Genetic variants associated with antithyroid drug-induced agranulocytosis: a genome-wide association study in a European population

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Summary

Background **Drug-induced agranulocytosis is a potentially life-threatening adverse reaction. Genome-wide association studies (GWASs) in ethnic Chinese people in Taiwan and Hong Kong have shown an association between agranulocytosis induced by antithyroid drugs and the HLA alleles *HLA-B*38:02* and *HLA-DRB1*08:03*. We aimed to identify genetic variants associated with antithyroid drug-induced agranulocytosis in a white European population.**

Methods **We did a GWAS in 234 European adults with any non-chemotherapy drug-induced agranulocytosis (absolute neutrophil count ≤0·5×10⁹/L [≤500/μL]) and 5170 population controls. 39 of the 234 patients had agranulocytosis that was induced by antithyroid drugs (thiamazole [methimazole], carbimazole, or propylthiouracil). After imputation and HLA allele prediction, 9 380 034 single nucleotide polymorphisms (SNPs) and 180 HLA alleles were tested for association. The genome-wide significance threshold was $p<5\times10^{-8}$.**

Findings **Agranulocytosis induced by non-chemotherapy drugs in general was significantly associated with the HLA region on chromosome 6, with odds ratios (ORs) of 3·24 (95% CI 2·31–4·55, $p=1\cdot20\times10^{-11}$) for *HLA-B*27:05* and 3·57 (2·61–4·90, $p=2\cdot32\times10^{-15}$) for the top SNP (rs114291795). Drug-specific analysis showed that the association with *HLA-B*27:05* was largely driven by cases induced by antithyroid drugs. In a multiple logistic regression model, the OR for *HLA-B*27:05* was 7·30 (3·81–13·96) when antithyroid drug-induced agranulocytosis was compared with population controls ($p=1\cdot91\times10^{-9}$) and 16·91 (3·44–83·17) when compared with a small group of hyperthyroid controls ($p=5\cdot04\times10^{-4}$). Three SNPs were strongly associated with antithyroid drug-induced agranulocytosis: rs652888 (OR 4·73, 95% CI 3·00–7·44, $p=1\cdot92\times10^{-11}$) and rs199564443 (17·42, 7·38–41·12, $p=7\cdot04\times10^{-11}$), which were independent of *HLA-B*27:05*, and rs1071816 (5·27, 3·06–9·10, $p=2\cdot35\times10^{-9}$) which was in moderate linkage disequilibrium with *HLA-B*27:05*. In heterozygous carriers of all three SNPs, the predicted probability of antithyroid drug-induced agranulocytosis was about 30% (OR 753, 95% CI 105–6812). To avoid one case of agranulocytosis, based on the possible risk reduction if all three SNPs are genotyped and carriers are treated or monitored differently from non-carriers, roughly 238 patients would need to be genotyped.**

Interpretation **In white European people, antithyroid drug-induced agranulocytosis was associated with *HLA-B*27:05* and with other SNPs on chromosome 6. In the future, carriers of these variants could be placed under intensified monitoring or offered alternative treatment for hyperthyroidism.**

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Introduction

Serious adverse drug reactions such as drug-induced agranulocytosis can severely limit the use of a drug. Agranulocytosis is defined as a decline in absolute neutrophil count to less than $0\cdot5\times10^9$/L (<500/μL).[1] It is causally related to more than 125 non-chemotherapy drugs, and among the most well documented are the thiourea drugs thiamazole (methimazole), carbimazole, and propylthiouracil, which are used for the treatment of hyperthyroidism.[2,3] The risk of agranulocytosis induced by antithyroid drugs is estimated to be 0·2–0·5%, and onset is typically in the first 3 months of treatment.[3,4] Patients often present with symptoms of infection, such as fever, chills, and myalgias.[5] Left untreated, sepsis will develop in roughly two-thirds of patients.[6] Even with appropriate management, the mortality rate for agranulocytosis induced by non-chemotherapy drugs is 4–5%.[6]

The pathogenic mechanism behind drug-induced agranulocytosis is not well established. Antibodies against circulating neutrophils have been identified in patients, which suggests an immunological mechanism.[7] Another postulated mechanism is induction of T-cell-mediated reactions against neutrophils and neutrophil precursors in the bone marrow by oxidative drug metabolites.[7]

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Research in context

Evidence before this study

We searched PubMed with the terms "agranulocytosis" or "neutropenia", in combination with "drug-related side effects" or in combination with "adverse reactions" for articles published until Jan 12, 2016. The pathogenic mechanism behind drug-induced agranulocytosis is not well established. Immunological mechanisms such as antibodies against circulating neutrophils and T-cell activation against neutrophils and neutrophil precursors in the bone marrow have been proposed, but the processes are not clearly understood. During the preparation of this report, two genome-wide association studies were reported describing a strong association between agranulocytosis induced by antithyroid drugs and *HLA-B*38:02* and *HLA-DRB1*08:03* in ethnic Chinese people in Taiwan and Hong Kong. The prevalence of these alleles in white European people is known to be much lower.

Added value of this study

To our knowledge, this is the first study to show an association between the HLA region and antithyroid drug-induced agranulocytosis in a white European population. The study is strengthened by bringing together cohorts from four European countries. Notably, the associated HLA types differed between white European people and previously studied ethnic Chinese people and Japanese people. In white European people, single nucleotide polymorphisms (SNPs) close to HLA were also associated with agranulocytosis, but linkage disequilibrium with HLA could contribute to these associations.

Implications of all the available evidence

Agranulocytosis induced by antithyroid drugs is associated with the HLA region on chromosome 6. We have shown that ethnic background determines which genetic variants need to be tested before the use of these drugs. In white European people, the predicted probability of antithyroid drug-induced agranulocytosis was roughly 30% and the odds ratio 753 (95% CI 105–6812) in heterozygous carriers of three identified SNPs on chromosome 6. We calculated that 238 patients would need to be genotyped to avoid a potentially life-threatening adverse drug reaction in one patient by consideration of alternative treatments or intensified monitoring. Our results represent a further step towards the concept of precision medicine in patients with hyperthyroidism.

An association between antithyroid drug-induced agranulocytosis and HLA alleles *HLA-B*38:02*[8,9] and *HLA-DRB1*08:03*[8] has been reported in ethnic Chinese people in Taiwan and Hong Kong. A similar association with *HLA-DRB1*08:03*, although not as strong, was previously reported in Japanese people.[10] Associations were strongest for the azole drugs thiamazole (methimazole) and carbimazole,[8–10] and in one study[9] the association with *HLA-B*38:02* was strengthened when patients with agranulocytosis induced by propylthiouracil were removed from the analysis.

