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Genome-wide mapping for clinically relevant predictors of lamotrigine- and phenytoin-induced hypersensitivity reactions

Aims: An association between carbamazepine-induced hypersensitivity and *HLA-A*3101* has been reported in populations of both European and Asian descent. We aimed to investigate *HLA-A*3101* and other common variants across the genome as markers for cutaneous adverse drug reactions (cADRs) attributed to lamotrigine and phenytoin. **Materials & methods:** We recruited patients with lamotrigine-induced cADRs (n = 46) and patients with phenytoin-cADRs (n = 44) and the 1958 British birth cohort was used as a control (n = 1296). *HLA-A*3101* was imputed from genome-wide association study data. We applied genome-wide association to study lamotrigine- and phenytoin-induced cADR, and total cADR cases combined. **Results:** Neither *HLA-A*3101* nor any other genetic marker significantly predicted lamotrigine- or phenytoin-induced cADRs. **Conclusion:** *HLA-A*3101* does not appear to be a predictor for lamotrigine- and phenytoin-induced cADRs in Europeans. Our genome-wide association study results do not support the existence of a clinically relevant common variant for the development of lamotrigine- or phenytoin-induced cADRs. As a predictive marker, *HLA-A*3101* appears to be specific for carbamazepine-induced cADRs.

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KEYWORDS: ■ epilepsy ■ GWAS ■ *HLA-A*3101* ■ hypersensitivity ■ lamotrigine ■ phenytoin

Carbamazepine (CBZ), lamotrigine (LTG) and phenytoin (PHT), among a class of antiepileptic drugs (AEDs) involved in sodium-channel modulation, are three of the leading first-line treatments for epilepsy. CBZ and PHT are among the earliest AEDs and are typically used to control partial seizures. LTG is well established as an effective drug for partial seizures, primary and secondary, generalized tonic-clonic seizures, as well as for the treatment of Lennox-Gastaut syndrome. All of them have also been found to be useful in other conditions, such as bipolar disorder and trigeminal neuralgia.

Despite being highly effective, CBZ, LTG and PHT are associated with cutaneous adverse drug reactions (cADRs), which encompasses a mild maculopapular eruption (MPE), a more serious hypersensitivity syndrome (HSS) with liver and other internal organ involvement to the potentially fatal Stevens-Johnson syndrome (SJS) and/or toxic epidermal necrolysis (TEN). The highest rate of AED-related cADRs occurs with CBZ, LTG and PHT, each of them are structurally related through an aromatic ring, yet each are targeted towards different seizure profiles [1].

CBZ has been the focus of numerous genetic studies, especially since the discovery of the genetic marker *HLA-B*1502* as a strong

predictor for CBZ-induced SJS/TEN in subjects of Han Chinese ancestry [2]. Studies by the same group have also shown that the *HLA-B*1502* association with SJS/TEN extends to PHT and oxcarbazepine (OXC) [3]. Further research has illustrated that *HLA-B*1502* also has a predictive ability for individuals of Malaysian, Indian, Thai and other Asian ethnicity [4–6]. The extent to which LTG-induced SJS/TEN associates with *HLA-B*1502* remains to be elucidated and requires further investigation. As noted by Shi and colleagues, of the seven reported cases of LTG-induced SJS/TEN, three were positive for *HLA-B*1502* [7]. To date, evidence from studies in Thai and Han Chinese populations suggests that there is no such association with the milder MPE phenotype triggered by either PHT or LTG [5,7,8]. However, a recent study has indicated that *HLA-B*1502* may have predictive value for OXC-induced MPE [9]. Although the authors only studied nine OXC-induced MPE cases, four of these were positive for *HLA-B*1502*, a significant observation when compared with population controls. These observations are particularly interesting given the close structural homology between OXC and CBZ.

However, the genetic association with *HLA-B*1502* appears to be limited to Asian

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populations. Studies in subjects of European ancestry have indicated that *HLA-B*1502* is not present in CBZ–SJS patients of European descent [10,11]. This is not surprising considering the decreasing frequency of the *HLA-B*1502* allele from a high frequency of 6% in Asia to virtually absent in Europe [12].

