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Systematic Review into the Role of Genomics in Profiling Overall Drug Performance and Variability

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ABSTRACT

Background and aim: The aim of this project was to conduct a systematic review of the current literature concerning the impact of genomics on drug performance and variability. The impact of genomics on drug performance is well pronounced, with variation in various Cytochrome P450 (CYP450) enzymes being significant. Due to this, within a large patient population treatment will only be successful for some. In addition, drug transporters regulate the movement of metabolites into and out of cells therefore, they can also influence drug performance. Clinical knowledge in such areas can be used to facilitate the possibility of individualized medicine, which attempts to tailor medication choices to suit the pharmacogenetic makeup of a patient; with the aims of increasing drug efficacy and reducing adverse events.

Methods: A search using common terms was conducted of 5 key databases, with articles assessed for eligibility before inclusion in the review paper. A total of 11 studies were identified covering a combined 2,163 patients aged from 18 to over 70 years old. All studies recruited human patients for clinical trials focusing on the efficacy of a selected drug, with regards to their specific genetic variants such as CYP450 enzymes.

Results and conclusion: A majority of the studies collected found a statistically significant impact for a particular genetic variant on drug performance; indicating that genomics is beneficial to future clinical practice. However, there is a need to assess the cost benefit implications of introducing genomic based testing across a wider healthcare network

INTRODUCTION

A universal truth known unanimously to healthcare professionals and patients alike is that every patient exhibits a varying response to drug therapy [1]. Scientists have been able to critically study this phenomenon surrounding different reactions, and strong evidence has been found for a genetic basis to this variability. The study of pharmacogenomics encompasses this very concept, as it looks at the genetic relation to inter-individual differences in drug responses, in particular: efficacy, medication doses and the incidence of adverse drug reactions (ADRs). As well as focusing on DNA, pharmacogenomics is also interested in mapping individual gene expression patterns that belong to an individual, with the goal of obtaining a clearer indication of their likelihood for particular diseases or chances of suffering ADRs [1]. The occurrence of ADRs has a negative impact on the individual's life, affecting their adherence to the medication and making them reluctant to take subsequent future drugs. During the time period 1990 to 2012, a total of 43 drugs have been withdrawn from the market due to ADRs [2]. They are the causative factor for approximately 6.7% of hospitalizations, with a ranking between fourth and sixth for the most common cause of inpatient death in the western world. Furthermore, the branch of genetics credited to the variation in drug response can broadly be split into two categories: germ line genetic variants and somatic mutations, with cancer tumours being a unique class of this [1].

A review conducted in 2002 looked at the genetic basis for variability in drug response, highlighting important concepts still

relevant today. It acknowledged that specific CYP gene alleles are required for drug metabolism hence, determining whether a drug will be successful, toxic or cause adverse reactions. For example, some tri cyclic antidepressants (TCA's) require a specific CYP2D6 to be metabolised to their inactive compounds; those who are poor metabolisers suffer side effects whereas extensive metabolisers receive no drug benefit. Furthermore, drug-drug interactions can be clinically damaging to a patient; e.g. serotonin re-uptake inhibitors taken with TCA's inhibit CYP2D6. Drug transport molecules are involved in drug uptake and efflux and they also exhibit allelic variability, which affects their functions. The blood brain barrier (BBB) has tight junctions in the capillary endothelium, efflux proteins and p-glycoprotein; which are all credited to being the primary reason as to why drug delivery to the brain is greatly reduced. These features of the BBB have resulted in a lack of clinically effective drugs for central nervous system (CNS) disorders. In addition, regulation of transcription over the genes coding for the production of drug metabolising enzymes and proteins, allow the creation of personalised concentration-time profiles for a drug [3].