To enable genome-wide association studies (GWASs) to investigate the genetic associations of this rare condition in Europe, we formed the European Drug-induced Agranulocytosis Consortium (EuDAC). Here, we present the overall results for all drugs, focusing on antithyroid drugs.

Methods

Study design and participants

EuDAC consists of a network of investigators in Sweden, Spain, France, Germany, the UK, and the Netherlands. Patients in Sweden and France were recruited through nationwide, spontaneous adverse drug reaction reports sent from health-care professionals to the national drug regulatory authorities. In Spain, patients were recruited both from spontaneous adverse drug reaction reports and through active surveillance at 17 hospitals in Barcelona. Patients in Germany were recruited through active surveillance at 50 hospitals in Berlin, as described previously.[11] No additional blood sampling or investigation was done for patients recruited through active surveillance compared with those recruited through spontaneous reports.

Eligible participants were aged 18 years or older and were able to provide informed consent. Cases were patients who had developed an absolute neutrophil count of $0{\cdot}5\times10^9$/L (≤500/μL) or less during non-chemotherapy drug treatment or within 7 days of stopping treatment. Each patient was required to have complete recovery after cessation of the drug, with an absolute neutrophil count of more than $1{\cdot}0\times10^9$/L (>1000/μL) or a compatible bone marrow aspirate or biopsy finding. Causality assessment was done in accordance with the WHO standard algorithm.[12] Exclusion criteria were anticancer chemotherapy within 1 month of onset of agranulocytosis; radiation therapy within the previous month; bone marrow transplantation at any time; ongoing infection with Epstein-Barr virus, hepatitis A virus, HIV, cytomegalovirus, or parvovirus B19; ongoing sepsis; ongoing miliary tuberculosis; current presence of chronic neutropenia (congenital cyclic or idiopathic); ongoing immunosuppressive therapy with cytotoxic drugs; current presence of malignant infiltration of bone marrow; haematological diseases (eg, myelodysplasia, aplastic anaemia, pancytopenia, and other blood dyscrasias, such as haemoglobin ≤100 g/L or platelets $\leq100\times10^9$/L); and current presence of systemic lupus erythematosus. There were no other restrictions relating to other drug treatments, primary diagnosis, or ancestry.

We collected clinical data (demographic information, medical history, drug treatment history, laboratory data,

and ancestry) by use of interviews with a standardised questionnaire and by obtaining and reviewing medical records. At each centre, patients were assessed by at least one senior investigator, and a final adjudication of the complete dataset was done by a specialist in haematology. DNA was extracted from peripheral venous blood. 234 patients fulfilled all requirements (94 patients from Sweden, 66 from Spain, 41 from Germany, 33 from France), and 39 of these patients had agranulocytosis that was induced by antithyroid agents (25 patients from Sweden, four from Spain, six from Germany, four from France).

Consenting control populations were available from Sweden, Spain, and Germany. 5170 controls were included in the study—4891 unrelated individuals from the Swedish Twin Registry,[13] 183 Spanish individuals, and 96 German individuals.[11] Of 183 Spanish controls, 147 were recruited in a previous study of upper-gastrointestinal bleeding,[14] whereas the remaining 36 were healthy controls. To match for hyperthyroidism, we used 49 controls from the Swedish Twin Registry who had been treated for hyperthyroidism according to the Swedish Prescribed Drug Register (available from 2005) or the Swedish National Patient Register (available from 1967).

The study was approved by the local ethics committees (2010/231, Uppsala, Sweden; Dec 22, 2014, Málaga, Spain; RTF011, Barcelona, Spain; Charité–Universitätsmedizin Berlin, Germany; and CPP Sud-Ouest et Outre-Mer I N°1–09–24, Toulouse, France) and was done in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants including controls. The study protocol has been indexed in the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) register.[15]

Genotyping and imputation

193 cases of drug-induced agranulocytosis from Sweden, Spain, and France, and 147 Spanish controls were genotyped with the Illumina HumanOmni 2.5M chip (Illumina, San Diego, CA, USA; figure 1). The remaining 36 Spanish controls were genotyped with the Illumina HumanOmni1-Quad 1M chip. Cases (n=41) and controls (n=96) from Germany were genotyped with the Illumina HumanOmniExpress 700K, as were controls from the Swedish Twin Registry (n=4891). Genotype calls were generated with GenomeStudio software (Illumina).

GWAS quality control and data management was done with PLINK (version 1.9). The resulting merged data included 596 010 single nucleotide polymorphisms (SNPs) on the autosomal chromosomes. Imputation of genotypes was done with Shapeit (version 2.r790)[16] and Impute (version 2.3.1).[17] The 1000 genomes project reference set was used for the imputations (appendix p 3). The total number of SNPs after imputation was 9 380 034. To account for possible population stratification, principal component analysis (PCA) was done (appendix p 15). Six genetic outliers were detected with PCA, all of which were cases (appendix p 16). These cases were not excluded from the data; however, sensitivity analyses were done by reanalysing each top hit with the data for these six cases excluded. Additional details about quality control, PCA, and imputation are reported in the appendix (pp 3–4).

See Online for appendix

All genome-wide analyses were adjusted for sex and the first four genetic principal components from the PCA. SNP effects were modelled as additive. The conventional genome-wide significance threshold $p<5\times10^{-8}$ was used to correct for multiple testing.[18] Results are presented as Manhattan plots and Q–Q plots. When genome-wide significant signals were identified, analyses were done sequentially by adjusting for each genome-wide significant signal until no genome-wide signals were left. Because of heterogeneity in the data, follow-up analyses were done stratified by country of inclusion (where patients were recruited) and drug class. Logistic regression was used to estimate univariate and multiple models. When a value of zero was present in one cell, odd ratios (ORs) and 95% CIs were estimated by adding 0·5 to all cells. The predictive ability of the univariate and multiple logistic regression models was expressed as the *c* statistic. Our definition of the optimum cutoff for deciding when to consider a patient for alternative treatment (using a prediction model) was the cutoff that maximised both sensitivity and specificity. Genome-wide analyses were done with PLINK (version 1.9) and individual SNP analyses were done with R 3.2.2.