A 2006 study by Hung and colleagues reported an association between CBZ-induced MPE with *HLA-A*3101* among patients of Han Chinese descent [13]. Using a genome-wide approach, we identified *HLA-A*3101* as a clinically relevant predictor for the full spectrum of CBZ-induced hypersensitivity reactions in Europeans, while a separate study by Ozeki and colleagues reported a significant association with *HLA-A*3101* and CBZ-induced cADRs in a Japanese population [14,15]. The relationship between *HLA-A*3101* and CBZ-induced HSS has now also been reported in patients of Korean descent [16].

Here we set out to investigate whether *HLA-A*3101* or any other common genetic variant identified through genome-wide association studies (GWAS) may act as a predisposing factor for two of the other common AEDs known to cause cADRs, namely LTG and PHT.

Materials & methods

■ Case subjects

We obtained approval for this study from the local research ethics committees of each site. Written, informed consent was obtained from all subjects prior to participation. Patients were recruited through centers affiliated with the EPIGEN Consortium; namely Duke University (NC, USA), University College London (London, UK), Université Libre de Bruxelles (Brussels, Belgium) and the Royal College of Surgeons in Ireland with Beaumont Hospital (Dublin, Ireland).

All patients were of European ancestry as determined by self-report and genetic-marker analysis. Cases were separated according to the causal drug and then phenotyped into distinct hypersensitivity states of MPE, HSS and SJS based on the extent and severity of their ADR and using disease criteria, as previously reported [14]. In total, there were 46 ADR cases relating to LTG, of which 42 were MPE cases, three were HSS and one was SJS. Of the 44 PHT-induced ADR cases, 40 were MPE cases and four were HSS cases. There were four patients who experienced MPE with both drugs on separate occasions and were thus included in each set of analyses. Given the low numbers of HSS and SJS patients and the evidence that a common marker may exist for all phenotypes (as evidence would suggest for *HLA-A*3101* and CBZ-induced cADRs) we analyzed all cases together as drug-specific cADR groups.

■ Control subjects

The control group consisted of a homogeneous subgroup of 1296 subjects from the 1958 British birth cohort that were selected by principal component analysis of GWAS. Controls were unscreened for AED-related ADRs.

■ Genome-wide analysis

Genome-wide data across >600,000 SNPs were available for 34 LTG and 42 PHT cases and all 1296 population control subjects. Genotyping for all cases was performed with the Illumina® Human610–610K Quad platform at the Duke University Center for Human Genome Variation (NC, USA). We removed subjects with a genotyping failure rate below 95%, SNPs with low minor allele frequency (<2%), those failing the Hardy–Weinberg equilibrium and any that had missing genotype information in >10% of all subjects.

Table 1. Distribution of *HLA-A*3101* among lamotrigine and phenytoin case subjects compared with population control subjects.

<i>HLA-A*3101</i>	Lamotrigine–cADR cases	Phenytoin–cADR cases	Controls
Carriers (n; %)	2 (4.35%)	2 (4.55%)	72 (5.56%)
Noncarriers (n; %)	44 (95.65%)	42 (95.45%)	1224 (94.44%)
Total subjects (n)	46	44	1296
p-value	0.698	0.746	
Odds ratio (95% CI)	0.756 (0.183–3.129)	0.791 (0.191–3.277)	
Relative risk for 80% power	2.204	2.229	

*HLA-A*3101* was found at a low average frequency of 4.45% across all cADR cases and at 5.56% in our population controls, which is comparable with reported European population datasets [23].
cADR: Cutaneous adverse drug reaction.

