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

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Research in context

Evidence before this study

The pathogenesis of antithyroid drug-induced agranulocytosis has previously been investigated with limited results. Immunological mechanisms such as antibodies to circulating neutrophils and T-cell activation against the blood marrow have been proposed, but the processes are not clearly understood. During the writing of this manuscript, two genome-wide association studies were published describing a strong association between agranulocytosis induced by antithyroid drugs and *HLA-B*38:02* and *HLA-DRB1*08:03* in ethnic Chinese. The prevalence of these alleles in Europeans is known to be much lower.

Added value of this study

This is the first study describing an association between the HLA region and antithyroid drug-induced agranulocytosis in Europeans. The study is strengthened by bringing together cohorts from four European countries. Notably, the associated HLA types differed between Europeans and the previously studied East-Asians (ethnic Chinese and Japanese). In Europeans, SNPs in a transcriptional repressor and a transcription factor close to HLA were also associated, but linkage disequilibrium (LD) 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 that need to be tested before the use of these drugs. In Europeans, the predicted probability of antithyroid drug-induced agranulocytosis was ~30% and the odds ratio 753 (95% CI [105 − 6812]) in heterozygous carriers of three SNPs on

chromosome 6. Genotyping of 238 patients offers a means to avoid a potentially life-threatening adverse drug reaction in one patient by considering alternative treatments or intensified monitoring. Our results represent a further step towards the concept of precision medicine in patients with hyperthyroidism.

Abstract

Background: Drug-induced agranulocytosis is a potentially life-threatening adverse reaction. Genome-wide association studies (GWAS) in ethnic Chinese have shown an association between agranulocytosis induced by antithyroid agents and the human leukocyte antigen (HLA) alleles *HLA-B*38:02* and *HLA-DRB1*08:03*, but this has not been studied in Europeans. **Methods:** We performed a GWAS on European adults with drug-induced agranulocytosis (n=234, absolute neutrophil count <0·5*10^9/L (<500/µL)) and population controls (n=5170). Thirty-nine of the agranulocytosis cases were induced by the antithyroid agents thiamazole (methimazole), carbimazole, or propylthiouracil. After imputation and HLA allele prediction, 9380034 single nucleotide polymorphisms (SNPs) and 180 HLA alleles were tested for association. The genome-wide significance threshold was p<5*10^{-8}. **Findings:** Overall, drug-induced agranulocytosis was significantly associated with the HLA region on chromosome 6 with odds ratio 3·24 for *HLA-B*27:05* (95% CI [2·31, 4·55] p=1·20*10^{-11}), and 3·57 for the top SNP rs114291795 (95% CI [2·61, 4·90] p=2·32*10^{-15}). Drug-specific analysis showed that the association with *HLA-B*27:05* was largely driven by cases induced by antithyroid agents. In a multiple model, the odds ratio for *HLA-B*27:05* was 7·30 when antithyroid drug-induced agranulocytosis was compared with population controls (95% CI [3·81, 13·96] p=1·91*10^{-9}), and 16·91 when compared with a small group of hyperthyroid controls (95% CI [3·44, 83·17] p=5·04*10^{-4}). Three SNPs were strongly associated with antithyroid drug-induced agranulocytosis: rs652888 and rs199564443 that were independent of *HLA-B*27:05,* and rs1071816 that 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 ~30% (OR=753 95% CI [105 – 6812]), and to avoid one case ~238 patients would need to be genotyped. **Interpretation:** In Europeans, antithyroid drug-induced agranulocytosis was associated with *HLA-B*27:05* and with other SNPs on chromosome 6. In the future, carriers could be placed under intensified monitoring or offered alternative treatment for hyperthyroidism.

Introduction

Serious adverse drug reactions (ADRs) such as drug-induced agranulocytosis can severely limit the use of a drug. Agranulocytosis is defined as a decline in absolute neutrophil count to <0·5x10^9/L (<500/μL).[1] It is causally related to over 125 non-chemotherapy drugs, and among the most well-documented are thiourea drugs thiamazole (methimazole), carbimazole and propylthiouracil used for the treatment of hyperthyroidism.[2,3] The risk of agranulocytosis induced by antithyroid agents is estimated to 0·2-0·5%, and onset is typically during the first three 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 approximately two-thirds of patients.[6] Despite appropriate management, the mortality rate of agranulocytosis induced by non-chemotherapy drugs is 4-5%.[6]

The current understanding of the pathogenic mechanism behind drug-induced agranulocytosis is minimal. Antibodies to circulating neutrophils have been found suggesting an immunological mechanism.[7] Another postulated mechanism is induction of T cell-mediated reactions against the myeloid cell line by oxidative drug metabolites.[7] Recently, an association between antithyroid drug-induced agranulocytosis and the human leukocyte antigen (HLA) alleles *HLA-B*38:02*[8,9] and *HLA-DRB1*08:03*[8] have been discovered in ethnic Chinese in Taiwan and Hong Kong. A similar association with *HLA-DRB1*08:03*, although not as strong, has previously been suggested in the Japanese.[10] Associations were the strongest for the azole drugs thiamazole (methimazole) and carbimazole,[8-10] and in one study the association with

HLA-B*38:02 was strengthened when cases induced by propylthiouracil were removed.[9] To enable genome-wide association studies (GWAS) of this rare condition in Europe, we formed the European Drug-induced Agranulocytosis Consortium (EuDAC). We here present the overall results for all drugs and focus in particular on antithyroid agents.