Imputation of two-digit (eg, *HLA-B*27*) and four-digit (eg, *HLA-B*27:05*) classical HLA alleles (n=180), aminoacid residues, and individual SNPs was done on

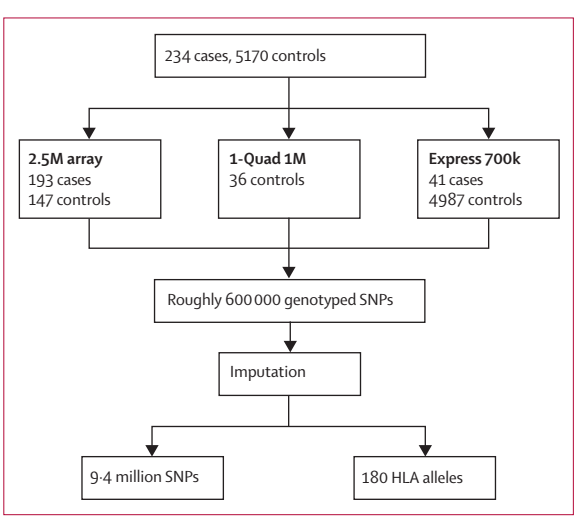

Figure 1: **Study design**
Cases and controls were genotyped on separate occasions using the following Illumina arrays: HumanOmni 2.5M, HumanOmni1-Quad 1M, and HumanOmniExpress 700K. 2.5M array=HumanOmni 2.5M, 1-Quad 1M=HumanOmni1-Quad 1M. Express 700K=HumanOmniExpress 700K. SNPs=single nucleotide polymorphisms.

the non-imputed, merged, and quality-controlled genome-wide data with SNP2HLA software (version 1.0.2), with a reference panel of 5225 individuals.[19] To avoid confounding by indication, top HLA signals were tested with a cohort of cases and controls matched for hyperthyroidism, which was available from Sweden.

Statistical analyses

Power calculations were made both for the total number of cases of drug-induced agranulocytosis (appendix p 14), and for the subset attributed to antithyroid drugs (appendix p 14). The power to detect an OR of 5 or more was 99% when using all 234 cases of drug-induced agranulocytosis and 5100 controls, with a minor allele frequency (MAF) of 5% or higher. The power to detect an OR of 5 or more was 80% when using the 39 cases of antithyroid drug-induced agranulocytosis and 5100 controls, with a MAF 20% or higher. These calculations are based on a genome-wide significance level of 5×10^{-8}, prevalence of drug-induced agranulocytosis of 1%, and an additive genetic model.[20]

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Of 243 cases of drug-induced agranulocytosis identified, nine were excluded after adjudication: seven Swedish cases (three because of exposure to chemotherapy, one because of negative rechallenge, two because of unknown white blood cell count, and one because of a diagnosis of chronic lymphatic leukaemia) and two Spanish cases (both because of missing clinical data). No German or French cases were excluded. Table 1 shows characteristics of all 234 included cases. 39 cases were induced by the antithyroid agents thiamazole (29 [74%]), carbimazole (five [13%]), and propylthiouracil (five [13%]). Six of the 234 cases, including

	All cases (n=234)	Antithyroid drug-induced cases (n=39)
Sex		
Men	96 (41%)	8 (21%)
Age group, years		
<25	19 (8%)	2 (5%)
25–29	13 (6%)	4 (10%)
30–34	18 (8%)	7 (18%)
35–39	21 (9%)	4 (10%)
40–44	14 (6%)	3 (8%)
45–49	16 (7%)	3 (8%)
50–54	25 (11%)	3 (8%)
55–59	30 (13%)	4 (10%)
60–64	22 (9%)	3 (8%)
65–69	16 (7%)	3 (8%)
70–74	13 (6%)	1 (3%)
>74	27 (12%)	2 (5%)
Ethnicity		
Swedish	84 (36%)	22 (56%)
Spanish	58 (25%)	3 (8%)
German	41 (18%)	6 (15%)
French	21 (9%)	1 (3%)
Other European	18 (8%)	6 (15%)
Non-European	8 (3%)	1 (3%)
Unknown	4 (2%)	0
Indication for treatment		
Infection	119 (1%)	2 (5%)
Pain condition	115 (49%)	..
Cardiovascular disease	53 (3%)	..
Thyroid disease	40 (17%)	..
Graves' thyrotoxicosis	13 (6%)	13 (33%)
Hyperthyroidism unspecified	26 (11%)	26 (67%)
Hypothyroidism	1 (<1%)	..
Rheumatic disease	40 (17%)	..
Gastrointestinal disease	39 (17%)	..
Psychiatric disease	31 (13%)	..
Neurological disease	19 (8%)	..
Other	34 (15%)	..

(Table 1 continues in next column)

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	All cases (n=234)	Antithyroid drug-induced cases (n=39)
Drug type		
β-lactam antibiotics	59 (25%)	2 (5%)
Metamizole (dipyrone)	43 (18%)	0
Antithyroid drugs	39 (17%)	39 (100%)
Thiamazole (methimazole)	29 (12%)	29 (74%)
Propylthiouracil	5 (2%)	5 (13%)
Carbimazole	5 (2%)	5 (13%)
Sulfasalazine	36 (15%)	0
Other	57 (24%)	3 (8%)
Daily dose, mg		
Thiamazole (methimazole)		28 (7·5–45·0)
Carbimazole		20*
Propylthiouracil		250 (100–300)
Agranulocytosis caused by more than one drug		
Proportion with co-suspected drugs	..	5 (13%)
Disease onset		
Time to onset, days	..	45 (34)†
Cell count		
Lowest neutrophil count, 10^9 cells per L	..	0·11 (0·14)

Data are n (%) or mean (SD). *Daily dose missing for four of five cases. †One outlier with time to onset of 900 days excluded.

Table 1: **Patient characteristics**

one case induced by an antithyroid drug (thiamazole), were of non-white ethnic origin (appendix pp 15–16).

In the complete cohort of 234 cases and 5170 controls, we identified genome-wide significant associations with SNPs in the MHC region (HLA region) on chromosome 6 (figure 2A). The Q–Q plot is shown in the appendix (p 17). The SNP with the best evidence for association was rs114291795, located in an intron of the *MICA* gene (OR 3·57, 95% CI 2·61–4·90, p=$2·32\times10^{-15}$; table 2, appendix p 6). After adjusting for this SNP, a strong signal remained for rs1811197, which flanks *HLA-B* (2·40, 1·89–3·06, p=$9·42\times10^{-13}$). No SNP reached genome-wide significance after adjusting for both rs114291795 and rs1811197. Stratification by country of inclusion (appendix p 18) showed a similar pattern in Sweden, Germany, and Spain. Patients from France were not tested separately because there were no French controls.