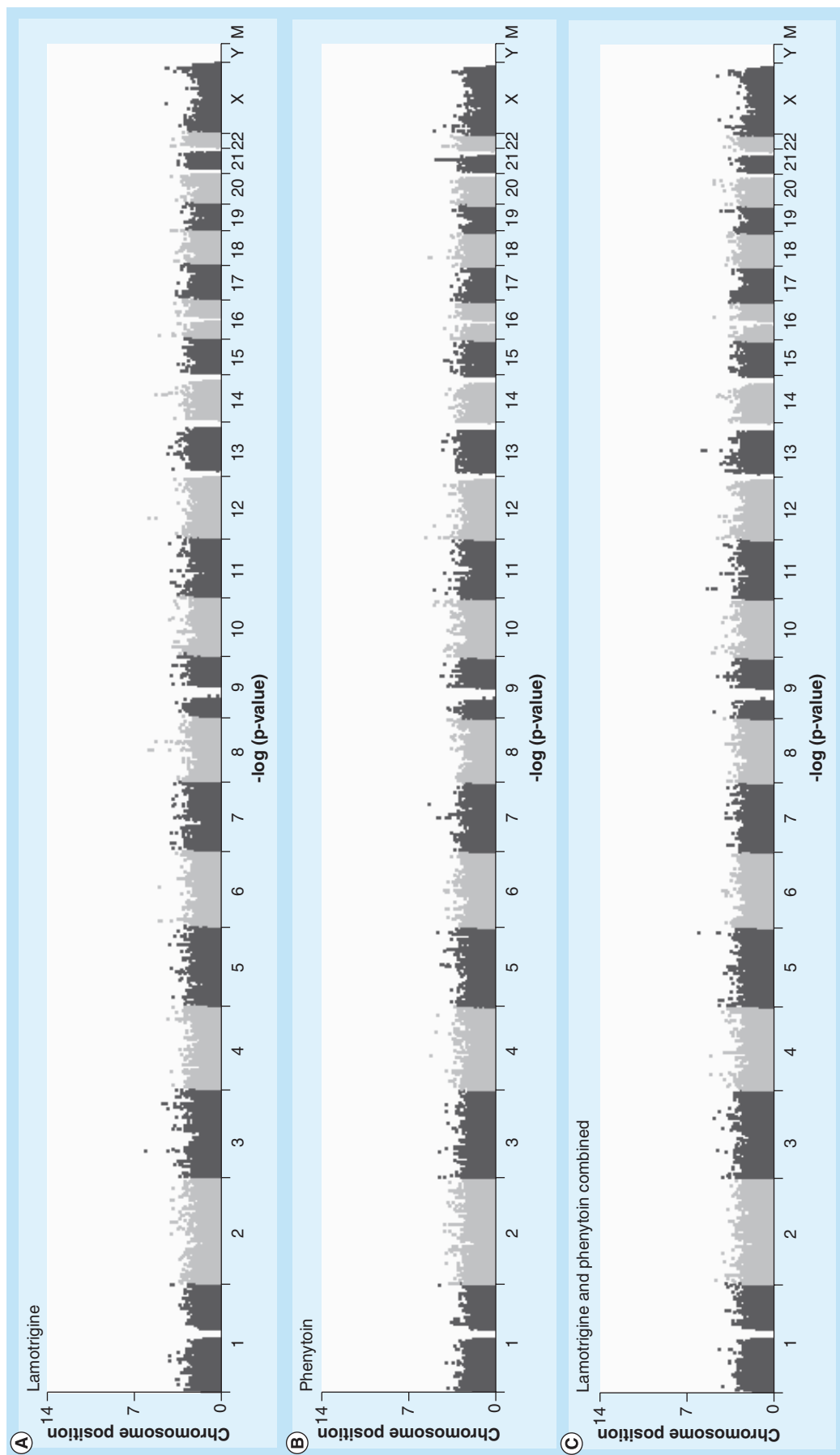


Figure 1. Manhattan plots of association results. Manhattan plots of genome-wide association studies analysis of (A) lamotrigine, (B) phenytoin and (C) combined drug-induced cutaneous adverse drug reaction vs controls. Genome-wide significance was taken as 5.0×10^{-8} . Figure generated in WGAViewer.

■ Imputation of *HLA* alleles

We imputed classic *HLA* alleles for all cases and controls using MACH 1.0 [17]. This method has been validated as an accurate method for imputing *HLA* subtypes in our cohort as described previously [14]. The *HLA*-typed 1958 British birth cohort constituted the reference population for imputation. For those samples for which GWAS data were not available we used PCR with sequence-specific primers to determine the presence of *HLA-A*3101*. The PCR conditions were as follows: the forward primer sequence

was 5'-GATAGAGCAGGAGAGGCCT-3', reverse primer sequence was 5'-AGCGCAGGTCCTCGTTCAA-3' and the cycling parameters were 95°C for 3 min, five cycles of 95°C for 15 s, 70°C for 15 s and 72°C for 30 s, two cycles of 95°C for 15 s, 65°C for 15 s and 72°C for 30 s, four cycles of 95°C for 15 s, 55°C for 1 min and 72°C for 2 min, finally 72°C for 7 min.