Methods

Ethical statement

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; CPP Sud-Ouest et Outre-Mer I N°1-09-24, Toulouse, France). Research was carried out in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The study protocol has been indexed in The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) register at www.encepp.eu (The EuDAC Study).

Sample description

EuDAC consists of a network of investigators in Sweden, Spain, France and Germany. The basis for case recruitment in Sweden and France was through nation-wide spontaneous ADR reports sent from health care professionals to the respective national drug regulatory authority. In Spain, cases were recruited both from spontaneous ADR reports and through active surveillance at 17 hospitals in Barcelona. German cases were recruited through active surveillance at 50 hospitals in Berlin as described.[11] No extra blood sampling or investigation was undertaken for cases collected through active surveillance as compared to those collected through spontaneous reports.

Each included subject was at least 18 years of age and able to give informed consent. Cases were patients who had developed an absolute neutrophil count $<0\cdot5*10^9$/L

(<500/μL) during drug therapy or within seven days of stopping medication. Each case was required to exhibit complete recovery after cessation of the drug with an absolute neutrophil count >1·0*10^9/L (>1000/μL) or a compatible bone marrow aspirate or biopsy. Causality assessment was according to the WHO standard algorithm.[12] Exclusion criteria were recent chemotherapy, radiation therapy within the last month, bone marrow transplantation, ongoing infection with Epstein-Barr virus, hepatitis A, HIV, CMV or parvovirus B19, sepsis, miliary tuberculosis, chronic neutropenia (congenital cyclic, idiopathic), immunosuppressive therapy with cytotoxic drugs, malignant infiltration of bone marrow, hematological diseases (e.g. myelodysplasia, aplastic anemia, pancytopenia, other blood dyscrasias, such as hemoglobin ≤100 g/L and platelets ≤100*10^9/L), and systemic lupus erythematosus. There were no other restrictions relating to other drug treatments, primary diagnosis, or ancestry.

We collected clinical data (demographics, medical history, drug treatment history, laboratory data, and ancestry) through interviews using a standardized questionnaire, and by obtaining and reviewing medical records. At each center, cases were evaluated by at least one senior investigator, and a final adjudication of the complete dataset was performed by a specialist in hematology. DNA was extracted from peripheral venous blood. The total number of cases fulfilling all requirements was 234 (Sweden, 94; Spain, 66; Germany, 41; France, 33), and 39 of these cases were induced by antithyroid agents (Sweden, 25; Spain, 4; Germany, 6; France, 4).

Consenting population controls were available from Sweden, Spain and Germany. In total, 5170 controls (4891 unrelated individuals from the Swedish Twin Registry,[13] 183

Spanish individuals, and 96 German individuals[11]) were utilized. Of the 183 Spanish

controls, 147 had been recruited in a previous study of upper gastrointestinal

bleeding,[14] while the remaining 36 were healthy control subjects. Matching for

hyperthyroidism was performed by using 49 controls in the Swedish Twin Registry that

had been treated for hyperthyroidism according to the Swedish Prescribed Drug

Register (available from 2005) or the Swedish National Patient Register (available from

1967).

Power calculation

Power calculations were made both for the total number of cases (Supplementary

Figure S1A), and for the subset attributed to antithyroid drugs (Supplementary Figure

S1B). The power to detect an odds ratio (OR) ≥5 was 99% when using all 234 cases and

5100 controls with a minor allele frequency (MAF) of ≥5%. The power to detect an OR

of ≥5 was 80% when using 39 cases and 5100 controls with MAF ≥20%. These

calculations are based on a genome-wide significance level of 5×10^{-8}, an ADR

prevalence of 1% and an additive genetic model.[15]

Genome-wide array data and analyses

A total of 193 cases of drug-induced agranulocytosis from Sweden, Spain and France

and 147 Spanish controls were genotyped with the Illumina HumanOmni 2.5M chip

(Figure 1). The remaining 36 Spanish controls had been 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 using the Genome Studio software from Illumina.

GWAS quality control (QC) and data management was performed using PLINK v1.9. The resulting merged data included 596010 SNPs on the autosomal chromosomes. Imputation of genotypes was performed using PhaseIT[16] and Impute v2.[17] The total number of SNPs after imputation was 9380034. In order to account for possible population stratification, principal component analysis (PCA) was performed (Supplementary Figure S2). Six genetic outliers were detected using PCA, all were cases (Supplementary Figure S3). These cases were not excluded from the data, however, sensitivity analyses were performed by reanalyzing each top hit with the six cases excluded. See supplementary methods for additional details on QC, PCA and imputation.

All genome-wide analyses were adjusted for sex and the first four genetic principal components from the PCA. SNP effects were modeled as additive. The conventional genome-wide significance threshold $p < 5*10^{-8}$ was used to correct for multiple testing.[18] Results are presented as Manhattan plots and QQ-plots. When genome-wide significant signals were found, analyses were performed sequentially by adjusting for each genome-wide significant signal until no genome-wide signals were left. Due to the heterogeneity in the data, follow up analyses were performed stratified by country of inclusion and drug class. Logistic regression was used to estimate univariate and multiple models. In the case when a value of zero was present in one cell, OR and CI were estimated by adding 0·5 to all cells. The predictive ability of the univariate and

multiple models was expressed as the C-statistic. Our definition of the optimal cutoff for deciding when to consider a patient for alternative treatment (using a prediction model) was the cutoff that maximized both sensitivity and specificity. Genome-wide analyses were performed using PLINK v1.9 and individual SNP analyses were performed using R 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria).

