Risk-conferring HLA variants in an epilepsy cohort: benefits of multifaceted use of whole genome sequencing in clinical practice


ABSTRACT

Background Whole genome sequencing is increasingly used in healthcare, particularly for diagnostics. However, its multifaceted potential for individually customised diagnostic and therapeutic care remains largely unexploited. We used existing whole genome sequencing data to screen for pharmacogenomic risk factors related to antiseizure medication-induced cutaneous adverse drug reactions (cADRs), such as human leucocyte antigen HLA-B*15:02, HLA-A*31:01 variants.

Methods Genotyping results, generated from the Genomics England UK 100 000 Genomes Project primarily for identification of disease-causing variants, were used to additionally screen for relevant HLA variants and other pharmacogenomic variants. Medical records were retrospectively reviewed for clinical and cADR phenotypes for HLA variant carriers. Descriptive statistics and the χ² test were used to compare frequencies of additional pharmacogenomic variants between HLA carriers with and without cADRs, respectively.

Results 1043 people with epilepsy were included. Four HLA-B*15:02 and 86 HLA-A*31:01 carriers were identified. One out of the four identified HLA-B*15:02 carriers had suffered antiseizure medication-induced cADRs; the point prevalence of cADRs was 16.9% for HLA-A*31:01 carriers of European origin (n=46) and 14.4% for HLA-A*31:01 carriers irrespective of ancestry (n=83).

Conclusions Comprehensive utilisation of genetic data spreads beyond the search for causal variants alone and can be extended to additional clinical benefits such as identifying pharmacogenomic biomarkers, which can guide pharmacotherapy for genetically-susceptible individuals.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Whole genome sequencing is becoming common practice in the clinical setting, due to its strong diagnostic potential, but its practical use on other aspects including pharmacogenomics is lagging behind.

⇒ Specific HLA variants are known pharmacogenomic risk markers for cutaneous adverse reactions with the use of antiseizure medications.

WHAT THIS STUDY ADDS

⇒ Utilisation of clinically-relevant pharmacogenomic markers, such as HLA variants, made available through whole genome sequencing, has the potential to improve pharmacotherapy and pharmacovigilance.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study emphasises that there is a need to develop mechanisms for implementation of routine pharmacogenomic testing in healthcare settings, which could translate into meaningful benefits for quality of care, healthcare costs and optimisation of pharmacotherapy.

INTRODUCTION

The clinical utility of genomics is increasingly recognised; whole genome sequencing (WGS) has been introduced as a first-line diagnostic genetic test in the UK’s National Health System and elsewhere. WGS reveals the complete genetic code, making it a powerful tool not only for diagnosis but also for informing personalised approaches to genetically tailored treatment strategies and comorbidity risk stratification. Pharmacogenomics (PGx), the influence of genetic variation on the outcome of drug treatment, is fundamental to personalisation of drug management. Variants in the genes responsible for pharmacokinetics and pharmacodynamics may serve as clinically actionable variants. Current progress in WGS and advanced bioinformatics approaches provide opportunities for profiling clinically actionable PGx variants, focused not only on new treatments but also on response to existing treatments in several fields, including the epilepsies.

The merits of WGS in the epilepsies have emerged mainly in diagnostics and have also revealed genetic associations, through single nucleotide polymorphisms or polygenic risk scores, carrying increased risk of comorbid conditions in epilepsy, potentially generating individualised information of value to management and contributing to a broad interpretation of ‘precision medicine’. Drug treatment in the epilepsies is characterised by significant

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interindividual variability both in terms of efficacy and susceptibility to adverse drug reactions (ADRs); genetic factors contribute to this variability. Most epilepsy PGx studies have focused on the search for genetic predictors of pharmacoresistance or idiosyncratic ADRs.

