*HLA-B\*38:02:01* Predicts Carbimazole/Methimazole-Induced Agranulocytosis

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**Abstract**

Thioamides antithyroid-drugs (ATD) are important in hyperthyroid disease management. Identification of the susceptibility locus of ATD-induced agranulocytosis is important for clinical management. We performed a genome-wide association study (GWAS) involving 20 ATD-induced agranulocytosis patients and 775 healthy controls. The top finding was further replicated. A SNP rs185386680 showed the strongest association with ATD-induced agranulocytosis in GWAS (odds ratio (OR)=36.4; 95% CI: 12.8-103.7; *P*=1.3x10-24) and replication (OR=37; 95% CI: 3.7-367.4; *P*=9.6x10-7). *HLA-B\*38:02:01* was in complete linkage disequilibrium with rs185386680. High-resolution HLA typing confirmed that *HLA-B\*38:02:01* was associated with carbimazole/methimazole-induced agranulocytosis (OR=265.5; 95% CI: 27.9-2528.0; *P*=2.5x10-14), but not associated with propylthiouracil. The positive and negative predictive values of *HLA-B\*38:02:01* in predicting carbimazole/methimazole-induced agranulocytosis were 0.07 and 0.999. Approximately 211 cases need to be screened to prevent one case. Screening for the risk allele will be useful in preventing agranulocytosis in populations where the frequency of the risk allele is high.

**Introduction**

Hyperthyroidism is a common disease with a prevalence of 1.3% in the United States[1](#_ENREF_1) and China,[2](#_ENREF_2) and 0.75% in the Europe.[3](#_ENREF_3) The thiourea antithyroid drugs (ATD), namely carbimazole (CMZ) and its active form, methimazole (MMI), and propylthiouracil (PTU), have been used for over 50 years. Although effective, they are associated with a rare but severe and life-threatening adverse drug reaction (ADR), agranulocytosis. It has been reported that around 0.2% to 0.5% of patients prescribed ATD develop agranulocytosis.[4](#_ENREF_4)

Many idiosyncratic reactions to drugs have now been shown to have a genetic basis. Genetic variation has been shown to be important in predicting ADRs and has been used clinically to prevent serious ADRs. For example, *HLA-B\*15:02* and *HLA-B\*57:01* have been identified as pharmacogenomic markers for serious ADRs induced by carbamazepine[5](#_ENREF_5" \o "Chung, 2004 #5) and abacavir,[6](#_ENREF_6) respectively. Screening for the risk allele before prescribing abacavir, carbamazepine, and allopurinol has been proven to be useful in reducing the risk of serious ADRs.[7-9](#_ENREF_7) However, not all pharmacogenomic markers can be used for screening, especially when the effect size in terms of numbers needed to test to prevent one case is high.

Since phenotype definition is crucial in genetic studies, especially when case numbers are small, a standardised phenotype definition with stringent inclusion and exclusion criteria usually enhances the power to detect the true variant.[10](#_ENREF_10) Recently, the first genome-wide association study (GWAS) of ATD-induced agranulocytosis conducted in Taiwanese population showed that combined *HLA-B\*38:02* and *HLA-DRB1\*08:03* genotype was associated with ATD-associated agranulocytosis with an odds ratio (OR) of 48.41.[11](#_ENREF_11) In this study, we present data showing that *HLA-B\*38:02:01* and its tagging SNP rs185386680 are associated with CMZ/MMI-induced agranulocytosis with an OR of 266, and 211 cases need to be screened to prevent one case of ATD-induced agranulocytosis.

