Pharmacogenetics and precision medicine: Is inflammation a covert threat to effective genotype-based therapy?

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Precision medicine, also referred to as personalized or stratified medicine, seeks to improve drug therapy by genotype-based prescribing to maximize efficacy rates and mitigate the risk of adverse drug reactions. With this aim, the Clinical Pharmacogenetics Implementation Consortium (CPIC) has promulgated a number of guidelines to facilitate genotype-based prescribing decisions for a number of actionable drug–gene pairs.[1] A key assumption underlying these guidelines is that clinical high-throughput and pre-emptive (pre-prescription) genotyping will become more widespread, and therefore clinicians will increasingly already have information on patients' genotypes when initiating drug therapy, or will be prompted to test for their genotype before prescribing. Hitherto, precision medicine has primarily focused on drug-metabolizing enzymes (DMEs) such as CYP2C9, CYP2C19, CYP2D6, TPMT and UGT1A1, among others. While *CYP2D6* genotype information is increasingly sought to guide the choice and dosage of its substrate drugs, such as antidepressants and antipsychotics, testing patients for their *HLA-B* genotype is obligatory, or strongly recommended, in certain countries before commencing treatment with drugs such as abacavir, allopurinol or carbamazepine to avoid potentially severe, if not life-threatening, adverse reactions.

Critically, however, genotype-based prescribing decisions are based on the assumption that the DME genotype accurately predicts the metabolic activity (phenotype) in each subject tested. While it is true that a poor metabolizer (PM) genotype (based on the detection of two no-function or severely reduced-function alleles) predicts a PM phenotype, the same cannot be claimed for individuals with genotypes predicting extensive or intermediate metabolizer (EM/IM, respectively) phenotype. Many extrinsic factors account for genotype–phenotype discordance in EM/IM subjects, but the two major factors are (1) co-medications that inhibit or alter the activity of a DME; or (2) inflammation-associated cytokines that impact expression levels of DMEs. Consequently, a genotypic EM can convert to phenotypic IM or PM during co-medication or inflammation;[2] likewise, genotypic IM subjects can convert to PM phenotype and those with genotype-predicted ultra-rapid metabolism can convert to EM, IM or PM phenotype status. This phenomenon, known as phenoconversion, threatens to undermine the appropriateness of genotype-based choice of dose and the anticipated plasma concentration of the prescribed drug.

Inflammatory conditions are associated with increased serum levels of pro-inflammatory cytokines, such as IL-1, IL-6 and TNF-α, that induce changes in liver protein expression which, typically, is downregulated. The precise mechanisms are not fully understood, and downregulation often does not correlate well with cytokine concentrations. However, these cytokines interact with their corresponding receptors on the cell surface in target organs, thereby activating intracellular signalling systems that regulate gene transcription and the biosynthesis of a wide range of enzymes and transporters, including those involved in drug metabolism and disposition.

Shah and Smith[3] have reviewed *in vitro* and *in vivo* non-clinical evidence that strongly suggests that increased exposure to certain pro-inflammatory cytokines, typically released during an infection or inflammatory condition, downregulate certain DMEs, particularly of the cytochrome P450 family, resulting in phenoconversion for the duration of the infection or inflammation. In this context, IL-6 has attracted the greatest interest. In non-clinical studies, IL-6 has been reported

to downregulate the expression of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP2E1 and a number of phase II (conjugating) DMEs as well as transporters. Conversely, administration of anti-IL-6 monoclonal antibodies such as tocilizumab, siltuximab, olokizumab and sirukumab may reverse IL-6-mediated suppression of CYP activities, particularly CYP3A, CYP2C9 and CYP2C19.[4,5] For example, *in vivo* studies with omeprazole, metabolized by CYP2C19 and CYP3A4, and simvastatin, metabolized by CYP3A4, showed up to a 28% and 57% decrease, respectively, in exposure one week following a single dose of tocilizumab.[6]

