Minireview

Inflammation-Induced Phenoconversion of Polymorphic Drug Metabolizing Enzymes: Hypothesis with Implications for Personalized Medicine

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MINIREVIEW

Phenoconversion transiently converts genotypic extensive metabolizers (EMs) into phenotypic poor metabolizers (PMs) of drugs, potentially with corresponding changes in clinical response. This phenomenon, typically resulting from coadministration of medications that inhibit certain drug metabolizing enzymes (DMEs), is especially well documented for enzymes of the cytochrome P450 family. Nonclinical evidence gathered over the last two decades also strongly implicates elevated levels of some proinflammatory cytokines, released during inflammation, in down-regulation of drug metabolism, especially by certain DMEs of the P450 family, thereby potentially causing transient phenoconversion. Clinically, phenoconversion of NAT2, CYP2C19, and CYP2D6 has been documented in inflammatory conditions associated with elevated levels of some proinflammatory cytokines, such as human immunodeficiency virus infection, cancer, and liver disease. The potential of other inflammatory conditions to cause phenoconversion has not been studied but experimental and anecdotal clinical evidence supports infection-induced down-regulation of CYP1A2, CYP3A4, and CYP2C9 as well. Collectively, the evidence supports a hypothesis that certain inflammatory conditions associated with elevated proinflammatory cytokines may cause phenoconversion of certain DMEs. Since inflammatory conditions associated with elevated levels of proinflammatory cytokines are highly prevalent, phenoconversion of genotypic EM patients into transient phenotypic PMs may be more frequent than appreciated. Since drug pharmacokinetics, and therefore the clinical response, is influenced by DME phenotype rather than genotype per se, phenoconversion (whatever its cause) can have a significant impact on the analysis and interpretation of genotype-focused clinical outcome association studies. There is a risk that focusing on genotype alone may miss important associations between clinical outcomes and DME phenotypes, thus compromising future prospects of personalized medicine.

Introduction

The concept of personalized medicine is underpinned by sound pharmacogenetic principles. Rather than prescribing medicines by the traditional one-size-fits-all approach, personalized medicine promises the right drug at the right dose the first time through genotype-based individualized therapy, thereby purporting to make medicines safer and more effective. Clinical response to a drug is believed to be typically related to the concentration of, or exposure to, the drug and/or its pharmacologically active metabolite(s). Therefore, polymorphisms of drug metabolizing enzymes (DMEs), which determine interindividual variability in the pharmacokinetics of a drug, have attracted considerable interest in the context of personalized therapy. The evidence for polymorphic drug metabolism is most clear for CYP2D6, CYP2C19, and TPMT, giving rise to three distinct genotype-based subpopulations with respect to each DME: extensive metabolizers (EMs), poor metabolizers (PMs), and a subgroup in between, the intermediate metabolizers (IMs). In addition, for CYP2D6 and CYP2C19, there is a fourth genotype, the ultrarapid metabolizer (UM) genotype (Brockmöller et al., 2000; Sim et al., 2006).

The observed inter-genotype differences in the pharmacokinetics, and therefore possibly the clinical response, following administration of a drug have stimulated a number of high-profile pharmacogenetic studies investigating potential associations between commonly prevalent DME genotypes and clinical outcomes. These studies have almost always focused on the DME genotypes of the study population with a view to establishing the association and genotype-specific safe

ABBREVIATIONS: DME, drug metabolizing enzyme; EM, extensive metabolizer; HIV, human immunodeficiency virus; IFN, interferon; IL, interleukin; IM, intermediate metabolizer; LKM-1, liver kidney microsomal type 1; LPS, lipopolysaccharide; PM, poor metabolizer; TNF, tumor necrosis factor; UM, ultrarapid metabolizer.
and effective dosing regimen (Kirchheiner et al., 2004). This focus on the DME genotype of the study population is based on the assumption that genotype is predictive of DME phenotype, and therefore the pharmacokinetics of, and the associated clinical response to, the drug.

However, predicting a patient’s DME phenotype from his/her genotype is highly complex (Gaedigk et al., 2008; Kirchheiner, 2008; Hicks et al., 2014) and not always reliable. Although a PM genotype correctly predicts the PM phenotype, quantitative prediction of drug metabolizing capacity among EM patients is not possible (Griese et al., 1998). In this context, CYP2D6 has attracted considerable research interest in view of its highly polymorphic nature and it being responsible for the metabolism of 20% of all drugs metabolized by cytochrome P450 enzymes. The activity of DMEs (phenotype) such as CYP2D6 is significantly altered in EM patients who are comedicated with its inhibitor(s). Integration of the inhibitory effect of concomitant medications with the CYP2D6 genotype to compute a composite CYP2D6 activity score greatly improves the ability to predict the CYP2D6 drug metabolizing phenotype (Borges et al., 2010), and therefore the clinical response. For example, a study in 87 Caucasian patients treated with antidepressant monotherapy with other comediations revealed genotype-phenotype mismatch in 10 (11.4%) of these patients and that the CYP2D6 phenotype, rather than the genotype, could better predict therapeutic response to the CYP2D6 substrate antidepressant (Gressier et al., 2015).

Therefore, not surprisingly, the results from many genotype-focused association studies, however well supported by sound mechanism-based pharmacologic principles, have been inconsistent, and often conflicting (Shah and Shah, 2012). Clearly, there are significant challenges to interpretation and clinical application of genotype-focused association studies and not least among these is the phenomenon of phenoconversion, whereby a genotypic EM of a DME is converted into a transient phenotypic PM of that DME. Since the DME genotype is immutable, any of the following findings after an intercurrent event or intervention suggests phenoconversion:

1. Mismatch between DME genotype and its phenotype in an individual;
2. Mismatch between DME genotypic and phenotypic structures of a population (in terms of percentage frequencies of UM, EM, IM, and PM individuals);
3. Down-regulation of mRNA and the corresponding DME activity in an individual; and
4. Unexpected and otherwise unexplained change in plasma concentration of, or clinical response to, a drug (potentially requiring dose adjustment) in an individual.

One major cause of phenoconversion is comedication, which typically inhibits a DME and affects its functional quality, the consequences of which have been reviewed previously (Shah and Smith, 2014). Phenoconversion has significant impact not only on the analysis and interpretation of genotype-focused clinical outcome association studies but also on personalizing therapy in routine clinical practice where phenoconversion due to comedication appears to be widely prevalent.

