Pharmacogenetics of Adverse Drug Reactions

Richard Myles Turner and Munir Pirmohamed

Abstract A large variation in drug response exists between patients, with susceptible individuals being at risk of experiencing an adverse drug reaction (ADR). This susceptibility is attributable to environmental, clinical and genetic factors although the contribution of each varies with the drug, ADR and ethnicity. The variation in drug response makes personalisation of pharmacological therapy appealing to minimise ADRs whilst promoting efficacy. Pharmacogenetics seeks to contribute through genetic-guided drug and dose selection strategies. ADR pharmacogenetics was first highlighted in the 1950s, but it is only in the last decade that it has seen a rapid expansion, aided by significant advances in our knowledge of the human genome and improved genotyping technologies. ADRs can be classified according to whether the dominant mechanism is immune- or nonimmune-mediated. Several ADRs have been strongly associated with specific human leukocyte antigen (*HLA*) alleles. There is growing evidence for a central role of these alleles in the pathogenesis of immune-mediated delayed hypersensitivity ADRs through facilitation of 'off-target' interactions that lead to the presentation of 'altered self,' drugs and/or their metabolites to the T-cell receptor in an HLA-restricted fashion. Genetic variation can also predispose to nonimmune-mediated ADRs through perturbing drug pharmacokinetics or by altering nonimmune pharmacodynamic processes. In particular, genetic variants of phase I and phase II biotransformation enzymes and drug transporters alter the availability of a drug at the site(s) responsible for the ADR. Depending on the drug and ADR, these sites may be the therapeutic target site, the same molecular site in another tissue or distinct off-target sites. A prominent example of pharmacogenetics improving drug safety and enhancing the cost-effective use of limited healthcare resources is the reduction in the incidence of the abacavir hypersensitivity syndrome. It is apparent though that the success of ameliorating the abacavir hypersensitivity syndrome by genetic screening is proving difficult to emulate for other drug-ADR combinations. This highlights the considerable hurdles

R. M. Turner (✉) · M. Pirmohamed
The Wolfson Centre for Personalised Medicine, Institute of Translational Medicine, University of Liverpool, Block A: Waterhouse Building, 1-5 Brownlow Street, Liverpool L69 3GL, UK
e-mail: rt34@liverpool.ac.uk

M. Pirmohamed
e-mail: munirp@liverpool.ac.uk

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encountered in translating a pharmacogenetic association into a clinical test that benefits patient safety. The development of international consortia alongside the potential of next generation sequencing technologies and other innovations offer tantalising prospects for future advances in pharmacogenetics to reduce the burden of ADRs.

Keywords Adverse drug reaction · Pharmacogenetics · Predictive genotyping · Translation · Abacavir · Hypersensitivity · Malignant hyperthermia · Codeine · Warfarin · Statin

1 Introduction

For many drugs, substantial evidence exists at the population level to advocate their use. However there is considerable inter-individual variability in drug response, affecting both drug efficacy and safety [1]. Over 961.5 million prescription items were dispensed in England in 2011 [2]. This high drug usage and the individuality of drug response contribute to the high frequency of adverse drug reactions (ADRs). A prospective study in England estimated that 6.5 % of hospital admissions for patients >16 years old were related to an ADR, with a median inpatient stay of 8 days [3]. This was contextualised through extrapolation to the entire hospital bed base of England to suggest that the equivalent of up to seven 800 bed hospitals in England could be occupied at any one time with patients admitted with ADRs [3]. Studies from other countries have reported similar ADR-related hospitalisation rates [4–8]. Clearly, ADRs pose a significant international challenge to the health and safety of individual patients and to the efficient use of limited resources by healthcare services.

An ADR, as defined by the World Health Organisation (WHO), is ‘a response to a drug that is noxious and unintended and occurs at doses normally used in man for prophylaxis, diagnosis or therapy of disease or for the modification of physiologic function’ [9]. Table 1 provides definitions and examples of the related pharmacovigilance terms [9–12]. In essence, an adverse event (AE) is an umbrella term for any harm occurring to a patient temporally associated with but not necessarily directly attributable to a therapeutic intervention [10, 13]. A subdivision of AE is an adverse drug event, which describes maleficence associated with the use of a drug and includes overdoses, medication error and ADRs [11, 14, 15].

There is considerable variability between ADRs in terms of presentation and level of current aetiological understanding. This poses a challenge to their accurate categorisation and so, different classifications have been developed. The most well-known system delineates ADRs into types A and B. Type A (‘augmented’) ADRs constitute over 80 % of ADRs; they are dose-dependent and predictable from the main pharmacological action of a drug [13, 16]. This is because Type A ADRs are ‘on-target’ and manifest through excessive drug action at the therapeutic target site. Type B (‘bizarre’) ADRs are dose-independent and are not predictable from a drug’s conventional pharmacology [13, 16] as they represent idiosyncratic ‘off-target’ drug

Table 1 Pharmacovigilance terminology for adverse effects

Adverse effect term	Definition	Example(s)
Adverse event	Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment [10].	In a clinical trial for a topical emollient for psoriasis a road traffic accident could be a serious, unexpected, not study related adverse event
Adverse drug event	An injury resulting from the use of a drug [11].	i) Intentional overdose
		ii) Medication error
		iii) Adverse drug reaction
Medication error	A medication error is any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the health care professional, patient, or consumer [12].	Decrease in consciousness following accidental insulin overdose due to a prescribing or administration error
Adverse drug reaction	A response to a drug that is noxious and unintended and occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of disease or for the modification of physiologic function [9].	Hypersensitivity reaction to allopurinol through standard clinical use of the drug

effects. This classification was first defined in 1977 [17] and has been variously extended subsequently to include additional categories as shown in Table 2 [13, 18]. However, it can prove difficult to categorise ADRs using this system. For example, some type B ADRs, including statin-induced muscle toxicity, are clearly dose related and other type B ADRs, such as hypersensitivity to abacavir, are now predictable. A second system is the DoTS classification, which categorises ADRs according to dose relatedness, timing and patient susceptibility factors [19]. This descriptive system improves the accuracy of ADR classification, but its complexity makes it more difficult to use.

In this chapter, ADRs will be classified as immune- or nonimmune-mediated. Immune-mediated ADRs result principally from a deleterious immune reaction mounted following drug exposure. Nonimmune-mediated ADRs encompass all other ADRs and as a point of clarification, include infections that result from predictable immunosuppression by biologics and disease-modifying agents. This is a simple classification, but it reflects the clinical presentation and predominant pathogenic processes of many ADRs and is helpful when considering pharmacogenetics.

The reasons for the heterogeneity in inter-individual drug response are often not known but there are a trilogy of implicated factors: environmental (e.g. drug-drug and drug-food interactions), clinical (e.g. age, co-morbidities, body mass index (BMI), pregnancy) and genetic [20]. The contribution of each postulated factor

Table 2 Adverse drug reaction pharmacovigilance classification and characteristics [13, 18]

ADR classification	Characteristics	Example
Major types		
A	*Augmented*	Hypotension with iloprost therapy
	Due to main pharmacological action of a drug	
	Common	
	Dose-related	
	Predictable from conventional pharmacology	
	Severity variable, but usually mild	
B	*Bizarre*	Achilles tendonitis with quinolone therapy
	Associated with off-target drug effects	
	Uncommon	
	No clear dose relationship	
	Unpredictable from conventional pharmacology	
	Variable severity; proportionately more serious than type A	
Supplemental Types		
C	*Continuing*; time-related; ADR persistence for a long duration	Osteonecrosis of the jaw with bisphosphonate therapy
D	*Delayed*; time-related; ADR of slow onset	Tardive dyskinesia with antipsychotic therapy
E	*End-of-treatment*; associated with dose reduction or therapy discontinuation	Benzodiazepine withdrawal syndrome after abrupt drug cessation
F	*Failure of therapy*; inadequate therapeutic drug action so it does not achieve its intended purpose	Ischaemic stroke second to atrial fibrillation whilst on warfarin

likely varies with the drug, ADR and patient ethnicity [21]. A genetic basis for specific ADRs was first suggested in the 1950s when perturbed drug metabolism was associated with abnormal drug responses, such as butyrylcholinesterase deficiency and prolonged apnoea after succinylcholine administration. Over the last decade there has been a rapid growth in our understanding of ADR pharmacogenetics. This has been facilitated by an increased knowledge of the human genome and its variation through the Human Genome Project and HapMap Projects. Further, advances in genetic technologies and a reduction in processing costs have increased the volume of pharmacogenetic research conducted and its capacity to yield associations. At present, disproportionately more is known about associations of strong individual effect size with specific ADRs. However for some ADRs it may be that

the genetic contribution is polygenic with distinct loci of individual low effect size collectively contributing. Despite the advances of the last decade, elucidation of potential complex interplays within and between different biological systems is proving challenging.

The rest of this chapter explores further the pharmacogenetics of immune- and nonimmune-mediated ADRs. A discussion about all known genetic associations is beyond the scope of this chapter and so a range of genetic associations with distinct ADRs have been selected, although the tables included provide additional examples. The selected associations facilitate expansion on the following key themes:

- the effect of specific genetic mutations on protein function,
- the variable extent of genetic contribution to ADRs,
- the pathogenesis of ADRs,
- the clinical application of specific genetic-ADR associations through predictive genotyping and
- the current variable evidence base supporting their use.

Lastly, the many challenges faced by pharmacogenetics in translating an observed genetic-ADR association from the 'bench' to the 'bedside' will be highlighted and contemporary strategies and future possibilities to overcome these obstacles and deepen our understanding of pharmacogenetics will be outlined.

2 Immune-Mediated Adverse Drug Reactions

Immune-mediated ADRs are off-target ADRs and more specifically, represent a form of hypersensitivity reaction. Hypersensitivity reactions can be classified according to the Gell and Coombs system into types I-IV representing IgE-mediated allergic reactions (type I), direct antibody-mediated (type II), immune complex-mediated (type III) and delayed-type hypersensitivity (DTH) reactions (type IV). At the time of writing, comparatively less is known about the pharmacogenetics of type I-III hypersensitivity reactions and therefore this section will concentrate on DTH reactions.

Over the last decade, the increasing use of genome-wide association studies (GWAS) in pharmacogenetic research has identified a growing number of ADRs that are strongly associated with specific human leukocyte antigen (*HLA*) haplotypes, genes and/or alleles. Table 3 provides an overview of *HLA*-ADR associations [22–59].