Stratification by the main drug classes showed that the associations on chromosome 6 were largely driven by the 39 patients with agranulocytosis induced by antithyroid drugs (figure 2B). The associations were similar when the five patients given propylthiouracil were excluded (appendix p 19). The top (most significantly associated) SNP for patients with agranulocytosis induced by all antithyroid drugs was rs652888, located in an intron of *EHMT2* (OR 4·73, 95% CI 3·00–7·44, p=$1·92\times10^{-11}$; table 2, appendix pp 7–8). After adjusting for this SNP, a strong signal remained for the deletion rs199564443, flanking the gene *FOXF2* (17·42, 7·38–41·12, p=$7·04\times10^{-11}$). After adjusting for both rs652888 and rs199564443, a signal remained for rs1071816, located in exon 2 of *HLA-B* (5·27, 3·06–9·10, p=$2·35\times10^{-9}$). Adjusting for all three SNPs revealed a significant association with rs111618861, in an intergenic region on chromosome 8 (13·75, 5·46–34·64, p=$2·71\times10^{-8}$; table 2, appendix pp 7–8).

The estimated univariate ORs for the chromosome 6 associations in the overall analysis (rs114291795 and rs1811197) and for the top signals in patients with agranulocytosis induced by antithyroid drugs (rs652888, rs199564443, and rs1071816) are shown in figure 3A. To avoid confounding by indication for treatment, the 25 Swedish cases induced by the antithyroid drug class were first tested against all 4891 Swedish controls and then against the 49 Swedish controls matched for hyperthyroidism. The ORs for all SNPs in figure 3A, apart from rs1811197, increased when matched hyperthyroid controls were used. Notably, six (24%) of the 25 Swedish cases induced by antithyroid drugs had the deletion caused by rs199564443, compared with none of the 49 matched controls (33·00, 1·77–614, p=0·019). A sensitivity analysis for the top five SNPs in which the six genetic outliers were excluded produced near identical results (appendix p 20).

After imputation of four-digit HLA alleles, the respective univariate ORs, 95% CIs, and p values were calculated for each HLA allele versus the outcome of agranulocytosis (table 2, appendix pp 9–10, 21). The top predicted allele was *HLA-B*27:05* (OR 3·24, 95% CI 2·31–4·55, p=$1·20\times10^{-11}$). After adjusting for this allele, the OR for *HLA-B*08:01* was 2·14 (95% CI 1·61–2·84, p=$1·48\times10^{-7}$). Agranulocytosis was not significantly associated with any four-digit HLA allele in the HLA region after adjusting for both *HLA-B*27:05* and *HLA-B*08:01*. The linkage disequilibrium between the classical HLA alleles *HLA-B*27:05* and *HLA-B*08:01*, and the SNPs rs114291795, rs1811197, rs652888, rs199564443, and rs1071816 is shown in the appendix (p 11). *HLA-B*27:05* was in high linkage disequilibrium with rs114291795 and in moderate linkage disequilibrium with rs1071816, and *HLA-B*08:01* was in moderate linkage disequilibrium with rs1811197 and rs652888. The deletion rs199564443, located outside the HLA region, was not in linkage disequilibrium with *HLA-B*27:05* or *HLA-B*08:01*.

Drug-specific analysis showed that the overall association with *HLA-B*27:05* was driven by patients with agranulocytosis induced by antithyroid drugs. The univariate OR for carrying *HLA-B*27:05* was 5·44 (2·94–10·06) in these patients (p=$6·88\times10^{-8}$; table 2, appendix p 12). After HLA imputation, the top variant was still rs652888—ie, the same SNP as in the genome-wide SNP analysis (appendix p 22). After adjusting for *HLA-B*27:05*, the top variant rs652888 remained, and the top four-digit HLA signal was *HLA-B*08:01* (OR 3·88, 95% CI 2·21–6·81, p=$2·20\times10^{-6}$; appendix p 22). After adjusting for both *HLA-B*27:05* and *HLA-B*08:01*, all

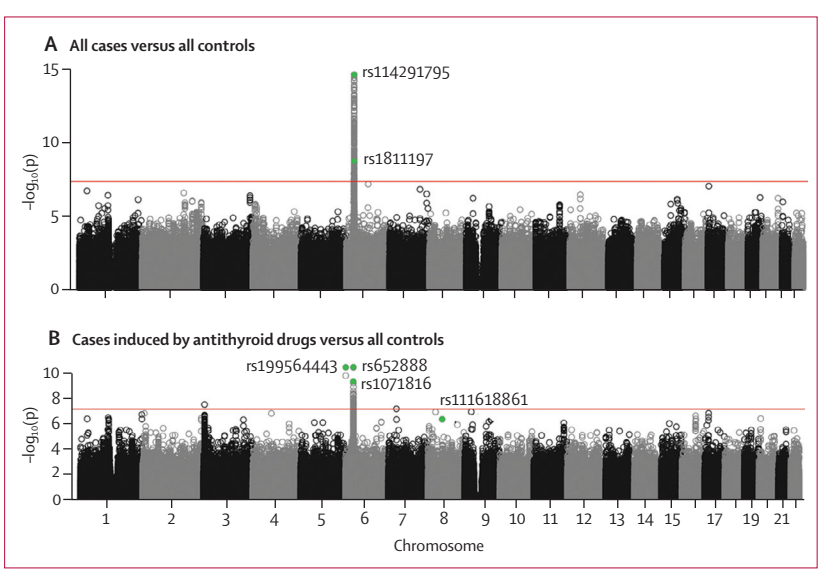

Figure 2: **Manhattan plots of genome-wide association analyses**
9 380 034 SNPs after imputation, adjusted by sex and genetic principal components 1–4. The red line shows the threshold for genome-wide significance of 5×10^{-8}. (A) Analysis of all 234 cases versus all 5170 controls. The top SNP was rs114291795, located in the intron of the MICA gene on chromosome 6. After adjustment for this SNP, a strong signal remained for rs1811197. (B) Analysis of 39 cases induced by antithyroid drugs versus all controls. The top SNP was rs652888, located in the intron region of the EHMT2 gene on chromosome 6. After adjustment for this SNP, a strong signal remained for rs199564443, which is a deletion variant flanking FOXF2 on chromosome 6. After adjustment for both rs652888 and rs199564443, a strong signal remained for a coding SNP in HLA-B, rs1071816. After adjustment for all three SNPs, a significant association with rs111618861, located in an intergenic region on chromosome 8, was identified. SNP=single nucleotide polymorphism.