The weight of evidence gathered over the last two decades, both non-clinical (in vitro and in vivo in animal models) and some clinical studies, suggests that increased exposure to certain proinflammatory cytokines, typically released during an infection or an inflammatory condition, down-regulate certain DMEs, particularly of the cytochrome P450 family, and result in transient phenoconversion. In contrast to comedication, proinflammatory cytokines suppress biosynthesis of the DMEs and affect their quantities, and thereby their drug metabolizing capacity.

Here, we review the evidence that collectively is consistent with a hypothesis that certain inflammatory conditions, particularly when associated with elevated levels of proinflammatory cytokines, may cause phenoconversion of certain DMEs. Following a review of the complexity of the inflammatory process and the myriad of mediators involved, we summarize data from three sources to support this hypothesis—namely, (1) data from in vitro studies in hepatocyte preparations and from in vivo studies in animal models of inflammation; (2) data from formal clinical studies in man investigating genotype-phenotype mismatch in inflammatory conditions such as infection with human immunodeficiency virus (HIV), cancer, and liver disease; and (3) anecdotal clinical evidence suggestive of down-regulation of drug metabolism during inflammation typically associated with infections.

Inflammation, Biochemical Mediators, and Drug Metabolism

Complexity of Inflammation. Inflammation is part of a complex biologic response to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a protective response, involving a whole range of host cells, blood vessels, and proteins and other mediators, intended to eliminate the initial cause of cell injury, necrotic cells, and tissues resulting from the original insult, and to initiate the process of repair. In absence of an inflammatory response, progressive destruction of tissue threatens the survival of the organism. Inflammation can be classified as either acute or chronic. The process of acute inflammation is initiated by cells already resident in all tissues such as macrophages, Kupffer cells, and mast cells. Chronic inflammation is characterized by simultaneous destruction and healing of the tissue from the inflammatory process and might give rise to a host of diseases, such as hay fever, asthma, atherosclerosis, diabetes, renal failure, rheumatoid arthritis, and even cancer.

A cascade of biochemical events, triggered by a myriad of chemical mediators, is associated with the inflammatory response and involves the local vascular system, immune system, and various cells within the injured tissue. These mediators include a variety of substances, a majority of which can be divided into (1) vasoactive amines such as histamine and serotonin; (2) plasma endopeptidases comprising three interrelated systems (kinin, complement, and clotting systems); (3) prostaglandins; (4) cytokines; and (5) a number of other hitherto unidentified factors. Of these, it would appear that certain specific cytokines are primarily responsible for the down-regulation of some DMEs as part of the inflammatory process.

Cytokines, acting through their respective receptors, are a broad group of low-molecular-weight proteins. They are produced by a range of cell types, including immune cells like macrophages, B lymphocytes, T lymphocytes, and mast cells, as well as endothelial cells, fibroblasts, and various stromal cells. A given cytokine may be produced by more than one cell type. A variety of cytokines such as chemokines, interleukins (ILs), tumor necrosis factor (TNF), and interferons (IFNs) are released in response to infection and other inflammatory diseases. They are important in cell signaling and affect the behavior of other cells, and sometimes the behavior of the releasing cell itself. Cytokines are important not only in health but also in disease states, specifically in host responses to infection, immune responses, inflammation, trauma, sepsis, cancer, and reproduction. Some cytokines enhance or inhibit the action of other cytokines through a complex interaction. Against this highly complex cellular, biochemical, and molecular background of inflammation and the myriad of mediators involved, we review how inflammation may down-regulate drug metabolism and propose a hypothesis that certain inflammatory conditions, associated with elevated levels of proinflammatory cytokines, may cause phenoconversion of certain DMEs, with implications for the implementation of personalized medicine.
Drug Metabolism and Pivotal Proinflammatory Cytokines. Some cytokines are proinflammatory (e.g., IL-1β, IL-2, IL-6, IL-12, IL-18, TNF-α, TNF-β, IFN-α, and IFN-γ) while others are anti-inflammatory (e.g., IL-4, IL-5, IL-10, IL-11, and IL-13). IL-1, IL-6, and TNF-α are believed to be the principal proinflammatory cytokines that induce changes in liver protein expression; typically, they down-regulate their expression. The precise mechanisms are not fully understood but these cytokines bind to their corresponding receptors on the cell surface in target organs and activate intracellular signaling pathways that regulate gene transcription and biosynthesis of a whole range of enzymes and transporters, including those involved in drug metabolism and disposition (Shedlofsky et al., 1994, 1997; Morgan, 1997; Morgan et al., 1998; Renton, 2004, 2005; Aitken et al., 2006; Le Vee et al., 2008, 2009; Fardel and Le Vée, 2009).

Although IL-1, IL-6, and TNF-α are believed to be the three principal proinflammatory cytokines, IL-6, which acts through its receptor (IL-6R) in various organs including the liver, has attracted the greatest interest. Clinically, in vivo treatment with IL-6 induces systemic symptoms of inflammation and has been most widely studied with respect to regulation of DMEs and drug transporters. Typically, it suppresses their biosynthesis (Morgan, 1997; Morgan et al., 1998; Renton, 2004, 2005; Aitken et al., 2006). When studied for an effect on CYP3A4, IL-6 also appears to be more potent than IL-1 in this respect (Dickmann et al., 2012). In contrast to IL-1 and IL-6, others such as IL-12 and IL-23 have been shown not to alter the expression or activity of cytochrome P450 enzymes (Dallas et al., 2013). Similarly, administration of IL-10 to healthy volunteers did not alter CYP1A2, CYP2C9, or CYP2D6 activities but it did reduce CYP3A activity, as measured by use of probe drugs caffeine (CYP1A2), tolbutamide (CYP2C9), dextromethorphan (CYP2D6 and CYP3A), and midazolam (CYP3A) (Gorski et al., 2000).