The *HLA* class I and II genes, located on chromosome 6, are the most polymorphic of the human genome and over 7000 classical alleles have been identified between them [60]. There is strong linkage disequilibrium between the alleles [61]. Classical HLA class I molecules (encoded on 3 loci: *HLA-A, -B, -C*) are expressed on the surface of most nucleated cells and present peptide antigen to the T-cell receptor (TCR) of CD8+ T-cells [62]. The peptides presented by HLA class I molecules are mostly derived from the degradation of intracellular proteins, although

Table 3 Examples of *HLA* associations to hypersensitivity adverse drug reactions

Reaction	Drug	HLA- association(s)	Reference(s)
Hypersensitivity syndrome/DRESS/DIHS	Abacavir[a]	*B*57:01*	[22, 23]
	Allopurinol[a]	*B*58:01*	[24, 25]
	Carbamazepine	*A*31:01*	[26, 27]
	Nevirapine	*C*08:02-B*14:02* (*Italian*), *C*08* (*Japanese*), *B*35:05* (*Thai*)	[28–30]
Stevens-Johnson syndrome/Toxic epidermal necrolysis	Allopurinol[a]	*B*58:01*	[24, 31]
	Carbamazepine[a]	*B*15:02*[a], *A*31:01*	[32–34]
	Lamotrigine	*B*38*	[35]
	Methazolamide	*B*59:01*	[36]
	Nevirapine	*C*04:01 (Malawian)*	[37]
	Oxicam NSAIDs	*B*73:01*	[35]
	Phenytoin	*B*15:02*	[33, 38]
	Sulfamethoxazole	*B*38*	[35]
Delayed exanthem without systemic features	Allopurinol	*B*58:01* (*Han Chinese*)	[39]
	Aminopenicillins	*A2, DRw52*	[40]
	Carbamazepine	*A*31:01*	[27, 41]
	Nevirapine	*DRB1*01:01* (*French*) *B*35:05* (*Thai*) *C*04* (*Thai*)	[42] [30] [43]
Drug-induced liver injury	Antituberculosis drug therapy	*DQB1*02:01*	[44]
	Co-amoxiclav	*DRB1*15:01-DQB1*06:02, A*02:01*	[45, 46]
	Flucloxacillin	*B*57:01*	[47]
	Lapatinib	*DQA1*02:01*	[48]
	Lumiracoxib	*DQA1*01:02*	[49]
	Nevirapine	*DRB1*01*	[50]
	Ticlopidine	*A*33:03* *A*33:03* with *CYP2B6*1H or *1J*[b]	[51, 52]
	Ximelagatran	*DRB1*07, DQA1*02*	[53]
Agranulocytosis	Clozapine	*DQB1* 6672G > C	[54]
	Levamisole	*B*27*	[55]
Asthma	Aspirin	*DPB1*03:01*	[56, 57]
Pneumonitis	Gold	*B*40, DRB1*01*	[58]
Proteinuria, Thrombocytopaenia	Gold	*DRB1*03*	[59]
Urticaria	Aspirin	*DRB1*13:02-DQB1*06:09*	[56]

DRESS drug reaction with eosinophilia and systemic symptoms, *DIHS* drug-induced hypersensitivity syndrome, *NSAID* non-steroidal anti-inflammatory drug

[a] odds ratio > 50 and reproduced in > 1 study. Adapted from Phillips et al. [78]

[b] *CYP2B6* is not an *HLA* gene

HLA class I molecules on specific dendritic cell subsets are additionally capable of presenting extracellular peptides through 'cross-presentation' [63]. Classical HLA class II molecule expression (encoded on 3 loci: *HLA-DP, -DQ, -DR*) is restricted to professional antigen-presenting cells (e.g. dendritic cells, macrophages, B-cells) and they present extracellular-derived peptides to the TCR of CD4+ T-cells [62]. *HLA* polymorphisms localise to the sequence motifs that encode residues of the peptide-binding groove [60, 64]. These polymorphisms alter the stereochemistry of pockets within the groove, creating individual HLA allotypes with distinct peptide-binding portfolios [62, 65]. The HLA system is integral to the development of T-cell tolerance to 'self' and to the development of adaptive immunity in response to 'non-self' peptide. *HLA* incompatibility is also known to be important in the pathogenesis of allogeneic transplant rejection and several HLA associations have been previously reported for autoimmune diseases including ankylosing spondylitis (with *HLA-B27*) and rheumatoid arthritis (e.g. with *HLA-DRB1* alleles [66]).

Most of the hypersensitivity ADRs with *HLA* associations, including the specific reactions to abacavir, carbamazepine, allopurinol and flucloxacillin discussed below, are considered DTH reactions. In keeping with DTH reactions, they normally present $\geq$72 h after drug exposure, may resolve with drug cessation and often re-present more rapidly and with a more severe phenotype following drug re-exposure. A T-cell mediated immunopathogenesis is thought to underlie this temporal pattern. Analogous to the development of pathogen-induced adaptive immune responses, it is thought that a T-cell clone(s) can be primed by presentation of culprit antigen on an HLA molecule during primary drug exposure and effector memory T-cells are rapidly activated with secondary exposure [62, 67, 68]. The isolation of drug-specific T-cells from patients that have suffered DTH ADRs supports T-cell involvement [69, 70].

Two hypotheses have conventionally been proposed to describe potential off-target pharmacodynamic processes that may lead to the neo-antigen formation necessary for DTH drug-specific T-cell development: the hapten (or pro-hapten) model and the pharmacologic interaction with immune-receptors (p-i) model [71]. The hapten model proposes that drugs and their metabolites are too small to be independently immunogenic and so covalently bind to self-protein and the resulting *de novo* hapten-self peptide adduct is antigenic [71, 72]. The p-i hypothesis proposes that drugs may interact directly with HLA molecules, without specific self-peptides, to elicit a T-cell response [73]. Regardless of the mechanism of neo-antigen formation, it is widely assumed that additional 'danger' signals are required to overcome the immune system's default tolerance and permit generation of an adaptive immune response. This concept is referred to as the 'danger hypothesis' [74]. Amongst the other key themes of this chapter, the following ADR examples illustrate how prior understanding of genetic susceptibility can facilitate elucidation of underlying mechanisms of antigen formation and presentation.

2.1 *HLA-B*57:01* and Abacavir Hypersensitivity Syndrome

Abacavir represents the epitome of translational pharmacogenetics as the loop from laboratory observation to improved patient care for the genetic association between *HLA-B*57:01* and the abacavir hypersensitivity syndrome (AHS) has been closed [75]. Abacavir is a nucleoside reverse transcriptase inhibitor indicated to treat HIV and is prescribed as a constituent of highly active antiretroviral treatment (HAART). AHS occurs in 2.3–9% of patients [76] with a median time to onset of 8 days therapy [77]. The clinical diagnostic criteria require ≥2 of: fever, rash, nausea, vomiting, arthralgia, myalgia, headache, lethargy or gastrointestinal symptoms and importantly, onset must occur within 6 weeks of commencing therapy and remit within 72 h of abacavir cessation [76]. Unlike other drug hypersensitivity reactions, the mild to moderate rash is not a consistent feature [67] and eosinophilia is unusual [78]. Although the initial reaction is unpleasant, the significant morbidity and mortality occurs upon rechallenge [67, 78], consistent with a DTH reaction.

In 2002, two groups independently reported an association between AHS and *HLA-B*57:01* [22, 23] and subsequent further observational research confirmed the association [79, 80]. The Prospective Randomised Evaluation of DNA Screening in a Clinical Trial (PREDICT-1) study was a multicentre, double-blind randomised controlled trial (RCT) that demonstrated pre-therapy *HLA-B*57:01* screening significantly decreased the incidence of AHS [77]. Briefly, 1956 patients were enrolled and randomised on a 1:1 basis. The interventional group received pre-therapy *HLA-B*57:01* genotyping and either HAART with abacavir for *HLA-B*57:01* negative patients or HAART without abacavir for *HLA-B*57:01* positive patients. The control group received HAART with abacavir and retrospective *HLA-B*57:01* genotyping from blood samples taken pre-therapy. All participants with clinically diagnosed hypersensitivity reactions underwent skin patch testing for immunological corroboration to improve the specificity for the hypersensitivity phenotype. The study demonstrated that avoiding abacavir in *HLA-B*57:01* positive patients in the prospective screening interventional group eliminated immunologically confirmed hypersensitivity reactions (0 vs. 2.7% in control group, $p < 0.001$) with positive and negative predictive values of 47.9% (PPV) and 100% (NPV), respectively [77]. An estimate of the number needed to screen (NNS) to prevent one case of AHS, given an *HLA-B*57:01* carriage prevalence of 6%, was ~25 [77]. However 84% of participants were Caucasian, limiting generalisation. The Study of Hypersensitivity to Abacavir and Pharmacogenetic Evaluation (SHAPE) was a retrospective case-control study that addressed this and demonstrated that *HLA-B*57:01* has 100% sensitivity for immunologically confirmed AHS in both US White and Black patients [81]. Pharmacoeconomic evaluations have demonstrated a cost effectiveness to pre-prescription *HLA-B*57:01* screening [80, 82, 83]. Observational data from open-screening studies has addressed practical matters of implementation [84–86] and shown genotyping to reduce the frequency of abacavir discontinuation due to clinically suspected as well as true immunological hypersensitivity reactions [85, 87]. This is most likely because the former clinician strategy of over-diagnos-

ing AHS to ensure high sensitivity to avoid AHS maleficence at the expense of lower specificity [78] is no longer required given the exclusivity of the association between *HLA-B*57:01* and AHS. In accordance with the substantial evidence base, the drug label has been updated and clinical guidelines either mandate or strongly recommend prospective screening [76]. To summarise, abacavir represents a pioneering example of ADR translational pharmacogenetics and has charted a course that other genetic-ADR associations might follow from initial observations to a RCT to studies that address generalisation, pharmacoeconomics and applicability in widespread clinical practice.