HLA allele imputation

Imputation of two and four digit classical HLA alleles (n=180), amino acid residues and individual SNPs was performed on the non-imputed merged and quality controlled genome-wide data using the software SNP2HLA with a reference panel of 5225 individuals.[19] To avoid confounding by indication, top HLA signals were tested using a cohort of cases and controls matched for hyperthyroidism that was available from Sweden.

Results

Overall 243 cases were collected for the study. Nine were excluded after adjudication: seven Swedish cases (6.9%; 3 due to exposure to chemotherapy, 1 due to negative rechallenge, 2 due to unknown white blood cell count, 1 due to a diagnosis of chronic lymphatic leukaemia) and two Spanish cases (2.9%; both due to missing clinical data). No German or French cases were excluded. Characteristics of all included cases are shown in Table 1. Among them, 39 cases were induced by the antithyroid agents thiamazole (n=29), carbimazole (n=5), and propylthiouracil (n=5). Six of the 234 cases, including one case induced by antithyroid agents, were of non-Caucasian origin (Supplementary Figure S3).

Genome-wide association analyses – overall results

In the complete cohort of 234 cases and 5170 controls, we found genome-wide significant associations with SNPs in the major histocompatibility complex (MHC) region (HLA region) on chromosome 6 (Figure 2A). The QQ-plot is shown in Supplementary Figure S4. The SNP with the best evidence for association was rs114291795 located in an intron of the gene MHC class I polypeptide-related sequence A (*MICA*), OR 3·57 (95% CI [2·61, 4·90] p=2·32*10^{-15}, Table 2-I, Supplementary Table S1). After adjusting for this SNP, a strong signal remained for rs1811197, which flanks *HLA-B*, OR 2·40 (95% CI [1·89, 3·06] p=9·42*10^{-13}). No SNP reached genome-wide significance after adjusting for both rs114291795 and rs1811197. Stratification by country of origin (Supplementary Figure S5) showed a

similar pattern in Sweden, Germany and Spain. Cases from France were not tested separately due to lack of French controls.

Genome-wide association analyses by drug class

Stratification by the main drug classes revealed that the associations on chromosome 6 were largely driven by the 39 cases induced by antithyroid agents (Figure 2B). The associations were similar when the 5 cases on propylthiouracil were excluded (data not shown). The top SNP for cases induced by all antithyroid agents was rs652888 located in an intron of euchromatic histone-lysine N-methyltransferase 2 (*EHMT2*), OR 4·73 (95% CI [3·00, 7·44] *p*=1·92*10^{-11}, Table 2-II, Supplementary Table S2). After adjusting for this SNP, a strong signal remained for the insertion/deletion rs199564443 flanking the gene forkhead box F2 (*FOXF2*), OR 17·42 (95% CI [7·38, 41·12] *p*=7·04*10^{-11}). After adjusting for both rs652888 and rs199564443, a signal remained for rs1071816 located in exon 2 of *HLA-B*, OR 5·27 (95% CI [3·06, 9·10] *p*=2·35*10^{-9}). Adjusting for all three SNPs revealed a significant association with rs111618861 in an intergenic region on chromosome 8, OR 13·75 (95% CI [5·46, 34·64] *p*=2·71*10^{-8}, Table 2-II, Supplementary Table S2).

The estimated univariate ORs for the chromosome 6 associations in the overall analysis (rs114291795 and rs1811197) as well as for the top signals in cases induced by antithyroid agents (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 using all 4891 Swedish controls and then using the 49 Swedish controls matched for hyperthyroidism. The odds ratios for all SNPs,

except rs1811197, increased when using matched controls. It should be noted that 24% of the 25 Swedish cases induced by antithyroid agents had the deletion caused by rs199564443, compared with none of the 49 matched controls, OR 33·00 (95% CI [1·77, 614] *p*=0·019). A sensitivity analysis for the top five SNPs, excluding six genetic outliers, produced near identical results (Supplementary Figure S6).

Analysis of four digit classical HLA alleles - overall results

After imputation of four digit HLA alleles, the respective univariate OR, 95% CI, and p-value was calculated for each HLA allele vs the outcome agranulocytosis (Table 2-III, Supplementary Table S3 and Supplementary Figure S7). The top predicted allele was *HLA-B*27:05*, OR 3·24 (95% CI [2·31, 4·55] *p*=1·20*10^{-11}). After adjusting for this allele the OR with *HLA-B*08:01* was 2·14 (95% CI [1·61, 2·84] *p*=1·48*10^{-7}). Agranulocytosis was not significantly associated with any HLA allele in the HLA region after adjusting for both *HLA-B*27:05* and *HLA-B*08:01*. The linkage disequilibrium (LD) between the classical HLA alleles *HLA-B*27:05* and *HLA-B*08:0*1, and the SNPs rs114291795, rs1811197, rs652888, rs199564443 and rs1071816 is presented in Supplementary Table S4. In summary, *HLA-B*27:05* was in high LD with rs114291795 and in moderate LD with rs1071816*,* and *HLA-B*08:01* was in moderate LD with rs1811197 and rs652888. The insertion/deletion rs199564443, located outside the HLA region, was not in LD with *HLA-B*27:05* or *HLA-B*08:01.*

