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PHARMACOGENOMICS AND THERANOSTICS IN PRACTICE

A summary of the Euromedlab-ESPT (The European Society of Pharmacogenomics and Theranostics) satellite symposium, May 2013

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INTRODUCTION

Genomic variation can lead to differential drug responses in individual patients, affecting drug absorption, metabolism, pharmacodynamics and excretion. Very often, polymorphisms in genes regulating these mechanisms are responsible for individual variability. The use of genome-wide association studies (GWAS) has enhanced the ability to identify such genetic variation, highlighting specific subgroups of patients who may either obtain particular benefit from, or who may be at risk of serious side effects associated with particular treatments (1). Targeted therapy or adaptations of therapy to tailor doses and timings of treatment have therefore become important parameters for achieving personalized healthcare (PHC). The increased understanding of disease provided by pharmacogenetic research is now also being incorporated into the drug development process in order to optimize therapeutic benefits and minimize the risk of toxicity in the individual patient (2).
Personalized healthcare represents a paradigm change in the management of patients, from a clinical definition of disease to a molecular definition of disease. The medical need for a more patient-tailored approach has resulted in a greater number of guided therapies focused on the patient. Theranostics, the association of a diagnostic test with a drug treatment, requires the expertise of the clinical chemist and clinical biochemist to use and interpret these tests. A satellite meeting, held by The European Society of Pharmacogenomics and Theranostics (ESPT) in conjunction with Euromedlab 2013 (Florence, May 24th, 2013) was held to support the continuing education of members of the clinical laboratory team who play in key role in ensuring the appropriate application of PHC. Opened by its President, Professor Gérard Siest, presentations were provided on current and future considerations for pharmacogenomics and theranostics in practice. This article provides an overview of the presentations at this meeting, the abstracts of which have also been published online (3).

PERSONALIZED HEALTHCARE IN ONCOLOGY

The implementation of PHC has been very successful in the field of oncology. A greater understanding of genetics is likely to have important ramifications for the prediction, prognosis and treatment of cancer, with the potential to expand PHC still further (4). An example of this was presented by Doctors Del Re, Michelotti, Roncella and Danesi, from Pisa, Italy, who explained how the genotyping of women with estrogen receptor positive (ER+) premenopausal breast cancer for deficient variants of the

cytochrome P450 enzyme CYP2D6 (*4 and *10) could identify those for whom treatment with standard dose tamoxifen is likely to fail. Tamoxifen is metabolised by CYP2D6 to the active metabolite endoxifen and in patients with limited metabolism, increases in tamoxifen dose may be beneficial. The development of a treatment dosing algorithm encompassing adaptations to tamoxifen dose based on categorization of a patient as an extensive, intermediate or poor tamoxifen metabolizer would allow more effective treatment to be implemented (5).
Protein tyrosine kinase inhibitors (TKIs) are an most important class of cancer drugs and one of the most impressive examples of targeted cancer therapy (6). Although they have revolutionized the treatment of several malignancies including chronic myeloid leukemia (CML), a subset of patients still exhibit a sub-optimal response.
Plasma concentrations of the TKI imatinib, a first-line treatment for CML, are known to correlate well with efficacy (7), but are highly variable and difficult to measure (6). Furthermore, intracellular concentrations (ICC) of imatinib in peripheral blood mononuclear cells and granulocytes are not routinely evaluated. Doctors Marc and Zakelj from Ljubljana, Slovenia described a dried blood spot (DBS) technique to investigate the correlation between imatinib ICC in leukocytes, plasma imatinib levels, and drug transporter genotypes. The specific inhibition of the organic cation transporter 1 was found to significantly reduce the ICC of imatinib, while inhibition of the P-glycoprotein or breast cancer resistance protein (BCRP) transporters did not (6). The novel and very simple DBS technique for imatinib therapeutic drug monitoring (TDM) also provides a sensitive technique for measuring imatinib ICC, and may be beneficial for use in future pharmacogenomics research to better understand the rationale for inter-individual differences in response to imatinib.