Nonclinical Evidence for Proinflammatory Cytokines Suppressing Biosynthesis of P450 DMEs

Cytokine-induced suppression of biosynthesis of DMEs has been most extensively studied for its effects on isozymes of the cytochrome P450 system. The effects of cytokines on other important metabolic pathways such as hydrolysis (mediated by human carboxylesterases) and conjugation (for example, those mediated by N-acetyltransferase-2, uridine 5'-diphospho-glucuronosyltransferases, glutathione S-transferases, or sulphotransferase) have not yet been well characterized, and therefore will not be discussed any further here. The evidence for the effects of various cytokines on individual CYP enzyme expression and activity (investigated either in vitro or in vivo) is summarized below. For a detailed discussion of the complex mechanisms involved and the differential modulation of different DMEs and drug transporters by different cytokines, the reader is referred to reviews by other authors (Morgan, 1997, 2009; Renton, 2000, 2004; Aitken et al., 2006, Aitken and Morgan, 2007; Morgan et al., 2008; Huang et al., 2010; Christensen and Hermann, 2012; Gandhi et al., 2012).

In Vitro Evidence. In vitro studies in hepatocyte preparations, with and without cytokines, have provided persuasive evidence that pro-inflammatory cytokines down-regulate biosynthesis of certain hepatic cytochrome P450 enzymes commonly involved in drug metabolism. Cytokines have been shown to down-regulate cytochrome P450 expression in cultures of rodent and human hepatocytes (Abdel-Razzak et al., 1993; Morgan, 1997; Summan et al., 2004; Aitken et al., 2006). In vitro studies using human hepatocytes indicate that although the effect of different cytokines is gene specific, IL-6 down-regulates the mRNAs of all cytochrome P450 enzymes studied so far. A comprehensive in vitro study using human hepatocytes has investigated the expression of mRNA levels and characterized the regulation of CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, and CYP3A4 by a number of agents associated with inflammation in human hepatocytes, including IL-1, IL-6, and TNF-α (Aitken and Morgan, 2007). IL-1 down-regulated CYP2C8 and CYP3A4 mRNA expression by 75% and 95%, respectively, but had no effect on CYP2B6, CYP2C9, and CYP2C19. Similarly, TNF-α also caused down-regulation of CYP2C8 and CYP3A4 mRNA expressions but had no effect on CYP2B6, CYP2C9, and CYP2C19. In contrast, IL-6 caused a decrease in CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 mRNA expressions. CYP2C19, typically expressed at very low levels in liver, was unaffected by cytokine treatments. In this study, analysis of P450 protein expression broadly supported the mRNA data, although there were some inconsistencies. IL-6-induced suppression of the activities of CYP3A4 and CYP1A2 activities in human primary hepatocytes was shown to be partially blocked by an anti-IL-6 monoclonal antibody (Dickmann et al., 2011).

In Vivo Nonclinical Evidence. In vivo studies have used various models of inflammation in different species of animals, including that induced by bacterial endotoxin [lipopolysaccharide (LPS)], infection with Citrobacter rodentium, and local injection of turpentine or adjuvant-induced arthritis. The following observations from these animal models indicate that proinflammatory cytokines are important mediators of cytochrome P450 expression/regulation in the liver during inflammation:

1. Significant decrease has been reported in gene expression levels and enzyme activities of hepatic CYP3A and CYP2B subfamily enzymes in an animal model of rheumatoid arthritis (Sanada et al., 2011).
2. The decreases in the gene expression levels and activities of these enzymes were closely correlated with increases in the expression levels of the inflammatory cytokines, TNF-α, IL-1 and IL-6 (Sanada et al., 2011).
3. In a study of wild-type, IL-6−/−, and IFN-γ−/− female C57BL/6J mice infected with C. rodentium, the majority of P450 mRNAs were equally affected by infection in each genotype. This would suggest that IL-6 and IFN-γ are not the only mediators of down-regulation of P450 enzymes in this disease model (Nyagode et al., 2010).
4. Mice with null mutations in cytokine or cytokine receptor genes display diminished down-regulation of cytochrome P450 in response to some inflammatory stimuli (Siewert et al., 2000; Ashino et al., 2004; Nyagode et al., 2010).
5. LPS-treated mice show suppressed transcription and activity of certain xenobiotic-induced hepatic CYP enzymes (Moriya et al., 2012).

In general, there is reasonable agreement between in vitro and in vivo nonclinical studies, although results from different animal models are not always consistent with each other, often due to differences in species (Aitken et al., 2006) and/or the nature of the inflammatory challenge.

Table 1, compiled from data in the sources indicated, represents a broad assessment of the potential effects of various cytokines on enzymes of the cytochrome P450 system. Collectively, these data suggest that, in response to inflammation, the effects of cytokines on human cytochrome P450 enzymes are regulated independently and may have a critical effect on clinical responses to certain drugs in disease states. In summary, evidence from the nonclinical studies reviewed above suggests the CYP enzymes that appear to be highly susceptible to cytokine-induced-down-regulation during inflammation,
The studies discussed below provide direct clinical evidence of genotype-phenotype mismatch (phenoconversion) of NAT2, CYP2C19, and CYP2D6 in inflammatory conditions such as HIV infection, cancer, and liver disease (summarized in Table 2). Although there are no similar clinical studies investigating or reporting specifically on phenoconversion of other major DMEs (CYP1A2, CYP2C8, CYP2C9, CYP3A4, and CYP2E1) or in other widely prevalent inflammatory diseases such as diabetes, cardiac failure, chronic kidney disease, or rheumatoid arthritis, there is sufficient anecdotal evidence (discussed below) to suggest that inflammation-induced phenoconversion may be more frequent than appreciated hitherto.