	Chromosome	SNP	Position	Alleles (minor/major)	n	MAF (patients)	MAF (controls)	OR (95% CI)	p value	Nearby gene
Genome-wide associations for all cases										
Adjusted by sex and genetic principal components 1–4	6	rs114291795	31377640	G/C	5376	0·14	0·06	3·57 (2·61–4·90)	$2·32×10^{-15}$	*MICA-HCP5*
Adjusted by sex and genetic principal components 1–4 and rs114291795	6	rs1811197	31335997	A/G	5366	0·29	0·16	2·40 (1·89–3·06)	$9·42×10^{-13}$	..
Genome-wide associations for cases induced by antithyroid drugs										
Adjusted by sex and genetic principal components 1–4	6	rs652888	31851234	G/A	5203	0·54	0·20	4·73 (3·00–7·44)	$1·92×10^{-11}$	*EHMT2*
Adjusted by sex, genetic principal components 1–4, and rs652888	6	rs199564443	1388978	C/CTTTT*	5149	0·12	0·01	17·42 (7·38–41·12)	$7·04×10^{-11}$	*FOXF2*
Adjusted by sex, genetic principal components 1–4, rs652888, and rs199564443	6	rs1071816	31324536	C/T	4975	0·40	0·11	5·27 (3·06–9·10)	$2·35×10^{-9}$	*HLA-B*
Adjusted by sex, genetic principal components 1–4, rs652888, rs199564443, and rs1071816	8	rs111618861	57044382	C/CA	4715	0·11	0·01	13·75 (5·46–34·64)	$2·71×10^{-8}$	..
HLA associations for all cases										
Adjusted by sex and genetic principal components 1–4	6	*HLA-B*27:05*	31431272	P/A	5404	0·10	0·06	3·24 (2·31–4·55)	$1·20×10^{-11}$	..
Adjusted by sex, genetic principal components 1–4, and *HLA-B*27:05*	6	*HLA-B*08:01*	31431272	P/A	5404	0·16	0·11	2·14 (1·61–2·84)	$1·48×10^{-7}$	..
HLA associations for cases induced by antithyroid drugs										
Adjusted by sex and genetic principal components 1–4	6	*HLA-B*27:05*	31431272	P/A	5209	0·19	0·06	5·44 (2·94–10·06)	$6·88×10^{-8}$	..
Adjusted by sex, genetic principal components 1–4, and *HLA-B*27:05*	6	*HLA-B*08:01*	31431272	P/A	5209	0·26	0·11	3·88 (2·21–6·81)	$2·20×10^{-6}$	..

Top GWAS results based on 9 380 034 SNPs after imputation for all cases and patients with antithyroid drug-induced agranulocytosis versus all controls. No genome-wide significant signals were left after adjusting for two variants for all drugs, and after adjusting for four variants for antithyroid drugs. Univariate odds ratios for the top HLA alleles for all cases, and for patients with antithyroid drug-induced agranulocytosis. The effect is modelled per increase of one present HLA allele. Complete lists of associated SNPs and HLA types are available in the appendix. Deletion variants are truncated to a maximum of two alleles. SNP=single nucleotide polymorphism. MAF=minor allele frequency. OR=odds ratio. GWAS=genome-wide association study.

Table 2: **Top genome-wide associations and HLA associations with agranulocytosis induced by all drugs and by antithyroid drugs**

HLA signals disappeared, including rs652888, which is in moderate linkage disequilibrium with *HLA-B*08:01* (r^2=0·4; appendix p 11).

Multiple logistic regression models including *HLA-B*27:05* and *HLA-B*08:01* were compared between all cases and subsets of cases (figure 3B). The OR for *HLA-B*27:05* increased from 3·65 (95% CI 2·58–5·15, $p=2·02×10^{-13}$) to 7·30 (3·81–13·96, $p=1·91×10^{-9}$) when the analysis was restricted to agranulocytosis induced by antithyroid drugs. When Swedish cases of agranulocytosis induced by antithyroid drugs were compared with Swedish controls, the OR for *HLA-B*27:05* was 7·64 (3·71–15·71, $p=3·29×10^{-8}$). Figure 3B shows the corresponding ORs for *HLA-B*08:01*. The associations with *HLA-B*27:05* and *HLA-B*08:01* were similar when the five cases of agranulocytosis induced by propylthiouracil were excluded (appendix p 19). To avoid confounding by indication for treatment, the two top HLA-B signals were tested against controls matched for hyperthyroidism. The OR for carrying *HLA-B*27:05* increased to 16·91 (3·44–83·17, $p=5·04×10^{-4}$) when 25 Swedish antithyroid drug-induced cases were compared with 49 matched controls, while the OR for *HLA-B*08:01* decreased. A sensitivity analysis for the top HLA-B alleles in which the six genetic outliers were excluded produced near identical results (appendix p 23).

For the HLA variant *HLA-B*27:05*, which reached genome-wide significance, the *c* statistic was 0·625. The *c* statistics for the three individual SNPs were 0·773 for rs652888, 0·608 for rs199564443, and 0·757 for rs1071816; when combined into a prediction model, the *c* statistic was 0·889. Therefore, we focused on the predictive ability of a model that combines the three SNPs. Figure 4 shows a nomogram for estimating the probability of a patient having antithyroid-induced agranulocytosis according to the prediction model. As an example, the predicted probability of agranulocytosis in heterozygous carriers of all three SNPs would be about 30% and the estimated OR would be 753 (95% CI 105–6812), when the SNPs are combined. By comparison, the estimated OR for a person heterozygous for *HLA-B*27:05* would be about 7·3, and for a person homozygous for *HLA-B*27:05* the OR would be about 53 (figure 3B).