**Results**

**Genome-wide association analysis**

The clinical characteristics of cases are provided in Supplementary Tables 1 and 2. Among 20 cases included in the GWAS, 15, 4, and 1 of them were CMZ-, PTU-, and MMI-induced agranulocytosis cases. We first conducted an association study involving ~5M typed and imputed SNPs from 20 ATD-induced agranulocytosis cases and 775 healthy controls. The quantile-quantile plot is provided in Supplementary Figure 2. A significant signal located in the MHC region on chromosome 6p was observed (Figures 1 and 2), with the strongest association observed for the SNP rs185386680 (cases: 34.9% vs. controls: 4.3%; OR: 24.7; 95% CI: 9.2-66.3; *P*=3.6x10-29). After conditioning on this SNP, all significant association signals were attenuated (Figure 2). Since this was an imputed SNP, we validated this SNP by Taqman genotyping using the original samples (Table 1), an even higher OR was obtained (cases: 37.5% vs. controls: 3.8%; OR: 36.4; 95% CI: 12.8-103.7; *P*=1.3x10-24). Analysis using the Taqman assay in 4 additional independent cases which served as a replication set and 387 additional independent healthy controls showed a similar association (cases: 37.5% vs. controls: 3.7%; OR: 37.0; 95% CI: 3.7-367.4; *P*=9.6x10-7). Next, we validated the association using 75 ATD-tolerant controls, this led to a much stronger association (cases: 37.5% vs. controls: 2.7%; OR: 53.2; 95% CI: 13.6-208.9; *P*=9.0x10-13).

Assessment of the LD structure showed that this SNP is in high LD (*r2*>0.9) with 4 SNPs (rs28753035, rs200593554, rs28753036, rs28771382) in the *HLA-B* locus, whereas such high LD was not observed in controls subjects (*r2*<0.2).

**HLA association analysis**

Since the most significant association signal was observed in the HLA region, as well as the LD between rs185386680 and *HLA-B* locus, we investigated if the association signal was driven by a particular HLA allele, especially HLA-B. Association analysis using imputed HLA alleles showed that *HLA-B\*38:02* was significantly associated with ATD-induced agranulocytosis, with an OR of 29.29 (95% CI: 10.35-82.94). We then performed high resolution HLA typing of HLA-B, the concordance between the imputed and assayed *HLA-B\*38:02:01* was 100%, with rs185386680 and *HLA-B\*38:02:01* being in complete LD (*r2*=1). Using ATD-tolerant patients as controls, we also found that *HLA-B\*38:02:01* was significantly associated with ATD-induced agranulocytosis with an OR of 53.2 (95% CI: 13.6-208.9; *P*=9.0x10-13; Table 1).

**Association with CMZ/MMI-induced agranulocytosis**

Next, we performed drug-specific analysis on CMZ/MMI-induced agranulocytosis. Using CMZ/MMI-induced agranulocytosis cases (N=19) and CMZ/MMI-tolerant controls (N=59), *HLA-B\*38:02:01* was associated with agranulocytosis with an OR of 265.5 (cases: 47.4%; controls: 3.2%; 95% CI: 27.9-2528.0; *P*=2.5x10-14; Table 1).

**Association with PTU-induced agranulocytosis**

In the 5 PTU-induced agranulocytosis cases and 12 PTU-tolerant controls, none carried *HLA-B\*38:02:01*.

**Association with ATD-induced neutropenia**

We also performed an exploratory analysis to evaluate if *HLA-B\*38:02:01* was associated with CMZ/MMI-induced neutropenia (Supplementary Table 3); however none of the cases carried *HLA-B\*38:02:01*.

**Pharmacogenomic predictor of CMZ/MMI-induced agranulocytosis**

We evaluated the prediction accuracy of *HLA-B\*38:02:01*. The sensitivity and specificity in predicting CMZ/MMI-induced agranulocytosis was 94.7% (95% CI: 71.9%-99.7%) and 93.2% (95% CI: 82.7%-97.8%). The positive and negative predictive values were 0.07 and 0.999, whereas the posterior probability of having agranulocytosis after screening was 7.3% for the *HLA-B\*38:02:01* carrier and 0.028% for *HLA-B\*38:02:01* non-carrier. The positive and negative likelihood ratio was 14.92 and 0.056, respectively. Approximately 211 cases need to be screened to prevent one case of CMZ/MMI-induced agranulocytosis (Figure 3).