### Phenoconversion of DMEs in HIV Infection

Elevated levels of IL-6 (and other inflammatory biomarkers) have been reported in HIV patients and are associated with progression and prognosis of the disease (Nixon and Landay, 2010; Reuter et al., 2012; Catalfamo et al., 2012). One study investigated (four times over a 2 month period) the effect of HIV infection on the activity of various DMEs and correlated the phenotype with the genotype (Jones et al., 2010). Urinary caffeine and dextromethorphan metabolic ratios were used to phenotype for CYP1A2, NAT2, xanthine oxidase, and CYP2D6 activities and midazolam plasma clearance was used to phenotype for CYP3A activity. The data from the HIV-infected subjects were compared with those of historic age- and sex-matched healthy volunteer controls. Plasma concentrations of IL-6 and TNF-α were also measured. Compared with healthy volunteers, HIV-infected subjects had 90% lower CYP2D6 activity, 53% lower NAT2 activity, 18% lower hepatic CYP3A4 activity, and 22% higher xanthine oxidase activity. The activity of CYP1A2 was not significantly different between HIV-infected individuals and the nonsmoker healthy volunteers. Five (29%) of the 17 HIV-positive subjects were phenotypic CYP2D6 PM, but only one of these five was found to be genotypic PM. In contrast, the only phenotypic PM (7%) among the 14 healthy volunteers was also a genotypic PM of CYP2D6. With regard to NAT2, there was significant genotype-phenotype discordance. Sixteen (94%) of the 17 HIV-infected individuals who were phenotyped were phenotypic slow acetylators. Of these 16 individuals, 15 were also genotyped and only seven (47%) had slow acetylation genotype. In this study, concentrations of IL-6 and TNF-α were significantly higher in HIV-infected individuals than in healthy volunteers. Although higher plasma TNF-α concentrations correlated with lower CYP2D6 and CYP3A4 activities, no significant relationship was found between IL-6 concentrations and hepatic CYP3A4, CYP2D6, or CYP1A2 enzyme activity.

### Clinical Evidence Suggestive of Phenoconversion of DMEs in Inflammatory Comorbidities

An in vivo study of healthy male volunteers has shown that inflammatory response to even a very low dose of LPS significantly decreases cytochrome P450-mediated drug metabolism by CYP1A2 (using theophylline as the probe drug) and CYP2C19 (using hexobarbital as the probe drug) (Shedlofsky et al., 1994). This effect evolved over a 24 hour period and its intensity correlated with the intensity of the inflammatory response, although there was no statistically significant correlation between the observed effects and levels of any of the cytokines. A later study by the same investigators confirmed a similar effect in vivo in female volunteers (Shedlofsky et al., 1994). However, an inverse correlation has been reported between plasma IL-6 levels and cytochrome P450-dependent drug clearance of erythromycin (measured by breath test) by CYP3A4 in patients with advanced cancer (Rivory et al., 2002) and of caffeine and mephentoin by CYP1A2 and CYP2C19, respectively, in patients with congestive heart failure (Frye et al., 2002).

Alterations in drug pharmacokinetics have been reported in patients with infections and other inflammatory conditions including cancer (Slaviero et al., 2003; Aitken et al., 2006; Morgan et al., 2008; Morgan, 2009; Gandhi et al., 2012). For example, HIV infection has long been reported to change the pharmacokinetics of caffeine, sulfamethoxazole, and fluconazole (Levy, 1997). Patients with rheumatoid arthritis have been reported to have increased verapamil concentrations (Mayo et al., 2000), and patients with sepsis to have a significantly higher Cmax and area under the curve of atorvastatin compared with healthy volunteers (Kruger et al., 2009). All these conditions are known to be associated with raised levels of IL-6.

### Table 1

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>CYP1A2</th>
<th>CYP2B6</th>
<th>CYP2C8</th>
<th>CYP2C18</th>
<th>CYP2C9</th>
<th>CYP2C19</th>
<th>CYP2D6</th>
<th>CYP3A4</th>
<th>CYP2E1</th>
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<td>IFN-α-2b</td>
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<td>IFN-γ</td>
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<td>GRO-α</td>
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The table shows the relationship between the observed effects and the intensity of the inflammatory response, although there was no statistically significant correlation between the observed effects and levels of any of the cytokines.
The distribution of the CYP2D6 phenotype and its relation to genotype, concomitant medication, and disease state in human HIV-infected patients was also investigated in another study, in which phenotype assignment was based on urinary metabolic ratio following a 30 mg oral dose of dextromethorphan (O’Neil et al., 2000b). A total of 108 patients were phenotyped, of whom 61 (20 with AIDS) were also genotyped. Genotype assignment was based on detection of wild-type (CYP2D6*1) and three mutant (CYP2D6*3, CYP2D6*4, and CYP2D6*5del) alleles. Fifty-nine (97%) of these 61 patients possessed the EM genotype and the other two (3%) possessed a PM genotype, frequencies consistent with those observed in demographically similar populations. Five patients with EM genotype were taking one or more comedications known to inhibit CYP2D6, and although they all expressed EM phenotypes these comedications did appear to reduce their CYP2D6 activity because these patients were clustered toward the impaired CYP2D6 metabolism side of the EM distribution. However, more importantly, despite not taking any medications known to inhibit CYP2D6 activity, two of the 59 genotypic EM subjects expressed a PM phenotype and four others were less EMs of dextromethorphan compared with those receiving medication known to inhibit CYP2D6. The reason for this phenoconversion from slow to fast acetylation phenotype is unclear but could be related to the fluctuating nature of the disease. Among the slow acetylators whose genotype was fast, the incidence of AIDS was higher (six out of 12) than that among the fast acetylators whose genotype was slow (two out of 14), suggesting that the disease progression in HIV infection and AIDS may alter expression of the NAT2 gene. For patients who were phenotyped more than once, changes in acetylation phenotype from fast to slow were associated with progression of HIV infection. Interestingly, an earlier report had noted that the prevalence of apparent slow acetylation phenotype was greater in AIDS patients with acute illnesses (27 out of 29, 93%) compared with control subjects (18 out of 29, 62%) (Lee et al., 1993). A subsequent study compared 30 AIDS patients (with and without an acute illness) with 30 healthy control subjects for their NAT2 acetylation phenotype and genotype and reported numerous discrepancies between phenotype and genotype (O’Neil et al., 2000a). Phenoconversion of DMEs in Cancer

Up to 20% of all cancers arise in association with chronic inflammation and most solid tumors contain inflammatory infiltrates (Grivennikov and Karin, 2011). Effects such as tumor initiation, growth, and progression are mediated by proinflammatory cytokines such as TNF-α and IL-6, and an increased expression of IL-6 is associated with an unfavorable prognosis in patients with various types of cancer (Suganuma et al., 2002; Moore et al., 2010; Culig and Puhr, 2012; Ataie-Kachoie et al., 2013; Waldner and Neurath, 2014). In cancer patients, there is discordance between CYP2C19 genotype and phenotype (as determined by omeprazole S'-hydroxylation activity) without any known cause for this discrepancy (Williams et al., 2000). Among the 16 cancer patients with CYP2C19 EM genotype, there were four (25%) with PM phenotype, and in the remaining 12 there was a general shift toward a slower metabolic activity. No two patients had received the same anticancer therapy and none of the

