Although clozapine is the most effective pharmacotherapy for treatment-resistant schizophrenia, it is under-utilized, and initiation is often delayed. One reason is the occurrence of a potentially fatal adverse reaction, clozapine-induced agranulocytosis (CIA). Identifying genetic variations contributing to CIA would help predict patient risk of developing CIA and personalize treatment. Here, we (1) review existing pharmacogenomic studies of CIA, and (2) conduct meta-analyses to identify targets for clinical implementation. A systematic literature search identified studies that included individuals receiving clozapine who developed CIA and controls who did not. Results showed that individuals carrying the HLA-DRB1*04:02 allele had nearly sixfold (95% CI 2.20–15.80, $p_{\text{corrected}} = 0.03$) higher odds of CIA with a negative predictive value of 99.3%. Previously unreplicated alleles, TNFβ5, HLA-B*59:01, TNFβ4, and TNFβ3 showed significant associations with CIA after multiple-testing corrections. Our findings suggest that a predictive HLA-DRB1*04:02-based pharmacogenomic test may be promising for clinical implementation but requires further investigation.

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INTRODUCTION

Schizophrenia is a debilitating condition that affects as many as 20 million people worldwide [1]. Approximately 20–30% of these individuals experience treatment-resistant schizophrenia (TRS), which is characterized by ongoing psychotic symptoms and functional impairments despite adequate trials with different antipsychotic medications [2]. At present, clozapine remains the standard treatment of choice for TRS recommended by international guidelines due to its superior efficacy compared to other existing antipsychotics [3–7]. Despite the abundance of robust evidence supporting the effectiveness of clozapine in improving outcomes for TRS patients, clozapine is underutilized due to concerns about tolerability and monitoring [8], and its initiation is commonly delayed for several years in many countries worldwide, including in the USA and Canada [9–11]. Studies have even suggested that the utilization of clozapine earlier in treatment, rather than waiting for multiple drug failures and subsequent severe TRS, results in better response [12–14]. Further, initiation of clozapine has been shown to reduce healthcare costs by decreasing the number of hospitalizations and shifting care from inpatient to outpatient [15].

The reasons for underuse and delay in clozapine initiation could be attributed to several factors, including highly variable and difficult to predict clinical outcomes. For example, roughly 40–70% of patients on clozapine experience persistent symptoms and remain treatment-resistant [16]. Further, side effects in patients taking clozapine vary greatly, ranging from none or mild to life-threatening side effects [16]. Particularly of concern is the development of clozapine-induced agranulocytosis (CIA), which is defined as an absolute neutrophil count (ANC) < 500 cells/mm$^3$. CIA is a severe and potentially fatal neutropenia with an overall prevalence of 0.4% (95% CI: 0.3%, 0.6%) and fatality rate of 0.05% (95% CI: 0.03%, 0.09%) [17]. The World Health Organization’s (WHO) Pharmacovigilance global database, VigiBase, containing more than 140,000 clinician reports of clozapine adverse drug reactions (ADRs) classified in over 5,000 ADR categories, showed that the “broad agranulocytosis” category is the third major cause of fatal outcomes after “broad pneumonia” and “sudden death and cardiac arrests” [18]. Although CIA is a rare hematological condition that represents only 2% of reported fatal outcomes within the VigiBase database [18], the U.S. Food and Drug Administration (FDA) along with the majority of global health authorities have mandated that patients taking clozapine receive regular blood draws to monitor neutrophil count. These authorities also require enrollment in the Clozapine Risk Evaluation and Mitigation Strategy (REMS) Program in order to reduce the risk of clozapine-induced neutropenia.

Existing strategies for regular long-term hematological monitoring in patients taking clozapine have been previously criticized for not being cost-effective, especially given that roughly 80% of CIA cases occur within 18 weeks of clozapine initiation, and after one year of clozapine treatment, incidence of CIA decreases to 0.07% or less [19]. One study reported that frequent and long-term monitoring of white blood cell counts increased quality-adjusted survival by less than one day per patient and was more costly compared to no monitoring [20]. The additional physical burden of regular blood monitoring and its related costs further discourages both patients and clinicians from choosing clozapine and may, in part, account for the suboptimal use of clozapine in clinical practice.

To broaden the usage of clozapine and improve outcomes for patients with TRS, researchers have focused on identifying predictive biomarkers for CIA that could be used to identify...
RESULTS

A total of 686 studies were identified, with 661 excluded following the screening of titles and abstracts. After screening and removal of duplicates, 21 studies met the selection criteria for being included into the literature review, and 13 of the 21 studies qualified for inclusion into the meta-analysis. The PRISMA flowchart with details of the search yield is shown in Fig. 1.

The characteristics of the included studies in the meta-analysis are summarized in Table S1. All of the studies included individuals receiving clozapine treatment who demonstrated CIA defined as an ANC < 500/mm³ (i.e., <0.5 × 10⁹/L or <500/μL), and comparison or control participants who had not developed any hematotoxic reactions to clozapine. The mean daily dosage of clozapine was 417.0 ± 144.8 mg/d and 482.4 ± 159.0 mg/d for the CIA and comparison groups, respectively, for studies that reported these data.