Over the last two decades, substantial clinical evidence has also emerged showing that a range of diverse inflammatory conditions, such as cancers and infections, can cause phenoconversion from genotype-anticipated EM phenotype to IM or PM status. Notably, such conditions are more prevalent in an ageing population. As reviewed by Shah and Smith,[3] some of the earliest indications of inflammation-mediated inhibition of DME activity were reports of toxicity to narrow therapeutic index drugs such as theophylline and clozapine, which are metabolized by CYP1A2, during inflammatory processes. Clinical evidence has also accumulated showing that activity of other clinically important CYP isoforms is reduced in inflammatory conditions such as liver disease,[7] rheumatoid arthritis,[8,9] Behçet's disease,[10,11] chronic renal failure,[12–14] acute visceral leishmaniasis[15] and cardiac failure.[16] Formal studies in inflammatory morbidities have also documented genotype–phenotype mismatch (phenoconversion) for NAT2, CYP2D6, CYP2C19 and CYP2C9.[3,10,11,17,18]

The last decade has witnessed many prospective pharmacogenetic association studies. The drugs attracting the greatest interest in this respect include warfarin (*CYP2C9* and *VKORC1* genotype and risk of haemorrhage 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 diarrhoea or myelosuppression) and thiopurines (*TPMT* genotype and/or phenotype and risk of myelosuppression). Broadly speaking, these studies have either failed to confirm, or yielded conflicting or only weak evidence of, the expected association between genotype and drug response. Although the prevalence of genotype–phenotype discordance in non-PM subjects in the population at large is unknown, it is not uncommon,[2] and hitherto has received little attention in pharmacogenetic association studies. Phenoconversion may be one reason for the disappointing outcomes of some association studies, despite being underpinned by mechanistically sound pharmacological hypotheses. With regard to the effect of inflammation on DME activity, it is interesting that many clinical conditions in which high-profile association studies have been conducted are known to have inflammatory components – cancer for three drugs (tamoxifen, irinotecan and thiopurines) and cardiovascular diseases for two (warfarin and clopidogrel). Presence of inflammation in these conditions may well undermine the appropriateness of genotype-determined dose and plasma concentration of the drug concerned. Warfarin and voriconazole are two good examples of the covert threat posed by inflammation.

The efficacy of genotype-guided warfarin dosing was investigated in two key randomized controlled studies published in 2013, the European Pharmacogenetics of Anticoagulant Therapy (EU-PACT) and Clarification of Optimal Anticoagulation through Genetics (COAG) trials.[19,20] The two came to divergent conclusions and various explanations have been advanced to account for this,[21] and the CPIC have recently updated their guideline on genotype-based warfarin dosing.[22] This 2017 update, like its original 2011 predecessor, acknowledges the need to account for co-medications that may inhibit or induce warfarin metabolism, but neither makes any reference to the effect of ongoing inflammatory processes. Atrial fibrillation (AF) was the indication for warfarin in 73%[19] and 22%[20] of patients enrolled in the above two studies. The other major indication was venous thromboembolism (27% and 58% respectively). Focusing for the present on AF only, emerging evidence suggests a significant role of inflammation in its pathogenesis. A positive correlation has long been known between CRP (a biomarker of inflammation) and IL-6 levels and AF, as well as the pathological impact and duration of AF before cardioversion.[23,24] Evidence for a role of inflammation and inflammatory biomarkers in the risk management and treatment of AF, and treatment outcomes, has been reviewed by others.[25–28] Available evidence suggests that inflammation causes AF or participates in its onset and continuation; other data suggest that AF

induces an inflammatory response.[29] A meta-analysis of available studies reported that increased CRP, IL-6 and TNF-α were significantly associated with the risk of AF.[30] In a subgroup analysis, CRP was significantly associated with persistent and permanent AF risk, but not with paroxysmal AF. More recently, plasma IL-6 level is reported to be an independent and consistent predictor of AF in patients with chronic kidney disease.[31]