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### TABLE 2

<table>
<thead>
<tr>
<th>DME</th>
<th>Comorbidity</th>
<th>Key Observation</th>
<th>Reference</th>
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<tr>
<td>NAT2</td>
<td>HIV</td>
<td>Prevalence of slow acetylation phenotype was greater in AIDS patients with acute illnesses (93%) compared with control subjects (62%).</td>
<td>Lee et al., 1993</td>
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<tr>
<td>HIV</td>
<td>HIV</td>
<td>18 discrepancies between genotype and phenotype; 12 slow acetylators with fast acetylation genotype and six fast acetylators with slow acetylation genotype. Among the slow acetylators whose genotype was fast, the incidence of AIDS was higher (six of 12) than that among the fast acetylators whose genotype was fast (two of 14).</td>
<td>O’Neil et al., 1997</td>
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<tr>
<td>HIV</td>
<td>HIV</td>
<td>Numerous discrepancies between phenotype and genotype.</td>
<td>O’Neil et al., 2000a</td>
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<td>CYP2D6</td>
<td>HIV</td>
<td>Two of the 59 genotypic EM subjects expressed a PM phenotype and four others were less EMs of dextromethorphan compared with those receiving medication known to inhibit CYP2D6.</td>
<td>O’Neil et al., 2000b</td>
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<td>Hepatitis C</td>
<td>There was a genotype-phenotype mismatch in 7 LKM-1 positive patients; six expressed an IM and one a PM phenotype.</td>
<td>Girardin et al., 2012</td>
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<td>Liver transplant</td>
<td>The phenotype of 13 subjects was changed by liver transplant; six EMs became PMs and seven PMs became EMs.</td>
<td>Monk et al., 1998</td>
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<tr>
<td>CYP2C19</td>
<td>Cancer</td>
<td>Among 16 patients with CYP2C19 EM genotype were four (25%) with PM phenotype, and in the other 12 there was a general shift toward a slower metabolic activity.</td>
<td>Williams et al., 2000</td>
</tr>
<tr>
<td>Cancer</td>
<td>Multiple myeloma</td>
<td>Phenotypic PM status in 37% of the patients who had EM genotype</td>
<td>Helsby et al., 2008</td>
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<td>Liver disease</td>
<td>Thirty (64%) of 47 patients with liver disease (of whom 41 were also comedicated) and 20 (18%) of 110 comedicated patients without liver disease had PM phenotype.</td>
<td>Burns et al., 2014</td>
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<td>Rost et al., 1995</td>
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patients was receiving any known CYP2C19 inhibitors or any known CYP3A4 inhibitors or inducers. Thus, no one drug could be identified as the source of the observed CYP2C19 genotype-phenotype mismatch. This study did not attempt to correlate these effects with changes in plasma cytokine concentrations.

Using omeprazole as the probe drug for determining the CYP2C19 phenotype, another more recent study has also reported severely compromised activity of CYP2C19 in patients with advanced cancer (Helsby et al., 2008). This resulted in a phenotypic PM status in 37% of the patients who had EM genotype. Serum cytokine levels (IL-1, IL-6, TNF-α, and TGF-β) were measured but there was no statistically significant correlation between CYP2C19 metabolic activity and the level of any individual cytokine.

The CYP2C19 genotype-phenotype relationship has also been investigated in patients with multiple myeloma (Burns et al., 2014), using proguanil as the metabolic probe for CYP2C19 activity. Circulating levels of CRP and IL-6 were also measured. Activity of CYP2C19 in the 25 patients studied was found to be severely compromised in some of these patients. No homozygous null CYP2C19 genotype subjects were detected in this cohort but seven (28%) of these 25 genotypic EM subjects had a proguanil metabolic ratio, which indicated no CYP2C19 activity, and were classified as phenotypic CYP2C19 PMs. This discordance was present in three (27%) of the 11 subjects with EM (*1/*1) and in four (57%) of the seven subjects with IM (*1/*2) genotype. Of the remaining seven genotypic EM subjects with a typical EM phenotype, six had UM genotype (*1/*17) genotype and one had IM (*3/*17) genotype, thus further emphasizing a shift toward reduced metabolic activity even in subjects with UM genotypes. This significant discrepancy between the CYP2C19 phenotype and genotype was not related to either of the two proinflammatory markers studied. Examination of circulating levels of CRP was seen in six patients and of circulating levels of IL-6 in four patients, and there was no significant difference in the mean CRP or IL-6 concentrations between concordant and discordant subjects.

Thus, in a cancer population, genotyping for CYP2C19 would significantly underestimate the number of phenotypic PM of CYP2C19 substrates. The lack of correlation in the two aforementioned in vivo studies (Helsby et al., 2008; Burns et al., 2014) between plasma IL-6 concentrations and CYP2C19 genotype-phenotype mismatch is difficult to explain in view of the robust in vitro evidence (Aitken and Morgan, 2007) (see Table 1) and in vivo data (Frye et al., 2002) showing down-regulation of CYP2C19 activity by IL-6. It could be due to high variability in the circulating levels of IL-6 in the two studies. Notwithstanding this lack of correlation with the inflammatory marker, there is little doubt that there is significant CYP2C19 genotype-phenotype mismatch, which could impact interpretation of genotype-focused association studies that enroll patients with cancer.

One study of CYP2C9-mediated metabolism in cancer patients has reported that CYP2C9 activity, as measured by apparent oral clearance and urinary metabolic ratio following oral tolbutamide, appeared similar in people with (n = 10) and without (n = 10) cancer (Shord et al., 2008). IL-1β was not detected in the sera of subjects with or without cancer. Although the median serum cytokine values for IL-6 and TNF-α were 5- or 7-fold higher, respectively, for subjects with cancer, the differences did not reach statistical significance and there was no significant association between serum cytokine values and apparent oral clearance or urinary metabolic ratio of tolbutamide. This study is difficult to interpret in view of its small size and the extent to which the two groups of patients were matched. As reported with the down-regulation of CYP3A4 activity in cancer patients (Rivory et al., 2002), differences in disease severity could be one explanation for the differential effects observed on CYP2C9 in this study in patients with no measurable disease and on CYP2C19 in the studies in patients with advanced cancers (Williams et al., 2000; Helsby et al., 2008; Burns et al., 2014). Other explanation may be that CYP2C9 and CYP2C19 are differentially regulated by cancer.