Of the 13 studies included in the meta-analysis, two were genome-wide association studies (GWAS) [27, 28] and the rest were candidate gene studies conducted in different populations, including Ashkenazi Jewish [29–31], Europeans [27, 32–38], Japanese [28], and others [39]. Of the 13 studies, eight (61.5%) included only non-Jewish European, three (23.1%) included only Jewish, one (7.7%) included a mix of non-Jewish and Jewish Europeans, and one (7.7%) included Japanese samples. One study included participants with diagnoses other than schizophrenia or schizoaffective disorder [29].

Tables 1 and 2 summarize the findings for fifty-three alleles and seven haplotypes, respectively, of individual studies for which no previous replication was found (i.e., these studies reported on allelic markers which have not been investigated in other independent studies). Twelve additional alleles and one additional haplotype were evaluated in at least two studies and were analyzed via meta-analysis shown in Table 3. Therefore, Bonferroni correction for multiple testing (m = 73) was applied to each of the 73-total analyses. After correction for multiple testing, four of the non-replicated alleles remained significant predictors of CIA, including TNFβ5 (OR = 0.08; 95% CI 0.04, 0.20; p = 1.64 × 10⁻⁵), HLA-B*59:01 (OR = 7.21; 95% CI 3.56, 14.61; p = 3.06 × 10⁻⁴), TNFβ4 (OR = 7.69; 95% CI 3.55, 16.65; p = 1.71 × 10⁻³), and TNFβ3 (OR = 4.61; 95% CI 2.17, 9.82; p = 5.23 × 10⁻³). None of the non-replicated haplotypes were significant predictors of CIA after Bonferroni correction.

After correction for multiple testing (m = 73), one of the meta-analyzed alleles (Table 3) remained a significant predictor of CIA, HLA-DRB1*04:02 (OR = 0.89; 95% CI 2.20, 15.80; p = 0.03). The sensitivity and the specificity values of the HLA-DRB1*04:02 allele for prediction of CIA were 26.0% and 94.0%, respectively. The PPV and NPV were estimated to be 3.88% and 99.52%, respectively. The number of new clozapine users needed to genotype to prevent one case of CIA is three in individuals of European ancestry, which may vary in other ancestral groups. Forest plots for each meta-analysis are available in the supplementary materials (Fig. S1).

Additional alleles have reached genome wide significance such as rs149104283 [27, 40], rs3129891 [40], rs41549217 [40], and HLA-B 158 T [41]. However, HLA-B 158 T was not found to be significantly associated with CIA in a second study [42].

DISCUSSION

We systematically summarized and quantified available evidence on genetic variants contributing to CIA and conducted several
meta-analyses. We found that one genetic variant within the human leukocyte antigen (HLA) locus (major histocompatibility complex [MHC] in humans) was significantly associated with CIA after correction for multiple testing. Specifically, individuals carrying the HLA-DRB1*04:02 allele had nearly sixfold (95% CI 2.20, 15.80) increased odds of CIA. For this variant, the probability that CIA was not present in individuals without the HLA-DRB1*04:02 allele (i.e., NPV) was 99.3%, corrected for the prevalence of CIA in the USA. A high NPV indicates potential clinical utility of the HLA-DRB1*04:02 allele in stratifying patients based on risk of developing CIA, with those that are low risk (i.e., carriers of the variant) monitored more closely while on clozapine or considered for alternative treatment options.

HLA-DRB1*04:02 genotyping prior to the initiation of clozapine, if clinically implemented, would not be the first HLA predictive test for assessing risk of drug-related adverse reactions. Currently, the U.S. FDA recommends prospective screening for specific HLA alleles that are strongly associated with hypersensitivity reactions to carbamazepine, abacavir, and allopurinol prior to their initiation in populations where the allele is common [43–45]. In comparison to these existing predictive tests, the NPV of HLA-DRB1*04:02 (99.3%) is higher than the NPV of HLA-B*15:11 and HLA-B*57:01 genotyping for carbamazepine (98.9%) [46] and abacavir (82%)
Table 1. Summary statistics of individual studies for non-replicated alleles.