The optimum cutoff for deciding when to consider a patient for alternative treatment was at a predicted probability of 0·005, giving an estimated sensitivity of

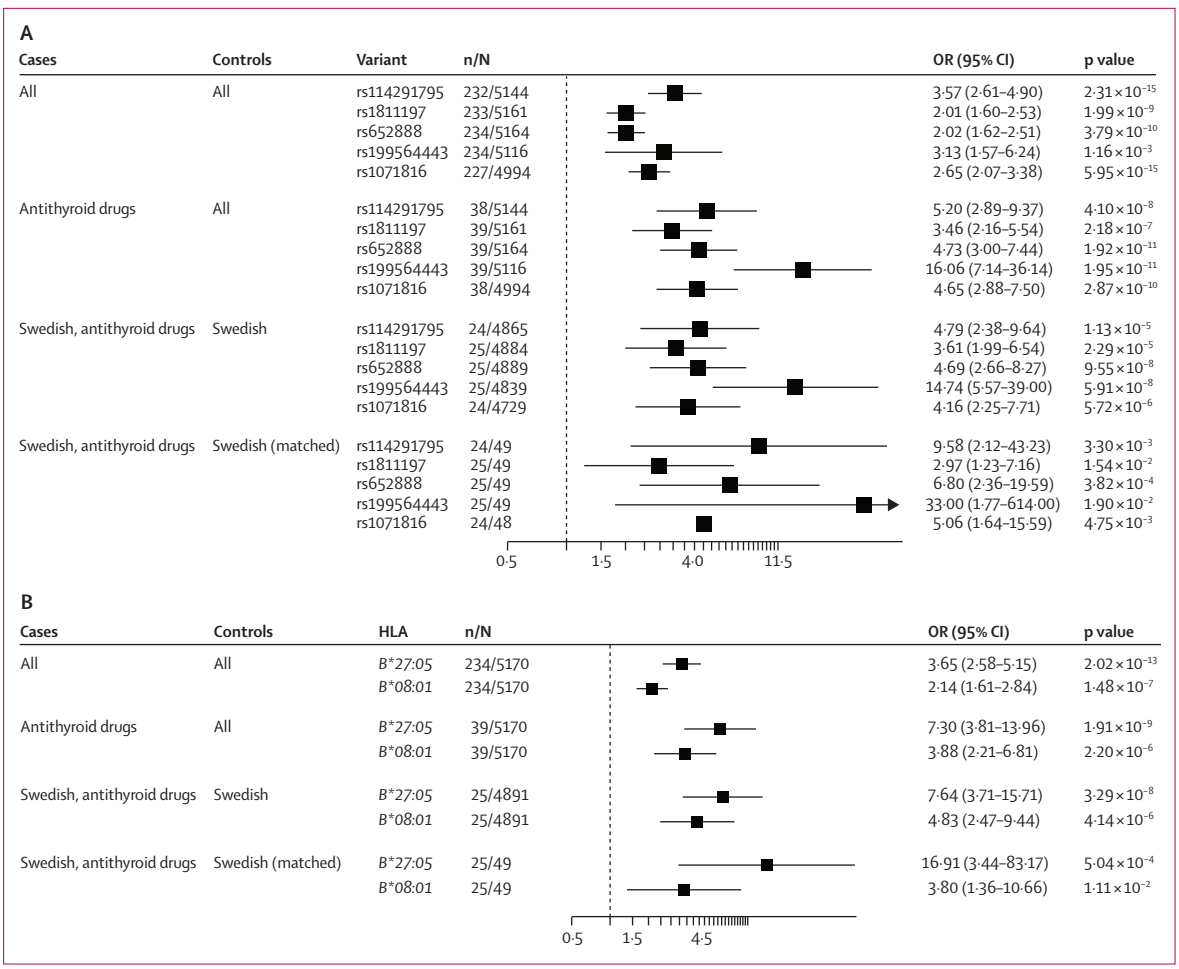

***Figure 3*: Estimated odds ratios for associations on chromosome 6**

(A) Forest plot of estimated univariate ORs with 95% CIs, showing the top SNPs on chromosome 6, rs114291795, rs1811197, rs652888, rs19956443, and rs1071816, for all cases versus all controls, and antithyroid drug-induced cases versus all controls. Also shown are Swedish patients with antithyroid drug-induced agranulocytosis versus all Swedish controls and Swedish antithyroid drug-induced cases versus Swedish controls matched for hyperthyroidism. None of the matched Swedish controls had the variant rs199564443, and ORs, 95% CIs, and p values were calculated manually. Adjustment for sex and genetic principal components 1–4 was, therefore, not possible for rs199564443. (B) Forest plot of estimated ORs with 95% CIs based on a multiple logistic regression model for the top HLA-B alleles showing all cases versus all controls, all cases induced by antithyroid drugs versus all controls, all Swedish cases induced by antithyroid drugs versus all Swedish controls, and all Swedish cases induced by antithyroid drugs versus matched Swedish controls. Matched controls have been treated for hyperthyroidism. Numbers of patients (n) or controls (N) are given in the n/N column. OR=odds ratio. SNP=single nucleotide polymorphism.

84·2% and a specificity of 86·1%, which means that 13·9% of patients treated with antithyroid drugs would be falsely predicted to have an increased risk of agranulocytosis (appendix p 24). In terms of individual SNPs, being heterozygous for only rs652888 or rs1071816 does not give a predicted probability above this cutoff; however, all other combinations give predictions above 0·005 (figure 4). Assuming that one in 200 patients starting antithyroid drugs will get agranulocytosis (ie, an incidence of 0·005)[4] and a sensitivity of 84·2%, we could theoretically reduce the incidence to 0·00079 (0·005–0·842×0·005) by genotyping three SNPs. The number needed to genotype to avoid one case of antithyroid-induced agranulocytosis would be about 238, which is the reciprocal of the absolute risk reduction (ie, 1/[0·005–0·00079]).

Discussion

In our GWAS, drug-induced agranulocytosis was associated with the HLA region on chromosome 6. Our finding adds to the growing number of drug-induced type B reactions that have been associated with the HLA region, including Stevens-Johnson syndrome, drug-induced liver injury, and clozapine-induced agranulocytosis.[21] The HLA region is challenging to study because of high gene density,[22] high degree of polymorphism,[23] and an extended linkage disequilibrium that makes the causative variant difficult to identify.[22]

Genetic susceptibility traits for rare serious adverse drug reactions are drug specific, as previously shown in genome-wide studies of drug-induced liver injury.[21] In our study, drug-induced agranulocytosis was associated