**Discussion**

In this study, we found a significant association between CMZ/MMI-induced agranulocytosis and *HLA-B\*38:02:01.* This was not seen with CMZ/MMI-induced neutropenia, and additionally, was also not seen with PTU-induced agranulocytosis. Assuming the prevalence of CMZ/MMI-induced agranulocytosis is 0.5% (1 in 200 patients), a positive genetic test would increase the risk to 0.5% to 7.3% (1 in 14 patients), whereas a negative test would reduce the risk from 0.5% to 0.028% (1 in 3572 patients). Importantly, the negative predictive value of 0.999 is important if this is to be used as a screening test, as has been shown with abacavir.[6](#_ENREF_6)

HLA-B alleles have been implicated in multiple serious adverse drug reactions, including *HLA-B\*57:01* in abacavir-induced hypersensitivity syndrome,[6](#_ENREF_6) *HLA-B\*58:01* in allopurinol-induced serious cutaneous adverse drug reactions[12](#_ENREF_12) and *HLA-B\*15:02* in carbamazepine-induced SJS/TEN.[5](#_ENREF_5) Interestingly, *HLA-B\*38* has previously been implicated in clozapine-induced agranulocytosis in Israeli Jewish schizophrenic patients,[13](#_ENREF_13) suggesting that *HLA-B\*38* may be involved in agranulocytosis caused by multiple drugs. Our study is in agreement with a very recent GWAS of ATD-induced agranulocytosis conducted in Taiwanese population showing that *HLA-B\*38:02* as a susceptibility locus.[11](#_ENREF_11) However, *HLA-B\*38:02* was suggested as the susceptibility locus for all ATD-induced agranulocytosis cases, whereas our findings suggest that *HLA-B\*38:02*, or specifically *HLA-B\*38:02:01*, is responsible for CMZ/MMI-, but not for PTU-, induced agranulocytosis. In addition, our data for predictive accuracy were found to be higher than in the Taiwanese study. These discrepancies could be due to the fact that the cases included in the Taiwanese study were scored as “probable” adverse drug reactions, as they only excluded patients who were on concomitant drugs known to be associated with agranulocytosis, whereas we applied stringent exclusion criteria to avoid agranulocytosis due to many other causes. Moreover, all CMZ/MMI-induced agranulocytosis patients in our study developed agranulocytosis during the first course of CMZ/MMI treatment without prior exposure to PTU. Thus the false positive rate for the CMZ/MMI-induced agranulocytosis ascertainment is expected to be very low. This highlights the importance of case definition in pharmacogenomics, as shown by our pharmacogenetic study of *SLCO1B1* in statin-induced myopathy,[10](#_ENREF_10) where we showed that the effect size for the association with *SLCO1B1* c.521 C allele was higher with severe myopathy (defined as plasma creatine kinase>10 x upper limit of normal or rhabdomyolysis) when compared with myopathy (defined as plasma creatine kinase>4 x upper limit of normal), despite the number of cases being reduced from 76 (myopathy) to 23 (severe myopathy). Stringent case selection reduces the false positive rate and hence improves the power of the study. To validate the role of *HLA-B\*38:02:01*, and provide further information on the role of the immune system, ex vivo drug testing using patients’ peripheral blood mononuclear cells, is currently underway. It is important to note that there is some, albeit limited, evidence that MMI-induced agranulocytosis is immune-mediated.[14](#_ENREF_14)

The recent Taiwanese study also showed that *HLA*-*DRB1\*08:03* was associated with ATD-induced agranulocytosis. We also evaluated the association using high-resolution HLA typing but found that this allele was only marginally associated with ATD-induced agranulocytosis and did not reach the GWAS significance threshold (Supplementary Table 4). However, this non-association could be due to the small effect size and hence our study has limited power to detect its association.