Identifying the genetic basis for AHS has directly benefitted patient care but until recently, insight into the underlying immunopathogenesis has been limited. However 3 recent independent studies have begun to expose the pharmacodynamic off-target molecular mechanisms [88–90]. Native abacavir can bind non-covalently with exquisite specificity to *HLA-B*57:01* at the base of its peptide-binding groove, extending into the deep F pocket [88, 89]. The specificity for the interaction is accounted for by the F-pocket architecture and in particular residue 116 [88]. In the absence of abacavir, the C-terminus of peptides that bind to the F-pocket of *HLA-B*57:01* have large hydrophobic residues, such as tryptophan and less commonly phenylalanine [89]. In the presence of abacavir, the peptide repertoire of *HLA-B*57:01* shifts, so that around 20–25% of recoverable peptides are novel [88] and have alternative residues including isoleucine or leucine at their C-terminus [88, 90]. In essence, abacavir alters the stereochemistry of *HLA-B*57:01* to create an HLA neo-allotype with a novel peptide portfolio. This model of antigen presentation is distinct from both the hapten and conventional p-i models. It is proposed that T-cells will not have been exposed to the novel range of *HLA-B*57:01*-restricted peptides during thymic maturation and so will lack tolerance. The formation of memory T-cells will lead to systemic AHS that is more deleterious upon abacavir re-exposure. In support of the large peptide shift and subsequent large array of 'altered immunological self', the observed CD8+ T-cell response is polyclonal [65]. Further, the effector T-memory cells from *HLA-B*57:01* positive patients with a clinical history of AHS respond preferentially in the presence of specific peptide and abacavir together rather than to peptide or abacavir alone [89]. This indicates that the memory T-cell response to self-peptide requires abacavir for efficient presentation.

Although the exact intracellular site(s) where abacavir associates with HLA-B*57:01-peptide complexes is currently unclear, there is evidence to suggest the endoplasmic reticulum [65]. However, a minority of T-cell clones *in vitro* appear to react to abacavir too quickly to be explained by *de novo* HLA-B*57:01-novel peptide assembly [91]. This suggests that abacavir may additionally bind to HLA-B*57:01-native peptide complexes already present on the cell surface, possibly distorting their stereochemistry [65]. This mechanism is in keeping with the conventional p-i hypothesis. An inadequately resolved question is why the PPV of *HLA-B*57:01* for AHS is <50% [77]. Postulated mechanisms to account for this include (a) the inter-individual polygenic influence on the novel peptide portfolio itself [89]; and (b) heterologous immunity as a result of pre-existing viral infections, in keeping

with data which show that hypersensitivity reactions are often associated with reactivation of viruses such as Epstein-Barr virus [92].

2.2 *HLA-B*15:02, HLA-A*31:01 and Carbamazepine Hypersensitivity*

Carbamazepine is indicated in the treatment of epilepsy, trigeminal neuralgia and bipolar affective disorder but in up to 10% of patients, it can provoke a cutaneous ADR [93]. Drug-induced skin injury (DISI) encompasses a spectrum of manifestations and can be caused by a diverse range of drugs including anticonvulsants, allopurinol and β-lactam antibiotics [94]; Table 3 lists drugs with known associations between DISI and genetic variants. There exists both inter- and intra-drug heterogeneity in DISI presentation but fortunately most reactions are mild [94]. Standardising phenotypic definitions for serious DISI conditions has been challenging and required an international collaborative approach. Carbamazepine itself can cause DISI ranging from mild maculopapular exanthema (MPE) of increasing severity to the hypersensitivity syndrome (HSS), also referred to as drug reaction with eosinophilia and systemic symptoms (DRESS) or drug-induced hypersensitivity syndrome (DIHS) [94], to the distinct Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis (TEN) [95].

HSS/DRESS/DIHS (herein referred to as HSS) and SJS-TEN represent severe cutaneous adverse reactions (SCARs) [96]. HSS is a multisystem disorder that carries a mortality rate of 10% [96]. HSS can be diagnosed by the presence of at least 3 of: cutaneous involvement, internal organ involvement, fever, lymphadenopathy and eosinophilia (and/or atypical lymphocytes) with at least 1 of the first 2 criteria listed being present [94]. The skin manifestation is most commonly an exanthematous eruption and the internal organ involvement includes hepatic dysfunction and interstitial nephritis [94, 97]. SJS and TEN represent different severities along a spectrum of the same disease and are characterised by epidermal detachment involving the skin and mucous membranes with systemic manifestations including fever, intestinal and pulmonary involvement [94, 98]. SJS is diagnosed when epidermal detachment affects ≤10% of the body surface area, TEN is diagnosed when >30% is affected and an overlap syndrome exists for 10–30% epidermal detachment [99]. SJS and TEN have estimated mortality rates of up to 5 and 50%, respectively [98].

Genetic association studies have shown *HLA-B*15:02* to be a susceptibility factor for carbamazepine-induced SJS-TEN in people of Han Chinese descent [32] and certain other Asian ethnicities including Thai, Malaysian and Indian [33, 100–102]; a meta-analysis of studies with Asian patients derived a pooled odds ratio (OR) of 113.4 (95% confidence interval (CI) 51.2–251.0) [34]. A recent open-label prospective study in Taiwan has demonstrated the beneficence of pre-therapy *HLA-B*15:02* screening in Han Chinese patients and reported no cases of SJS-TEN in both the carbamazepine-taking *HLA-B*15:02* negative cohort and *HLA-B*15:02* positive cohort administered alternative medication or advised to continue their pre-

study medication [103]. Due to ethical and sample size considerations, an estimated historical annual incidence of carbamazepine SJS-TEN (0.23%) was used as the comparator rather than a prospective non-screened control group (with appropriate blinding), unlike the PREDICT-1 study for AHS [77]. Furthermore this genetic correlation is both phenotypically restricted to SJS-TEN [34, 41] and ethnically restricted: it has not been reproduced in Europeans [104, 105] or other specific Asian populations including South Koreans [26] and Japanese [106–108]. The reason(s) for the latter are not clear but allelic frequency should be considered since *HLA-B*15:02* is present in 8.6% of the Han-Chinese population but in <1% of Europeans, Koreans and Japanese [26, 109], reducing the power of studies in these populations to detect statistically significant associations [109].

A more recently reported carbamazepine DISI association is with *HLA-A*31:01* and importantly, it is associated with MPE, HSS and SJS-TEN and is present in multiple diverse ethnicities, including Han Chinese [41], Koreans [26], Japanese [107] and Europeans [27]. Interestingly, the allele frequency of *HLA-A*31:01* for these ethnic groups is 1.8, 10.3, 9.1 and 2–5%, respectively [26, 109]. The magnitude of the association though is smaller than with *HLA-B*15:02*, with a meta-analysis pooled OR for *HLA-A*31:01* of 9.5 (95% CI 6.4–13.9) [34]. However, the estimated NNS to prevent one case of carbamazepine DISI with *HLA-A*31:01* is lower and ranges from 47–67 depending on ethnicity. This is in contrast to the estimated NNS of 461 Asian patients with *HLA-B*15:02* to specifically prevent one case of SJS-TEN [34]. This difference in NNS is largely attributable to the higher incidence of ADRs (up to 10% [93]) that are associated with *HLA-A*31:01* compared to *HLA*B15:02* (circa 0.23% [103]), due to the relationship of *HLA-A*31:01* with a broader range of phenotypes. These findings form a credible foundation for a future prospective study to assess the clinical benefit of pre-therapy *HLA-A*31:01* screening.

It has been shown that carbamazepine can non-covalently associate with HLA-B*15:02 molecules and in the presence of carbamazepine, there is a shift in the peptide repertoire of HLA-B*15:02 with a novel preference for smaller residues at the 4th and 6th peptide positions and an increase in hydrophobic residues at several positions [88]. The scale of this peptide shift is smaller (approximately 15%) than for abacavir [88]. This off-target pharmacodynamic effect does resonate with the novel model proposed for abacavir and *HLA-B*57:*01, but overall the mechanisms leading to carbamazepine hypersensitivity ADRs are likely to be more complex. This is because firstly, it has been shown that carbamazepine and its metabolite, carbamazepine-10,11-epoxide, can associate with other structurally related *HLA-B75* family members [110]. Secondly, the availability of a restricted range of T-cell clonotypes with specific TCR rearrangements has been demonstrated to be an important determinant in the pathogenesis of *HLA-B*15:02*-associated carbamazepine-induced SJS-TEN [111]. This indicates that both the TCR repertoire and *HLA* genotype modulate the risk of carbamazepine-induced SJS-TEN and likely explains why some *HLA-B*15:02* carriers tolerate carbamazepine [109]. Further research is still required to understand other complicating observations. These include how two seemingly disparate *HLA* alleles, *HLA-B*15:02* and *HLA-A*31:01*, are linked to

carbamazepine ADRs and furthermore, how *HLA-A*31:01* can be associated with multiple phenotypes.

2.3 HLA-B*58:01 and Allopurinol Hypersensitivity

Allopurinol, an analogue of hypoxanthine, inhibits xanthine oxidase (XO) and is indicated in the management of gout and other hyperuricaemic conditions including tumour lysis syndrome. Although generally well tolerated, ~2–3% of patients suffer mild hypersensitivity reactions including MPE [39, 112] and crucially, ~0.1–0.4% of patients develop HSS or SJS-TEN [112]. The SCARs, HSS and SJS-TEN, normally present within weeks to months of commencing allopurinol but may take considerably longer [112]. Allopurinol is a major cause of SCARs [113, 114] and the combined mortality from allopurinol-induced SCARs approaches 25% [112].

In 2005, a strong genetic association between *HLA-B*58:01* and allopurinol-induced SCARs (HSS/SJS-TEN) was described in the Taiwan Han-Chinese population (OR 580.3, 95% CI 34.4–9780.9); all 51 cases (100%) carried *HLA-B*58:01* in comparison to only 20 of 135 allopurinol-tolerant controls (15%) [24]. This association has been replicated in Thai [31], Korean [25], European [35] and Japanese patients [115], although its magnitude was more modest for the latter 3 ethnic groups, possibly reflecting the lower prevalence of *HLA-B*58:01* in these populations. A meta-analysis has confirmed the association between allopurinol-induced SJS-TEN and *HLA-B*58:01* in both Asian and non-Asian patients compared to allopurinol-tolerant controls (combined OR 96.6, 95% CI 24.5–381.0) [116]. Based on data from the Han Chinese and Thai populations, current estimates for the PPV and NPV for *HLA-B*58:01* are ~1.5 and 100%, respectively [112], although these values will be lower for other ethnic groups. An exciting development is the ongoing prospective study in Taiwan to assess the clinical benefit of pre-therapy genotyping for *HLA-B*58:01* prior to commencing allopurinol [112]. Similarly to the prospective study discussed above for *HLA-B*15:02* screening prior to initiating carbamazepine [103], an estimated historical ADR incidence is being used for the control. At the time of writing, no results from this study have been published.