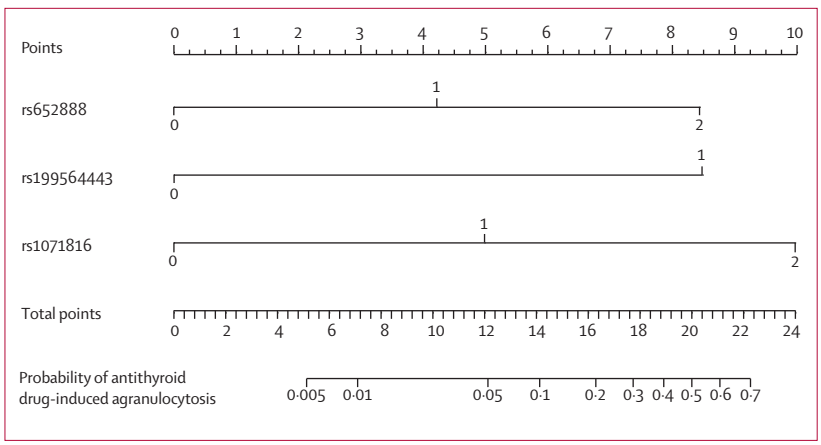

Figure 4: **Nomogram for predicting the individual risk of antithyroid drug-induced agranulocytosis based on the three SNPs**
The model used to estimate the predicted probabilities was logit(p)=7·0908 + 1·4938 × rs652888 + 3·0046 × rs199564443 + 1·7725 × rs1071816, where the logit(p) value needs to be transformed to a probability by taking elogit(p)/(1+ elogit(p)). For information on how to use the nomogram see appendix (p 4).

with *HLA-B*27:05*. This HLA type has previously been associated with agranulocytosis induced by levamisole, which was not included in our study.[24] In our cohort, no other single drug class apart from antithyroid drugs was significantly associated with *HLA-B*27:05*. The association with *HLA-B*27:05* was unchanged when cases induced by the antithyroid propylthiouracil were removed.

The indication for antithyroid treatment is most commonly Graves' disease—an autoimmune disease that causes hyperthyroidism.[25] Because Graves' disease has been associated with specific HLA types in white people, particularly *HLA-C*07* and *HLA-B*08*,[26] it was necessary to control for confounding by indication. This was done by comparing antithyroid drug-induced cases with controls matched for hyperthyroidism, and as expected the OR for *HLA-B*08:01* decreased because of the association of *HLA-B*08* with Graves' disease. However, the OR for *HLA-B*27:05* simultaneously increased, suggesting that the association with antithyroid drug-induced agranulocytosis is genuine.

Because of known differences in HLA structure across populations, it is not unexpected that HLA types associated with a specific adverse drug reaction might differ between populations. Antithyroid drug-induced agranulocytosis has been strongly associated with *HLA-B*38:02* in ethnic Chinese people in Taiwan and Hong Kong, and with *HLA-DRB1*08:03* in Japanese people and ethnic Chinese people in Taiwan.[8–10] *HLA-B*38:02* and *HLA-DRB1*08:03* are fairly common in Asian people (allele frequencies 0·036 and 0·048), but are rare in white people (allele frequencies 0·004 and 0·002), and are therefore unlikely to be detected as risk alleles in Europe.[27] Similarly, allele frequencies of *HLA-B*27:05* and *HLA-B*08:01* in Han Chinese people are estimated as 0·005 and 0·007,[28] whereas they were common in the controls in our study (0·078 and 0·114, respectively).

Biological mechanisms for adverse drug reactions associated with HLA proteins are largely not well understood. Chen and colleagues[8] used three-dimensional structure modelling to show how HLA-B*38:02 and HLA-B*38:01 proteins interact with antithyroid drugs. However, since the peptides involved in the process were unknown, the authors concluded that the binding modes and affinities of the HLA–peptide complex could not be determined.

In our study, we noted interesting genome-wide significant associations between antithyroid drug-induced agranulocytosis and the *EHMT2* and *FOXF2* genes. Although we identified associations between antithyroid drug-induced agranulocytosis and classical HLA alleles, we cannot exclude the possibility that nearby regulatory variants are the genuine causative factors. The top SNP rs652888 is intronic in *EHMT2*, which encodes the transcriptional repressor G9a. The *FOXF2* SNPs rs199564443 (flanking the promoter) and rs115308096 (in intron 1) are both located close to gene regulatory elements. *FOXF2* is a transcription factor expressed in many cells including lymph nodes. The predictive ability of our model increased when rs652888 and rs199564443 (alternatively rs115308096, which is easier to genotype) were used in combination with the HLA-B marker rs1071816. For example, heterozygosity for all three SNPs increased the risk of agranulocytosis to roughly 30% and the estimated OR to 753. Conversely, homozygosity for *HLA-B*27:05* was associated with an estimated OR of 53, which suggests an added value of SNPs in genes involved in transcriptional regulation.

This study has some limitations. First, the small sample size decreased the power to detect uncommon variants associated with the adverse drugs reaction. To alleviate this limitation, we could have actively recruited matched controls, but to find more patients with antithyroid drug-induced agranulocytosis would have been difficult. Second, HLA imputation was used instead of direct HLA genotyping. The quality of imputed HLA variants are highly dependent on the reference panel, which in our study was 5225 European individuals from the Type 1 Diabetes Genetics Consortium (T1DGC).[19] These individuals were mostly self-identified white European people.[29] With the T1DGC reference panel, the imputation at four-digit resolution was shown to be accurate (96·7%) in the British 1958 Birth Cohort (n=918). However, there are errors even in established methods for HLA typing that might have limited the assessment of accuracy.[23] Additionally, our cohort was from four countries across Europe whereas the reference panel was from countries in Asia, North America, and Europe, but since most participants were from northern Europe and North America, we believe the T1DGC reference panel to be representative for our HLA imputation.

We have shown that drug-induced agranulocytosis in people of European ancestry, particularly when induced by antithyroid agents, is associated with *HLA-B*27:05* and with nearby genes. These are not the same risk markers as reported in ethnic Chinese people and Japanese people.[8–10] Both patient ethnicity and genotype should therefore be taken into account before starting antithyroid treatment. It has been predicted that every person will have their pharmacogenome in their medical record in the future.[30] We propose that white European people known to carry combinations of *HLA-B*27:05* or rs652888, rs199564443, and rs1071816 should be offered an alternative treatment for hyperthyroidism, such as radioiodine or surgery.[31] It will not be possible to avoid treatment with antithyroid drugs in all carriers of *HLA-B*27:05* or rs652888, rs199564443, and rs1071816, and for these patients, intensified monitoring is warranted. This individualisation would be a further step towards precision medicine.

Contributors

PH, MW, NE, EB-G, LI, RK, AC, MIL, QYY, and MM designed the study. PH, MW, LI, EB-G, RK, AC, MIL, QYY, JM, and PKM were responsible for recruitment of study participants, data acquisition, and sample preparation. ESP was responsible for case adjudication. TA did genotyping. NE analysed the data. PH, MW, and NE interpreted the data and wrote the draft report. LI, EB-G, RK, AC, MIL, QYY, JM, PKEM, MM, ESP, and TA revised the draft report.