ADRs are considered a major problem in healthcare. A large prospective study in the UK estimated that up to 6.5% of all hospital admissions are related to ADRs; additionally, they account for up to 30% of emergency department visits and hospital admissions. Moreover, ADRs generate substantial healthcare costs. ADRs may add USD $2284–5640 per individual to hospitalisation costs and account for up to 9.5% of all direct healthcare costs in primary, outpatient and hospital care. Cutaneous ADRs (cADRs) are one of the most common secondary to CBZ or PHT exposure in Asian populations, where the variant has the highest prevalence. The strongest association has been reported in Han Chinese from Taiwan with OR 4.35 to 8.05 for HLA-B*15:02 and/or HLA-A*31:01. In addition to direct health consequences, the burden of cADRs on hospital healthcare expenditure is high. The annual direct costs for ASM-induced severe cADRs range between 2460 and 7910 Rupees (USD $27–77) in India and USD $3720–8061 in Korea, where the authors estimated that the costs are comparable to other major health problems such as chronic renal disease and lung cancer. There are two main categories of risk factors for cADRs: drug-related factors such as drug exposure, duration of exposure and polytherapy and host-related factors such as age, gender, concomitant medical conditions, immune system disorders and genetic factors. Human leucocyte antigen (HLA) variants are the most well-established genetic markers for predisposition to cADRs, especially with the use of aromatic ASMs. The HLA-B*15:02 variant is strongly associated with SJS secondary to CBZ or PHT exposure in Asian populations, where the variant has the highest prevalence. The strongest association has been reported in Han Chinese from Taiwan with OR of 1357 (95% CI 193.4 to 8838.3); an association confirmed in Malay, Thai and Indian populations. In contrast to the Asian-specific HLA-B*15:02, the HLA-A*31:01 variant shows population variability. Moreover, it predisposes to various cADR phenotypes, including maculopapular exanthema, SJS, TEN and drug reaction with eosinophilia and systemic symptoms (DRESS). A recent meta-analysis investigating this association showed an increased aggregated risk, with OR of 5.92 (95% CI 4.35 to 8.05) for HLA-A*31:01-mediated cADRs, corroborating the results of previous meta-analyses.

As WGS gains ground in healthcare settings, the potential for mining relevant information, which spreads beyond diagnostics, such as PGx variants, is significant. Its clinical value remains largely unexplored, as transformation of such information into clinical tools has been complex and slow. Nonetheless, utilising WGS data comprehensively can add layers of clinically meaningful information, with the prospect of more individually customised diagnostic and therapeutic clinical care. Here, we used existing WGS data, generated from the Genomics England UK 100 000 Genomes Project (GEL) to identify causal variants, to additionally screen for HLA-B*15:02 and HLA-A*31:01 variants, in a cohort of people with epilepsy from a single tertiary epilepsy centre. We reviewed the medical records for drug history and occurrence of aromatic ASM-induced cADRs. As a secondary aim, we screened additional PGx variants associated with the pharmacokinetics of aromatic ASMs to investigate the modulatory effects of genetics on ASM-induced cADRs in individuals carrying HLA-B*15:02 or HLA-A*31:01 variants.

**METHODS**

**Study cohorts**

Individuals with a diagnosis of epilepsy from a single tertiary centre (National Hospital for Neurology and Neurosurgery, University College London Hospital NHS Foundation Trust), for whom WGS was performed through GEL, were included in the study. Unrelated individuals without epilepsy, who were recruited to GEL as unaffected relatives of probands with non-neurological rare diseases considered to be unrelated to epilepsy, were used as controls for comparison of HLA variant frequencies. We defined our cohort of interest based on HLA carrier status. We used this cohort to conduct reverse phenotyping and analysis of additional PGx variants.

**HLA-A*31:01 and HLA-B*15:02 variant genotyping**

HLA genotyping results, already available within the GEL Research Environment by the HLA-typer software, which is part of the Illumina NSV4 pipeline, were used to screen for HLA-B*15:02 and HLA-A*31:01 variants. Additionally, the proxy polymorphisms rs10484555 and rs1061235 were used to confirm the presence of HLA-B*15:02 and HLA-A*31:01, respectively, and were examined in the Integrative Genomics Viewer (IGV) tool.

We selected further genes and single nucleotide polymorphisms reported to be associated with drug and/or metabolite level or maintenance dosing of aromatic ASMs. Genotype results of 13 PGx variants involving eight genes (CYP2C19 (rs192154563), CYP3A4 (rs2242480), EPHX1 (rs2234922; rs1051740), UGT1A4 (rs2011425; rs6755571), UGT2B7 (rs7668258; rs7439366), ABCB1 (rs1045642), ABCCC2 (rs3871779); rs2273697; rs717620), SCN1A (rs3812718)) were extracted from WGS data of all HLA-B*15:02 and/or HLA-A*31:01 carriers.