■ Statistical analysis

Statistical analyses were performed using PLINK (version 1.05) [18] and EIGENSTRAT [19] software packages. For the GWAS, we applied logistic regression (using an additive model) to each of the cADR case groups in turn versus the 1958 British birth cohort controls. We adjusted for population stratification by including significant principal components (generated using EIGENSTRAT) as covariates in the logistic-regression model and used a p-value of less than 5.0×10^{-8} as a threshold for genome-wide significance. SUPPLEMENTARY FIGURE S1 (see online, www.futuremedicine.com/doi/suppl/10.2217/PGS.11.165) for a plot of the first and second principal components between cases and controls. Post-GWAS annotation analysis was performed with WGAViewer [20]. We calculated study power using the program Power for Genetic Association Analyses [21]. We estimated the relative risk (RR) required for 80% power to detect an allelic association (under an additive disease model) with the following parameters: a prevalence of 10% of the ADR in the population, a minor allele frequency of 5%, an experiment-wide α level of 0.05 and the assumption that a genetic marker is causal or in complete linkage with the causal variant.

Results

■ *HLA-A*3101* as a predictor of LTG- and PHT-induced cADRs

TABLE 1 illustrates the results of *HLA-A*3101* genotyping in LTG- and PHT-induced cADR cases and population controls. The allele does not appear to be enriched in any of the cADR patient groups. For each of the analyses considered, our power calculations indicate that we had reasonable power to detect effect sizes of clinical relevance (RR >2).

■ GWAS results

We next conducted a GWAS for each of the drug-specific cADR groups as well as a total combined case cohort. The results of the logistic regression analyses are depicted as Manhattan and quantile–quantile plots in