Phenocverting of DMEs in Liver Disease and Following Liver Transplantation

Various hepatic diseases such as chronic viral hepatitis (Spanakis et al., 2002), alcoholic liver disease (Martinez et al., 1992; An et al., 2012; Gao, 2012), cirrhosis, and hepatocellular carcinoma (Soresi et al., 2006) are also associated with high circulating levels of IL-6. The metabolism of dipyrone has been reported to be selectively impaired in asymptomatic carriers of hepatitis B virus (Levy et al., 1997). Oxidative pathways to produce 4-aminoantipyrine and 4-formylaminantipyrine were significantly affected, whereas acetylation was not impaired. Available evidence suggests that with the increasing severity of liver disease, there is a progressive decline in CYP1A2, CYP2C19, CYP2D6, and CYP2E1 activities (Larrey et al., 1989; Joanne et al., 1994; Frye et al., 2006). The effect of liver disease is also isoform selective, CYP2C19 being more sensitive than CYP2D6 (Adeooyin et al., 1998). Review of the data suggests that the amounts and the activities of CYP1A, CYP2C19, and CYP3A appear to be particularly vulnerable to the effect of liver disease, while CYP2D6, CYP2C9, and CYP2E1 are less affected (Villeneuve and Pichette, 2004). Therefore, it is reasonable to conclude that liver disease could be another important cause of phenocversion of some DMEs, especially CYP2C19.

One study compared the frequency of CYP2C19 phenotype in Caucasian patients without liver disease or concomitant drug intake with patients with liver disease or medicated patients without liver disease (Rost et al., 1995). In this study, only one (3.3%) of the 30 subjects without liver disease or concomitant drug intake had CYP2C19 PM phenotype. In contrast, 20 (18%) of the 110 medicated patients without liver disease and 30 (64%) of 47 patients with liver disease (of whom 41 were on comedication) had PM phenotype, both frequencies highly exceeding the frequency of 3%-4% of genotypic CYP2C19 PMs in Caucasians. This study provides evidence of phenocconversion not only due to comedication but also due to liver disease per se and that liver disease may be a more potent factor than comedications in causing phenocversion. This study did not determine the plasma levels of cytokines of the patients studied.

Liver kidney microsomal type 1 (LKM-1) antibodies are present in a minority of patients with chronic hepatitis C infection. A comparison of 10 genotypic CYP2D6 EM patients with LKM-1 antibodies with matched patients without LKM-1 antibodies also revealed evidence of phenocconversion in many of the patients with LKM-1 antibodies (Girardin et al., 2012). Patients with PM genotype were excluded from this study. All the patients were phenotyped using dextromethorphan and classified into four activity phenotypes. The observed phenotype was concordant with the CYP2D6 genotype in most LKM-negative patients and three LKM-1 positive patients. There was a genotype-phenotype mismatch in the remaining seven LKM-1 positive patients; six expressed an IM and one a PM phenotype. Although cytokine levels were not measured, this study indicated that in chronic hepatitis C patients with LKM-1 antibodies, there is a high probability of phenocconversion with marked reduction in CYP2D6 activity on average by 80%.

Within the context of genotype association studies, it should be noted that liver transplant can also result in DME phenocconversion, and it has been reported to alter the DME phenotype of the recipient patient. It might reasonably be expected that the DME phenotype of
the recipient would correspond to that of the genotype of the donor liver, bearing in mind the dominant role of this organ in determining the profile of overall drug metabolism. However, this does not always seem to be the case for reasons that are not obvious, and other factors may contribute to determining post-transplant DME phenotype (Mitchell et al., 1994; Monek et al., 1998; Carcillo et al., 2003).

Evidence for Potential Phenoconversion of DMEs in Other Inflammatory Comorbidities

Raised levels of proinflammatory cytokines have been reported in the progression and complications associated with other inflammatory or immune conditions such as myocarditis and cardiac failure (Heymans et al., 2009; Watanabe et al., 2011; Gullestad et al., 2012; Cialdella et al., 2013), chronic kidney disease (Stenvinkel et al., 2005; Carrero et al., 2008, 2009; Filippoupolous and Vlassopoulos, 2009; Rosner et al., 2012), diabetes (Pickup et al., 2000; de Galan et al., 2003; Tuttle et al., 2004; Konukoglu et al., 2006), and rheumatoid arthritis (Holt et al., 1991; Feldmann, 1996; Vervoordeldonk and Tak, 2002). Because raised levels of certain cytokines down-regulate certain DMEs, it seems reasonable to anticipate that drug metabolism may also be down-regulated in many of these widely prevalent inflammatory conditions, potentially giving rise to phenoconversion and genotype-phenotype mismatch, often reflected as unexpected and otherwise unexplained increases in plasma concentrations of the parent drug.

Clinical evidence also suggests that down-regulation of certain DMEs, and therefore transient phenoconversion, may occur in conditions associated with inflammation, such as cardiac failure, infections, renal failure, and rheumatoid arthritis. Thus, following administration of a metabolic probe cocktail consisting of caffeine, mephenytoin, dextromethorphan, and chlorzoxazone to assess the activities of the CYP enzymes 1A2, 2C19, 2D6, and 2E1, respectively, to 16 patients with congestive heart failure, there was a significant inverse relation-

Down-Regulation of CYP1A2 during Clinical Inflammation. CYP1A2 catalyzes oxidative biotransformation of up to 10%–15% of clinically relevant drugs including theophylline, clozapine, and caffeine. In one study, the plasma half-life of theophylline was determined during and 1 month after serologically confirmed upper-respiratory-tract viral illness in six children with chronic asthma. In this group the plasma theophylline half-life (mean = 419.8 minutes) was significantly longer during the acute stage of illness than 1 month later (mean = 249.9 minutes) (Chang et al., 1978). Serum theophylline levels also increase, often to toxic levels, following influenza vaccination (Renton et al., 1980). During an influenza B outbreak in King County (Washington) in 1980, 11 children, whose asthma had been previously controlled with a stable theophylline dose, developed theophylline toxicity at the same dose (Kraemer et al., 1982). Following recovery, decreased theophylline clearance gradually returned to preillness levels over a period of one to three months. This toxicity has been attributed to the inflammatory mediators produced in response to the virus, causing a dramatic loss in CYP1A2 activity, which metabolizes theophylline.