**Phenotypic information**

Demographic and clinical variables for the HLA carriers were obtained from medical records and included age, ancestry, type of epilepsy, drug history specifically including exposure to aromatic ASMs (CBZ, PHT, LTG, oxcarbazepine (OXC), eslicarbazepine (ESL)) and presence of cADRs including rash (maculopapular or unspecified), SJS, TEN and DRESS. Clinical variables to explore systemic involvement in conjunction with cADRs other than the previously described syndromes were also collected where available, including presence of fever, liver or haematological abnormalities. For HLA-A*31:01 and HLA-B*15:02 carriers with ASM-induced cADRs, additional phenotypic features were included as relevant risk factors, where available, comprising age at time of exposure to culprit ASM, duration of exposure to culprit ASM, monotherapy/polytherapy at time of cADR (including ASM and non-ASM medications if on polytherapy), allergy to non-ASMs and presence and type of comorbidities.

**Statistical analyses**

Descriptive statistics were employed to analyse phenotypic data as well as genotype data for HLA-B*15:02 and/or HLA-A*31:01 and the 13 additional PGx variants. The $\chi^2$ test was used to compare the prevalence of HLA-A*31:01 between people with...
epilepsy and controls and to compare the frequency of 13 PGx variants in HLA-A*31:01 carriers of European ancestry with or without ASM-induced cADRs. Bonferroni correction was applied to p values to correct for multiple testing.

RESULTS

A total of 1043 individuals with epilepsy were included (558 women, 53.5%). The control cohort included 1187 individuals (722 women, 60.8%). We identified 86 individuals with epilepsy and 76 controls who carried the HLA-A*31:01 variant. Four individuals with epilepsy, and no controls, carried the HLA-B*15:02 variant.

All HLA-A*31:01 and HLA-B*15:02 carriers were heterozygotes. The IGV analysis confirmed the presence of both proxies (rs1061235 for all 86 HLA-A*31:01 carriers and rs10484555 variant for all four HLA-B*15:02 carriers). Comparison between European individuals with epilepsy (n=862) and ancestry-matched controls (n=1187) showed no significant difference in the HLA-A*31:01 allele frequency (AF) distribution (AF: 0.036 vs 0.033, respectively, p=0.62). Due to the small number of non-European patients (n=92) and modest number of patients of mixed ancestral background (n=89) in our study cohort, we did not formally compare the HLA-A*31:01 AF of these samples with matched controls. Additionally, formal statistical comparison for the HLA-B*15:02 cohort was not undertaken given the very small sample size.

The clinical characteristics of HLA-A*31:01 and HLA-B*15:02 carriers with epilepsy are summarised in table 1. 62/86 HLA-A*31:01 carriers (72.1%) were Europeans. Most HLA-A*31:01 carriers had been exposed to at-risk aromatic ASMs (83/86, 96.5%); among those, 68/83 (82%) were exposed to CBZ alone or in combination with other aromatic or non-aromatic ASMs. Twelve out of 83 HLA-A*31:01 carriers had developed cADRs, yielding a point prevalence of 14.4%.

Demographic and phenotypic characteristics of HLA-A*31:01 and HLA-B*15:02 carriers with aromatic ASMs-induced cADRs are listed in table 2. All 12 HLA-A*31:01 carriers with cADRs had been exposed to CBZ. The majority of them (10/12, 83.3%) had a cADR as a result of CBZ exposure (rash; n=8, rash with fever; n=1 and SJS; n=1), whereas 2/12 (16.7%) had a cADR secondary to LTG exposure but not to CBZ. In two individuals with CBZ-induced cADRs (patients #3 and #7), the same type of cADR (rash) was reported additionally with the separate use of PHT or OXC, respectively. The majority of HLA-A*31:01 carriers were on monotherapy at the time of reported cADR. For 7/12 (58.3%) HLA-A*31:01 carriers who had developed a cADR on exposure to an at-risk ASM, no allergy information was stated in the dedicated allergy section of their electronic or previous paper health records, even after the documented occurrence of cADR.

European-specific and CBZ-specific cohort

The majority of HLA-A*31:01 carriers of European ancestry (59/62, 95.2%) had been exposed to at-risk aromatic ASMs, with reported cADRs in 10/59, yielding a point prevalence of 16.9%. Most of the European HLA-A*31:01 carriers (46/59, 77.9%) had been exposed to CBZ alone or in combination with other aromatic or non-aromatic ASMs. Of 9/46 (19.6%), had CBZ-induced cADRs, whereas 37/46 (80.4%) did not (CBZ-tolerant).