Analysis of four digit classical HLA alleles by drug class

Drug-specific analysis showed that the overall association with *HLA-B*27:05* was driven by agranulocytosis induced by antithyroid agents. The univariate OR for carrying

HLA-B*27:05 was 5·44 among these cases (95% CI [2·94, 10·06] *p*=6·88*10^{-8}, Table 2-IV, Supplementary Table S5). The top HLA imputed variant, rs652888, was the same SNP as in the genome-wide SNP analysis (Supplementary Figure S8A). After adjusting for *HLA-B*27:05*, the top variant rs652888 remained, while the top four digit HLA signal was *HLA-B*08:01,* OR 3·88 (95% CI [2·21, 6·81] *p*=2·20*10^{-6}, Supplementary Figure S8B). After adjusting for both *HLA-B*27:05* and *HLA-B*08:01*, all HLA signals disappeared including rs652888 that is a moderate tag for *HLA-B*08:01* (r^2=0·4, Supplementary Table S4).

Multiple models of four digit classical HLA alleles

Multiple 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 (95% CI [3·81, 13·96] *p*=1·91*10^{-9}) when restricting analysis to agranulocytosis induced by antithyroid agents. When Swedish antithyroid cases were compared with Swedish controls, OR for *HLA-B*27:05* was 7·64 (95% CI [3·71, 15·71] *p*=3·29*10^{-8}). The corresponding ORs for *HLA-B*08:01* are shown in Figure 3B. The associations with *HLA-B*27:05* and *HLA-B*08:01* were similar when the five cases on propylthiouracil were excluded (data not shown). To avoid confounding by indication for treatment, the two top HLA-B signals were tested using controls matched for hyperthyroidism. The OR for carrying *HLA-B*27:05* increased to 16·91 (95% CI [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, excluding the six genetic outliers, produced near identical results (Supplementary Figure S9).

Predictive ability and clinical implications for cases induced by antithyroid agents

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 rs652888, rs199564443 and rs1071816 were 0·773, 0·608 and 0·757, respectively, and when combined into a multiple prediction model, the C-statistic was 0·889. Hence we focused on the predictive ability of a model combining the three SNPs. A nomogram for estimating the probability of experiencing antithyroid-induced agranulocytosis from the multiple prediction model is presented in Figure 4. As an example, the predicted probability of agranulocytosis in heterozygous carriers of all three SNPs would be ~30% and the estimated OR 753 (95% CI [105 – 6812]) when combining the SNPs in a logistic regression model. In comparison, the estimated OR for a person heterozygous or homozygous for *HLA-B*27:05* was ~7 and ~53 respectively (Figure 3B).

The optimal cutoff for deciding when to consider a patient for alternative treatment was at a predicted probability of 0·005. This gives an estimated sensitivity of 84·2% and a specificity of 86·1%, which means that 13·9% would be falsely predicted to be cases (Supplementary Figure S10). 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 an incidence of antithyroid-induced agranulocytosis of 0·005 (1 in 200 patients starting antithyroid agents)[4] and a sensitivity of 84·2%, we could theoretically reduce the incidence to 0·00079 (0·005–0·842*0·005). The number needed to genotype (NNG) to

avoid one case of antithyroid-induced agranulocytosis was estimated to 238, which is

the reciprocal of the absolute risk reduction, i.e. 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 associated with the HLA region, including Stevens-Johnson syndrome, drug-induced liver injury and clozapine-induced agranulocytosis.[20] The HLA region is challenging to study due to high gene density,[21] high degree of polymorphism[22] and an extended LD that makes the causative variant difficult to identify.[21]

Genetic susceptibility traits for rare serious ADRs are drug specific, as previously shown in genome-wide studies on drug-induced liver injury.[20] Overall, drug-induced agranulocytosis was associated with *HLA-B*27:05*. *HLA-B*27:05* has previously been associated with agranulocytosis induced by another azole drug, levamisole.[23] In our cohort, no other single drug class apart from antithyroid agents 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.[24] As Graves' disease per se has been associated with certain HLA types in Caucasians, in particular HLA-C*07 and HLA-B*08,[25] it was necessary to control for confounding by indication. This was done by comparing antithyroid-induced cases with controls matched for hyperthyroidism, and as expected the OR for *HLA-B*08:01* decreased due to the association of HLA-B*08 with Graves' disease. However, the OR for *HLA-B*27:05* simultaneously increased, indicating that the association with antithyroid-induced agranulocytosis is genuine.

Due to known differences in HLA-structure across populations it is not unexpected that HLA-types associated with a specific ADR may differ between populations. Antithyroid drug-induced agranulocytosis has been strongly associated with *HLA-B*38:02* in ethnic Chinese, and with *HLA-DRB1*08:03* in the Chinese and Japanese.[8-10] *HLA-B*38:02* and *HLA-DRB1*08:03* are relatively common in Asians (allele frequencies 0·036 and 0·048), but rare in Caucasians (allele frequencies 0·004 and 0·002), and hence unlikely to be detected as risk alleles in Europe.[26] Similarly, allele frequencies of *HLA-B*27:05* and *HLA-B*08:01* in Han Chinese are estimated to only 0·005 and 0·007,[27] while they were common among controls in our study, 0·078 and 0·114, respectively.