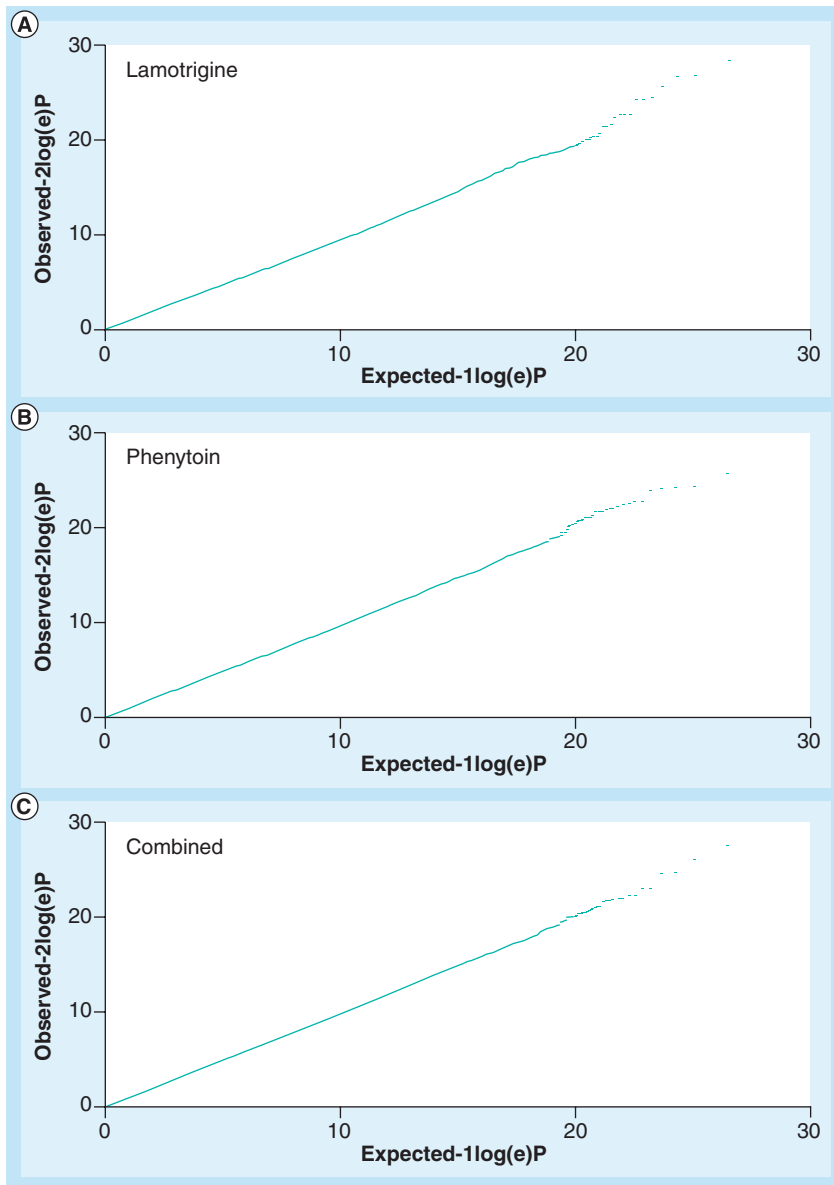


Figure 2. Quantile–quantile plot of the association results. Quantile–quantile plots of p-values from genome-wide association studies of (A) lamotrigine, (B) phenytoin and (C) total drug-induced cutaneous adverse drug reactions vs controls calculated using logistic regression and including significant EIGENSTRAT axes as covariates. The expected chi distribution is represented along the x-axis, while the observed chi distribution is represented along the y-axis. Figure generated in WGAViewer.

FIGURES 1 & 2. The ten most significant markers from the logistic regression analyses are listed in SUPPLEMENTARY TABLES S1–S3. We did not detect any variants satisfying our threshold for genome-wide significance (p -value of 5.0×10^{-8}), nor did we observe any significant deviations from the expected distribution in quantile–quantile plots (results shown in FIGURES 1 & 2). From power calculations (FIGURE 3) we estimated that we had 80% power to detect a variant with a minor allele frequency $>10\%$ and a RR >2.5 when considering all cases together. We feel this RR is within the realm of clinical relevance.

Discussion

The results presented here suggest that *HLA-A*3101* is not a clinically relevant predictor of cADRs caused by LTG and PHT in populations of European descent.

It has been shown in previous studies by ourselves and others that *HLA-A*3101* is a strong predictor for the clinical spectrum of CBZ-induced hypersensitivity and we reported an odds ratio of 8.33 for CBZ-induced MPE for a population of European descent, while the corresponding value for a population of Japanese descent was 10.8 [15].

We calculated that the current study had 80% power to detect a RR of 3.27 for cADRs based on a frequency of 5% for *HLA-A*3101*, which gives this study ample power to detect an effect size similar to that reported previously for CBZ-induced MPE. If *HLA-A*3101* is a predictor for either LTG- or PHT-induced cADRs, we were well powered to detect it. Of the 44 cases of PHT-induced cADRs, only two were positive for *HLA-A*3101*. Similarly, only two of the 46 cases of LTG-induced cADRs were positive for the allele. Incidentally, one of the subjects positive for *HLA-A*3101* in the LTG case group had a historical report of a rash with CBZ.

Although we included HSS and SJS, the overwhelming majority of cases were of the MPE type. Given the small number of HSS and SJS cases, we pooled all cases into a single group of hypersensitivity ADR cases per drug for a better powered genome-wide analysis. We chose to analyze all cases together as cADR groups as we did not have enough subjects to run a powered GWAS for HSS or SJS alone. The GWAS results remain negative when the HSS and SJS cases are removed. Further studies of PHT- and LTG-induced HSS and SJS in larger patient cohorts is warranted, especially given that the genetic predictors (e.g., *HLA-B*1502*) can be selective for a given hypersensitivity reaction [5,13,22], and