| Author          | Ethnicity     | Allele       | CIA   | CIA− | Control | Control− | Sensitivity | Specificity | NPV* | PPV* | OR [95% CI] | Z    | p value p | p<sup>a</sup> |
|-----------------|---------------|--------------|-------|------|---------|----------|-------------|-------------|-------|-------|-------------|------|----------|-------------|
| Turbay 1997c    | European      | TNFb5       | 9     | 57   | 43      | 23       | 14%         | 35%         | 97.8% | 0.2%  | −2          | 0.08 | 5.6      | 2.25E-08   |
| Saito 2016      | Japanese      | HLA-B*5901  | 19    | 53   | 18      | 362      | 26%         | 95%         | 99.3% | 4.9%  | 3           | 7.21 | 5.5      | 4.19E-08   |
| Turbay 1997c    | European      | TNFb4       | 48    | 18   | 17      | 49       | 73%         | 74%         | 99.7% | 2.5%  | 3           | 7.69 | 5.2      | 3.24E-07   |
| Turbay 1997c    | European      | TNFb3       | 51    | 15   | 28      | 38       | 77%         | 58%         | 99.6% | 1.6%  | 3           | 4.61 | 4.0      | 7.16E-05   |
| Lahdelma 2001   | Caucasian     | HLA-A1      | 3     | 23   | 11      | 8        | 12%         | 42%         | 98.1% | 0.2%  | −2          | 0.09 | 3.1      | 2.22E-03   |
| Yunis 1995      | Non-Jewish    | HLA-DQA1*01:02 | 15   | 27   | 3       | 29       | 36%         | 91%         | 99.4% | 3.4%  | 3           | 5.37 | 2.4      | 1.40, 20.63 |
| Yunis 1995      | Ashkenazi Jewish | HLA-DR4    | 9     | 1    | 12      | 20       | 90%         | 63%         | 99.9% | 2.2%  | 3           | 15.00| 2.4      | 1.68, 13.56 |
| Turbay 1997c    | Ashkenazi Jewish | HLA-DQA1*03:01 | 12   | 12   | 13      | 41       | 50%         | 76%         | 99.4% | 1.9%  | 4           | 3.15 | 2.2      | 1.14, 8.70  |
| Yunis 1995      | Non-Jewish    | HLA-DR2     | 13    | 8    | 5       | 14       | 62%         | 74%         | 99.5% | 2.1%  | 3           | 4.55 | 2.2      | 1.18, 17.52 |
| Turbay 1997c    | Non-Jewish    | HLA-DRB1*02 | 14    | 26   | 4       | 28       | 35%         | 88%         | 99.3% | 2.5%  | 4           | 3.77 | 2.1      | 1.10, 12.93 |
| Ostousky 2003   | Jewish        | NQO2 372 T>C | 14    | 4    | 40      | 40       | 78%         | 50%         | 99.6% | 1.4%  | 6           | 3.50 | 2.1      | 1.06, 11.56 |
| Ostousky 2003   | Jewish        | NQO2 202 G>A | 17    | 1    | 54      | 26       | 94%         | 33%         | 99.8% | 1.3%  | 5           | 8.19 | 2.0      | 1.03, 64.89 |
| van der Weide 2017 | Dutch     | A8C81 2677 G>T | 16    | 15   | 167     | 73       | 52%         | 30%         | 98.6% | 0.7%  | −13          | 0.47 | 2.0      | 0.22, 0.99  |
| Lahdelma 2001   | Caucasian     | HLA-A28     | 8     | 18   | 1      | 18       | 31%         | 95%         | 99.3% | 5.1%  | 3           | 8.00 | 1.9      | 0.91, 70.71 |
| Dettling 2001   | German        | HLA-DQB1*02:01 | 13   | 17   | 20      | 57       | 43%         | 74%         | 99.3% | 1.5%  | 7           | 2.18 | 1.7      | 0.90, 5.27  |
| van der Weide 2017 | Dutch     | A8C81 3435 C>T | 26    | 5    | 166     | 75       | 84%         | 31%         | 99.5% | 1.1%  | 14          | 2.35 | 1.7      | 0.87, 6.36  |
| Lahdelma 2001   | Caucasian     | HLA-A9      | 6     | 20   | 1       | 18       | 23%         | 95%         | 99.3% | 3.9%  | 4           | 5.40 | 1.5      | 0.59, 49.26 |
| van der Weide 2017 | Dutch     | TNFα -308 G>A | 12    | 19   | 63      | 178      | 39%         | 74%         | 99.2% | 1.3%  | 16          | 1.78 | 1.5      | 0.82, 3.88  |
| van der Weide 2017 | Dutch     | Hsp70-2 1267 G>A | 22    | 9    | 195     | 43       | 71%         | 18%         | 98.5% | 0.8%  | −14         | 0.54 | 1.4      | 0.23, 1.25  |
| Lahdelma 2001   | Caucasian     | HLA-B16     | 6     | 16   | 2       | 17       | 27%         | 89%         | 99.3% | 2.3%  | 4           | 3.19 | 1.3      | 0.56, 18.16 |
| Lahdelma 2001   | Caucasian     | HLA-A11     | 5     | 21   | 1       | 18       | 19%         | 95%         | 99.2% | 3.2%  | 4           | 4.29 | 1.3      | 0.46, 40.16 |
| Lahdelma 2001   | Caucasian     | HLA-B27     | 1     | 21   | 3       | 16       | 5%          | 84%         | 99.0% | 0.3%  | −4          | 0.25 | 1.1      | 0.02, 2.68  |
| Yunis 1995      | Ashkenazi Jewish | HLA-DQ3    | 9     | 1    | 23      | 9        | 90%         | 28%         | 99.7% | 1.1%  | 6           | 3.52 | 1.1      | 0.39, 31.95 |
| Mosyagin 2005   | Caucasian     | FcyRlIla R8H | 41    | 7    | 59      | 16       | 85%         | 21%         | 99.4% | 1.0%  | 10          | 1.59 | 0.9      | 0.60, 4.20  |
| Yunis 1995      | Non-Jewish    | HLA-DQ1     | 13    | 8    | 14      | 5        | 62%         | 26%         | 98.7% | 0.8%  | −8          | 0.58 | 0.8      | 0.15, 2.24  |
| Mosyagin 2005   | Caucasian     | FcyRlIib NA2/NA1 | 25   | 23   | 44      | 31       | 52%         | 41%         | 98.9% | 0.8%  | −16         | 0.77 | 0.7      | 0.37, 1.59  |
| Lahdelma 2001   | Caucasian     | HLA-B18     | 1     | 21   | 2       | 17       | 5%          | 89%         | 99.0% | 0.4%  | −5          | 0.40 | 0.7      | 0.03, 4.85  |