The Human Genome Project gave rise to pharmacogenetics which has been touted as an area which can provide great clinical advances [4]. Personalized medicine is tailored to an individual's genetic makeup to ensure that medication will give the desired response, reducing the risk of unwanted ADRs. The use of technology such as phenotyping and genotyping strategies can provide a new gateway for a more personalized medicine approach. Hence, this will encourage financially favourable practice as it would reduce excess expenditure on drugs that won't work for a patient. The Food and Drug Administration (FDA) has relabelled over 100 drugs to also include genetic information, in response to recent developments in pharmacogenomics [1]. Another breakthrough attributed to this is cancer therapy, as doctors can examine at the level of genomic changes to better categorise some cancers. The difficulties in achieving a balance between efficacy and toxicity with cancer medication are well known, with most drugs benefitting a minority of patients [5].

This systematic review will gather recent literature to provide an indication of the role of genomics in drug performance and variability. It will cover the clinically relevant impact of genomics on drug performance, as well as limitations in the development of highly sensitive predictive research tools and ultimately individualized medicine.

SUMMARY OF AIMS AND OBJECTIVE

The primary aim of this research project is to produce a systematic review on the role of genomics in drug performance and variability. This includes the effectiveness of genomic techniques in drug discovery, areas which benefit most from this technology and pitfalls in limiting the development of highly sensitive predictive research tools and eventually individualised medicine. The objectives are as follows:

- In order to carry out the systematic review: perform searches of electronic databases to collect relevant literature that discusses the role of genomics in drug performance and variability. Using the guidelines from the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart and checklist to compile a final set of relevant articles.

- Understand how genomics affects medicine and the possible limitations.

- Discuss the impact genomics has had on drug performance with clinical examples.

- If possible carry out a meta-analysis on the collected data in order to reach a conclusion which is statistically significant.

METHODS

A systematic review was carried out to assess the role of genomics on drug performance and variability. To ensure the quality of the data obtained, Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was used. PRISMA sets out a 27 point checklist and a flow diagram **(Figure 1)**, which provides a minimum standard that needs to be adhered to for a systematic review and meta-analysis [6]. The articles were identified via extensive database searching using a common set of key words giving a total of 10,335 articles; duplicates across databases were then removed leaving 10,149. After these records were screened for eligibility which included both full text availability and relevance of the article to the project; titles and abstracts were searched to ensure that the article related to human genomics and drug variability only. This left 25 articles which were then analysed in full to ensure that they involved the collection of primary data from participants taking a medication, with the effects of that medication studied in relation to a genetic variant e.g. CYP450 enzymes. Studies were broadly required to relate to: genomics and the direct impact this has on drug performance or discovery. At the end this left a total of 11 articles to be used in the systematic review.