Interestingly, unlike carbamazepine and *HLA-A*31:01*, it is less clear at the current time whether *HLA-B*58:01* also predisposes to MPE in patients taking allopurinol. A study in Australia demonstrated no association between *HLA-B*58:01* and MPE [117], but a study of Han-Chinese patients in mainland China reported *HLA-B*58:01* as a risk factor for both allopurinol-induced MPE and SCARs [39]. More research into this area is required, but one hypothesis from the available literature is that the risk of MPE with *HLA-B*58:01* may be ethnically-restricted. If this association is confirmed, it will increase the PPV further for the affected ethnic groups and so augment the potential utility of pre-therapy *HLA-B*58:01* screening in these groups to reduce the burden of allopurinol-induced ADRs.

The exact underlying mechanism(s) by which allopurinol, or its long-circulating active metabolite oxypurinol, interact with *HLA-B*58:01* for the generation

of drug-specific T-cells has yet to be elucidated. However, as the PPV of *HLA-B*58:01* for SCARs is low (~1.5%) [112], this alludes to other contributing factors in their pathogenesis. It has long been thought that viruses play a role in drug hypersensitivity and there is increasing recognition that the reactivation of herpes viridae is important in the aetiology of HSS [92, 118]. However, any interaction(s) between allopurinol and/or oxypurinol and viruses is poorly understood. Prior to the discovery of *HLA-B*58:01*, several non-genetic risk factors were espoused including renal dysfunction, higher allopurinol doses, diuretic use and concomitant antibiotic therapy [112]. Although verification of these variables is difficult as SCARs are fortunately rare events, patients on allopurinol with renal insufficiency have been shown to be almost 5 times more likely to develop SCARs [24]. In addition, patients on a daily dose of ≥200 mg allopurinol seem to be at an increased risk of SJS-TEN compared to lower doses [113]. By assimilation of these 2 observations, it can be hypothesised that increasing the plasma concentration of allopurinol and/or oxypurinol increases the risk of drug-specific T-cell development [109]. To mitigate the risk of ADRs the dose of allopurinol could be reduced, but it is well established that the most commonly used doses of allopurinol (≤300 mg daily) are frequently ineffective already for the long term treatment of hyperuricaemia in gout [119].

Nevertheless, *HLA-B*58:01* is the single largest predictor of allopurinol-induced SCARs and this makes genetic screening appealing to directly prevent *HLA-B*58:01*-associated SCARs. In addition, it is conceivable that a successful genetic screening programme may indirectly improve the overall benefit: harm ratio of allopurinol further. This is because genotyping may empower clinicians to titrate allopurinol doses up to optimise efficacy in *HLA-B*58:01* negative patients with normal renal function. However, any benefit derived from genetic screening in the ongoing Taiwan study will require follow up studies to determine the extent of generalisation. This is because for other ethnic groups and especially Europeans, allopurinol-induced SCARs also occur in *HLA-B*58:01* negative patients. Furthermore, the identification of other (non)-genetic risk factors may be required to improve the PPV of the test, as currently many *HLA-B*58:01* patients will be unnecessarily denied allopurinol in place of other urate-lowering therapies, with unmeasured effects as yet on cost-effectiveness and treatment efficacy.

*2.4 HLA-B*57:01 and Flucloxacillin-Induced Liver Injury*

Flucloxacillin is a narrow-spectrum beta-lactam antibiotic indicated in Gram-positive bacterial infections and in particular, is used to treat non-methicillin resistant *Staphylococcus aureus* infections. In approximately 8.5 per 100,000 patients treated with flucloxacillin, cholestatic liver injury occurs [120]. Drug-induced liver injury (DILI) is associated with a structurally disparate range of drugs but notably these include non-steroidal anti-inflammatory drugs (NSAIDs) and certain antimicrobials including flucloxacillin [121]. Although rare, drug-induced liver injury (DILI) can be severe and accounts for up to 15% of all cases of acute hepatic failure [122–124].

Analogous to DISI, the type and severity of DILI vary between causative drugs and for a given drug, presentation is variable [121]. Consequently, standardising the DILI phenotype is not straightforward but the diagnosis can be made from clinical, biochemical and histopathological parameters [125].

The aetiology of DILI can be divided into immune- and nonimmune-mediated processes [121]. Pharmacogenetic associations with DILI have now been identified for several drugs and the associated genetic variants reflect both immune and non-immune aetiologies (see Tables 3 and 4, respectively for examples). However, DILI can be difficult to categorise by this means. This is because, although recognition of clinically suggestive features of hypersensitivity is relatively easy, the absence of such features, such as eosinophilia, does not preclude immune system involvement [126].

To date, the strongest DILI genetic association described is between flucloxacillin and *HLA-B*57:01* with an OR of 80.6 (95% CI 22.8–284.9) [47]. This is intriguing as the *HLA-B*57:01* allele is also strongly associated with AHS, yet AHS rarely involves hepatitis [78].

Unlike AHS, it is improbable that this association will lead to a screening test for clinical practice because, despite an adequate estimated sensitivity and specificity (84 and 94%, respectively) [47], the rarity of flucloxacillin-induced liver injury diminishes the PPV to 0.12% [127]. An estimate of the NNS to prevent one flucloxacillin-induced liver injury case is 13,513 and the screening approach would unnecessarily deny almost 7% of patients first line flucloxacillin therapy, with unmeasured adverse effects on infectious disease treatment efficacy and cost effectiveness [127]. However genetic testing may help establish the diagnosis of flucloxacillin-induced liver injury when the underlying cause of liver dysfunction is unclear [47].

The off-target pharmacodynamics that underpin flucloxacillin-induced liver injury are being unravelled. Flucloxacillin can adduct covalently to proteins to form neo-antigen drug-protein conjugates and specific flucloxacillin-modifiable lysine residues on albumin, the major circulating protein, have been identified [128]. It is predicted that several albumin-derived peptides containing flucloxacillin-modifiable lysine residues have high-affinity for HLA-B*57:01 [129] and could be presented on HLA-B*57:01 by professional antigen presenting cells through cross-presentation. Flucloxacillin-responsive CD4+ and CD8+ T-cells have been characterised *in vitro* from patients who have previously suffered flucloxacillin cholestatic liver injury and their activation is dependent on peptide processing. *In vitro*, the CD8+ T-cell activation is restricted to HLA-B*57:01 and the very similar allotype, HLA-B*58:01 [129]. The proposed model, which aligns with the hapten hypothesis, suggests that immunogenic peptide neo-antigens are derived from natural processing of flucloxacillin-protein conjugates and can be presented on HLA-B*57:01 to generate an adaptive immune response [129]. Interestingly, besides this proposed alternative mechanism of neo-antigen formation, this immunopathogenesis differs to that of abacavir in at least 2 ways. Firstly, CD4+ as well as CD8+ flucloxacillin-responsive T-cells have been cloned from patients and secondly, the T-cell clones

Table 4 Examples of associations between adverse drug reactions and nonimmune-related genetic variants

Reaction	Drug	Gene association(s)	Variant(s)	Reference(s)
i) Drug metabolizing enzyme and drug transporter variants				
Increased risk of bleeding	Clopidogrel	*CYP2C19*	*17	[130, 131]
	Warfarin	*CYP2C9*	*3	[132–135]
Increased risk of opioid toxicity	Codeine	*CYP2D6*	Ultrarapid metabolisers	[136, 137]
	Tramadol	*CYP2D6*	Ultrarapid metabolisers	[138, 139]
Drug-induced liver injury	Antituberculosis drug therapy	*NAT2*	Slow acetylator	[140–142]
		GSTM1	null/null	
		CYP2E1 (East Asians)	*1A/*1A	
	Diclofenac	*UGT2B7*	*2	[143]
		ABCC2	rs717620	
		CYP2C8	Different haplotypes	
	Tacrine	*GST T1* *GST M1*	Double null/null	[144]
	Troglitazone	*GST T1* *GST M1*	Double null/null	[145]
Diarrhoea, neutropaenia	Irinotecan	*UGT1A*	*Poor metabolisers*	[146]
Drug discontinuation	Risperidone	*CYP2D6*	Poor metabolisers	[147]
Muscle toxicity	Simvastatin	*SLCO1B1*	rs4149056	[148]
Myelosuppression	Azathioprine, 6-mercaptopurine, thioguanine	*TPMT*	Poor metabolisers	[149]
Peptic ulcer disease	NSAIDs	*CYP2C19*	*17	[150]
Prolonged apnoea	Succinylcholine, mivacurium	*BCHE*	rs1799807, other variants	[151]
Stent thrombosis	Clopidogrel	*CYP2C19*	*2	[152]
Therapy-induced toxicity[a]	5-fluorouracil/ capecitabine	*DPD*	rs3918290, rs55886062, rs67376798	[153]
ii) Other variants				
Drug-induced liver injury	Methotrexate	*MTHFR*	rs1801133	[154]
Malignant hyperthermia	Halogenated inhalation anaesthetics	*RYR1*	rs118192163 >30 other variants	[155, 156]
		CACNA1S	rs1800559 rs80338782	[157, 158]

Table 4 (continued)

Reaction	Drug	Gene association(s)	Variant(s)	Reference(s)
i) Drug metabolizing enzyme and drug transporter variants				
Metabolic syndrome	Clozapine, risperidone	*5HTR2C*	rs1414334	[159]
Nonimmune haemolytic anaemia	Primaquine, dapsone, methylene blue, others	*G6PD*	Mediterranean, A-(202A), >150 other variants	[160, 161]
Therapy-induced toxicity[a]	5-fluorouracil/ capecitabine	*TYMS*	rs45445694	[162]

NSAID non-steroidal anti-inflammatory drug

[a] Toxicity from 5-fluorouracil-based therapy includes diarrhoea, mucositis, nausea, neutropaenia

show cross-reactivity *in vitro* with other commonly prescribed beta-lactam antibiotics including amoxicillin and piperacillin [129].

In summary, this section illustrates that immune-mediated DTH reactions are an emerging prominent type of off-target ADR with the potential for significant morbidity and mortality. However, pharmacogenetics has been pivotal in reducing the healthcare burden associated with abacavir, may have important future roles in the prevention of carbamazepine and allopurinol DISI and is facilitating elucidation of underlying immune-mediated aetiologies.