| Lahdelma 2001   | Caucasian     | HLA-B37     | 1     | 21   | 2       | 17       | 5%          | 89%         | 99.0% | 0.4%  | −5          | 0.40 | 0.7      | 0.03, 4.85  |
| Lahdelma 2001   | Caucasian     | HLA-B12     | 4     | 18   | 2       | 17       | 18%         | 89%         | 99.2% | 1.6%  | 7           | 1.89 | 0.7      | 0.31, 11.68 |
| Author          | Ethnicity      | Allele      | CIA+ | CIA− | Control+ | Control− | Sensitivity | Specificity | NPV a | PPV b | NNG | OR [95% CI] | | Z | p value b,a |
|-----------------|----------------|-------------|------|------|----------|----------|-------------|------------|-------|-------|-----|-------------|-------|-------------|
| Lahdelma 2001   | Caucasian      | HLA-B8     | 5    | 17   | 6        | 13       | 23%         | 68%        | 99.0  | 0.7   | 9   | 0.64 [0.16, 2.56] | 0.6   | 0.52        |
| Lahdelma 2001   | Caucasian      | HLA-A2     | 10   | 16   | 9        | 10       | 38%         | 53%        | 98.9  | 0.7   | 12 | 0.69 [0.21, 2.30] | 0.6   | 0.55        |
| Lahdelma 2001   | Caucasian      | HLA-B5     | 5    | 17   | 3        | 16       | 23%         | 84%        | 99.2  | 1.3  | 10 | 1.57 [0.32, 7.66] | 0.6   | 0.58        |
| Ostrosky 2003   | Jewish        | NQO2 -394 G > C | 16 | 1    | 63       | 2        | 94%         | 3%         | 98.3  | 0.9  | 8  | 0.51 [0.04, 5.96] | 0.5   | 0.59        |
| Lahdelma 2001   | Caucasian      | HLA-A3     | 9    | 17   | 8        | 11       | 35%         | 58%        | 99.0  | 0.7  | 13 | 0.73 [0.22, 2.46] | 0.5   | 0.61        |
| Lahdelma 2001   | Caucasian      | HLA-A813    | 2    | 20   | 1        | 18       | 9%          | 95%        | 99.1  | 1.6  | 8  | 1.80 [0.15, 21.57] | 0.5   | 0.64        |
| Lahdelma 2001   | Caucasian      | HLA-B22     | 2    | 20   | 1        | 18       | 9%          | 95%        | 99.1  | 1.6  | 8  | 1.80 [0.15, 21.57] | 0.5   | 0.64        |
| van der Weide 2017 | Dutch         | GSTM1null  | 16   | 15   | 113      | 125      | 52%         | 53%        | 99.2  | 1.0  | 60 | 1.18 [0.56, 2.50] | 0.4   | 0.67        |
| Mosyagin 2005   | Caucasian      | FcγRIIIa F/V | 28   | 20   | 41       | 34       | 58%         | 45%        | 99.2  | 1.0  | 29 | 1.16 [0.54, 2.41] | 0.4   | 0.69        |
| Lahdelma 2001   | Caucasian      | HLA-A10    | 2    | 24   | 1        | 18       | 8%          | 95%        | 99.1  | 1.3  | 11 | 1.50 [0.13, 17.86] | 0.3   | 0.75        |
| Dettling 2001   | German         | HLA-DQB1*03 | 16   | 14   | 43       | 34       | 53%         | 44%        | 99.0  | 0.9  | 49 | 0.90 [0.19, 2.11] | 0.2   | 0.81        |
| van der Weide 2017 | Dutch         | GSTT1null  | 27   | 4    | 204      | 34       | 87%         | 14%        | 99.2  | 0.9  | 87 | 1.13 [0.37, 3.42] | 0.2   | 0.84        |
| Lahdelma 2001   | Caucasian      | HLA-B815   | 4    | 18   | 3        | 16       | 18%         | 84%        | 99.1  | 1.0  | 24 | 1.19 [0.23, 6.12] | 0.2   | 0.84        |
| Mosyagin 2004   | Caucasian      | CYPBA C242T | 42   | 38   | 39       | 37       | 53%         | 49%        | 99.1  | 0.9  | 85 | 1.05 [0.56, 1.97] | 0.1   | 0.88        |
| Ostrosky 2003   | Jewish        | NQO2 -367 A > G | 15  | 2    | 65       | 8        | 88%         | 11%        | 99.0  | 0.9  | 80 | 0.92 [0.18, 4.80] | 0.1   | 0.92        |
| van der Weide 2017 | Dutch         | GSTP1 313A > G | 28   | 3    | 211      | 24       | 90%         | 10%        | 99.1  | 0.9  | 166 | 1.06 [0.30, 3.76] | 0.1   | 0.93        |
| Lahdelma 2001   | Caucasian      | HLA-A19    | 4    | 22   | 3        | 16       | 15%         | 84%        | 99.1  | 0.9  | 133 | 0.97 [0.19, 4.95] | 0.0   | 0.97        |
| van der Weide 2017 | Dutch         | GSTA1 -49 C > T | 26   | 5    | 199      | 39       | 84%         | 16%        | 99.1  | 0.9  | 522 | 1.02 [0.37, 2.82] | 0.0   | 0.97        |
| Ostrosky 2003   | Jewish        | NQO2 1536 C > T | 18  | 0    | 41       | 39       | 100%        | 49%        | 100.0 | 1.8  | 4  | 35.22 [2.05, 664.57] | 2.59  | 0.014       |
| Yunes 1995     | Ashkenazi Jewish | HLA-DQB1*03:01 | 0  | 16   | 18       | 36       | 0%          | 67%        | 98.6  | 0.0  | 4  | 0.06 [0.01, 1.01]  | 1.99  | 0.054       |
| Lahdelma 2001   | Caucasian      | HLA-B801    | 5    | 17   | 0        | 19       | 23%         | 100%       | 99.3  | 100.0| 2  | 12.26 [0.63, 2388.00] | 1.79  | 0.104       |
| Yunes 1995     | Ashkenazi Jewish | HLA-DRB1*11 | 0  | 16   | 13       | 41       | 0%          | 76%        | 98.8  | 0.0  | 4  | 0.09 [0.01, 1.66]  | 1.64  | 0.114       |
| Lahdelma 2001   | Caucasian      | HLA-B817   | 0    | 22   | 1        | 18       | 0%          | 95%        | 99.0  | 0.0  | 2  | 0.27 [0.01, 7.13]  | 0.08  | 0.444       |
| van der Weide 2017 | Dutch         | NQO1 609 C > T | 31  | 0    | 234      | 7        | 100%        | 3%         | 100.0 | 0.9  | 9  | 2.01 [0.11, 36.04] | 0.53  | 0.636       |