PRISMA 2009 Flow Diagram

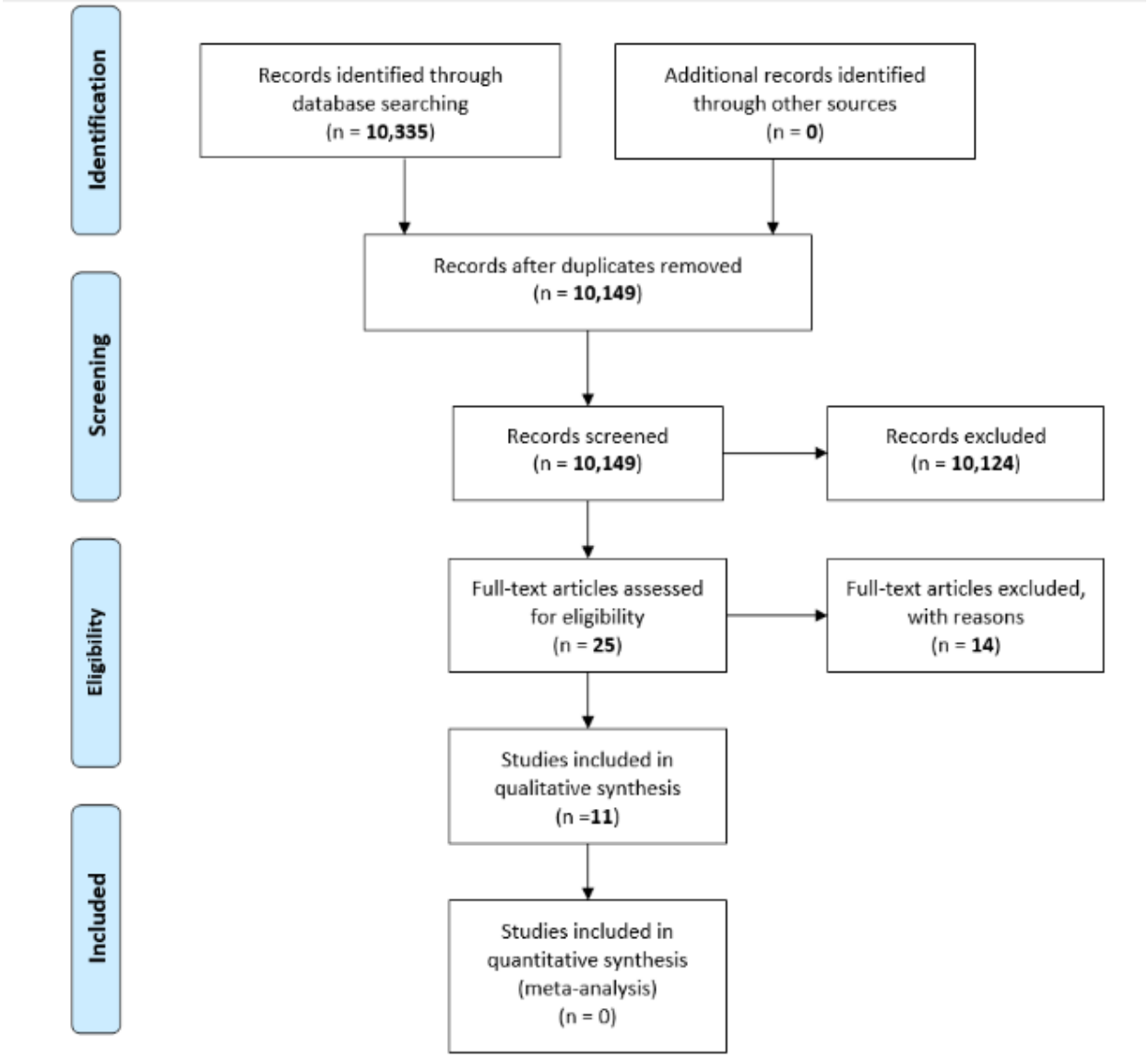

Figure 1. The flow chart above shows the process for article screening and selection with data completed for this application.

Articles were identified via searching 5 databases with the removal of duplicates. Inclusion and exclusion criteria were applied to the articles to assess their relevance. e.g. English language, time period 2012-2017, human studies, availability and original articles only. Full text articles were assessed to ensure that they included data related to human genomics impacting drug variability, leaving a total of 11 articles [6].

Eligibility Criteria

In order to ensure that all the relevant studies possible were collected, various inclusion and exclusion criteria were applied. A general search in the Google Scholar database for the keywords “genomics AND medicine” generated over a million search results. This confirmed that pharmacogenomics is indeed an advancing area of study, therefore various criteria needs to be applied to obtain only the relevant articles amongst them. The year of publication is a significant factor in determining the eligibility status of an article; the more recent the date the more relevance the article will hold to the current landscape of pharmacogenomics. Across all the databases studies were limited to the past 5 years; this allows only the most relevant studies post the human genomic project era to be used. To ensure that all the articles are understandable only those written in the English language are preferred, due to the difficulties in translating foreign articles accurately within the time limit. In addition, original articles instead of reviews are selected as this gives the primary source detailing the methods and raw results. These articles were then used to generate this review in light of the conclusions drawn by the authors. The primary focus is on the impact of genomics on drug performance in the human population, so articles regarding any other animal species were not used. The availability of the studies was the key deciding factor for study selection, as only those accessible in full text format could be put forward to be used in the review. Other factors resulting in the exclusion of articles included:

- The use of other ‘omic’ techniques alongside genomics, such as: proteomics, metabolomics or transcriptomics.