The clinical use of voriconazole, indicated for many invasive fungal infections including aspergillosis and candidiasis, is complicated by hepatotoxicity, neurotoxicity and visual disturbances in some patients. Voriconazole is principally metabolized by CYP2C19[32] with significant exposure differences between CYP2C19 ultra-rapid metabolizers (UMs), EMs, IMs and PMs. Trough concentrations of <0.5 mg/l are associated with reduced rates of efficacy and trough concentrations >3.0 mg/l and >4.0 mg/l are associated with higher rates of hepatotoxicity and neurotoxicity, respectively,[33] placing the CYP2C19 UMs at higher risk of therapeutic failure. However, the exposure–toxicity relationship is weak and contentious.[32,34] In paediatric patients receiving haematopoietic stem cell transplantation, the time and the dose required to reach the adequate concentration reportedly show a trend towards correlation with individual *CYP2C19* genotypes, although voriconazole concentrations showed large interpatient variability in EM subjects with *CYP2C19*1/*1* genotype.[35] Median times to reach the target concentration using genotype-guided dosing were reportedly 9, 6.5, and 4 days for UMs, EMs and IMs, respectively. Overall, the median time to reach the target concentration with genotype-guided dosing was 6.5 days compared with a median time of 29 days when all patients were started on the same dose regardless of their *CYP2C19* genotype.[35] Notwithstanding these tantalizing data, association studies have not revealed any consistent or significant relationship between *CYP2C19* genotype and voriconazole toxicity.[32,36–38] As with *CYP2C9* genotype and variability in warfarin concentrations, *CYP2C19* genotype accounts for only a fraction (30–50%) of variability in voriconazole concentrations.[2] The recently published CPIC guideline on genotype-based voriconazole dosing summarizes the current evidence in support of using *CYP2C19* genotype information and clinical outcomes and provides recommendations for genotype-based dosing strategies.[39] The guideline acknowledges various non-genetic factors including phenoconversion due to concomitant medications and/or inflammation that may impact voriconazole concentrations and wide interpatient variability thereof. Inflammation has been shown to play a significant role in the largely unpredictable pharmacokinetics of voriconazole, especially in patients with high inflammatory response, as reflected by high CRP levels.[40] For every 1 mg/l increase in CRP levels, the voriconazole trough concentration increased by 0.015 mg/l,[40] and it has been suggested that the CRP value may be helpful in therapeutic drug monitoring of voriconazole during severe infection.[41] This is hardly surprising given that CYP2C19, CYP3A4 and CYP2C9, the three DMEs involved in metabolism of voriconazole, are all highly susceptible to cytokine-induced downregulation of their expression. Interestingly enough, clopidogrel, an antiplatelet agent widely used as an antithrombotic agent, requires bioactivation by CYP2C19 to its therapeutically active metabolite.[42] Dose–response analysis in patients undergoing percutaneous coronary interventions and treated with clopidogrel showed a significant 12% increase in the risk of major adverse cardiac events with every 1 mg/l increment in pre-procedural serum CRP level.[43] This observation also suggests the contribution of phenoconversion to therapeutic failure.

It seems reasonable to conclude that inflammation-mediated phenoconversion of an unquantified, but likely substantial, subset of study populations in various association studies cannot be ruled out. It has been recommended that the impact of inflammation on the variability in the pharmacokinetics and pharmacodynamics of drugs should be considered in the design, analysis and interpretation of clinical pharmacology studies.[44] This sound advice should be taken under consideration when designing prospective pharmacogenetic studies, especially if anecdotal associations are to be tested and clinically relevant associations are not to be missed. Furthermore, there is a pressing need for association studies to include actual measured DME phenotype in addition to genotype of the subjects enrolled.[2]

In conclusion, apart from co-medications, inflammatory processes are also likely to impact drug exposure. Their effect on genotype-based dosing decisions requires further investigations if the aspirations of genotype-based precision medicine are to be realized.

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