CIA clozapine-induced agranulocytosis, CIA+, number of variant positive CIA subjects, CIA− number of variant negative CIA subjects, Control+ number of variant positive control subjects, Control− number of variant negative control subjects, NNG number needed to genotype, NPV negative predictive value, OR odds ratio, PPV positive predictive value.

aNPV and PPV were corrected for the prevalence of CIA in the US.
bBonferroni correction (m = 73) was applied based on the number of alleles/haplotypes analyzed in this review.
cStudy included both Jewish and Non-Jewish individuals.
dHaldane correction was applied for case-control pairings which had 0 subjects in at least one cell.
| Author          | Ethnicity       | Haplotype                                                                 | CIA+ | CIA− | Control+ | Control− | Sensitivity | Specificity | NPV* | PPV* | NNG | OR [95% CI] | Z      | p value | p<sup>b,a</sup> |
|-----------------|-----------------|---------------------------------------------------------------------------|------|------|----------|----------|-------------|-------------|------|------|-----|-----------|--------|---------|-----------------|
| Yunis 1995      | Ashkenazi Jewish| HLA-B38, -DR4, -DQ3                                                       | 9    | 1    | 6        | 26       | 90%         | 81%         | 99.9%| 4.2% | 2  | 39.00 [4.12, 369.53] | 3.2    | 0.00    | 0.10            |
| Yunis 1995      | Ashkenazi Jewish| DRB1*04:02, DRB4*01:01, DQB1*03:02, DOA1*03:01                          | 7    | 9    | 6        | 48       | 44%         | 89%         | 99.4%| 3.5% | 3  | 6.22 [1.69, 22.88] | 2.8    | 0.01    | 0.43            |
| Turbay 1997     | European        | HLA-DRB1*04:02, DRB4*01:01, DQB1*03:02, DOA1*03:01, HSP70-2*A, HSP70-1*9, TNFε3, TNF-d, TNFα (0.308*1), TNFβ (0.308*1) | 12   | 52   | 4        | 62       | 19%         | 94%         | 99.2%| 2.8% | 4  | 3.58 [1.09, 11.76] | 2.1    | 0.04    | 1               |
| Theodoropoulou 1997 | Non-Jewish     | HLA-B8, -DR4, -DQ3                                                        | 1    | 2    | 1        | 39       | 33%         | 98%         | 99.4%| 10.9%| 3  | 19.50 [0.87, 439.35] | 1.9    | 0.06    | 1               |
| Turbay 1997     | European        | HLA-DRB1*02, DRB5*02, DOA1*05:02, DQB1*05:02, HSP70-2*A, HSP70-1*9, TNFε3, TNF-d, TNFα (0.308*1), TNFβ (0.308*1), TNFα11, TNFβ4 | 10   | 54   | 0        | 66       | 16%         | 100%        | 99.2%| 100.0%| 2  | 25.62 [1.47, 447.25] | 2.2    | 0.03    | 1<sup>c</sup> |
| Yunis 1995      | Non-Jewish      | HLA-DRB1*16:01, DRB5*02, DOA1*05:02, DOA1*03:02, DOA1*01:02              | 10   | 32   | 0        | 32       | 24%         | 100%        | 99.3%| 100.0%| 2  | 21.00 [1.18, 373.52] | 2.1<sup>c</sup> | 0.04<sup>c</sup> | 1<sup>c</sup> |
| Yunis 1995      | Non-Jewish      | HLA-B8, -DR2, -DQ1                                                       | 5    | 16   | 0        | 19       | 24%         | 100%        | 99.3%| 100.0%| 2  | 13.00 [0.67, 252.99] | 1.7<sup>c</sup> | 0.09<sup>c</sup> | 1<sup>c</sup> |