- Articles not being accessible.
- Studies based on data generated from animals or simulation software only.
- Studies related to genomic techniques or mapping only.
- Did not involve the collection of any primary data from patients regarding drug therapy.
- Did not relate the drug responses observed to any type of genetic variant.

Information Sources

Online databases were used to search for all the studies collected. An extensive search of 5 major databases was carried out, using a uniform set of key words and eligibility criteria across them all. This allowed a range of relevant articles to be collected, those of which all relate to the same key words, with the removal of duplicates giving a total of 10,149 articles. By using the same key words the articles found all related to similar concepts and therefore could be used within the review article as they all related to similar topics. The dates covered across them all was the time period 2012-2017, with the last search taking place on 18/02/17. The databases included:

1. Cochrane- The Cochrane library is a collection of 6 databases which aim to provide high quality evidence to allow health care professionals to make evidence based decisions [7].

2. Google Scholar- A large database allowing the search of scholarly literature from a range of sources [8].

3. PubMed- Contains 26 million citations for biomedical literature from MEDLINE, life science journals and online books [9].

4. Scopus- A large collection of the world's collection of: peer-reviewed literature, scientific journals, books and conference proceedings [10].

5. Web of Science- Subscription based and contains 7 online databases to give a comprehensive literature search [11].

Search

In order to ascertain the final search terms a range of combinations were tested, with the articles and number of hits recorded. This helped to inform the next search as articles need to be relevant, but due to the time constraints it was not possible to search through thousands of literature papers. To maintain the relevance of the review, the publication date was set to the past 5 years. Key word sets such as: (pharmacogenomics OR genomics* AND drug performance OR drug discovery OR drug* AND variability OR Individualised medicine OR personalised medicine) gave up to 95,000 results, therefore this was not realistically manageable due to time constraints which is a limitation. The key words were refined to include only the main concepts of the question, which still encompass the effect of genomics on drug behaviour. The use of 'AND' and 'OR' words can allow respectively refinement of results, or in the latter case broadening of results. The term 'variability' is vital to the question, so alongside 'pharmacogenomics' it was included; whereas either drug performance or discovery were both applicable. The key words used across all the databases were: (pharmacogenomics AND drug performance OR drug discovery AND variability). This covers the main aspects of the research question, as well as being flexible enough to apply to a vast range of articles.

Article Selection

In addition to the eligibility criteria set out above, the studies were required to relate to genomics impacting a factor which provides a measure for drug variability. These factors can range from: drug clearance values, drug dose amounts, metabolism of drugs and the response to a particular drug. With regards to the genomics aspect this is broadly represented by the family of Cytochrome P450 enzymes (CYP450) which are responsible for drug metabolism hence, genetic variation in them is key to understanding drug variability in mankind. Others can include drug transporters, such as the ATP-binding cassette transporters (ABC transporters) or even P-glycoprotein. The data collected within the studies was required to involve the recruitment of patients taking a particular medication, with an association made to their genotype (e.g. CYP450 enzymes) which linked to the drug response reported. There were no restrictions placed upon: the drug studied, dosage forms, parameter measured to assess drug efficacy, gender, ethnicity, number of participants or geographical location; as this enables the collection of data that studies the effect across the wider population. It is also apparent that heavy restrictions placed upon study requirements would further hinder the amount of studies that would be recruited, reducing the data available.