The CT genotype of the ABCC2: c.3972C>T variant (RR=7.73; 95% CI 1.04 to 57.24; unadjusted p=0.011) was the only nominally enriched variant in European HLA-A*31:01 carriers with CBZ-induced cADRs (77.78%) compared with the CBZ-tolerant carriers (32.3%), when comparing the 13 additional PGx variants (online supplemental table 1), but significance was not sustained after correcting for multiple comparisons. Analysis of the additional PGx variants was not conducted in other ancestry-specific groups due to the small sample size.

DISCUSSION

In this retrospective study, we utilised existing WGS data from a cohort of people with epilepsy, which were primarily produced for diagnostic purposes, to look for well-established PGx markers of ASM-induced cADRs. Out of 1043 individuals with epilepsy, we identified 86 carriers of HLA-A*31:01 and four carriers of HLA-B*15:02 variants. HLA-A*31:01 prevalence varies across populations, with the highest prevalence in populations of South and Central America (ranging from 2.6% to 28.8%).

Table 1

Demographic and clinical characteristics of HLA-A*31:01 and HLA-B*15:02 carriers with epilepsy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HLA-A*31:01</th>
<th>HLA-B*15:02</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA carriers; N</td>
<td>86</td>
<td>4</td>
</tr>
<tr>
<td>Age (in years)</td>
<td>48.1 (SD 14.4)</td>
<td>49.2 (SD 15.1)</td>
</tr>
<tr>
<td>Sex; N: female/male</td>
<td>41/45</td>
<td>2/2</td>
</tr>
<tr>
<td>Ancestry; N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>European/White background</td>
<td>62 (72.1)</td>
<td>0</td>
</tr>
<tr>
<td>Black background</td>
<td>3 (3.5)</td>
<td>0</td>
</tr>
<tr>
<td>Black African</td>
<td>2 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Black Caribbean</td>
<td>1 (1.1)</td>
<td>0</td>
</tr>
<tr>
<td>Asian background</td>
<td>12 (13.9)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Asian/Indian</td>
<td>8 (9.3)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Asian/Bangladeshi</td>
<td>2 (2.3)</td>
<td>0</td>
</tr>
<tr>
<td>Asian/Pakistani</td>
<td>2 (2.3)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Asian/Chinese</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixed background</td>
<td>4 (4.7)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Background not specified</td>
<td>5 (5.8)</td>
<td>0</td>
</tr>
<tr>
<td>Epilepsy type; N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Focal epilepsy</td>
<td>78 (90.7)</td>
<td>3 (75)</td>
</tr>
<tr>
<td>Generalised epilepsy</td>
<td>5 (5.8)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (3.5)</td>
<td>0</td>
</tr>
<tr>
<td>Exposure to aromatic ASMs; N (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash alone</td>
<td>10 (12)</td>
<td>1 (25)</td>
</tr>
<tr>
<td>Rash with systemic involvement</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>Fever</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>Liver abnormalities</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Haematological abnormalities</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other organ involvement</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>SJS</td>
<td>1 (1.2)</td>
<td>0</td>
</tr>
<tr>
<td>TEN</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DRESS</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Age (in years) at the time of inclusion to the study: mean and SD.
†Ancestry information based on self-report as documented in electronic health records.
‡Percentages for CADR phenotypes were calculated using only the number of HLA carriers exposed to aromatic ASMs, rather than all HLA carriers, as denominator: n=83 for HLA-A*31:01 carriers and n=4 for HLA-B*15:02 carriers.
§ASMs, antiseizeure medications; cADR, cutaneous adverse drug reaction; DRESS, drug reaction with eosinophilia and systemic symptoms; HLA, human leucocyte antigen; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

frequencies across other populations such as Europeans (1%–5.9%), Japanese (7%–11%), South Koreans (5.6%) and with the lowest prevalence in African populations (0.4%–1.1%).19 In our cohort, HLA-A*31:01 carriers were of different ancestral backgrounds, with the majority being of European descent (72.1%), most likely reflecting the make-up of individuals seen at our centre. In contrast, the prevalence of HLA-B*15:02 is highest in Asian populations, found in up to 6.9% and 8% of East and Southeast Asians, respectively, whereas it is rare in Europeans, South American or African populations (<1%).14 All four HLA-B*15:02 carriers in our cohort were of Asian/mixed ancestry. We did not expect our epilepsy cohort to be enriched or protected with respect to the HLA variants as a selection (natural, clinical or ADR-related) of participants was not based on these variants. Indeed, on formal comparison, there was no significant difference in the HLA-A*31:01 distribution between individuals with epilepsy and controls of European ancestry (p=0.62). Formal statistical comparison was not undertaken for the HLA-B*15:02 cohort given the very small sample size.