CIA clozapine-induced agranulocytosis, CIA+ number of variant positive CIA subjects, CIA− number of variant negative CIA subjects, Control+ number of variant positive control subjects, Control− number of variant negative control subjects, NNG number needed to genotype, NPV negative predictive value, OR odds ratio, PPV positive predictive value.

<sup>a</sup>NPV and PPV were corrected for the prevalence of CIA in the US.

<sup>b</sup>Bonferroni correction (m = 73) was applied based on the number of alleles/haplotypes analyzed in this review.

<sup>c</sup>Haldane correction was applied for case-control pairings which had 0 subjects in at least one cell.
Table 3. Summary statistics of meta-analyses.

| Authors                        | Allele/Haplotype | CIA+ | CIA− | Control+ | Control− | Sensitivity | Specificity | NPV* | PPV* | NNG | OR [95% CI] | Z   | I²   | p value | p value b,a |
|--------------------------------|------------------|------|------|----------|----------|-------------|-------------|-------|------|-----|------------|-----|------|---------|-------------|
| Dettling 2001, Turbay 1997     | HLA-DRB1*04:02   | 14   | 40   | 8        | 123      | 26%         | 94%         | 99.3% | 3.8% | 3   | 5.89 [2.20, 15.80] | 3.5 | 0%   | 4.00E-04 | 0.03         |
| Legge 2016, Yunis 1995, van der Weide 2017, Athanasioi 2011 | HLA-DQ2*05:02   | 33   | 178  | 9        | 521      | 16%         | 98%         | 99.2% | 7.8% | 2   | 7.12 [1.91, 26.51] | 2.9 | 53%  | 3.00E-03 | 0.22         |
| Theodoropoulou 1997, Yunis 1995 | HLA-DR2, -DQ1   | 15   | 9    | 18       | 41       | 63%         | 69%         | 99.5% | 1.8% | 4   | 5.40 [1.58, 18.43] | 2.7 | 0%   | 0.01    | 0.511        |
| Dettling 2001, Yunis 1995     | HLA-DRB5*02     | 13   | 59   | 2        | 107      | 18%         | 98%         | 99.2% | 8.3% | 2   | 6.44 [1.57, 26.39] | 2.6 | 0%   | 0.01    | 0.73         |
| Dettling 2001, Yunis 1995     | HLA-DRB1*16:01  | 13   | 59   | 5        | 104      | 18%         | 95%         | 99.2% | 3.5% | 3   | 3.62 [1.15, 11.45] | 2.2 | 0%   | 0.03    | 1            |
| Yunis 1995, Valevski 1998, Dettling 2001 | HLA-B38       | 27   | 29   | 22       | 137      | 48%         | 86%         | 99.5% | 3.1% | 3   | 10.01 [1.13, 88.55] | 2.1 | 82%  | 0.04    | 1            |
| Ostrowsky 2003, van der Weide 2017 | NQO2 1541 G > A | 40   | 9    | 154      | 167      | 82%         | 52%         | 99.7% | 1.5% | 7   | 7.16 [0.52, 98.34] | 1.5 | 70%  | 0.14    | 1            |
| Dettling 2001, Turbay 1997     | HLA-DQ8*015:02  | 16   | 38   | 20       | 111      | 30%         | 85%         | 99.2% | 1.8% | 6   | 2.31 [0.53, 10.09] | 1.1 | 71%  | 0.26    | 1            |
| Mosyagin 2004, van der Weide 2017 | CYBA 640 A > G     | 78   | 31   | 246      | 70       | 72%         | 22%         | 98.8% | 0.8% | −16 | 0.70 [0.36, 1.38] | 1.0 | 27%  | 0.31    | 1            |
| Lahdelma 2001, Yunis 1990      | HLA-B7          | 10   | 32   | 5        | 33       | 24%         | 87%         | 99.2% | 1.6% | 6   | 2.17 [0.14, 34.50] | 0.6 | 75%  | 0.58    | 1            |
| Dettling 2001, Yunis 1995     | HLA-DRB4        | 20   | 26   | 53       | 78       | 43%         | 60%         | 99.1% | 1.0% | 42  | 1.27 [0.35, 4.55] | 0.4 | 69%  | 0.72    | 1            |
| Dettling 2001, Lahdelma 2001  | HLA-B35         | 10   | 42   | 19       | 87       | 19%         | 82%         | 99.1% | 1.0% | 52  | 1.08 [0.45, 2.57] | 0.2 | 0%   | 0.87    | 1            |
| Mosyagin 2004, van der Weide 2017 | MPO −463 G > A     | 42   | 70   | 125      | 193      | 38%         | 61%         | 99.1% | 0.9% | −69 | 1.03 [0.63, 1.68] | 0.1 | 0%   | 0.92    | 1            |

