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Pharmacogenetics: transforming clinical medicine

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ABSTRACT Pharmacogenetics, the study of genetic variation relevant to drug metabolism, is a rapidly evolving area of medicine. This brief review will consider some of the recent advances where inherited genetic variants have been associated with either drug efficacy or toxicity. Examples of where pharmacogenetic testing has been adopted into clinical practice will be provided as well as a look at its likely development over the next decade. Finally, the large increase in genetic testing of tumour tissue samples to predict response to molecularly targeted treatments in cancer will be considered.

KEYWORDS Pharmacogenetics, adverse drug reactions, oncology, pharmacogenomics, efficacy

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Terms such as pharmacogenetics, pharmacogenomics, personalised medicine and stratified medicine have all been used interchangeably over the past few years to describe a revolution that is occurring in medicine. Every day throughout the UK thousands of individuals undergo genetic tests to establish the most effective or safest medication for their condition. However, pharmacogenetics is not a new concept. Over fifty years ago reports emerged of haemolytic anaemia in Americans on antimalarial medication and its relationship with glucose 6-phosphate dehydrogenase deficiency.[1] It was at this time that Friedrich Vogel first coined the term 'pharmacogenetics', defining it as the 'study of the role of genetics in drug response'.[2] Significant advances were made in the 1970s with studies undertaken to characterise the cytochrome P450 enzyme family. These liver enzymes are key factors in the first pass metabolism of the majority of drugs and overactivity or deficiency of specific members of this family have been associated with a range of adverse drug reactions. It is only over the last decade that the knowledge gained through thousands of studies has started to be applied in clinical practice.

The major motivation behind pharmacogenomics is to use genetic information to reduce the likelihood of adverse drug reactions (ADRs) and to improve the likelihood of individuals responding effectively to their medication. Pharmaceutical companies have invested millions of dollars undertaking genetic profiling to improve the drug development pipeline to manufacture drugs less likely to cause serious ADRs, notably drug induced liver injury and long QT syndrome. These ADRs cause their drugs to fail at a late stage in trial development or to be withdrawn from the marketplace after licensing. There is also the possibility that drugs that have already failed during development or been withdrawn may be reinstated if a patient subgroup likely to respond and at low/no risk of side-effects is identified.

ADVERSE DRUG REACTIONS

Adverse drug reactions are a significant cause of morbidity and mortality. A large prospective study of over 18,000 patients attending hospitals in the North West of England revealed that 6.5% of patients presented due to an ADR.[3] Furthermore, a study of hospital inpatients identified ADRs in nearly 15% of patients following admission.[4] Most of these adverse reactions are due to non-adherence, polypharmacy leading to drug interactions, inaccurate doses or prescription of the wrong medication for the clinical indication. However, an important and increasingly recognised subset of serious ADRs is due to genetic factors.

THIOPURINE PHARMACOGENETICS

The pharmacogenetics of the thiopurine drugs azathioprine and 6-mercaptopurine have been at the forefront of this discipline. Thiopurines are used routinely in the management of inflammatory diseases, including inflammatory bowel disease, and in the maintenance phase of the treatment of childhood leukaemia. However, the use of thiopurines is limited by intolerance to a range of side-effects including nausea, vomiting, flu-like symptoms and, importantly, neutropenia. The initial description of individuals deficient in thiopurine methyltransferase (TPMT) enzyme activity who experience severe neutropenia emerged over 30 years ago.[5,6] Over the

ensuing years controversy continued about the clinical utility of testing and the reluctance to adopt routine testing prior to thiopurine prescription.[7] Approximately one in 300 individuals are deficient for TPMT enzyme activity. The evidence is compelling that these people will experience a serious ADR (neutropaenia) if exposed to standard doses of thiopurines. Therefore, genotyping or enzyme assays should be performed to identify individuals deficient in TPMT and they should be given very low doses of thiopurines or an alternative drug. However, not all neutropaenia secondary to thiopurines can be predicted by TPMT testing and routine full blood count monitoring is therefore still required for all patients on this group of drugs. Many reports indicated that individuals heterozygous for a TPMT loss of function variant and therefore with intermediate enzyme activity, were also at an increased risk of ADRs.[8] However, in a prospective randomised controlled trial of adults with inflammatory diseases treated with azathioprine, there was no evidence of an increased frequency of ADRs in this group.[9] Despite the initial delay in the use of TPMT testing in the UK, over the past five years there has been a dramatic increase due to changes in professional clinical guidelines, technological advances facilitating cheap and rapid enzyme analysis and growing physician awareness.[10]