Pharmacogenomics of antithrombotics

Antithrombotics are extensively used in clinical practice, in particular in CVD. Professor Siest from Nancy, France, reviewed how clopidogrel inhibits platelet aggregation and, in combination with aspirin, is widely used to treat patients with a variety of CVDs. However, the pharmacodynamic response to clopidogrel varies widely from subject to subject such that approximately 25% of patients treated with a standard dose of clopidogrel display a poor response to treatment. This has been associated with low ex vivo inhibition of adenosine diphosphate (ADP)-induced platelet aggregation (8), and an increased risk of recurrent ischemic events.
Although the exact mechanisms underlying clopidogrel resistance are not entirely defined, genetic factors are likely to play a part. The metabolism of clopidogrel to its active metabolite is required for inhibition of platelet aggregation and a number of functional polymorphisms have been found in genes encoding CYP isoforms involved in clopidogrel metabolism. In particular, genetic variations in CYP2C19 or the use of concomitant medications that interfere with CYP2C19 appear to influence the response to clopidogrel, and this has now been incorporated into labeling recommendations for this agent by the US Food and Drug Administration (FDA). The recent development of point-of-care (POC) tests for clopidogrel is also an important evolution. The Spartan RX system will allow CYP2C19 genotyping in 2 hours or less without the need for specialized technicians. This could improve the use of genotyping before administration of clopidogrel (9). Clinical trials of clopidogrel (of which there have been approximately 400 during the last 4 years) continue to be designed and run to compare its use with other thienopyridines, to define the influence of pathologies (such as diabetes, inflammation, etc) and ethnic differences on response, and to evaluate potential drug interactions. Continuous education and training of clinicians are therefore necessary for an informed pharmacogenetic strategy for clopidogrel.
Response to therapy with coumarin derivatives such as warfarin, a mainstay of anticoagulation therapy, varies significantly among patients. This is thought to be influenced by genetic differences in the CYP2C9 enzyme and the vitamin K reductase VKORC1 genes (10). Dr Manolopoulos from Alexandroupolis, Greece, explained that these differences affect coumarin pharmacokinetics (with CYP2C9*2 and *3 variant alleles resulting in decreased CYP2C9 enzymatic activity), and pharmacodynamics (with the VKORC1 1639G>A polymorphism influencing the pharmacodynamic response) (11). It is anticipated that reductions in coumarin dosing in patients with variations in these genes will lead to reduced bleeding or thrombotic events, and increased effectiveness of anticoagulant agents. To this end, pharmacogenetic-based dosing algorithms are being tested in large prospective, randomized, clinical trials in Europe and the USA, including the European Union-Pharmacogenetics of Anticoagulant Therapy (EU-PACT) Trial. It is hoped that the results of these trials will provide a clear evidence base for incorporating genotype-guided dosing of anticoagulant therapy into the clinic.
New oral anticoagulant (NOAC) agents are now being used which specifically inhibit either thrombin (dabigatran) or factor Xa (rivaroxaban, apixaban). Unlike coumarin derivatives, NOACs have a wide therapeutic index, a rapid onset of action and do not require routine coagulation monitoring (11). However, pharmacogenetics may still play a role in NOAC treatment and this was explored by Doctors Gaussem, Gouin-Thibault, Mismetti, Azizi, Siguret, and Loriot from Paris, France (12). Variations in genes encoding P-glycoprotein efflux transporters may impact the absorption of dabigatran and rivaroxaban, while differential metabolism by CYP3A4/5 and/or CYP212 enzymes may have an influence on rivaroxaban pharmacokinetics. Variability such as

this is thought to contribute to the clinically significant drug–drug interactions and inter-individual pharmacokinetic variations that have been reported for dabigatran and rivaroxaban in both healthy subjects and patients with various cardiovascular diseases (12). Furthermore, GWAS undertaken as part of the Randomized Evaluation of Long Term Anticoagulant Therapy (RE-LY) Trial in patients with atrial fibrillation recently identified genetic determinants in ABCB1 and CES1 loci that were associated with a significant decrease in dabigatran concentration and a lower risk of bleeding (13). The presentation concluded with the design of the ongoing DRIVING study, which aims to determine the impact of the ABCB1 genotype and other genetic polymorphisms on the pharmacokinetics/pharmacodynamics of dabigatran and of rivaroxaban in healthy Caucasian subjects (12). Studies such as these may more clearly define the potential role of NOAC genetic variation in clinical practice.