CIA clozapine-induced agranulocytosis, CIA+ number of variant positive CIA subjects, CIA− number of variant negative CIA subjects, Control+ number of variant positive control subjects, Control− number of variant negative control subjects, NNG number needed to genotype, NPV negative predictive value, OR odds ratio, PPV positive predictive value.

NPV and PPV were corrected for the prevalence of CIA in the US.

Bonferroni correction (m = 73) was applied based on the number of alleles/haplotypes analyzed in this review.
hypothesis testing for observed associations, making the use of FDR correction methods less common in meta-analysis. For example, Bonferroni corrections were applied (Table 3). Furthermore, Legge et al. (2017) [27] and Konte et al. (2021) [54] provided independent replications for the association between HLA-DQB1 6672 G > C and CIA risk in individuals of European ancestry. These results implicate HLA-DQB1 in the pathophysiology of CIA and indicates that polymorphisms within this gene may be associated with risk of CIA in European populations.

In the GWAS by Legge et al. (2017), an association between genes at chromosome 12p12.2 with CIA was reported in a sample of European patients with the top SNP being rs149104283 (OR = 4.32, P = 1.79 × 10⁻⁵), which is intrinsic to transcripts of the sulfonylurea receptor genes, SLC01B3 and SLC01B7 [27]. A replication analysis was conducted by Saito et al. (2017) in a Japanese sample as a part of the Clozapine Pharmacogenogenetics Consortium of Japan, which found no significant association of 12p12.2 with CIA [55]. Instead, in their GWAS, Saito et al. (2016) showed HLA-B*5901 (OR = 10.7, 95% CI 4.8–22.4) as a risk factor for CIA in a sample of Japanese patients with schizophrenia (CIA = 50, Controls = 2905) [28]. A combined GWAS meta-analysis in a sample of patients of Chinese ancestry identified a nominal association between the HLA-B and clozapine-induced neutropenia; however, this GWAS was not included in the meta-analysis as the results are not specific to CIA [56]. Findings from these GWAS taken together demonstrate that risk alleles for CIA may vary by ancestral group given that some variants lie in variation-rich genomic regions and demonstrate large differences in allele frequencies across populations, and population-specific recombination sites contribute to the high diversity of haplotypes further influencing CIA risk [54, 57].

Several well-known alleles and genetic variants are localized within the MHC region and show LD; therefore, it complicates whether conclusions about specific associations between HLA alleles with CIA represent a true genetic association or whether the HLA allele is in LD, or closely located to the true causative gene [58]. Goldstein et al. (2014) found that HLA-DRB1*04:02 and HLA-DQB1*05:02 were not in strong LD according to r², yet the D’ between these two alleles may be quite high [41]. Limited haplotype frequency data is available for HLA-DRB1*04:02 and HLA-DQB1*05:02, including a bone marrow registry for a Polish population whose LD statistics were calculated. Given a high enough D’, the association between HLA-DRB1*04:02 and CIA may not be sufficient independent from that of HLA-DQB1*05:02 and makes it difficult to conclude the respective contribution of a given allele to predisposition to CIA. Therefore, further analysis is required to confirm this in other populations, especially considering the rarity of this haplotype in the Polish population.