In contrast to the delay in TPMT testing, there was a rapid adoption of testing related to the highly active antiretroviral therapy, abacavir. It was introduced for human immunodeficiency virus (HIV) treatment in 1999 as single agent or in combination. Approximately 5–8% of patients exposed to abacavir developed drug hypersensitivity in the first six weeks of use. Individuals presented with characteristic nausea, fever, vomiting, rash and headache. Importantly, the drug was potentially fatal on re-exposure. Mallal and colleagues identified a strong association between the *HLA-B*5701* allele and hypersensitivity.[11] The test was rapidly adopted as a pre-screen in Australia and to reinforce the evidence of the association, GlaxoSmithKline designed and executed a randomised controlled trial. A total of 1,800 individuals were randomised equally to either HLA genotyping or standard care. Individuals in the intervention arm identified as carrying the *HLA-B*5701* allele were given an alternative antiretroviral agent. The results showed a complete absence of genuine hypersensitivity reactions in the genotyping arm and led to recommendations by the US Food and Drug Administration (FDA) and European Medicines Agency (EMEA) to mandate *HLA-B*5701* genotyping prior to abacavir prescription.[12]

ANTI-EPILEPTIC DRUGS

A study among Han Chinese in 2004 demonstrated that all individuals affected by the rare and severe skin reaction Stevens-Johnson syndrome (SJS) that can occur on exposure to the anti-epileptic drug carbamazepine carried the *HLA-B*1502* allele.[13] This allele is not present in people of European ancestry. The results led to recommendations by the FDA and EMEA that all patients of East Asian origin should have *HLA-B*1502* testing prior to receiving carbamazepine treatment. A recent study in Europeans with a range of different side-effects from carbamazepine demonstrated an association with another HLA genotype, *HLA-A*3101*.[14]

ANTICOAGULANTS

Warfarin is the second most common cause of ADRs.[3] Thousands of patients undergo anticoagulation to prevent thrombosis-related disease. Standard initial treatment previously involved a dose of 10 mg followed by doses of 5 mg for the next two days, prior to testing the international normalised ratio (INR). This approach led to erratic under-anticoagulation in some and dramatic over-anticoagulation in others. Incorporation of clinical parameters including smoking status, age, gender and co-medication helped to refine the dosing amount and optimise outcome. Recent association studies, including genome-wide association studies (GWAS), have indicated that variants in *VKORC1* and *CYP2C9* especially contribute to the effectiveness of anticoagulation with warfarin.[15] Online algorithms (www.warfarindosing.org) and downloadable apps including genotypic data are available to quickly predict the optimum dose of warfarin. An evaluation by the International Warfarin Pharmacogenetic Consortium compared the value of adding genotypic information into the clinical algorithm with outcomes based on either standard dosing or dosing using clinical parameters.[16] This study showed a significant reduction in ADRs when the pharmacogenetic data was added to predict the starting doses of warfarin. A number of randomised controlled trials are currently underway worldwide to examine the clinical utility of introducing pharmacogenetic testing for warfarin. One issue that is particularly important for testing warfarin and many other drugs is the turnaround time required to generate genetic testing results. Ideally, for simple analyses, point of care tests could generate results within minutes, leading to minimal delays before accurate, safe prescription.

POINT OF CARE TESTING

In 1993 a mutation in the mitochondrial genome (m.1555A>G) was found to be associated with profound, irreversible sensorineural deafness following exposure to aminoglycosides.[17] Approximately one in 500 individuals carries this mutation which confers a very high risk of aminogycoside-induced deafness.[18] Currently, gentamicin is mostly used in children on neonatal units where delays of two or three days for a genetic test

result are unacceptable for a child who is septicaemic. Therefore point of care testing, where a genotype can be rapidly realised from a blood spot or saliva sample at the bedside would provide this key information in a clinically relevant time and ensure gentamicin or other aminoglycosides could be avoided in at-risk children.

ADVANCES IN PHARMACOGENETIC TESTING

Over the coming years it is likely that the most significant advances will be made in defining the causes of severe ADRs. As we have seen, these pose a major health burden. The International Serious Adverse Event Consortium (www.saeconsortium.org) is a public-private investor partnership undertaking an ambitious programme of work to define the causes of some of the ADRs that lead to drugs being withdrawn from the market, including drug-induced liver injury (DILI), long QT syndrome and serious skin reactions. Notable successes have already been achieved through the collection of highly phenotyped cohorts where genome-wide association studies (GWAS) have revealed strong genotypic relationships. A recent example is the identification of the association between *HLA-B*5701* and flucloxacillin, causing DILI.[19] Interestingly, this is not a relationship that can be extrapolated, as carrying the *HLA-B*5701* allele is not associated with DILI caused by other medications, including non-steroidal anti-inflammatory drugs.