3 Nonimmune-mediated Adverse Drug Reactions

Nonimmune-mediated ADRs are a heterogeneous group in aetiology and presentation. However, over the last decade it has been increasingly recognised that susceptibility to many nonimmune ADRs is associated with gene variants of drug metabolising enzymes (DMEs) and less frequently, with drug transporters. It is thought that perturbed pharmacokinetics increases the availability of drug/metabolite(s) at the target site(s), increasing the likelihood of developing an ADR. The sites that mediate nonimmune ADRs include both on-target and off-target sites. On-target ADRs manifest through excessive drug/metabolite(s) action either at the therapeutic target site or at the same molecular site located in other tissues. The latter occurs for instance with NSAID-induced upper gastrointestinal ADRs.

It is important to note that, although the majority of ADRs with a genetically-influenced pharmacokinetic-mediated susceptibility found to date are nonimmune ADRs, perturbed pharmacokinetics is also relevant in the genesis of a few immune-mediated ADRs. This was described earlier for the case of allopurinol-induced SCARs and non-genetic pharmacokinetic factors. Furthermore, genetic susceptibility to ticlopidine-induced hepatotoxicity has been demonstrated to be greatest in patients with *HLA-A*33:03* in combination with variants of a DME (Table 3).

In the following section, the effects of gene variants of phase I and phase II biotransformation enzymes on susceptibility to ADRs will be discussed in the context of codeine/warfarin and azathioprine, respectively. Then, the effects of gene variation for a drug transporter will be illustrated for statin-induced muscle toxicity. However as the first example of malignant hyperthermia shows, genetic susceptibility to nonimmune-mediated ADRs can occur through plausible pharmacodynamic mechanisms too. Table 4 lists examples of ADRs associated with nonimmune-related genetic variants.

3.1 RYR1 and Anaesthesia-Induced Malignant Hyperthermia

In 1962, a paper was published about a pedigree that contained 10 relatives who had unfortunately and unexpectedly died during or shortly following general anaesthesia [163]. The deaths were associated with core body temperatures, when measured, in excess of 41 °C and followed an autosomal dominant inheritance pattern [163]. Other pedigrees have since been described [164, 165] and over 500 cases of malignant hyperthermia (MH) have now been reported in the medical literature [166].

MH is precipitated by volatile anaesthetics in genetically susceptible individuals. All halogenated inhalation anaesthetics have been implicated including halothane, isoflurane, sevoflurane and desflurane [167]. The depolarising neuromuscular blocker, succinylcholine, augments the adverse response to these potent inhalation anaesthetics but its role as an independent precipitant of fulminant MH is controversial [167, 168]. Rarely, non-pharmacological stressors including environmental heat [169, 170], infections [170] and severe exercise or emotional strain [171] have been implicated in MH-like episodes.

The incidence of anaesthetic-induced MH is approximately 1 per 50,000 adults and 1 per 15,000 paediatric patients [172] and it occurs in all ethnic groups [173]. The basis of MH is hypermetabolism which can present as tachypnoea, a rise in end-tidal carbon dioxide exhalation, tachycardia, cyanosis, cardiac arrhythmias, skeletal muscle rigidity, hyperthermia [174], convulsions and eventual death [163]. Associated electrolyte complications include acidosis, hyperkalaemia, elevated creatine kinase (CK) and acute kidney injury (AKI) [174]. Timely intervention improves prognosis [175]. However, an early diagnosis of MH can be challenging as the initial clinical signs are nonspecific and variable in their time course, making them easily mistaken for other pathologies (e.g. sepsis, thyrotoxic crisis) [176]. Nevertheless, the mortality from MH has dramatically fallen from 70 % in the 1970s [169] to < 5 % today [173]. This reduction has been aided by the introduction of the muscle relaxant dantrolene for treatment of suspected MH [166] and testing for susceptible relatives (see later) [169]. A clinical grading scale has been introduced to help researchers retrospectively assess the likelihood of MH following an adverse anaesthetic event, which enables accurate phenotyping and determination of future susceptibility [177].

RYR1 is located on chromosome 19 and encodes ryanodine receptor 1 (RyR1). There are 3 RyRs isoforms (RyR1–3) and each forms a homotetrameric assembly within the endoplasmic (or sarcoplasmic) reticulum and functions as a Ca^{2+} channel [178]. They have evolved into the largest ion channels found to date (~2.2MDa) [179]. This is undoubtedly to facilitate tight channel regulation through interaction with numerous regulatory small molecules and proteins, which is important as Ca^{2+} is a potent intracellular mediator of several cell processes [179]. RyR1 is widely expressed in skeletal muscle and is pivotal to excitation-contraction coupling [180]. RyR1 opens in response to nerve impulses and releases Ca^{2+}, from the sarcoplasmic reticulum where it has been sequestered, into the cytoplasm to drive muscle contraction. It is thought that a direct physical connection exists between the voltage-gated Ca^{2+} channel $Ca_V1.1$ (the skeletal dihydropyridine receptor) in the transverse tubule and RyR1 [181, 182], which induces conformational changes that open RyR1 when the wave of depolarisation from the neuromuscular endplate is detected by $Ca_V1.1$ [183].

Approximately 70% of MH susceptible families carry *RYR1* variants [156]. A nonsynonymous mutation of *RYR1* was found to cause the porcine stress syndrome in inbred pigs, which is an animal model of MH [184]. The analogous C1843T mutation in humans was subsequently identified in an analysis of 1 of 35 MH susceptible pedigrees [185]. Currently, over 200 *RYR1* mutants have been described and most are single nucleotide polymorphisms (SNPs), but only 31 have been designated as causative of MH according to the specific criteria set out by the European Malignant Hyperthermia Group (EMHG) [155].

Impaired Ca^{2+} homeostasis underlies the pathogenesis of MH [173]. Gain-of-function *RYR1* mutations have been shown *in vitro* to lead to RyR1 hyperactivation [186, 187]. The increase in intracellular Ca^{2+} concentration results in sustained muscle contraction and heat generation [173]. Attempts to restore the Ca^{2+} balance and the contracting muscle filaments deplete the cell of adenosine triphosphate resulting in muscle rigidity, loss of integrity to the sarcolemma and leakage of intracellular contents (e.g. K^+, myoglobin) out into the extracellular fluid predisposing to systemic sequelae [172]. However, the exact mechanism(s) by which volatile anaesthetics precipitate this potentially fatal cascade has not been clearly elucidated [188].

Interestingly, ~20% of patients have undergone previous uneventful general anaesthesia with potent inhalation agents before experiencing MH [166]. The reasons for this incomplete penetrance are not fully understood but hypotheses include dose and/or duration dependency effects of the volatile anaesthetic agents [167], the ambient temperature and the simultaneous use of possible mitigating drugs [173].

The majority of MH occurs in asymptomatic individuals and they are considered to have a genetically-determined subclinical myopathy [176]. However, there are at least 3 rare clinical myopathies likely associated with MH susceptibility: central core disease (CCD), multiminicore disease (MmD) and King-Denborough syndrome [189, 190]. Within each syndrome there is clinical, genetic and histological variability and considerable overlap exists, in particular, between CCD and MmD [189]. Importantly, the majority of CCD cases are associated with *RYR1* variants

and furthermore, *RYR1* mutations have also been found in cases of MmD [189] and King-Denborough syndrome [191], although these links are less certain [192]. Of the 200 *RYR1* variants, ≥150 are associated with MH alone (subclinical myopathy), ~100 with CCD and ≥20 with both MH and CCD [192]. *RYR1* alleles are also implicated in instances of exercise-induced rhabdomyolysis [193, 194]. Clearly, *RYR1* is involved in a spectrum of muscle disorders, but at the present time the degree of genotype to phenotype concordance is incompletely understood.

The gold standard for MH diagnosis in patients and unaffected relatives is the *in vitro* muscle biopsy contracture test (IVCT), which assesses muscle contraction in response to caffeine and halothane [195]. However, the IVCT is invasive, costly and confined to specialist centres. Therefore, genetic testing has been increasingly used since 2001 [156] to determine MH susceptibility in family members of MH patients that have been shown to carry a causative *RYR1* mutation, as classified by the EMHG [196]. A relative not carrying the familial *RYR1* mutation should still undergo an IVCT though as the absence of a *RYR1* mutation does not exclude MH susceptibility [176].

~75% of MH events occur in patients with no reported family history [166] and therefore universal pre-anaesthetic genetic screening is appealing. However, genetic screening for MH is currently untenable, due to the heterogeneous and incompletely understood genetics underpinning MH susceptibility. The complexity of the RyR1 molecule makes structural and functional predictions of *RYR1* variants challenging [197] and regardless, 30% of MH cases are not associated with *RYR1*. At least 5 other genetic loci have been implicated [172] but of these to date, only nonsynonymous SNPs in *CACNA1S*, the gene encoding the α1 subunit of $Ca_V1.1$, have been linked to MH and in only 1% of cases [157, 158, 172].

In summary MH is a potentially fatal disorder with a strong genetic predisposition, although the full spectrum of genetic risk variants and associated genotype-phenotype correlations are incompletely characterised. However, this strong genetic susceptibility lends itself to the future prospect of successful genetic screening to reduce the incidence of drug-induced MH.

3.2 CYP2D6 and Codeine Analgesia and Safety

Codeine is a weak opioid that is indicated for analgesia in mild to moderately severe pain and as an antitussive and anti-diarrhoeal agent. Although it has been used for many years, recent concerns are mounting over its variable efficacy and safety.