Our study shows that *HLA-B\*38:02:01* may be specific to CMZ/MMI-induced agranulocytosis, but appears not to be associated with PTU-induced agranulocytosis. A similar observation was found in the Taiwanese study showing that HLA-B\*38:02 was less associated with PTU-induced agranulocytosis with an OR of 5.81 (P=0.0019), when compared to MMI-induced agranulocytosis.[11](#_ENREF_11) Although cross-reactivity among thioamides is common,[15](#_ENREF_15) using PTU as an alternative treatment in patients with CMZ/MMI-induced agranulocytosis, or vice-versa, has been successful in many cases,[16](#_ENREF_16) suggesting that the predisposing genetic factors for agranulocytosis induced by CMZ/MMI and PTU could be different. However, it is worth-noting that we defined PTU as the culprit drug if PTU was the ATD being prescribed when agranulocytosis developed. Among 5 of the PTU-induced agranulocytosis cases, 4 of them had previous exposure to CMZ (Supplementary Table 2). In addition, all CMZ/MMI developed agranulocytosis during the first course (ARANfirst; Supplementary Table 1), whereas 4 out of the 5 PTU cases developed agranulocytosis during later courses of ATD treatment (AGRANlater). A recent study showed that 9 out of 10 AGRANlater patients had experienced minor adverse effects when treated with the other ATD before the diagnosis of agranulocytosis, whereas 0 out of 4 AGRANfirst patients had the same experience,[17](#_ENREF_17) suggesting that the mechanism leading to AGRANfirst and AGRANlater could be different. In this case, whether *HLA-B\*38:02:01* may be more associated with AGRANfirst requires further study. Similarly, *HLA-B\*38:02:01* was absent in patients with ATD-induced neutropenia, this further suggests that the mechanism of ATD-induced agranulocytosis could be different from ATD-induced neutropenia, which is similar to the study of statin-induced myopathy aforementioned. Since ATD-induced agranulocytosis and neutropenia are rare, future collaborative effort will be required to elucidate the role of genetic variants in these ADRs.

There are limitations to the current study. We did not have the absolute neutrophil count (ANC) measurements in ATD-tolerant controls. We only defined ATD-tolerant controls if they were prescribed ATD for more than 3 months without a diagnosis of agranulocytosis. However, the false-negative rate is expected to be low as only 0.5% of ATD users develop agranulocytosis. Similarly, compliance to ATD was unclear. Our numbers of cases are relatively low, but despite this, we have found a strong association. This is unlikely to be a false-positive result since we were able to replicate with only 4 cases, the same finding has been reported in another Southeast Asian population from Taiwan[11](#_ENREF_11), there is evidence that ATD-induced agranulocytosis has an immune basis[14](#_ENREF_14) consistent with the HLA association and the finding is in keeping with the very large effect sizes demonstrated with other immune-mediated adverse drug reactions.[18](#_ENREF_18)

We therefore propose that *HLA-B\*38:02:01* is a clinically relevant predictor of CMZ/MMI-induced agranulocytosis. On the assumption that the prevalence of CMZ/MMI-induced agranulocytosis is 0.5% (1 in 200) among ATD-users, the presence of the *HLA-B\*38:02:01* allele increases the risk of agranulocytosis to 7.3% (1 in 14), whereas its absence reduces the risk to 0.028% (1 in 3,572). Screening for *HLA-B\*38:02:01* itself would help in preventing CMZ/MMI-induced agranulocytosis, especially the prevalence of *HLA-B\*38:02:01* is common in some Southeast Asian countries and CMZ/MMI are the most prescribed ATD in many Asian countries including, Japan,[19](#_ENREF_19) China, and Hong Kong. Interestingly, homozygous *HLA-B\*38:02:01* was found in a Filipino CMZ-induced agranulocytosis patient (Supplementary Table 5), further highlighted the usefulness of screening *HLA-B\*38:02:01* in other Southeast Asian countries. Although CMZ/MMI are also the most prescribed ATDs in other non-Asian countries, such as United States,[20](#_ENREF_20) the prevalence of *HLA-B\*38:02:01* appears to be low outside Southeast Asia according to the HLA Allele Frequency Net Database (Supplementary Figure 3)[21](#_ENREF_21) – for instance, the allele frequency of *HLA-B\*38:02:01* is 4% in Chinese, 5.7% in Indonesian, and 2% in Thai populations, but was 0% in South Korean, Bulgarian, and Moroccan populations. Similar finding is observed for *HLA-B\*38:02* (Supplementary Figure 4 and Supplementary Table 6). This observation is reminiscent of carbamazepine-induced Stevens-Johnson Syndrome, which is associated with *HLA-B\*15:02*[5](#_ENREF_5) in Southeast Asian populations but not in Northern Europeans where another allele, *HLA-A\*31:01*, has been found to be more important.[22](#_ENREF_22) Further work is needed to identify the predisposing HLA alleles in other populations outside Southeast Asia.