Previously unreplicated alleles, TNFB5 (OR = 0.08; 95% CI 0.04–0.20), TNFB4 (OR = 7.69; 95% CI 3.55–16.65), and TNFD3 (OR = 4.61; 95% CI 2.17–9.82), showed significant associations with CIA after corrections for multiple testing. TNF microsatellites d3 and b4 were associated with increased susceptibility to CIA, while microsatellite b5 showed a protective effect in both Jewish and non-Jewish individuals with schizophrenia [30]. TNF are immune-regulating non-HLA genes in the MHC region located between the complement cluster region and the HLA-B gene, which has more recently been demarcated as the Class IV region, and have shown a LD with HLA-B and HLA-DR alleles [59]. As a result, for studies showing an association between TNF or HLA-B alleles with CIA noted above, strong LD between these genes complicates unraveling the relative contributions of genetic variation in the TNF locus or the HLA-B locus to CIA.

The limitations of the present study include a lack of bias analysis, which may lead to an overestimation of the true effect size of the results, since studies with higher effects are more likely to be published, and thus be included in the meta-analysis. To reduce the effects of publication bias, we performed a comprehensive search to identify all relevant, published literature. An additional limitation is that we cannot confirm with certainty that
the sample in the GWAS conducted by Legge et al. (2016) does not overlap with samples in the other studies included in this meta-analysis. For the GWAS by Saito et al. (2016), since it is the only study conducted in patients of Japanese ancestry, it can be safely deduced that it does not overlap with the other studies which are conducted in patients of European ancestry (Table S1). Finally, translating findings from pharmacogenomic investigations, such as the present study, into clinical practice may not necessarily increase the usage of clozapine to treat TRS patients, but may instead be a deterrent to its usage as was the case with carbamazepine, which was substantially less prescribed to patients with epilepsy and bipolar disorder following the introduction of HLA-B*15:02 screening in Taiwan to prevent carbamazepine-related SCARs [60].

CONCLUSION
Currently, there is no predictive test for CIA. For a predictive test to be useful within clinical practice, it should reliably be able to identify individuals who are at low risk of developing CIA in non-carriers of the risk allele, such that hematological monitoring is either unnecessary or reduced in frequency. NPV or the proportion of patients with a negative test (i.e. non-carriers of the risk allele) who truly do not have the condition is a reliable diagnostic for the clinical usefulness of a predictive test [61]. Test results with a high NPV are useful to clinicians when considering treatments which can potentially be unnecessary, costly or even risky, such as clozapine pharmacotherapy [61]. Based on the results of the meta-analyses, the HLA-DRB1*04:02 variant demonstrates a potential for pharmacogenomic prediction of CIA within clinical practice with a high NPV (99.3%). Estimates of NPV, PPV, sensitivity, and specificity for HLA-DRB1*04:02 genotyping for CIA risk assessment are comparable to other existing HLA screening tests for drug-induced hypersensitivity reactions that are used clinically. However, since allele frequencies and haplotypes vary substantially by ancestral groups, further research is needed to investigate the association between the HLA-DRB1*04:02 allele and CIA susceptibility in different populations. Furthermore, the results of the meta-analysis indicate immunogenetic variations, specifically relating to the MHC genomic region, may be involved in the pathophysiology of CIA, and therefore are potential targets for pharmacogenomic investigations to date suggest the involvement of multiple genetic variations with varying levels of impact on CIA. Therefore, further research is necessary to identify reliable and reproducible genetic variants in diverse populations with large effects related to CIA that can be incorporated into a predictive pharmacogenomic test. Clinical application of predictive pharmacogenomic tests with high NPV may increase the safe utilization of clozapine, decrease costs associated with regular long-term hematologic monitoring, and most importantly, improve patient outcomes.

DATA AVAILABILITY
All data generated or analysed during this study are included in this published article and its supplementary information files.

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AUTHOR CONTRIBUTIONS

FI: Conceptualization, Investigation, Data Curation, Writing—Original Draft, Writing—Review & Editing. DH: Conceptualization, Methodology, Validation, Formal Analysis, Investigation, Data Curation, Writing—Original Draft, Writing—Review & Editing, Visualization, Supervision. DL: Conceptualization, Writing—Review & Editing, Supervision. RL: Conceptualization, Validation, Writing—Review & Editing. LCB: Writing—Review & Editing. LD: Writing—Review & Editing, Supervision. JT: Writing—Review & Editing, Supervision. DMJ: Writing—Review & Editing, Supervision.

COMPETING INTERESTS

DH, DL, RL, LCB, and JAT were employed by Myriad Neuroscience (formerly Assurex Health) at the time of this study. DMJ was a co-inventor on two patents assessing risk for antipsychotic-induced weight gain at the time of this study and was also a co-investigator on two pharmacogenetic studies where genetic test kits were provided in in-kind contribution by Myriad Neuroscience but did not receive any salary, equity, stocks, or options from any pharmacogenetic companies. All other authors declare no conflicts of interest in relation to this work.

ADDITIONAL INFORMATION

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