Figure 1 shows the principal pharmacokinetic pathways for codeine. Codeine is considered a prodrug whose function is derived from conversion into 2 active metabolites: morphine and morphine-6-glucuronide (M6G). Both are agonists for the widespread μ-opioid receptor, which is largely responsible for the therapeutic effects and opioidergic ADRs [198, 199]. The affinity of morphine for μ-opioid receptors is 200-fold stronger than compared to codeine [200]. The polymorphic cytochrome 2D6 enzyme (CYP2D6) catalyses the O-demethylation of codeine into

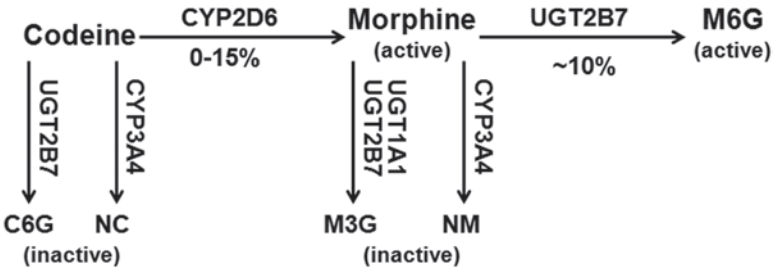

Fig. 1 Codeine metabolism. *C6G* codeine-6-glucuronide, *NC* norcodeine, *M6G* morphine-6-glucuronide, *M3G* morphine-3-glucuronide, *NM* normorphine, *CYP2D6* cytochrome P450 2D6, *CYP3A4* cytochrome P450 3A4, *UGT2B7* uridine diphosphate glucuronosyltransferase 2B7, *UGT1A1* uridine diphosphate glucuronosyltransferase 1A1

morphine. Uridine diphosphate glucuronosyltransferase 2B7 (UGT2B7) catalyses morphine into M6G and, in conjunction with UGT1A isoforms, into inactive morphine-3-glucuronide (M3G). Only a minority of codeine biotransformation (≤15%) [201] is via CYP2D6; the majority of codeine is converted directly into the inactive metabolites codeine-6-glucuronide and norcodeine by UGT2B7 and CYP3A4, respectively. The major codeine metabolites, including morphine and M6G, are excreted renally [201].

CYP2D6 belongs to the superfamily of cytochrome P450 (*CYP*) genes. They encode haemoproteins that catalyse oxidative, phase I metabolism [202] and account for ~75% of all drug metabolism reactions [203]. Although 57 *CYP* genes have been identified, ~95% of these reactions are catalysed by just 5 isoenzymes including CYP2D6 [203]. Direct clinical measurement of CYP2D6 phenotypic activity is unfeasible as it is primarily expressed in the liver and indirect measurements of CYP2D6 metabolites in the plasma or urine are susceptible to other factors including renal dysfunction and drug interference. Consequently, *CYP2D6* genotyping as a phenotype surrogate is appealing for clinical practice.

CYP2D6 is located on chromosome 22 and over 80 alleles have been identified [204]. They are formed by a range of genetic alterations including SNPs, insertions and deletions and can be grouped functionally into increased, normal, reduced and non-functional alleles [137]. An individual's *CYP2D6* genotype can in turn be categorised into 1 of 4 predicted phenotype classes based on the combination of *CYP2D6* alleles they carry: an extensive, intermediate, poor or ultrarapid metaboliser (EM, IM, PM and UM, respectively) [205]. The EM is the wild-type CYP2D6 phenotype, IMs have reduced activity and PMs have no enzymatic activity as they carry no functional alleles. If multiple copies of functional alleles are detected this is denoted the UM phenotype as high enzymatic activity is expected [137]. There is considerable variability in the prevalence of *CYP2D6* alleles and in the prevalence of the extreme phenotypes in different ethnic groups (0–10 and 0–29% for PMs and UMs, respectively) [137].

It has been shown that following codeine administration, PMs have significantly lower plasma morphine concentrations, reduced urinary active metabolite excretion

and decreased analgesia compared to EMs [206, 207]. Conversely, plasma morphine concentrations and urinary active metabolite excretion are significantly higher in UMs compared to EMs [208]. Furthermore although there is no definitive study, a growing series of case reports are documenting severe ADRs after standard codeine use associated with the UM phenotype [136, 209–213]. These case reports are from neonatal [209], paediatric [210–212] and adult populations [136, 213] and the documented on-target (opioidergic) ADRs include: severe epigastric pain, euphoria and dizziness [213], central nervous system/respiratory depression [136, 211] and death [209, 210, 212]. One especially poignant case was the death of a 13-day old neonate who was breastfed by a mother taking codeine (and paracetamol) for episiotomy pain [209]. The autopsy found an extremely high level of morphine in the neonate's blood and a sample of stored maternal breast milk from day 10 showed an elevated morphine concentration. The mother was found to have a C*YP2D6* gene duplication indicative of the UM phenotype [209]. Following this report, the US Food and Drug Administration (FDA) issued a warning on codeine use by nursing mothers [214].

Although there is increasing concern regarding the efficacy and safety of codeine, several barriers exist that hamper the translation of *CYP2D6* genotyping into widespread clinical practice. Firstly, the ADR profile of PMs is incompletely understood [137]. Secondly, when compared to the prevalence of the UM phenotype (0–10%), the documented case reports of severe ADRs are rare, suggesting that there are additional genetic and non-genetic susceptibility factors. The pharmacogenetic influence of *UGT2B7* is controversial at present [201]. Other risk factors may include renal dysfunction [136, 201, 215], drug inhibitors of CYP3A4 [136, 201], ontogeny [215, 216] and repeated episodes of hypoxia [215]. The paediatric case reports are from children receiving codeine after adeno(tonsillectomy) for recurrent tonsillitis and obstructive sleep apnoea (OSA) [210–212]. OSA leads to intermittent sleep hypoxia and it has been shown that opioid analgesia sensitivity increases in children after recurrent hypoxia [217]. Another factor is potential publication bias favouring selection of case reports documenting extreme but fortunately uncommon ADRs with codeine. 10 of 11 UM participants in a pharmacokinetics study felt sedation (91%) compared to 6 of 12 (50%) EMs ($p=0.03$) suggesting that ADRs in UMs may occur more frequently than is reported [208]. Other potential barriers include the absence of prospective studies that demonstrate clinical benefit of *CYP2D6* genotyping, scarce cost-effectiveness data, lack of clinician knowledge and no clear guidelines on what constitutes a suitable substitute for codeine in CYP2D6 PMs and UMs. This is important because CYP2D6 is involved in the metabolism of other opioid drugs including oxycodone, hydrocodone and tramadol. There is evidence at least for tramadol that CYP2D6 PMs experience reduced analgesia [218] and UMs a higher risk of nausea [138] when compared to EMs. There is also a case report of respiratory depression following tramadol in a UM patient with renal dysfunction [139]. Tramadol and codeine are step 2 'weak' opioid drugs on the WHO analgesia ladder [219] and are often used interchangeably in clinical practice for a patient that does not tolerate one. However if tramadol is also undesirable in CYP2D6 PMs and UMs, clinical guidance regarding suitable alternative analgesic agents is warranted.

3.3 *CYP2C9 and the Risk of Haemorrhage with Warfarin*

Warfarin is the most frequently prescribed oral anticoagulant worldwide [220] and is indicated in the prophylaxis and treatment of venous thromboembolism (VTE) and in the prophylaxis of systemic embolism in predisposing conditions such as atrial fibrillation and following mechanical heart valve insertion [221]. It is a coumarin-derived therapeutic that is administered as a racemic mixture; the S-warfarin enantiomer is more potent than R-warfarin [222]. They disrupt the vitamin K cycle by antagonising vitamin K epoxide reductase, resulting in a decrease in vitamin K-dependent post-translational γ-carboxylation of protein glutamate residues [223, 224]. This notably diminishes the activity of clotting cascade proteins including the procoagulant factors II, VII, IX and X and anticoagulant molecules protein C and protein S [225]. The overall anticoagulant effect is quantified by the prothrombin time-derived international normalised ratio (INR); the usual desired therapeutic INR is 2.5 [226]. However, certain high thrombotic risk conditions such as recurrent VTE(s) on warfarin and mechanical heart valves warrant higher anticoagulation levels (e.g. a desired INR range of 3.0–4.0) [226].

Epidemiological evidence has implicated warfarin as a major cause of ADRs; it is the therapeutic associated with the greatest number of preventable ADRs in Sweden [227] and the third most common cause of ADR-related hospitalisations in the UK [3]. Haemorrhage is an on-target ADR and is the predominant ADR associated with warfarin [221], especially during therapy initiation [228]. It is highly correlated to the intensity of anticoagulation [229, 230] and the risk of clinically significant bleeding increases when the desired INR range is higher [221]. The safe management of warfarin therapy is notoriously challenging because of the wide inter-individual range of optimal dose requirements (0.6–15.5 mg/day) and its narrow therapeutic index [231]. It is worth noting also that there is evidence to suggest a pharmacogenetic association between *CYP2C19*17* carriage and increased bleeding risk in patients taking clopidogrel (Table 4) [130, 131], although for now, the genetic susceptibility to haemorrhage on warfarin will be outlined.

CYP2C9, like CYP2D6, is 1 of the 5 main human CYP DMEs [203]. CYP2C9 is the principal enzyme involved in the metabolism of the potent S-warfarin stereoisomer, while R-warfarin is cleared via CYP1A1/CYP1A2/CYP3A4 [228]. Over 30 allelic variants of *CYP2C9* are known, but their relative prevalence varies with ethnicity [220]. The *CYP2C9* reference genotype **1/*1* produces the normal (EM) phenotype [220] and a resultant estimated warfarin half-life of 30–37 h [232]. The 2 most frequent reduction-of-function minor alleles amongst people with European ancestry are *CYP2C9*2* (rs1799853) and *CYP2C*3* (rs1057910) [202]. Both are characterised by one nonsynonymous SNP, prolong the half-life of warfarin (up to 92–203 h in **3/*3* homozygotes [233, 234]) and are associated with reduced maintenance warfarin dose requirements [235].