Our study provided strong evidence that *HLA-B\*38:02:01* is a clinically useful pharmacogenetic predictor for CMZ/MMI-induced agranulocytosis. Our results are consistent with an independent study in Taiwan, suggesting that they are robust and that this genetic marker is a highly important in the Chinese and may have clinical utility in some Southeast Asian populations. Further investigations of the mechanisms involved, as well as determination of other parameters required for clinical utility such as cost-effectiveness will be important in assessing the possible future implementation of screening for this allele.

**Materials and methods.**

**Case subjects Recruitment**

Case subjects were recruited at several regional hospitals and specialist clinics in different districts in Hong Kong since 2013. All subjects were of southern Chinese descent. All subjects gave their informed consent. The study was approved by the ethics committee and was conducted according to the Declaration of Helsinki.

**Definition of ATD-induced agranulocytosis**

Agranulocytosis was defined as ANC<0.5x109/L. ATDs studied included CMZ, MMI, and PTU. We define ATD-induced agranulocytosis according to the international consensus meeting on drug-induced cytopenia:[23](#_ENREF_23) onset of agranulocytosis during ATD treatment, and complete recovery with ANC>1.5 x 109/L within 1 month of discontinuation of the drug. Subjects with any history of congenital neutropenia or immune neutropenia, recent infectious disease (particularly recent viral infection), recent chemotherapy or radiotherapy or immunotherapy and existence of an underlying hematological disease were excluded. In addition to the consensus definition, patients who had concomitant treatment with a drug that is known to be associated with agranulocytosis, or patients with any systemic diseases that are associated with neutropenia such as aplastic anaemia, AIDS, leukemia, malignant lymphoma, systemic lupus erythematosus, osteomyelofibrosis, vitamin B12/folic acid deficiency, and hypersplenism were also excluded. All subjects with hyperthyroidism were confined to those with Graves’ disease. A total of 24 cases (20 cases in the discovery cohort and 4 cases in the replication cohort) were included in the current study.

**Definition of control subjects**

There were 2 sets of control subjects:

(i) Healthy female subjects without Graves’ disease[24](#_ENREF_24) served as controls in the discovery and replication phases, which is a convenient sample from our previous GWAS and genetic study of osteoporosis: genome-wide genotyping data were available in 775 subjects and therefore they were selected for discovery phase; whereas 387 controls used for replication were randomly selected. These control subjects were originally used for studying genetics of osteoporosis and thus free of any thyroid diseases and major chronic diseases that would affect bone and mineral metabolism; All healthy controls were drawn from the Hong Kong Osteoporosis Study.[24](#_ENREF_24) The 775 controls were used in our previous GWAS of bone mineral density,[24](#_ENREF_24) whereas the 387 controls were randomly selected from the Hong Kong Osteoporosis Study for various genetic studies. All these controls were free of any thyroid diseases and major chronic diseases that would affect bone and mineral metabolism at baseline. Given the nature of the pharmacogenomic study and all controls were randomly selected from the database, the selection bias should be minimal; and