There have been a number of reports of toxic plasma concentrations of clozapine, a CYP1A2 substrate, during inflammation due to infection in patients who were on chronic medication and normally had stable plasma clozapine levels (Haack et al., 2003; van Gool et al., 2010; Darling and Huthwaite, 2011; Espnes et al., 2012; Leung et al., 2014). Decreased CYP1A2 activity in these patients is suggested by increased serum clozapine levels and lowered norclozapine-to-clozapine ratios (Raaska et al., 2002) and increased concentration-to-dose ratios (de Leon and Diaz, 2003) during the infection.

Down-Regulation of CYP2C9 during Clinical Inflammation. An in vivo study has shown that the plasma concentrations of EXP 3174, the active CYP2C9-generated metabolite of losartan, are significantly reduced in patients with rheumatoid arthritis (Daneshtalab et al., 2006). The elimination of warfarin is almost entirely by stereoselective metabolism, independent of renal function. CYP2C9 is the principal enzyme that metabolizes the pharmacologically active S-warfarin. In patients with end-stage renal disease, there is a 50% increase in the plasma warfarin S/R ratio relative to control subjects, reflecting reduced activity of CYP2C9 (Dreisbach et al., 2003). Consistent with this observation is the finding that compared with patients with normal or mildly impaired renal function patients with moderate impairment have been reported to require 7.2%–10.9% lower dose, and patients with severe impairment required 13.9%–21.3% lower dose, of warfarin (Lindli et al., 2010). This association remained significant even after adjustment for clinical and genetic factors. In a further study of 1245 patients, 35% had impaired renal function, and as the glomerular filtration rate decreased the incidence rate of hemorrhage increased (Lindli et al., 2013).

Down-Regulation of CYP2C19 during Clinical Inflammation. Voriconazole is principally metabolized by CYP2C19, and its plasma concentrations and hepatotoxic potential are modulated by polymorphism of this enzyme (Wang et al., 2014). Voriconazole plasma concentrations display large variability, which cannot completely be explained by known factors. In a study of 128 patients, there was a significant correlation between inflammation, reflected by CRP concentrations, and voriconazole plasma trough concentrations (van Wanrooy et al., 2014). A significantly higher trough concentration was observed in patients with severe inflammation (6.2 mg/l; n = 20) compared with patients with moderate inflammation (3.4 mg/l; n = 60) and no-to-mild inflammation (1.6 mg/l; n = 48). For every 1 mg/l increase in CRP concentration, the voriconazole trough concentration increased by 0.015 (95% confidence interval: 0.011–0.019 adjusted for other factors) mg/l.

As a corollary to down-regulation and potential phenoconversion of hepatic cytochrome P450 enzymes during inflammation, relief of the
latter by cytokine-targeted therapeutic protein therapy should restore (by up-regulation) normal cytochrome P450 activity, thereby increasing the clearance of coadministered drugs, leading to decreased comedication exposure and potentially parent drug-related therapeutic failure or metabolite-induced toxicity. Interestingly, in vitro studies showed that tocilizumab (an anti-IL-6R humanized monoclonal antibody) has the potential to affect expression of multiple CYP enzymes including CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, and CYP3A4 [Food and Drug Administration product reviews and label for ACTEMRA (tocilizumab) (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125472s000lbl.pdf)]. Exposure to omeprazole, which is a CYP2C19 and CYP3A4 substrate, is approximately 2-fold higher in patients with rheumatoid arthritis, and this decreased by 12% in PM and IM and by 28% in EM of CYP2C19, 1 week after a single infusion (8 mg/kg) of tocilizumab [Food and Drug Administration product reviews and label for ACTEMRA (tocilizumab) (http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/125472s000lbl.pdf)].

Down-Regulation of CYP3A4 during Clinical Inflammation. A study on the effect of persistent inflammation in patients with renal failure and who were undergoing hemodialysis on the pharmacokinetics of alprazolam, a CYP3A4 substrate, revealed that after a single dose of alprazolam, there was a significant correlation between CYP3A4 activity and CRP level, which suggested that inflammation may clinically down-regulate CYP3A4 activity (Molanaei et al., 2012). Studies in human liver microsomes have shown that oxatomide, an H1-antihistamine, is mainly metabolized by CYP3A4 and CYP2D6, and there has been a report of a severe long-lasting impaired consciousness induced by therapeutic doses of oxatomide in a child affected by acute gastroenteritis (Antoniazzi et al., 2012). Other causes of impaired consciousness were excluded and this child was receiving no other medication. Since genotyping excluded the presence of common nonfunctional allelic variants of these two P450 enzymes, the authors concluded that toxicity was due to inflammation-induced depression of their activities.

Within the context of the pharmacokinetic effects of inflammation and its relief, exposure to simvastatin, a CYP3A4 substrate, is 4- to 10-fold higher in patients with rheumatoid arthritis, compared with exposures observed in healthy subjects—and a single infusion of tocilizumab reduces this by 57% (Schmitt et al., 2011). In this study, mean plasma CRP levels normalized within 1 week after tocilizumab was initiated and the time course of the CRP-reducing effect of tocilizumab paralleled that of simvastatin pharmacokinetics. The decreased exposure to simvastatin was linked to changes in CYP3A4 expression reported by the same group following anti-IL-6R treatment in cultured human hepatocytes (Zhang et al., 2009a).