A recent meta-analysis has reported hazard ratios for the risk of bleeding in patients on warfarin with **1/*3* or **3/*3* genotypes, compared to **1/*1* patients, to be 2.05 (95% CI 1.36–3.10) and 4.87 (95% CI 1.38–17.14), respectively, suggestive of a gene-dose effect [135]. Although *CYP2C9*2* was also significantly associated

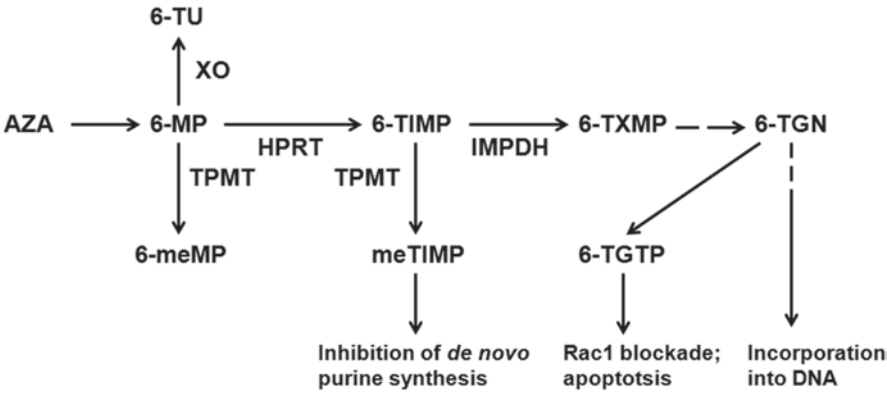

Fig. 2 Azathioprine (*AZA*) and 6-mercaptopurine (6-MP) metabolism (simplified). *me prefix* methyl, *XO* xanthine oxidase, *TU* thiouric acid, *TPMT* thiopurine methyltransferase, *HPRT* hypoxanthine phosphoribosyltransferase, *TIMP* thioinosine monophosphate, *IMPDH* inosine monophosphate dehydrogenase, *TXMP* thioxanthosine monophosphate, *TGN* thioguanine nucleotides, *TGTP* thioguanine triphosphate

with bleeding, albeit with a lower pooled effect size than *CYP2C9*3*, after stratification into **1/*2* and **2/*2* genotypes through synthesis of studies that reported these individual genotypes, neither genotype was significantly associated with bleeding. Overall, *CYP2C9*3* is the main risk factor for bleeding on warfarin, which is biologically plausible as the **3* allele has a more deleterious effect than **2* on CYP2C9 enzyme function [135]. For a more comprehensive account of overall warfarin pharmacogenetics, dosing strategies to incorporate multiple environmental, clinical and genetic factors and a discussion regarding the recently published prospective warfarin pharmacogenetic RCTs, the reader at this point is referred to Chap. 11.

3.4 TPMT, Azathioprine- and 6-Mercaptopurine-Induced Myelosuppression

The immunosuppressive agent azathioprine (AZA) is a pro-drug of 6-mercaptopurine (6-MP). AZA is indicated in both the prophylaxis of transplant rejection and in the treatment of many autoimmune conditions including inflammatory bowel disease (IBD), rheumatoid arthritis and severe eczema [236]. 6-MP is conventionally used with haematological malignancies and in particular acute lymphoblastic leukaemia [237], although it also has a role in IBD [238]. AZA/6-MP can induce several ADRs including myelosuppression (predisposing to neutropaenic sepsis), DILI, pancreatitis, nausea and vomiting [239]. Although ADRs occur in 10–28 % of patients [240], the rate of fatal ADRs among AZA users is estimated at 1 in 10,000 [241].

Approximately 90 % of AZA is converted to 6-MP by ubiquitous non-enzymatic processes [242, 243]. Figure 2 depicts the 3 main competing enzyme pathways for

metabolism of 6-MP: thiopurine methyltransferase (TPMT), XO and the main anabolic pathway via hypoxanthine phosphoribosyltransferase (HPRT) [244]. Both therapeutics are subject to extensive intestinal and hepatic first pass metabolism following oral dosing [244, 245]. Although there is an incomplete understanding of the modes of action of AZA/6-MP [246], the accumulation of 6-thioguanine nucleotide (6-TGN) metabolites formed *in vivo* via HPRT is thought to contribute to both their efficacy [247] and when in relative excess, the increased risk of myelosuppression [242, 248]. The immunosuppressive mechanisms include incorporation of 6-TGNs into DNA inhibiting leukocyte DNA synthesis [244, 249] and blockade of Rac1 protein by the 6-TGN derivative, 6-thioguanine triphosphate (6-TGTP), inducing T-cell apoptosis [246]. TPMT can methylate both 6-MP and the intermediate metabolite, 6-thioinosine monophosphate (6-TIMP), to give 6-methylmercaptopurine (6-meMP) and methyl-TIMP (meTIMP), respectively. meTIMP may be efficacious through *de novo* purine synthesis inhibition [240, 250] whilst high levels of TPMT methylated thiopurine metabolites (and further phosphorylated metabolites) may be associated with DILI [251–255].

TPMT is a phase II biotransformation enzyme, encoded by *TPMT* on chromosome 6 [243], and is a major pharmacokinetic determinant for active 6-TGN metabolite levels [240], which are inversely related to TPMT activity [244, 256, 257]. It is variably expressed in several tissues; the highest levels of TPMT are present in the liver and the lowest in the brain and lung [240]. Erythrocyte TPMT activity correlates with hepatic TPMT activity [258] permitting direct TPMT phenotypic assessment of patients in clinical practice, which is unusual for a DME [259]. TPMT enzymatic activity follows a trimodal distribution; ~90% of individuals have high activity, ~10% intermediate and 0.3% low/undetectable enzyme activity [260, 261].

Around 30 allelic variants of *TPMT* have been reported [20] and despite ethnic variability, 3 account for >90% of the minor alleles: *TPMT*2*, *TPMT*3A* and *TPMT*3C* [254]. They are caused by one (*TPMT*2*, *TPMT*3C*) or two (*TPMT*3A*) nonsynonymous SNPs that reduce enzymatic activity through enhancing the rate that the TPMT variant is catabolised [262–264]. Analogous to *CYP2D6* and *CYP2C9*, *TPMT* genotype correlates with the variable TPMT enzymatic activity levels: heterozygotes have intermediate activity (IM) and individuals carrying no normally functioning alleles have low/absent activity (PM) [254]. Like *CYP2D6* and *CYP2C9*, homozygous deficient individuals include both those homozygous for 1 variant allele and compound heterozygotes with 2 distinct inactivating alleles [243]. *TPMT *1/*1* individuals have normal phenotypic activity (EM).

Clinically, ~27% of AZA/6-MP-induced myelosuppression cases are explained by inactivating *TPMT* alleles [265], although little correlation exists with other specific ADRs including DILI [239, 266]. A meta-analysis of patients with chronic inflammatory diseases has reported a gene-dose effect for this on-target ADR: homozygous deficient individuals carry a higher risk of leukopaenia (OR 20.84, 95% CI 3.42–126.89) than heterozygotes (OR 4.29, 95% CI 2.67–6.89) when compared with **1/*1* individuals [149] and in general the myelosuppression onset is earlier [265, 267] and more severe [267]. A second systematic review, not limited to a

specific class of disease, has reported that 86% of *TPMT* homozygous deficient patients develop myelosuppression and the pooled OR for patients with intermediate TPMT activity or one *TPMT* variant allele, compared with wild-type, was 4.19 (95% CI 3.20–5.48) [268]. For both studies, their results were primarily derived from synthesis of observational studies.

As clinical evidence has grown, consensus national clinical guidelines have been published that recommend and interpret pre-therapy TPMT testing, including the UK dermatology [269] and rheumatology guidelines [270]. In patients identified as TPMT deficient (by either genotyping of homozygous deficiency or TMPT phenotypic analysis of low/absent activity), guidance advises selection of alternative immunosuppressive therapy in non-malignant conditions and a reduction in starting dose to 10% of normal when treating malignancy [254]. For heterozygous variant/intermediate activity patients commencing AZA/6-MP therapy, a dose reduction of 30–70% is suggested [254]. TPMT analysis has been adopted into clinical practice and a national survey reported that 94% of dermatologists, 60% of gastroenterologists and 47% of rheumatologists in England requested TPMT testing [271].

Despite the relatively high, albeit variable, clinical uptake of TPMT testing, outstanding issues remain. Firstly, there is a lack of robust prospective randomised evidence assessing the utility of pre-therapy TPMT analysis in reducing myelosuppression. An RCT ($n=333$) was undertaken but the recruitment target ($n=1000$) was not met due to guideline-driven pre-existing routine TPMT testing at some centres adversely impacting study recruitment [272]. The one patient in the non-genotyped arm found at study completion to be *TPMT* homozygous deficient developed severe, early onset neutropaenia. However overall, the study found no difference in the rates of AZA cessation due to ADRs between the *TPMT* genotyped arm (with recommended AZA dose reduction and avoidance in heterozygous and homozygous *TPMT* deficient patients, respectively) and the non-genotyped arm, and no increase in AZA cessation in *TPMT* heterozygous patients compared to wild-type patients [272].

Secondly, whilst the evidence and recommendations for *TPMT* homozygous deficient individuals are relatively clear, the optimal management strategy for heterozygous patients is less certain. Although overall they appear to be at a modest increased risk of myelosuppression [149, 268], complicating factors include the observation that only ~30–60% of heterozygous patients do not tolerate full doses of AZA/6-MP [254, 257, 273] and the benefit: harm ratio attributable to different thiopurine starting doses for heterozygotes likely varies depending on the disease-specific necessity for rapid therapeutic action. A higher risk of myelosuppression with a higher starting dose in a heterozygote might be justifiable for treating malignancy, but not chronic, stable immunological disease.

Thirdly, *TPMT* can be analysed by phenotype or genotype and the screening test protocol remains incompletely standardised. Erythrocyte TPMT activity is predominantly offered to clinicians in the UK, but it can be affected by patient ethnicity, concurrent use of interacting drugs (e.g. mesalazine, sulfasalazine, allopurinol), allogeneic erythrocyte transfusions during the preceding 120 days, and in haematological malignancies, it can be affected by disease-related influences [274]. Whilst

the overall genotype to phenotype test concordance is 98.4% in healthy volunteers, it decreases to 86% in the intermediate TPMT activity range, attributable to both non-genetic influences on TPMT activity, as described above, and to a lesser extent, novel mutations [275]. Therefore, neither test is 100% sensitive to correctly identify TPMT deficiency, but research from a National Centre suggests that genotyping is more accurate and should be used as the primary test, in contrast to current UK practice [276].

Therefore, a pharmacogenetic association exists between *TPMT* and myelosuppression and there is strong evidence, affirmed by clinical guidelines, for avoiding thiopurine drugs or significantly reducing their dose in *TPMT* homozygous deficient patients, given their near universal experience of myelosuppression at conventional doses [254]. Further research is required to clarify optimal management for heterozygous patients. However, it is already cost-effective to routinely test TPMT status to identify homozygous deficient patients alone [274]. Pre-therapy TPMT testing is not a substitute for routine on-therapy blood test monitoring, given that several thiopurine ADRs are not associated with TPMT and the majority of myelosuppression cases are still not accounted for by *TPMT* variants [265]. Finally, in addition to TPMT testing, there is also a growing role for thiopurine metabolite level monitoring (e.g. 6-TGNs) to individualise thiopurine doses soon after starting treatment; prospective studies to evaluate this proactive approach are ongoing [277].