(ii) subjects with Graves’ disease who had been treated with ATD for at least 3 months but without the development of agranulocytosis served as “ATD-tolerant controls”. Diagnosis of Graves' disease was confirmed by an elevated serum concentration of free T4, reduced concentration of thyroid-stimulating hormone and positive thyroid antibodies. All controls (both healthy and ATD-tolerant controls) were recruited from the Queen Mary Hospital, Hong Kong. Since the prevalence of hyperthyroidism is around 2% in the female population, and the prevalence of ATD-induced agranulocytosis is only 0.2-0.5% in ATD users, the chance of misclassification of controls in the discovery phase was expected to be extremely low. This is a cost-effective approach that was also adopted in our previous GWAS of thyrotoxic periodic paralysis.[25](#_ENREF_25)

**Power calculation of the sample size required for the GWAS and replication**

The current study was initiated in 2013. When the study was started, no literature related to the topic was available. Thus, we estimated the effect size based on a pharmacogenomic study of clozapine-induced agranulocytosis (CIA), in which an HLA-DRB5\*0201 was associated with CIA with an effect (OR) of 22.[26](#_ENREF_26) Thus, we assumed that the effect size of the risk allele of ATD-induced agranulocytosis would be 22, and we found that 20 cases and 775 controls would have >90% power to test a susceptibility locus with a large range of MAF (from 0.03 to 0.45; Supplementary Table 7). Therefore, 20 cases were selected for the discovery phase. Based on the finding of the discovery phase, the odds ratio of the susceptibility locus was found to be 24.7. Based on the actual odds ratio and MAF of the locus, we performed power calculation and found that 4 cases and 387 controls had >90% to replicate the SNP. These calculations were performed using QUANTO.[15](#_ENREF_15)

**Genotyping**

In the discovery phase, cases and controls were genotyped using Illumina Omni2.5 and HumanHap 610 Quad chip, respectively. In the replication phase, single-SNP genotyping was performed using TaqMan® genotyping assay (ABI). All assays had a discordance rate of <0.5% among built-in technical duplicates.

**Quality control and power calculation**

In the GWAS data set, quality control filtering for SNPs was applied to remove SNPs that were not autosomal, were missing in >5% of samples, had minor allele frequency<1% or showed significant deviation from Hardy-Weinberg equilibrium in controls (*P*<0.0001). In addition, sex-check and genetic relatedness were also checked as described previously.[25](#_ENREF_25)After filtering, 25 healthy controls were removed, and 20 cases and 775 healthy controls were retained for analysis. The power calculation for the discovery cohort is provided in Supplementary Table 7.

**Pre-phasing and imputation**

Pre-phasing was performed using SHAPEIT[27](#_ENREF_27) v2 with reference to the 1000 Genome Project Phase 3 variant set (October 2014 release). Imputation based on the same reference panel was performed using IMPUTE[28](#_ENREF_28) v2.3.1. Quality control after imputation included the exclusion of indels and structural variants, SNPs with minor allele frequency <5%, SNPs with imputation certainty (info score)<0.9 and SNPs with significant deviation from Hardy-Weinberg equilibrium in the controls (*P*<0.0001). Finally, a total of 5,063,698 imputed SNPs and 276,242 genotyped SNPs that were common across the studies were used in the association analysis.

**Statistical analysis.**

Underlying population structure was assessed by principal component analysis using PLINK v1.9. The first 10 principal components were checked to see if there were deviation between cases and controls. All ATD-induced agranulocytosis cases did not show deviation from the 775 healthy controls (Supplementary Figure 1). Association analysis of the genotyped and imputed genotype dosages was performed using SNPTEST[29](#_ENREF_29) v2.4.1 with the frequentist test under the additive model. Cochran-Armitage trend test was used to evaluate the association in validation and replication analyses. Since our initial discovery phase was done using genotyped and imputed SNPs, we adopted a stricter genome-wide significance threshold of *P*<1×10−8, which accounted for the SNPs included in the initial analysis (~5M).