Our cohort was selected based on available genetic data, irrespective of the presence of cADRs. We performed reverse phenotyping, using the presence of the risk-confering HLA variants as a starting point to identify genetically susceptible individuals and then search for cADR phenotypes from medical records. We identified a cADR point prevalence of 14.4% for all HLA-A*31:01 carriers exposed to aromatic ASMs irrespective of ancestry (n=83) and 16.9% for European HLA-A*31:01 carriers (n=46). Irrespective of HLA carrier status, it is estimated that cADRs affect 1%–8% of individuals on prescribed medications with variable rate ranges of 2%–8% for hospitalised patients29 and 1%–2.6% for outpatients,30 reaching up to 10% for ASM-specific cADRs, with CBZ being the principal offender.12 13 Here, the majority of the carriers on aromatic ASMs, 68/83 (82%), were exposed to CBZ monotherapy or polytherapy. HLA-A*31:01 risk association is well established for CBZ and OXC-induced cADRs;20 data are less robust for PHT or LTG-induced cADRs.31 Of 10/12 (83.3%) HLA-A*31:01 carriers who had developed any cADR did so with exposure to CBZ. The single HLA-B*15:02 carrier had suffered recurrent cADRs after exposure to several aromatic ASMs, despite clinical evidence of previous cADRs. Had the information about PGx risk been available earlier, repeated trials of aromatic, potentially offending, ASMs may have been avoided. This reverse phenotyping approach differs from other studies investigating the HLA association, which starts with the phenotype (of cADRs) and, therefore, formal comparison of our prevalence results with other studies is difficult. Defining the prevalence of cADRs in HLA carriers could help understand why only a proportion of carriers develops cADRs.

Besides the HLA-A*31:01 and HLA-B*15:02 association with cADRs, emerging data have highlighted the potential role of additional PGx variants. Combining different PGx biomarkers can shed light on the nuances of interindividual variability related to cADR susceptibility. In a recent study by Mullan et al.,12 five novel genetic variants were found to increase the risk of cADRs.
cADRs irrespective of HLA carrier status, in Chinese individuals with epilepsy. Moreover, in the same study, a non-protein coding variant was shown to reduce the risk of CBZ-induced cADRs in HLA-B*15:02 carriers. Here, we sought additional PGx variants to explore why some of the HLA-B*15:02 and/or HLA-A*31:01 carriers developed or did not develop aromatic ASIM-induced cADRs. We identified an enrichment of the ABC2:c.3972C>T variant in HLA-A*31:01 carriers with CBZ-induced cADRs compared with CBZ-tolerant carriers, which was not significant after correcting for multiple comparisons. Variants in the ABC2 gene have been reported to affect CBZ pharmacokinetics, poten-

tially contributing to interindividual variability in response to CBZ treatment. The presence of the ABC2:c.3972C>T variant has been associated with higher serum levels of CBZ, 10,11-

epoxide, the active CBZ metabolite and the need for higher CBZ maintenance doses in Caucasian and Han Chinese individuals with epilepsy, whereas it was not reported to affect CBZ clear-

ance in an epilepsy cohort from the UK, for which ancestry was not specified. Further work is needed in this area.

In none of the individuals with cADRs had post-cADR genetic screening for HLA carrier status been undertaken. Moreover, for 7/12 (53.8%) HLA-A*31:01 and the single HLA-B*15:02 carriers with reported cADRs, no relevant information was available in the dedicated allergy section of their medical records. Proper documentation of drug hypersensitivity in electronic health records is of crucial importance as it not only supports more informed treatment strategies but it may also prevent read-

ministration of the offending medication. Readministration of cADR-provoking drugs has been reported in 27% of patients who have previously suffered ADRs in the hospital setting, solely due to insufficient documentation. Following our findings, allergy information was updated in the electronic health records of the individuals with clinically established cADRs stating the offending medication.