3.5 SLCO1B1 and Statin-Induced Muscle Toxicity

Statins are the most commonly prescribed class of medication worldwide [278] and are highly efficacious in the primary and secondary prevention of cardiovascular disease [1]. They reduce plasma low-density lipoprotein (LDL) cholesterol through competitive inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, the rate limiting enzyme in *de novo* cholesterol synthesis. This in turn leads to an upregulation of hepatic LDL receptors, increasing cholesterol influx into hepatocytes and reducing the plasma burden [279].

The currently licensed statins have a good safety profile, but carry a small risk of skeletal muscle toxicity [280]. The spectrum of muscle pathology varies from the most common manifestation of asymptomatic elevations in plasma CK level, to myopathies with pain and high plasma CK levels through to rhabdomyolysis with the potential sequelae of AKI and death. Alternatively, statin therapy can cause myalgias with no detectable plasma CK rise [21]. Depending on precise definitions, myopathy and rhabdomyolysis occur at frequencies of ~1/1000 and ~1/100,000, respectively [281], although this is modulated by other risk factors including higher statin dose, female gender, older age, low BMI, untreated hypothyroidism and other drug therapies, for example concomitant use of gemfibrozil [281].

The solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) belongs to the superfamily of solute carrier (*SLC*) influx transporter genes and encodes the organic anion-transporting polypeptide 1B1 (OATP1B1) [282]. OATP1B1

is one of the most highly expressed influx transporters within the human liver [283]. It facilitates hepatic uptake of a variety of xenobiotic compounds and endogenous substances [284] and so affects the level of exposure of substrate drugs to intracellular hepatic DMEs [285].

Although the effects of statins on the off-target muscle tissue are incompletely defined at present [286], there exists a significant association between gene variants of *SLCO1B1* and the risk of statin-induced muscle ADRs. A seminal statin GWAS used data from the Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH) RCT in the UK; 85 cases of definite or incipient myopathy were contrasted with 90 controls [148]. Both the cases and controls for the GWAS had been prescribed 80 mg simvastatin daily. Only an intronic SNP variant, rs4363657, was strongly correlated with myopathy and further regional genetic analysis showed it to be in near complete linkage disequilibrium with the nonsynonymous SNP, rs4149056, in exon 6 (*SLCO1B1*5*; 521T > C; V174A). Further, a gene-dose relationship was demonstrated for rs4149056: the OR for myopathy in heterozygotes and homozygotes for the minor C allele was 4.5 (95% CI 2.6–7.7) and 16.9 (95% CI 4.7–61.1), respectively, when compared to the ancestral TT genotype. Overall, greater than 60% of the myopathy cases in this study were attributable to the C variant [148]. The association with rs4149056 has been replicated [148, 287, 288] but the incidence of severe myopathy and the magnitude of correlation were lower in a second UK randomised trial population [289], attributable to the smaller 40 mg daily simvastatin dose used [148]. The rs4149056 variant has been subsequently associated with more mild, statin-induced muscle ADRs [290], reduced simvastatin adherence [290] and general intolerance to simvastatin defined as a composite endpoint of prescribing +/− mild biochemical changes [291]. The weight of evidence to date for rs4149056 is with simvastatin and the evidence with other statins is less compelling [287, 290, 292], suggesting that rs4149056 may represent a simvastatin-specific effect.

Mechanistically, rs4149056 may interfere with localisation of the transporter to the hepatic plasma membrane reducing its activity [284]. It is associated with higher statin, and especially simvastatin acid, plasma concentrations [293–295] that conceivably increase skeletal muscle drug exposure. However, the relationship between plasma simvastatin acid concentration and muscle toxicity is not straightforward. Clinically, current FDA guidance recommends against the 80 mg simvastatin dose unless a patient has tolerated the higher dose for over 12 months [296].

Overall, the rs4149056 variant is a plausible candidate for a predictive test to reduce simvastatin-induced skeletal muscle ADRs. Current guidance suggests that when initiating simvastatin therapy in CT or CC genotype patients, simvastatin 20 mg daily is selected rather than the normal 40 mg daily dose, possible routine CK surveillance is utilised and alternative statin therapy is commenced rather than increasing the dose of simvastatin if lipid goals are not reached. However, the effects of these recommendations on the incidence of simvastatin ADRs and adherence are currently unknown [281].

4 Outlook and Recommendations

The aspiration of pharmacogenetics is to individualise drug treatment to minimise harm and promote efficacy. Pre-therapy predictive genetic testing seeks to tailor therapy to reduce ADRs primarily through guiding drug or dose selection and has impacted positively upon clinical practice, notably with abacavir. Genetic screening may also find a role in identifying patients for whom regular biomarker surveillance may be indicated to minimise the incidence of severe ADRs. In addition to the direct patient benefit of reducing ADRs, there are at least 3 other potentially favourable spin-offs from understanding the pharmacogenetics of ADRs. Firstly, genetic-ADR associations provide novel insights that facilitate investigation into underlying pathological processes and the extrapolation of new knowledge regarding hypersensitivity reactions may have implications for cancer, autoimmune and infectious disease management. Secondly, the safety profile of new therapeutics may be improved through screening of drug candidates for affinity to high risk *HLA* alleles, for example *HLA-B*57:01* and *HLA-B*58:01* [71]. Thirdly, the beneficial side effects of some drugs have resulted in new therapeutic indications, for example with sidenafil (Viagra) and its fortuitous alleviation of erectile dysfunction. Pharmacogenetics has the potential to increase this 'drug repositioning' through identifying novel off target pharmacodynamic sites.

Abacavir has provided a blueprint for translational pharmacogenetics, but it has yet to be emulated. This is partly due to certain 'favourable' characteristics of AHS including: the high relative prevalence of AHS [76], the exclusivity of the association between *HLA-B*57:01* and immunologically-mediated AHS, the reduction of false-positive clinical diagnoses mediated by the screening programme [78], the vocal patient lobby, and a physician community who were relatively amenable to changing their prescribing and clinical behaviour. It is also because there are multiple obstacles encountered when attempting translation. It is important to first understand these hurdles, and then to have a systematic approach to both developing the ADR-genotype evidence base and to implementing it in clinical practice [297].

Many ADRs are rare and some, such as the HSS, consist of varying constellations of non-specific features. As a result, international consortia using standardised definitions for these ADRs are advisable so patient samples of sufficient size with well demarcated phenotypes that are generalisable across ethnic groups can be pooled together. The 'International Serious Advent Consortium' and their 'Phenotype Standardisation Project' are both steps in the right direction [298]. These coordinated efforts are a prerequisite to reducing the risk of type I and type II errors in genetic association studies of rare and variable ADRs.

Pharmacogenetics has traditionally harnessed the candidate gene approach, whereby genes predicted to be relevant, typically through knowledge of a drug's pharmacology, are selectively studied. However, this approach is limited to contemporary knowledge and so has largely been superseded by GWAS, which has no stipulation for *a priori* hypotheses [20] and can test at least 10^6 SNPs concurrently. However GWAS increases sample size requirements and data capture, increasing

the complexity of study data management and statistical processes and potentiates the threat of selective publication reporting. Further, the lack of a preformed hypothesis augments the importance of confirming biological causality for GWAS putative associations.

Nevertheless, GWAS is a valuable asset: it can confirm in a 'blinded' fashion the results of previous candidate gene studies [20] and offer a novel foothold into the idiosyncratic processes of off-target ADRs. For polygenic ADRs, GWAS may detect new loci of individual small effect size and assess genotype-phenotype associations of larger haplotype signatures. The '1000 Genomes Project,' which has recently described the genomes of 1092 individuals, is in turn increasing the resolution of GWAS [299]. The 1000 Genomes Project should additionally provide a baseline reference for normal human genetic variation, enable fine mapping of existing GWAS associations and aid discovery of new genetic associations, partly through its detailed identification of indels and larger deletions as well as contemporary SNPs [299]. In the near future, next generation sequencing technologies that provide high throughput whole genome capability will offer the pinnacle of DNA resolution whilst advances in our understanding of epigenetic imprinting and microRNA regulation promise new directions for the study of ADR pharmacogenetics. As genetic variation does not usually account for all of the inter-individual variation in drug response, incorporation of data from transcriptomics, metabolomics and proteomics may further improve predictive values [127].

After identification and validation of a statistically significant genetic association(s) for an ADR, several hurdles still bar adoption into clinical practice. Large, well-conducted prospective studies represent the gold standard to confirm clinical outcome benefit, although given the rarity of some ADRs these are not always practical. For other ADRs, genetic sub-studies of clinical trials and registries will likely offer the highest attainable level of evidence [300]. Subsequent pharmacoeconomic studies should base their analyses on this high quality data rather than expert opinion and retrospective data [301].

Logistical and knowledge barriers to the implementation of ADR pharmacogenetics also exist. On-demand genotyping, where the treating physician requests a specific pharmacogenetic test for a patient when seeking to prescribe a drug with a clinically established ADR-genotype association, relies on both a physician's knowledge of pharmacogenetics and a system for following-up and acting on the pharmacogenetic test result. Robust and validated point-of-care genotyping tests may be necessary. An alternative proposed method is pre-emptive genotyping, where multiple relevant SNPs are routinely genotyped together and this genetic data is incorporated into a patient's electronic medical record, with subsequent access by automated clinical decision support (CDS) algorithms to provide a clinically relevant alert regarding a potential drug-genotype interaction specific to the individual patient, at the point in time when the physician is seeking to prescribe the drug of interest. This approach provides the pharmacogenetic information at the most pertinent time and secondly, the CDS approach is likely better suited to keep up with our rapidly expanding understanding of

ADR pharmacogenetics. However, the associated computational challenges are considerable [302].

Finally, a genetic test should be ethically acceptable to patients, clinicians and society. The emphasis of pharmacogenetics is for the beneficial personalisation of medicine, yet paradoxically the realisation of this goal requires not only very large international research collaborations but also active engagement with society as a whole. This is not least because genetic information harbours potential adverse implications, such as individual discrimination by insurance firms based on high risk genotype carriage and neglect of ethnic minorities by pharmaceuticals opting to segregate research initiatives to benefit the majority to maximise profit margins [303]. Open dialogue between patients, healthcare services, insurance providers, pharmaceuticals and the wider public is required to address these risks. If society chooses pharmacogenetics, it must safeguard against encroachment on the rights of individuals and minority groups. Ultimately, the widespread application of pharmacogenetics throughout clinical practice to ameliorate ADRs remains far off, but the examples in this chapter and the promises inherent in the new technologies foreshadow a future potential.

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