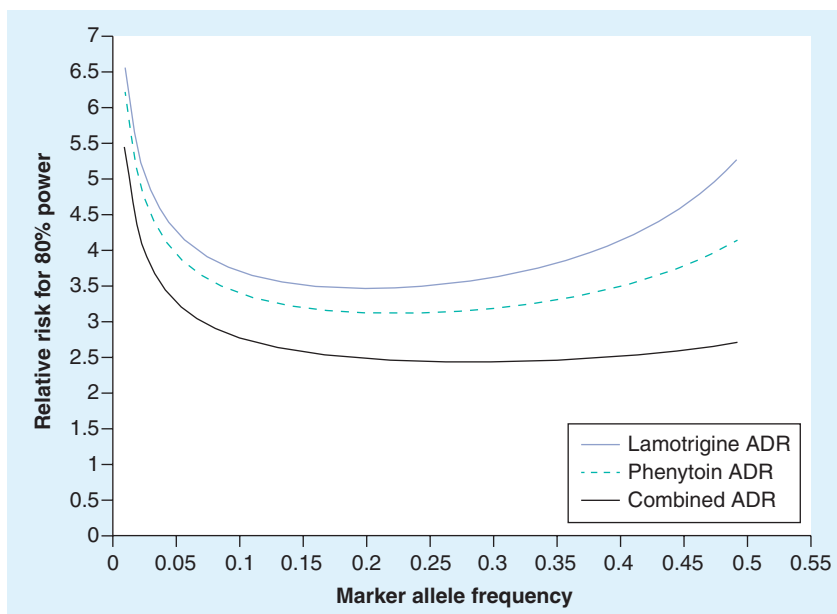


Figure 3. Power calculation for genome-wide association results.

The three analyses are represented with lamotrigine, phenytoin and combined drug cutaneous ADR groups with 1296 controls. Taking all cases together, we estimated we had 80% power to detect a variant with a minor allele frequency $>10\%$ and a relative risk >2.5 .

ADR: Adverse drug reaction.

the effect size correlates with the severity of the phenotype across the hypersensitivity spectrum (as appears to be the case for *HLA-A*3101* and CBZ [14,16]).

Our GWAS results are not suggestive of a common, clinically relevant predictor of LTG- or PHT-induced cADRs in a European population. None of the variants tested satisfied the GWAS significance threshold, nor was there any clear biological candidate from within the pool of the most significant SNPs (SUPPLEMENTARY TABLES S1–S3). Our study was powered to detect a variant of minor allele frequency $>10\%$ carrying a clinically relevant genetic effect (RR >2.5). We recognize that sample size is a major limitation of our study. This has limited our analysis to effect sizes in the realm of clinical relevance (for cADRs) and has prevented us from studying SJS/TEN specifically. We also cannot rule out genetic variants conveying smaller effects (RR <2.5) or rare variants of small or large effect, which were not captured by our GWAS chip.

With the increased availability of whole-exome and whole-genome sequencing, it should become possible and more affordable to identify rare variants not captured through GWAS. Discovering these genetic markers would further the understanding of the functional role of *HLA* in drug response, as well as helping clinicians screen patients susceptible to these ADRs. This study mainly focused on patients

with the less severe MPE hypersensitivity reaction; however, there is a clear motivation to investigate whether *HLA-A*3101* extends itself as a genetic marker for HSS and SJS/TEN caused by LTG and PHT.

Conclusion

*HLA-A*3101* does not appear to be a genetic marker for LTG- and PHT-induced MPE reactions in Europeans. The predictive application of *HLA-A*3101* as a clinically relevant genetic marker for cADRs in Europeans appears to be specific to CBZ.

Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Background

- *HLA-B*1502* is a genetic predictor for Stevens–Johnson syndrome/toxic epidermal necrolysis caused by carbamazepine (CBZ) and phenytoin (PHT) among Han Chinese and south-east Asian populations, while *HLA-A*3101* has recently been shown to associate with the full spectrum of CBZ-induced cutaneous adverse drug reactions (cADRs; maculopapular eruption, hypersensitivity syndrome and Stevens–Johnson syndrome/toxic epidermal necrolysis) in European and Japanese populations.
- With this knowledge we investigated whether *HLA-A*3101* or any other common genetic marker was associated with lamotrigine- (LTG) or PHT-induced cADRs.

Results

- There was no association between *HLA-A*3101* and LTG- or PHT-induced cADRs.
- We did not find a significant common genetic variant from our genome-wide analyses.

Conclusion

- We have shown that the association with *HLA-A*3101* does not extend to LTG- or PHT-induced cADRs. We also did not detect any other significant common variants of association with LTG- or PHT-induced cADRs from genome-wide association studies.
- *HLA-A*3101* thus appears to be a specific marker for CBZ-induced cADRs.

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- of considerable interest

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