Pharmacogenetics of immunosuppressive therapy

Adequate immune suppression is crucial in order to prevent graft rejection following solid organ transplantation. The calcineurin inhibitors tacrolimus or ciclosporin are frequently employed as immunosuppressants, but both have a narrow therapeutic window: the difference between the plasma concentrations that are high enough to prevent organ rejection, and low enough to avoid drug toxicity (particularly nephrotoxicity) and opportunistic infections is small. As such, TDM is currently critical in order to determine the drug concentration in the patient's blood and maintain effective and well-tolerated doses.
Doctors van Schaik, Elens, Hesselink, Haufroid and van Gelder from Rotterdam, The Netherlands/Brussels, Belgium explained how predictive genetic markers could potentially be of help in optimizing immunosuppressive therapy in the transplant scenario. Potential markers of variability were investigated in renal transplant patients receiving tacrolimus or ciclosporin in combination with myclophenolate mofetil (MMF) in the randomized, controlled Fixed-Dose Concentration-Controlled (FDCC) Study (14, 15). Inter-individual variability in MMF pharmacokinetics was associated with genetic polymorphisms in UGT1A9, with the -275T>A promoter polymorphism significantly associated with the risk of acute rejection in MMF/tacrolimus-treated patients. In addition, genetic variants in CYP3A5*3, CYP3A4*22 and POR*28 contributed to differences in the metabolism of tacrolimus: patients expressing CYP3A5 showed a 2-fold higher tacrolimus dose requirement compared with those who did not express this allele for example (15). For ciclosporin, patients with the CYP3A4*22 polymorphism had poorer renal function. These data provide an insight into the potential of genetic testing in order to optimize the efficacy and tolerability of immunosuppressive therapy.

Evaluating findings from GWAS

Vascular endothelial growth factor (VEGF) inhibitors are used as treatments in several pathologies, including cancer, macular degeneration and rheumatoid arthritis, although they have been associated with cardiovascular adverse events. Indeed, VEGF is also implicated in cardiovascular disease, having a role in angiogenesis, lymphangiogenesis, vascular permeability and hematopoiesis.
The biological determinants of response to VEGF inhibitors and the potential influence of genetic variants are not well described. Dr Visvikis-Siest from Nancy, France, presented how initial studies using the STANISLAS cohort identified that inter-individual variation in VEGF was associated with a significant degree of heritability. This led to the development of an integrated systems biology strategy aimed at better understanding the pharmacogenetics of VEGF (16). In healthy individuals, participating in the Framingham Heart Study, the STANISLAS family study and the PIVUS study, genetic variants in VEGF were evaluated using GWAS, and the relationship between these variants and blood lipids and with adhesion/inflammatory markers were further assessed. Four polymorphisms were identified in the study that were able to account for approximately 50% of the VEGF heritability. There were associations between these variants and the lipid and inflammatory markers studied that suggested the presence of molecular links between VEGF and cardiovascular disease biology (16). The identification of these VEGF genetic variants may be useful for future pharmacogenetic studies aimed at improving the prediction of response and/or toxicity to VEGF inhibition.
Despite the identification of some viral and host factors associated with viral clearance, response to therapy with pegylated-interferon (PEG-IFN)-ribavirin in patients infected with hepatitis C virus (HCV) remains highly unpredictable. Doctors Cariani, Rota and Trenti, from Modena, Italy reviewed data from GWAS which have identified an IL28B polymorphism as an important pre-treatment predictor of response to PEG-IFN treatment (17, 18). However, little is known about the biological relationship between the IL28B gene product IFNλ3 and the sensitivity of HCV to antiviral treatment, and the exact role of this marker in the treatment algorithm has not been established (18). Further investigation of the potential significance of the IL28B genotype for tailoring type, dose and duration of treatment is of interest, particularly in specific patient subgroups (such as those with cirrhosis, individuals co-infected with HIV, and in subjects infected with genotypes other than HCV1) and in the era of direct-acting antivirals that are rapidly becoming part of the anti-HCV therapeutic armamentarium.