As genetic information is becoming increasingly available in the clinical setting, identification of clinically relevant PGx vari-

ants will accelerate. In our study, identification of HLA variants in individuals with epilepsy became available en passant, and indeed revealed 90 HLA variant carriers; 14.4% had already suffered a relevant cADR. Had this genetic information been readily available to the treating clinical teams, it could translate to potential treatment benefits, as it could assist clinicians adopt a more informed approach on treatment selection and ensure more rigorous monitoring for genetically susceptible individuals. Notwithstanding the potential clinical benefits for our cohort, the prospect of large-scale implementation of such PGx screening in the clinical setting could help prevent cADRs by identifying genetically susceptible individuals and, thus, could facilitate more effective mitigation strategies for cADR-related adverse outcomes and related healthcare costs. A recent study comparing the cost-effectiveness between PGx-guided (including studies on PGx-guided CBZ treatment) and conventional phar-

macological treatment based on published economic evaluations estimated that, sequencing and genotyping costs aside, 75% of economic evaluations would support PGx-guided treatment, with 50% of these considering it the preferred, and even cost-
saving, treatment strategy. As genetic testing costs decrease and data become more widely available, incorporating genetic infor-

mation for all individuals as part of their healthcare may become a realistic prospect, where WGS has been undertaken to search for a cause, PGx data may be available effectively for free.

However, we also note that for HLA variants, there is a translational gap that hinders large-scale clinical implementa-

tion mainly attributed to economic barriers, conflicting data on cost-effectiveness, test availability and the variable predictive values emerging from studies investigating HLA associations with cADRs.11 There have been considerable advances in this context. HLA-B*15:02 screening before initi-

ation of CBZ treatment has been proven to be cost-effective for several Asian populations. HLA-B*15:02 pre-emptive screening prior to CBZ treatment is a requirement for Asians according to the Food and Drug Administration (FDA) and is routinely conducted in Thailand’s national healthcare system. Screening for HLA-B*15:02 carriers irrespective of ancestry may double the number of identified at-risk individuals compared with screening individuals of Asian ancestry alone. Nonetheless, its application in healthcare systems remains limited, even for Asian populations. In the UK, although the risk association with HLA-B*15:02 and cADRs is acknowledged, HLA-B*15:02 screening, even for Asian CBZ-naive individuals, is only stated as a recommendation and not a requirement. Although highly likely to be so at a global level, the cost-effectiveness of routine screening for HLA-A*31:01 has not yet been established. Neverthe-

less, official recommendations in the UK state that if people or European or Japanese ancestry are known carriers of the HLA-A*31:01, the use of CBZ should be considered only if the benefits outweigh the risks.

Our study had a number of limitations. The precise mapping of sequencing reads is one of the leading challenges in character-

ising HLA genes from next-generation sequencing data despite recent advances. Factors such as high sequence homology and the presence of multiple pseudogenes could influence the correct mapping of reads. These challenges may lead to ambiguous HLA genotyping from WGS data. We used a separate additional SNP proxy for each HLA variant to address this issue. Given the retrospective study design, phenotypes of cADRs could not be assessed based on established diagnostic criteria but rather were determined from the relevant documentation available in medical records, mainly because access to the contemporary primary clinical data was not possible. Therefore, further clas-

sification of the reported rashes was generally not possible. In addition, we were unable to collect and analyse data on serum concentrations for either the parent drug or drug metabolites, which could be valuable parameters in investigating the lack of cADRs in the majority of HLA carriers. Moreover, despite the clinical relevance and potential clinical utility of our PGx find-

ings, we were unable to implement them into the clinical setting for our cohort, mainly due to lack of clear mechanisms within the local national health system for streamlining clinical testing of PGx markers and incorporating them into clinical/treatment algorithms (the tests were unavailable on the UK National Genomic Test Directory).

In conclusion, we emphasise that comprehensive utilisation of WGS data spreads beyond the search for causal variants alone and can be extended to additional clinical benefits such as identifying PGx markers, which can guide pharmacotherapy for genetically susceptible individuals who are at risk for ASM-

induced cADRs, some of which are life threatening. As WGS is entering health systems as a routine test, PGx data will become increasingly accessible to clinicians with the realistic prospect of a meaningful multifaceted shift towards precision medicine. Nevertheless, as highlighted by the limitations in our study, allied work between the research community, regulatory agencies and national health systems is necessary for the successful incorpora-

tion of PGx procedures into medical practice, which could translate into meaningful benefits for quality of care, healthcare costs and optimisation of pharmacotherapy.