Discussion
The hypothesis that certain inflammatory conditions associated with elevated levels of proinflammatory cytokines may cause pharmacoresistance of certain DMEs is underpinned by the following observations:

1. Inflammation is associated with the release of proinflammatory cytokines in addition to a myriad of other biochemical mediators;
2. Nonclinical evidence of cytokine-induced down-regulation of mRNA and activities of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP3A4 and CYP2E1;
3. Formal studies in comorbidities, associated with inflammation and raised cytokine levels, demonstrate genotype-phenotype mismatch (phenoconversion) of NAT2, CYP2D6, and CYP2C19;
4. High prevalence of phenotypic PM of CYP2C19 in patients with inflammatory conditions, compared with normal population;
5. Down-regulation of CYP1A2, CYP2C9, and CYP3A4 by inflammation associated with LPS, cardiac failure, or advanced cancer, frequently correlating with IL-6 levels;
6. Up-regulation of CYP2C19 and CYP3A4 activities following administration of anti-IL-6R therapy with tocilizumab; and
7. Changes in dose requirements or plasma concentrations of and/or toxicity to substrates of CYP1A2, CYP2C9, CYP2C19, and CYP3A4 during clinical conditions associated with inflammation.

However, findings from various clinical studies have not always been consistent within themselves and with nonclinical evidence. Furthermore, clinical studies investigating the correlation between down-regulation of DMEs studied and plasma cytokine levels, particularly IL-6, have not always been conclusive. The reasons for this are not clear but it seems probable that the correlation depends on the DME studied, cytokine measured, and nature of the inflammatory condition (Ashino et al., 2004; Moriya et al., 2014). Inflammation is a complex response associated with myriads of chemical mediators, and substances other than cytokines may be responsible for the observed effects of inflammation on altered handling of drugs, but the current evidence strongly implicates a role of cytokines. Additionally, nonclinical evidence also suggests that the specificity of CYP regulation may be different in different models of infection and inflammation, in which the profile, time course, and sources of cytokines are different (Aitken et al., 2006). Findings from different in vivo models of inflammation suggest that effects are often species dependent, and therefore humans may respond differently. Similarly, different inflammatory comorbidities with different profiles of cytokine elevations may differentially down-regulate DMEs. For example, a number of studies have been conducted in diabetes on the clearance of various probe drugs considered to be specific for individual P450 enzymes but the data are not conclusive enough to draw any general conclusions (Cheng and Morgan, 2001).

Inflammatory conditions are highly prevalent, especially in aging populations. As far as we are aware, no systematic studies such as those in HIV infection and cancers have investigated genotype-phenotype mismatch in other inflammatory conditions such as diabetes, cardiac failure, chronic kidney disease, or rheumatoid arthritis, or in other P450 enzymes such as CYP1A2, CYP2C8, CYP2C9, or CYP2E1. The evidence summarized in this review suggests that inflammation-induced phenoconversion may be more frequent than appreciated hitherto.Clearly, further systematic prospective studies are called for on a variety of inflammatory conditions to investigate cytokine release profiles and to correlate their effect on a range of DMEs.

Regarding the potential implications of inflammatory comorbidities for personalized medicine, current high-profile pharmacogenetic association studies include warfarin (CYP2C9 genotype and risk of hemorrhage or stroke), tamoxifen (CYP2D6 genotype and risk of therapeutic failure), clopidogrel (CYP2C19 genotype and risk of thrombotic cardiovascular outcomes), irinotecan (UGT1A1 genotype and risk of diarrhea or myelosuppression), and thiopurines (TPMT genotype and/or phenotype and risk of myelosuppression) (Shah and Shah, 2012). The results from various studies examining each of these associations have not always been consistent and are at times conflicting. We hypothesize that apart from comedication, another explanation that may account for these discrepant results could be phenoconversion due to inflammatory comorbidity since all of the aforementioned association studies concern inflammatory conditions—three in cancers and two in cardiac diseases. The implication of potential inflammation-induced
phenoconversion as hypothesized above for the analysis and interpretation of data from these studies is self-evident. 

Pharmacogenetic studies on warfarin and other vitamin K antagonists, aimed at associating CYP2C9 genotype (in addition to VKORC1 genotype) with the risks of hemorrhage or stroke, illustrate the significance of inflammation as a potential variable that may confound the results. Of the three recently published large prospective studies, two failed to show an association (Kimmel et al., 2013; Verhoeft et al., 2013), while the third showed a weakly positive association of questionable clinical relevance (Pirmohamed et al., 2013). There is wide interindividual variability in maintenance dosages of warfarin and only approximately 55–60% of this variability is explained by the known genetic (genotypes of CYP2C9, VKORC1, and CYP4F2) and clinical factors (Turner and Pirmohamed, 2014). At present, there is no explanation for the residual variability, and inflammation with associated cytokine release could explain some of this variability. It is noteworthy that one of these studies (negative for an association) had enrolled a cohort, 45% of which had diabetes, cardiac failure, and/or myocardial infarction as comorbidities, all known to be associated with inflammation (Kimmel et al., 2013), while the other two (negative and one weakly positive for the association) included no information on comorbidities. Furthermore, and perhaps more crucially significant, there is a well-documented relationship between a DME phenotype and only approximately 55–60% of this variability is explained by the known genetic (genotypes of CYP2C9, VKORC1, and CYP4F2) and clinical factors (Turner and Pirmohamed, 2014). At present, there is no explanation for the residual variability, and inflammation with associated cytokine release could explain some of this variability. It is noteworthy that one of these studies (negative for an association) had enrolled a cohort, 45% of which had diabetes, cardiac failure, and/or myocardial infarction as comorbidities, all known to be associated with inflammation (Kimmel et al., 2013), while the other two (negative and one weakly positive for the association) included no information on comorbidities. Furthermore, and perhaps more crucially significant, there is a well-documented relationship between a DME phenotype and only approximately 55–60% of this variability is explained by the known genetic (genotypes of CYP2C9, VKORC1, and CYP4F2) and clinical factors (Turner and Pirmohamed, 2014). At present, there is no explanation for the residual variability, and inflammation with associated cytokine release could explain some of this variability. It is noteworthy that one of these studies (negative for an association) had enrolled a cohort, 45% of which had diabetes, cardiac failure, and/or myocardial infarction as comorbidities, all known to be associated with inflammation (Kimmel et al., 2013), while the other two (negative and one weakly positive for the association) included no information on comorbidities. Furthermore, and perhaps more crucially significant, there is a well-documented relationship between a DME phenotype and only approximately 55–60% of this variability is explained by the known genetic (genotypes of CYP2C9, VKORC1, and CYP4F2) and clinical factors (Turner and Pirmohamed, 2014). At present, there is no explanation for the residual variability, and inflammation with associated cytokine release could explain some of this variability.