The emerging role of clinical biochemistry in personalized healthcare

In a corporate sponsor presentation, Doctor Jordan from Roche Diagnostics International Ltd, Rotkreuz, Switzerland, described how recent advances in molecular science and technology are being increasingly translated into diagnostic, prognostic, and therapeutic tools that are allowing PHC to become a reality (19). In addition, the clinical development of targeted treatments is being linked to identification of companion diagnostics at an increasingly early stage, with the potential to improve the success rate of that development and benefit both patients and the economics of healthcare (20).

Aiming to match treatments with an individual's genetic and/or disease characteristics as closely as possible requires appropriate scientific, technologic and clinical expertise as well as the availability of technologies to evaluate protein expression, gene expression and gene copy number. This combination has led to the successful introduction of PHC into clinical practice, with particular impact to date in the fields of oncology and virology (21). However, there is potential for biomarker-guided treatment to enhance the efficacy of novel therapies in many other areas of unmet need such as coronary heart failure (HF), asthma, schizophrenia and Alzheimer's Disease (22-26). More detail on the potential of diagnostic testing within these areas is provided in specific articles in this issue of the journal.

Once diagnostic tests have been developed, there is an urgent need for standardization of methodology and reporting in order to provide consistent benefits of testing across diverse patient populations. Doctor Pazzagli from Florence, Italy (27) noted that molecular diagnostic methods can be performed using commercially available tests that are generally regulated as in vitro diagnostic devices. For these tests, a standard set of reagents are usually provided and the testing is performed using protocols developed and validated by the manufacturers, with recommendations for standardized reporting. By contrast, tests may also be developed for use by individual laboratories (laboratory-developed tests; LDTs).

Although there are generally accepted minimum requirements for laboratory testing, regulation of medical laboratories and of the tests they provide differ by country and region. LDTs may be offered without clearly defined evaluation of their analytic (evaluation of performance characteristics such as accuracy, precision, analytic sensitivity, analytic specificity and linear range) or clinical (determination of reference ranges or cut-off points) validity. A contributing factor is that, alongside the rapid application of highly complex and continuously developing technologies, laboratories can struggle to find guidance and material resources to establish these parameters. To address these challenges, a series of molecular-methods guidelines in the areas of genetics, hematopathology and infectious diseases have been published by the Clinical and Laboratory Standards Institute (28). These provide best practice strategies for validation, implementation and quality assurance. In addition, approaches are being evaluated to tackle the challenges surrounding the limited availability of reference materials.

Doctors Borro, Gentile, Lionetto and Simmaco from Rome, Italy, described how an integrated work-flow can be applied to enhance personalized medicine strategies in hospital practice (29). The Sant'Andrea Hospital Trust of Rome joined with the Faculty of Medicine and Psychology of the Sapienza University in Rome in the early 2000s, with both faculties relocating to the same building. This stimulated the development and experimentation of new concepts in medicine, with biochemists and molecular biologists joining forces with clinicians to create a shared, innovative laboratory, the Advanced Molecular Diagnostic Unit. The availability of advanced technologies, such as mass spectrometry and medium-to-high throughput DNA analysis paved the way for a 'real-time' evaluation of the benefits brought to clinical practice by implementation of new diagnostics aimed at guided therapy. The future of pharmacogenomics and theranostics may lie in an integrated approach, in which each patient is managed from a central point, encompassing a drug treatment, an accompanying diagnostic, a monitoring device and a patient support platform.

Conclusions

Pharmacogenetic research continues to improve the understanding of inter-individual variability, particularly regarding the contribution of genetic variants in predicting response to treatment. PHC is therefore a growing area of medicine; the number of clinical situations where the use, dose, timing and combination of therapies can be guided by pharmacogenomics is increasing, and will require ever-more innovative diagnostic tools to guide their use. In the future, the clinical biochemistry laboratory will continue to play pivotal and an increasingly important role in clinical decision making.

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