Biological mechanisms for ADRs associated with HLA proteins are in most cases not understood. Chen *et al.* performed 3D structure modelling in an effort to show how HLA-B*38:02 and HLA-B*38:01 proteins interact with antithyroid agents.[8] However, since the peptides participating in the process were unknown, the authors concluded that the binding modes and affinities of the HLA-peptide complex could not yet be determined.

In our study, interesting genome-wide significant associations were also observed between antithyroid drug-induced agranulocytosis and the *EHMT2* and *FOXF2* genes. Although we found associations between antithyroid drug-induced agranulocytosis and classical HLA alleles, we cannot exclude that nearby regulatory variants are the genuine causative factors. The lead SNP, i.e. rs652888, is intronic in *EHMT2* that encodes the transcriptional repressor G9a, while rs199564443 is located in the

promoter and rs115308096 in intron one of *FOXF2* close to 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 instance, heterozygosity for all three SNPs increased the risk of agranulocytosis to ~30% and the estimated OR to 753. In comparison, 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.

There are limitations of this study. First, the small sample size decreased the power to detect uncommon variants associated with the reaction. To alleviate this we could have actively recruited matched controls, but to find more cases of 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 had been recruited in Great Britain by the Wellcome Trust Case Control Consortium, and the vast majority were self-identified white Europeans.[28] Using 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 may have limited the evaluation of accuracy.[22] An additional impediment was that our cohort is from four countries across Europe, but since the majority is Northern European, we believe the T1DGC reference panel to be representative for our HLA imputation.

In conclusion, 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.[8,9] Both patient ethnicity and genotype should therefore be taken into consideration before starting antithyroid treatment. It has been predicted that in the future everyone will have the pharmacogenome in their medical record.[29] We advocate that Europeans known to carry combinations of *HLA-B*27:05* or rs652888, rs199564443 and rs1071816 are offered an alternative treatment for hyperthyroidism, such as radioiodine or surgery.[30] 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, intensified monitoring is warranted. This individualisation would be a further step towards precision medicine, which is proposed as an important part of future healthcare.

Acknowledgements

Authorship contributions

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Disclosure of conflicts of interest

None of the authors declare any conflicts of interest.

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Table 1. Patient characteristics. All cases, and cases induced by antithyroid drugs.

	All cases (n=234)	Antithyroid drug-induced cases (n=39)
Proportion male, n (%)	96 (41)	8 (20·5)
Age group, n (%)		
<25	19 (8·1)	2 (5·1)
25-29	13 (5·6)	4 (10·3)
30-34	18 (7·7)	7 (17·9)
35-39	21 (9·0)	4 (10·3)
40-44	14 (6·0)	3 (7·7)
45-49	16 (6·8)	3 (7·7)
50-54	25 (10·7)	3 (7·7)
55-59	30 (12·8)	4 (10·3)
60-64	22 (9·4)	3 (7·7)
65-69	16 (6·8)	3 (7·7)
70-74	13 (5·6)	1 (2·6)
>75	27 (11·5)	2 (5·1)
Ethnicity, n (%)		
Swedish	84 (35·9)	22 (56·4)
Spanish	58 (24·8)	3 (7·7)
German	41 (17·5)	6 (15·4)
French	21 (9·0)	1 (2·6)
Other European	18 (7·7)	6 (15·4)
Non-European	8 (3·4)	1 (2·6)
Unknown	4 (1·7)	0 (0)
Indication for treatment, n (%)		
Infection	119 (50·9)	
Pain condition	115 (49·1)	
Cardiovascular disease	53 (22·6)	
Thyroid disease	40 (17·1)	
· Graves' thyrotoxicosis		13 (33·3)
· Hyperthyroidism unspecified		26 (66·7)
· Hypothyroidism	1 (0·4)	
Rheumatic disease	40 (17·1)	
Gastrointestinal disease	39 (16·7)	
Psychiatric disease	31 (13·2)	
Neurologic disease	19 (8·1)	
Other	34 (14·5)	
Drug type, n (%)		
Beta-lactam antibiotics	59 (25·2)	
Metamizole (dipyrone)	43 (18·4)	
Antithyroid agents	39 (16·7)	
· Thiamazole (methimazole)		29 (74·4)
· Propylthiouracil		5 (12·8)
· Carbimazole		5 (12·8)
Sulfasalazine	36 (15·4)	
Other	57 (24·4)	
Mean daily dose, mg [range]		
· Thiamazole (methimazole)		28 [7·5-45]
· Carbimazole		20ł
· Propylthiouracil		250 [100-300]
Proportion with co-suspected drugs, n (%)		5 (12·8)
Mean time to onset, days [range]		45 [1-180]‡
Mean lowest neutrophil count, *10^{9}cells/l [range]		0.11 [0-0·5]

ł Daily dose missing for 4 of 5 cases.
‡ One outlier with time to onset of 900 days excluded.