**Validation of imputation accuracy and replication study**

Imputation accuracy was assessed by direct TaqMan genotyping. TaqMan genotyping assay (ABI, Foster City, CA, USA) was optimized for rs185386680. The concordance rate between assayed genotypes and imputed genotypes was 98.97%, whereas the allelic R2 measured between assayed and imputed genotypes were 0.87.

**HLA imputation, typing, and analysis**

To determine if specific coding variants within HLA genes contributed to the diverse association signals, we imputed all classical HLA alleles (A, B, C, DRB1, DQA1, DQB1, DPB1, DPA1) using SNP2HLA.[2](#_ENREF_2),[30](#_ENREF_30) The imputation was based on a reference panel from the Pan-Asian population consisting of genotype data from 530 individuals who were typed for ​HLA-A, B, C, DRB1, DQA1, DQB1, DPB1, and DPA1 alleles to 4-digits. Imputation accuracy of HLA alleles was assessed by comparing HLA alleles to the HLA sequencing data on a subset of samples (N=100) in the discovery cohort. High resolution HLA-typing of HLA-B and HLA-DRB1 was performed by Histogenetics, LLC (Ossining, NY, USA). The concordance rate obtained was 97.49% for HLA-B, suggesting robust performance of the imputation method. HLA alleles across the HLA region for association with Cochran-Armitage trend test was performed using PLINK.

**Study highlights**

**What is the current knowledge on the topic?**

Genetics of ATD-induced agranulocytosis is largely unknown. Recent study identified *HLA-B\*38:02* and *HLA-DRB1\*08:03* as the independent susceptibility loci. However, only one single study showed the association and the predictive performance of these loci may be suboptimal for clinical use. Moreover, whether these loci are associated with ATD-induced neutropenia remain unknown.

**What question did this study address?**

This study performed GWAS with subsequent high-resolution HLA-typing (6-digit). Subgroup analysis and calculation relating clinical prediction were also performed.

**What this study adds to our knowledge?**

*HLA-B\*38:02:01* was the susceptibility locus of CMZ/MMI-induced agranulocytosis, while the association with *HLA-DRB1\*08:03* was not independent. This locus was not associated with ATD-induced neutropenia. Using clinically well defined cases, the positive and negative predictive values of *HLA-B\*38:02:01* in predicting CMZ/MMI-induced agranulocytosis were 0.07 and 0.999. Approximately 211 cases need to be screened to prevent one case of CMZ/MMI-induced agranulocytosis.

**How this might change clinical pharmacology and therapeutics?**

The presence of the allele increased the risk of agranulocytosis among ATD-users from 0.5% to 7.3%, whereas its absence reduced the risk from 0.5% to 0.028%. Our study showed that screening of *HLA-B\*38:02:01* will be useful to prevent CMZ/MMI-induced agranulocytosis.

**Author Contributions**

CLC, MP, and AWK designed the study. CHC, CSH, EYL, KFL, MWM, JYL, TWW, AYH, KWC, VHH, VT, SCS, BMC, ICW, KCT, and AWK were involved in patient selection and recruitment. CSW and VKC did the DNA extraction and genotyping. CLC and CST developed the statistical analysis plan and did the analyses. CLC prepared the first draft of the manuscript, which all authors subsequently contributed to and approved.

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Figure 1. Manhattan plot of the GWAS of ATD-induced agranulocytosis.

-log10 P values on the y axis plotted against ascending physical position on the x axis. The dotted red line represents the genomewide

significance threshold P values 1x10-8.

Figure 2. Regional association plot of the region +/- 300kb of rs185386680.

The upper panel shows the original regional association plot; the middle panel shows the regional association plot after conditioning on the genotype of rs185386680; the lower panel indicates the physical location and location of genes in the region.

Figure 3. Flow diagram for calculating numbers needed to screen to prevent one CMZ/MMI-induced agranulocytosis.