, respectively. There was a statistically significant association between agranulocytosis and DQB1*0502 (P = .02) or DQA1*0102 (P = .01). Comparison of the frequency of the haplotype HLA-DRB1*1601, DRB5*02, DQB1*0502, DQA1*0301 (23.8%) was higher in patients versus controls (P < .004) with an odds ratio of 21 (95% CI, 1.2 to 373.8). HLA-DPB1 alleles were not different in patients with and without agranulocytosis.

**DISCUSSION**

Phenotypes of the HLA system have been associated with drug-induced adverse reactions in previous reports. For example, HLA-DR4 was found overrepresented in hydralazine-induced lupus patients, and the haplotype HLA-B8, DR3 was found increased in patients who developed toxicities to treatment with gold and D-penicillamine. Susceptibility to develop antinuclear antibodies, lupus anticoagulant, and elevated levels of IgM may also be associated with potential development of antibodies against central nervous system tissue. Although this relationship has not been established directly, it was reported that the presence of autoantibodies in association with HLA-B44 was a predictor of neurologic complications of long-term chlorpromazine therapy.

In preliminary studies, we reported that the haplotype HLA-B38, DR4, DQw3 was more frequent in Ashkenazi Jewish patients with CA. This result was partially registered in a native American patient. We have taken special care to classify the patients with strict criteria for diagnosis of agranulocytosis and to avoid inclusion of patients who may have had episodes of leukopenia. Although we had not performed family studies in our previous reports, we interpreted the results to indicate that the HLA haplotype, and not the individual generic types, were markers for risk of agranulocytosis in Ashkenazi Jewish patients. Our rationale was that the HLA-B38, DR4, DQw3 haplotype occurs characteristically in Jewish populations from the United States and Israel at a rate of 10% and 12%, and at ~0.4% to 0.8% in the general population.

We have now confirmed our prior studies by examining a new group of agranulocytosis patients and a control group without the complication. Furthermore, we have performed high-resolution studies of the alleles of the class II MHC, including family studies, and have identified the haplotype HLA-B38, DRB1* 0402, DRB4*0101, DQB1*0302, DQA1*0301 as the marker for CA in Ashkenazi Jewish patients. The alleles HLA-DR 11 and DQB1 *0301 are markers for protection in Ashkenazi Jewish patients.

The cumulative US incidence of agranulocytosis in patients treated with clozapine is 0.8% at 1 year of treatment. Because information on the ethnic or racial background of patients was not available from the Clozaril Monitoring System, we do not know the overall number of clozapine-treated Jewish patients in the United States, nor the number who may have developed CA. The rate in Jewish patients treated with clozapine is ~20% at our institution.

We have previously reported the presence of the haplotype HLA-DR2, DQ1 in four non-Jewish patients with CA, but others reported lack of association of HLA with the complication in patients of European ancestry. Here, we have confirmed the association of CA with HLA-DR2, DQ1 and have extended the work and defined the involvement of CA to the haplotype HLA-DRB*1601, DRB5*02, DQB1*0502, DQA1*0301 in non-Jewish patients. It is important to mention that CA in non-Jewish patients has the highest association with the DQA1*0102 and DQB1*0502, suggesting that genes of DQ and not DR are important. Others did not include high-resolution typing of DQ (definition of alleles) explaining their negative results.

Relevant to our findings is the fact that HLA alleles or haplotypes are associated with several autoimmune diseases and that these markers may vary in different ethnic groups. For example, rheumatoid arthritis is associated with HLA-DR4 in several ethnic groups, but with HLA-DR1 in the Ashkenazi Jewish group; multiple sclerosis is associated with DR2 in whites, but with DR6 in Japanese; pemphigus vulgaris is associated with DR4 in Ashkenazi Jewish patients and with DR6 in non-Jewish patients. The haplotype marker for pemphigus vulgaris in Ashkenazi Jewish patients is the same found in CA in our study. In non-Jewish patients, the HLA association found was with either the DRB1*0402, DQB1*0302 haplotype or the DRB1*1401, DQB1*0503 haplotype in non-Jewish patients, however, despite the fact that it has been suggested that immune mechanisms mediate CA, direct evidence for HLA involvement in immune reactions against clozapine has not been found.

It is not clear why different haplotypes are associated with CA in Ashkenazi Jewish and non-Jewish patients. Possibly there is heterogeneity in the mechanism that mediates the disorder that may differ in Jewish patients and patients of other ethnic groups. Alternatively, it is possible that the abnormal gene product that mediates agranulocytosis is located in the HLA-B, DR, DQ region, and would be common in patients of several ethnic groups.

The observation that several HLA alleles in two different haplotypes, one in Ashkenazi Jewish patients and one in non-Jewish patients, are associated with CA is in keeping with the concept that susceptibility genes are non-HLA genes within the MHC and are in nonrandom association with HLA alleles. Therefore, HLA associations with CA found by us suggest that any marker within these haplotypes or other selected candidate genes within the MHC could be involved in its pathogenesis because of linkage disequilibrium. There are cases of agranulocytosis with different HLA types (false-negative cases) and control patients with the HLA types associated with agranulocytosis (false-positive cases) favoring such a hypothesis. We believe that genes of the MHC other than class I and class II might be responsible for CA. Such genes could be heat-shock protein 70-2 (HSP70-2) or tumor necrosis factor (TNF), which are in linkage disequilibrium with HLA class II alleles. This disequilibrium is caused by the location of HSP-70 and TNF genes in the midportion of the MHC between the class I and class II regions: TNF at 250 kb from HLA-B and 850 kb from DRA (border locus of class II), and HSP-70 at 220 kb from TNF and 630 kb.
from DRA.\textsuperscript{35} We have found in a panel of homozygous typing cells that the 9.0-kb genetic variant of HSP70-2 is nonrandomly associated with HLA-DR4 (including DRB1*0402, DQB1*0302) and HLA-DR2. Of interest, HLA-B44 either with DR4 or DR7, was also associated with the 9.0-kb HSP70-2 marker that was increased in the group of non-Jewish patients with CA.\textsuperscript{36} We have postulated that certain HSP70 variants associated with HLA could be markers for the abnormal signal to activate apoptosis in polymorphonuclear cells or their precursors after exposure to clozapine. Polymorphism of TNF genes could also be common to Ashkenazi Jewish and non-Jewish patients with CA. Three polymorphic microsatellite allelic systems (TNFa, b, and c) have been identified in or near the TNF-\beta gene,\textsuperscript{37} and their linkage disequilibria with some MHC alleles and extended haplotypes have been described.\textsuperscript{38,39} Because TNF induces apoptosis in human neutrophils,\textsuperscript{40} it will be necessary to study whether patients with risk-associated haplotypes produce more TNF, putting them at a greater risk of developing agranulocytosis.

**ACKNOWLEDGMENT**

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