Table 2. I) Top GWAS results based on 9,380,034 single nucleotide polymorphisms (SNPs) after imputation for all cases. A) Adjusted by sex and genetic principal components 1-4; B) adjusted by sex and genetic principal components 1-4 and rs114291795. After adjusting for both rs114291795 and rs1811197 no genome-wide significant signals were left. **II).** Top GWAS results for antithyroid induced cases vs all controls based on 9,380,034 SNPs after imputation. Adjusted by A) sex and genetic principal components 1-4; B) sex, genetic principal components 1-4 and rs652888; C) sex, genetic principal components 1-4, rs652888 and rs199564443. D) sex, genetic principal components 1-4, rs652888, rs199564443 and rs1071816. After adjusting for variants in A, B, C and D no genome-wide significant signals were left. **III)** Univariate odds ratios for the top HLA alleles. The effect is modelled per increase of one present HLA allele. A) Adjusted by sex and genetic principal components 1-4; B) adjusted by sex, genetic principal components 1-4 and *HLA-B*27:05*. More results are available in the Supplementary Tables. **IV)** Univariate odds ratios for the top HLA alleles for antithyroid agents. The effect is modelled per increase of one present HLA allele. A) Adjusted by sex and genetic principal components 1-4; B) adjusted by sex, genetic principal components 1-4 and HLA-B*27:05. More results are available in the Supplementary Tables.

	CHR	SNP	BP	Alleles (minor/major)	N	MAF case	MAF control	OR [95% CI]	P	Nearby gene
I										
A	6	rs114291795	31377640	G/C	5376	0·14	0·06	3·57 [2·61, 4·90]	2·32E-15	*MICA-HCP5*
B	6	rs1811197	31335997	A/G	5366	0·29	0·16	2·40 [1·89, 3·06]	9·42E-13	
II										
A	6	rs652888	31851234	G/A	5203	0·54	0·20	4·73 [3·00, 7·44]	1·92E-11	*EHMT2*
B	6	rs199564443	1388978	C/CTTTT*	5149	0·12	0·01	17·42 [7·38, 41·12]	7·04E-11	*FOXF2*
C	6	rs1071816	31324536	C/T	4975	0·40	0·11	5·27 [3·06, 9·10]	2·35E-09	*HLA-B*
D	8	rs111618861	57044382	C/CA	4715	0·11	0·01	13·75 [5·46, 34·64]	2·71E-08	
III										
A	6	*HLA-B*27:05*	31431272	P/A	5404	0·10	0·06	3·24 [2·31, 4·55]	1·20E-11	
B	6	*HLA-B*08:01*	31431272	P/A	5404	0·16	0·11	2·14 [1·61, 2·84]	1·48E-07	
IV										
A	6	*HLA-B*27:05*	31431272	P/A	5209	0·19	0·06	5·44 [2·94, 10·06]	6·88E-08	
B	6	*HLA-B*08:01*	31431272	P/A	5209	0·26	0·11	3·88 [2·21, 6·81]	2·20E-06	

* Insertion/deletion variants truncated to max two alleles.
CHR=chromosome; SN*P*=single nucleotide polymorphism; B*P*=base pair; MAF=minor allele frequency; OR=odds ratio; CI=confidence interval.

Figure Legends

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. After quality control, the merged data included 596,010 genotyped single nucleotide polymorphisms (SNPs) that were further imputed using 1000 Genomes data and the software SNP2HLA.

Figure 2. A) Manhattan plot for the analysis of all 234 cases vs all 5170 controls in 9,380,034 SNPs after imputation adjusted by sex and genetic principal components 1-4. The red line displays the threshold for genome-wide significance of $5*10^{-8}$. The top single nucleotide polymorphisms (SNP) was rs114291795, located in the intron of the major histocompatibility complex (MHC) class I polypeptide-related sequence A (*MICA*) on chromosome 6. After adjustment for this SNP, a strong signal remained for rs1811197. **B)** Manhattan plot for the analysis of cases induced by antithyroid agents vs all controls in 9,380,034 single nucleotide polymorphisms (SNPs) after imputation, adjusted by sex and genetic principal components 1-4. The red line displays the threshold for genome-wide significance of $5*10^{-8}$. The top SNP was rs652888, located in the intron region of euchromatic histone-lysine N-methyltransferase 2 (*EHMT2*) on chromosome 6. After adjusting for this SNP, a strong signal remained for rs199564443, which is an insertion/deletion variation flanking forkhead box F2 (*FOXF2*) on chromosome 6. Adjusting for both rs652888 and rs199564443 showed a remaining strong signal for a coding SNP in HLA-B, rs1071816. Adjusting for all three chromosome 6 SNPs revealed a significant association with rs111618861 in an intergenic region on chromosome 8.

Figure 3. A) Forest plot of estimated univariate odds ratios (OR) with 95% confidence intervals (CI) showing the top single nucleotide polymorphisms (SNPs) on chromosome 6, rs114291795, rs1811197, rs652888, rs19956443 and rs1071816, for all cases vs all controls, and antithyroid-induced cases vs all controls. Also shown are Swedish antithyroid drug-induced cases vs all Swedish controls as well as Swedish antithyroid drug-induced cases vs Swedish controls matched for hyperthyroidism. Note that none of the matched Swedish controls had the variant rs199564443, and OR, CI and P were calculated manually why adjustment for gender and PC 1-4 was not possible. Numbers of cases/controls are given in the N column. **B)** Forest plot of estimated odds ratios (OR) with 95% confidence intervals (CI) based on a multiple model for the top HLA-B alleles showing A) all cases vs all controls, B) all cases induced by antithyroid agents vs all controls, C) all Swedish cases induced by antithyroid agents vs all Swedish controls, and D) all Swedish cases induced by antithyroid agents vs matched Swedish controls. The results are from a multiple model including both variants. Matched controls have been treated for hyperthyroidism. Numbers of cases/controls are given in the N column.