One of the big challenges of modern medicine is distilling the important information generated by research studies from those findings that have less evidence of clinical benefit. In pharmacogenetics there have been numerous association studies which have been underpowered, poorly designed and not replicated. The Clinical Pharmacogenetics Implementation Consortium (CPIC) of the Pharmacogenomics Research Network has been established where experts in genetics, pharmacology and medicine assess the evidence base for the adoption of pharmacogenetic testing and draw up specific guidelines.[20]

New DNA sequencing platforms are now able to generate affordable, rapid whole genome or exome sequence data from individuals. Some initial studies have highlighted the implications for pharmacogenetics where variants in genes known to be important in drug metabolism have been identified.[21] The challenges of effectively managing this data and providing people with accurate actionable information is yet to be realised.

MOLECULAR ONCOLOGY

The identification of acquired (somatic) mutations in tumours has also been facilitated by new sequencing and genotyping technologies. Large-scale international projects are underway to characterise the somatic mutations that occur in primary and metastatic tumours to establish their molecular profiles. Such information will help in the understanding of the pathogenesis of the different tumour types, the reasons predisposing to metastasis and provide novel targets for therapeutic intervention. The success of this approach has been seen with drugs to target the tyrosine kinase receptor family. The earliest example of molecularly-targeted treatments in cancer emerged with the development of trastuzumab (herceptin), a monoclonal antibody which is effective in the approximately 30% of women with breast cancer with overexpression (amplification) of the HER2 receptor. Breast tumour tissue is routinely analysed by immunohistochemistry and fluorescent *in situ* hybridisation (FISH) to determine HER2 status and the appropriateness of trastuzumab treatment.

In the late 1990s, activating mutations in the *CKIT* gene encoding a tyrosine kinase receptor were identified in rare cancers called gastrointestinal stromal tumours (GISTs).[22] These are soft-tissue sarcomas arising from mesenchymal stem cells in the gastrointestinal tract. Imatinib (gleevec) was identified as a small molecule inhibitor of c-Kit and reports emerged of its dramatic efficacy in patients with metastatic GIST and rapid regulatory approval followed.[23]

Activating mutations in *CKIT* (~85%) and PDGFRA (10–15%) have been identified in the majority of GISTs. Genetic testing of tumours for *CKIT* and *PDGFRA* can provide diagnostic and prognostic information (patients with mutations in *PDGFRA* have a better outcome and mutations in different exons in *CKIT* are associated with different outcomes). Furthermore, genetic testing can provide important information about the likelihood of treatment response, for example the specific position of the mutation in *CKIT* predicts the outcome of imatinib treatment, where patients with a mutation in exon 9 require an increased dose. This genetic information allows the selection of the optimum treatment and also avoids unnecessary costs in individuals with tumours bearing mutations that suggest resistance to treatment.

TABLE 1 Tumour testing to predict response to cancer treatment

Gene	Variant(s)	Cancer	Drug
CKIT	Exons 9 and 11	GIST	Imatinib
PDGFRA	Exon 18	GIST	Imatinib
EGFR	Exons 18–21	NSCLC	Gefitinib and erlotinib
KRAS	Codons 12,13 and 61	colorectal cancer	Cetuximab and panitumumab
BRAF	V600E	melanoma	Vemurafenib
EML4-ALK	Fusion oncogene	NSCLC	Crizotinib

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Numerous other targeted drugs have been developed over the past decade including those for non-small cell lung cancer,[24–26] colorectal cancer[27,28] and malignant melanoma[29] (Table 1). Testing of these tumour types for somatic mutations is a routine part of the oncology treatment workup. Testing for further tumour types and different genes will grow exponentially over the next few years. Technological advances mean that tumours will be profiled for mutations across a panel of genes to determine the optimum treatment pathway. Numerous challenges still remain however. Tumour biopsy samples are often very small or unobtainable and working with very small amounts of tissue, which is often formalin-fixed in paraffin, and a heterogeneous mix of normal and cancer cells requires further optimisation. Many groups are developing approaches whereby tumour DNA circulating freely in the blood or tumour cells in the blood can be isolated and genetic analyses undertaken as a surrogate of the primary tumour.

These are exciting times in molecular medicine where the power of new genetic technologies is being harnessed to provide optimum treatments to patients. The era of personalised medicine is upon us.

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