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Declaration of interests

RK obtained personal fees from Bayer Pharma AG during the course of the study. PH, NE, LI, EB-G, AC, MIL, ESP, MM, JM, TA, Q-YY, PKEM, and MW declare no competing interests.

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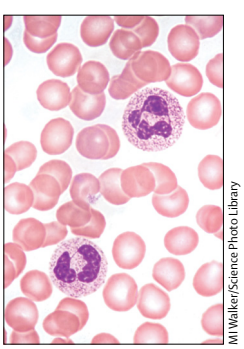
First step towards precision medicine for antithyroid drug-induced agranulocytosis

Antithyroid drugs are a cornerstone of treatment for hyperthyroidism, but can cause life-threatening agranulocytosis. In *The Lancet Diabetes & Endocrinology*, Pär Hallberg and colleagues[1] present results from the EuDAC study, a genome-wide association study of drug-induced agranulocytosis in European people, focusing on cases caused by antithyroid drugs. Their results are based on 39 agranulocytosis cases caused by antithyroid drugs and 5170 population controls from four European countries (Sweden, Spain, France, and Germany). They used genome-wide single nucleotide polymorphism (SNP) genotypes and the imputed HLA genotypes for the analysis. They identified *HLA-B*27:05* as the major susceptibility gene (odds ratio [OR] 7·30, 95% CI 3·81–13·96, $p=1·91\times10^{-9}$). Three SNPs (rs652888, rs19956443, and rs1071816) in the HLA region also showed association signals and were used to build a regression model for risk prediction.

This was a long awaited and reasonably powered study investigating the important issue of antithyroid drug-induced agranulocytosis in a mostly white European population. Previous genetic association studies in Taiwanese people,[2] in ethnic Chinese people in Hong Kong,[3] and in Japanese people[4] showed that *HLA-B*38:02* and *HLA-DRB1*08:03* were the susceptibility genes for antithyroid drug-induced agranulocytosis in these populations. White European people were postulated to have different susceptibility HLA genes from Asian people,[2] because the allele frequencies of the two known Asian HLA susceptibility genes were too low in Europeans. The identification of a susceptibility association of *HLA-B*27:05* in white European people confirms this assumption. A similar interpopulation difference had previously been reported for carbamazepine-induced Stevens–Johnson syndrome, for which *HLA-B*15:02* was initially shown to be the risk gene in Taiwanese people,[5] whereas *HLA-A*03:01* was identified as the risk gene in Japanese[6] and northern European people.[7] Therefore, identification of population-specific pharmacogenetic markers is important for precision medicine. Furthermore, comparison of the structures of these different risk HLA proteins might provide useful insights for future pathophysiological research.[8]

Hallberg and colleagues' study[1] illustrates the importance of finding appropriate control samples. Using the original cohort (39 agranulocytosis cases *vs* 5170 population controls), the researchers identified both *HLA-B*27:05* and *HLA-B*08:01* as the susceptibility alleles. However, to avoid confounding by indication for treatment[9] (which means associations with the reason for treatment, but not with the adverse effect of treatment), they later used only 49 Swedish controls matched for hyperthyroidism. Comparing agranulocytosis cases and controls with hyperthyroidism, the OR for carrying *HLA-B*27:05* increased, while the OR for *HLA-B*08:01* decreased. Since *HLA-B*08:01* had previously been shown to be associated with Graves' disease,[10] the investigators reasoned that the identified association of *HLA-B*08:01* in this study might be (at least partly) biased by confounding by indication. In our opinion, the possibility of *HLA-B*08:01* being a susceptibility gene for antithyroid drug-induced agranulocytosis has not been completely excluded. In European people, *HLA-B*08:01* could still be associated with both the risk



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of Graves' disease and the risk of agranulocytosis after antithyroid drug treatment. More samples are needed to address this issue. Notably, in the previous study done in Taiwanese people,[2] we used hyperthyroidism controls throughout the study, whereas, in Hallberg and colleagues' study,[1] 49 controls of this kind were used.

In addition to identifying *HLA-B*27:05* as the risk gene, Hallberg and colleagues[1] reported that three HLA-region SNPs (rs652888, rs19956443, and rs1071816) were also associated with antithyroid drug-induced agranulocytosis. In their analyses, these SNPs were in weak-to-moderate linkage disequilibrium with *HLA-B*27:05*, *HLA-B*08:01*, or both, but also had independent associations. Genetic signals in the HLA region are quite difficult to dissect because of the complexity of extended linkage disequilibrium structures and long haplotypes in this region. Although this study has reasonably good statistical power to detect major pharmacogenetic signals, the sample size might still be insufficient to fully delineate the complicated associations in the HLA region. Vigilance and caution are needed when applying knowledge of these three SNPs in precision medicine in other populations. The linkage disequilibrium and haplotype structures in the HLA region might be different across populations, and the tagging SNPs for specific HLA genes or alleles need to be established accordingly. Identification of the *HLA-B*27:05* association with antithyroid drug-induced agranulocytosis was based on imputation data without direct HLA typing. Although there was a high HLA imputation accuracy (96·7%) in the 1958 Birth Cohort used,[11] this cohort was a quite homogeneous group compared with the populations in Hallberg and colleagues' study, whether this level of imputation accuracy was attained given a heterogeneous sample[12] remains a concern. Direct HLA typing would be necessary to achieve accurate genotype-based precision medicine for antithyroid drug use.

However, Hallberg and colleagues' study,[1] as well as those done in Asian people,[2–4] do indeed herald the dawn of genotype-based precision medicine for antithyroid drug use. This is important because agranulocytosis is the most serious complication of antithyroid drugs and can potentially be fatal. However, to date, these studies have not been able to answer several crucial questions. Are there major genetic determinants other than the classical HLA genes in the HLA region? Are there susceptibility genes outside the HLA region? Are there interactions between the susceptibility genes? To tackle these issues, studies with substantially larger sample sizes and genome-wide genotyping will be necessary. However, for such an uncommon adverse drug reaction, we foresee difficulties for investigators in the recruitment of a large enough number of cases and appropriate controls, and in obtaining blood or DNA samples from all individuals. Perhaps the big breakthrough will come when a large enough proportion of general populations can be genotyped or sequenced. Genetic study will thus become a science enabling genetic researchers and clinicians to link accurate phenotypes with existing genotypes.

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P-LC, CS-JF, S-RS, W-SY, and T-CC have a patent pending for a method and device for assessing the risk of adverse drug reactions.

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