Figure 4. Nomogram for predicting the individual risk of antithyroid drug-induced agranulocytosis using 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 $e^{logit(p)}/(1+e^{logit(p)})$.

How to use the nomogram: Find the points each genotype renders in the top axis "Points", and plot the total sum on the axis "Total Points" which will show the predicted probability of agranulocytosis.

Examples: Heterozygosity for rs622888 (i.e. 1 minor allele G) and no other variants gives a sum of 4·2, which is below the threshold 0·005 on the "Total Points" axis. Heterozygosity for rs1071816 (i.e. 1 minor allele C) and no other variants gives a sum of 5, which is just at the threshold 0·005. All other possible combinations of variants are clearly above the threshold and indicate an increased risk of agranulocytosis. Heterozygosity for rs199564443 (i.e. deletion of TTTT) and no other variants gives 8·6 points and a predicted probability of ~0·15, heterozygosity for rs622888 *and* rs1071816 gives a total of 9·2 points and a predicted probability of ~0·02, heterozygosity for rs199564443 *and* rs622888 *and* rs1071816 gives a total of 17·8 points and a predicted probability of ~0·30, homozygosity for rs622888 *and* rs1071816 gives a total of 18·4 points and a predicted probability of ~ 0·35. In theory, heterozygosity for rs199564443 *and* homozygosity for rs622888 *and* rs1071816 would give a total of 28·5 points and a predicted probability of close to 1.
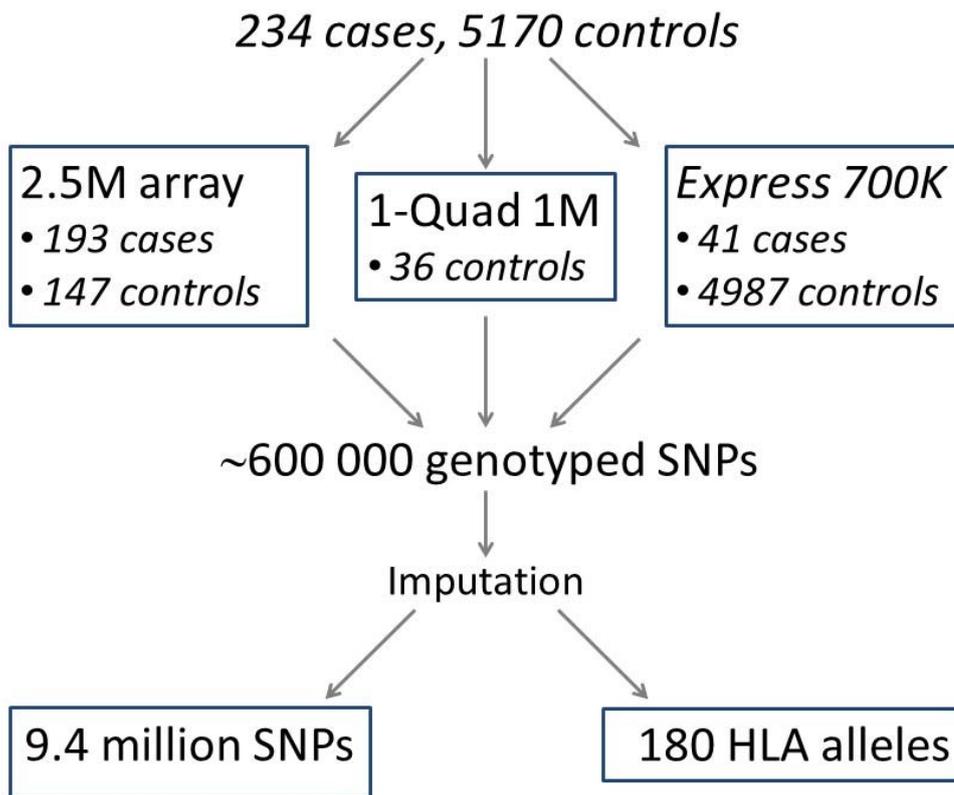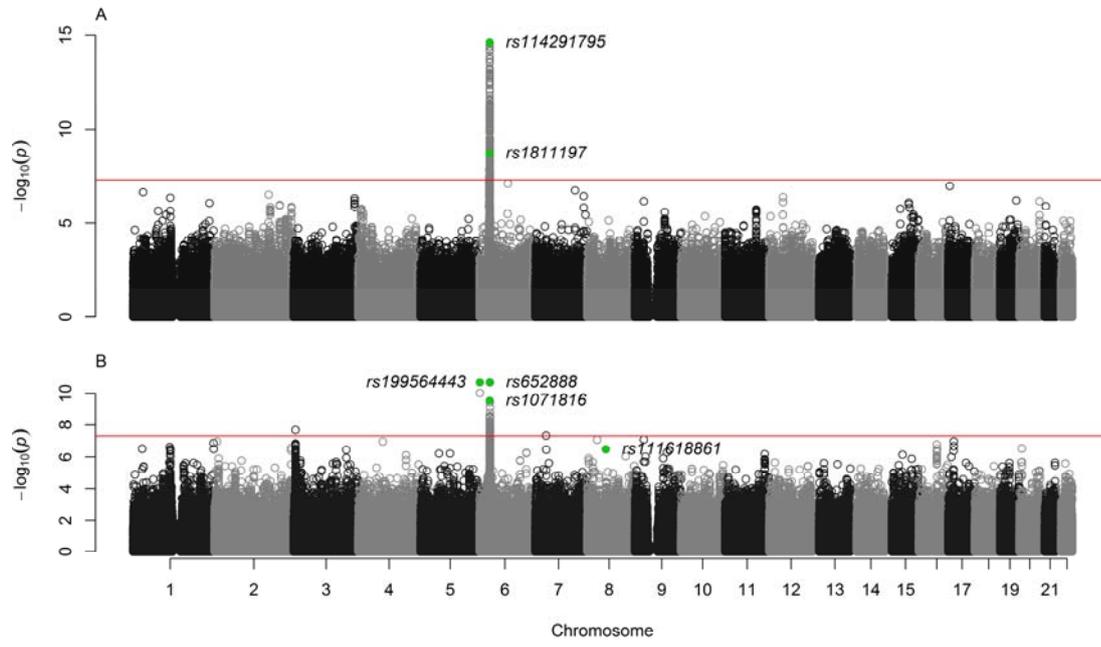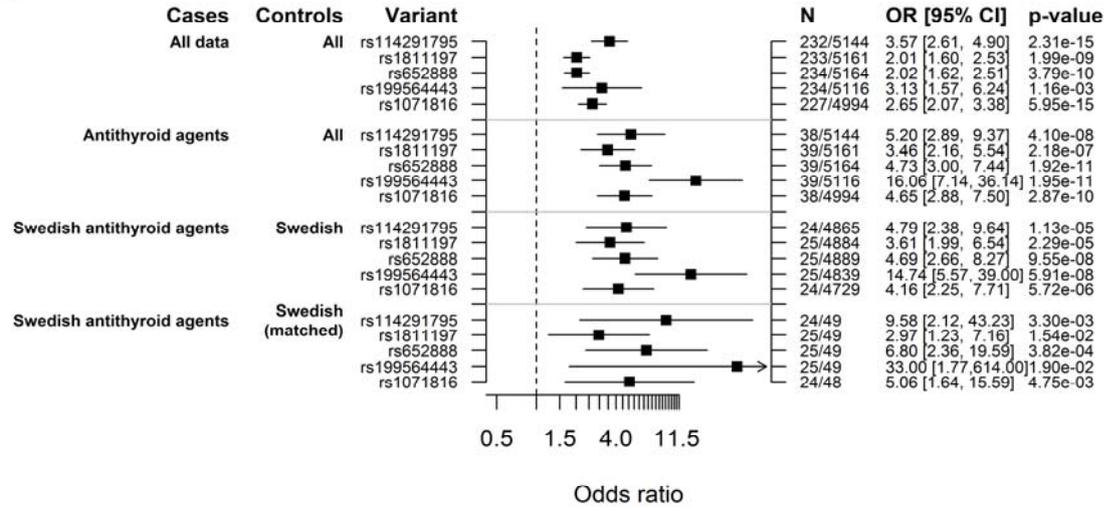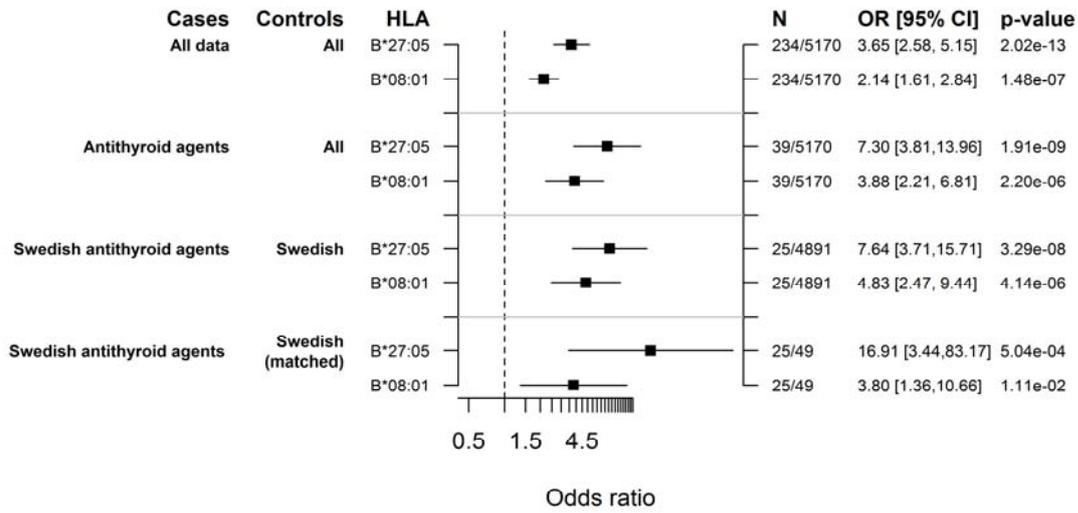
Figure 1.

Figure 2.

A

B

Figure 3.

Figure 4.