Risk of Bias

Bias is the deviation from the truth that can influence a set of results that are reported. As a consequence of this it can result in an underestimate or overestimate of the actual effect size, as a skewed representation of the results will be given. The heterogeneity amongst the results of studies that form a systematic review can be accredited to bias of varying degrees. The

process of a systematic review aims to reduce the impact of bias as much as possible, in order to come to a valid conclusion. It ensures that all articles within a database that fit the required topic will be analysed therefore, this eliminates a large amount of potential bias that can come from study selection. All the articles identified were put forward for the systematic review; with refinements made in the later stages, based upon the pre-selected eligibility criteria which were equally applied across all articles. Furthermore, only original sourced articles have been used, as this gives the raw methods and observed data as reported by the authors themselves. In contrast to a review article, which is a non-primary source and the data has already been interpreted by another individual whom may have introduced further bias.

Meta-Analysis

Residing at the top of the evidence based pyramid are systematic reviews and meta-analysis. A meta-analysis is the statistical combination of results from the individual studies found in the systematic review, which can be combined in order to answer a research question. Once the data from each individual study has been extracted an effect size from each will need to be calculated, and this will be pooled to obtain an overall summary of the effect size. Various statistical methods can be applied to the data to compare it, e.g. risk ratios. The notion of an effect size reports the average between scores in intervention and control groups; across studies this value needs to be standardized to produce comparable estimates of the effect. Finally, an appropriate model needs to be selected with the common ones being: Fixed effects and random effects models. Fixed effects assume that each study assesses a common treatment effect whereas, Random effects assumes that true treatment effects in individual studies differ. These results can be graphically displayed in a 'forest plot', for each study a horizontal line indicates the effect size whilst the box relates to the relative weights calculated by the meta-analysis. A diamond shape at the end gives the pooled result overall. In order for the meta-analysis to generate a meaningful result the data used for it must be assessed for heterogeneity to decide if it can be pooled [12].

For the 11 studies used in this systematic review there was some uniformity in the genetic variants studied and drug types, the outcomes measured and methodology varied between the individual studies however, when taking these differences into consideration it was decided that on this occasion a meta-analysis would not be suitable. The varying amount of heterogeneity between the methods for measuring outcomes in each individual study does not allow a justified overall effect size to be calculated.

RESULTS

The combined searches of the various databases identified a total of 11 eligible studies. The total number of participants across these studies is 2,163; this includes patients across varied geographical locations such as Spain, Turkey and America. In view of a genomics perspective this is desirable as a range of genetic variants can be assessed from different populations, with most of the articles reporting Hardy-Weinberg equilibrium analysis also. There is a vast age range span across the studies ranging from 18 years old to elderly patients over 70; this in itself may provide another confounding factor to the drug response variability seen e.g. older patients tend to have a decline in liver/kidney function which impacts drug behaviour. The main genetic variants covered have also been documented above, with their selection for inclusion inspired primarily by their relevance to the drug that was observed in that article.

The studies conducted by Chan et al. [13] and Mazzaccara et al. [14] are both based upon warfarin and as a result both focus on similar genetic variants, namely CYP2C9, CYP4F2 and VKORC1. The article by Arici and Ozhan [15] also contains data related to warfarin. The case study by Chan et al. [13] is based on 248 patients from a multi ethnic cohort in Asia (131 Chinese, 81 Malays and 36 Asian Indians) and patients were required to have a stable international normalized ratio between 2 and 3 for 3 months prior. Statistical analysis did indeed confirm a significant association between genetic variants and warfarin dose variation: CYP2C9*3 (P-value=0.000161), VKORC1 (P-value=0.00000719) and CYP4F2 (P-value=0.000033), explaining between 7% (CYP2C9*3) and 30% (VKORC1) of the dose variance [13].

The study by Mazzaccara et al. [14] recruited 266 patients from Southern Italy affected by cardiovascular disease. Again statistically significant differences ($p<0.001$) were observed in warfarin dose in response to different genetic variants. CYP2C9*1/*3, *2/*3 and *3/*3 genotypes require a lower warfarin dose than patients with the wild-type allele (22.03 mg/week ± 8.80; 13.4 mg/week ± 10.10; 9.74 mg/week ± 3.25), respectively vs. (32.11 mg/week ± 13.98). The mean weekly warfarin dose was also significantly lower in VKORC1 -1639 G>A mutated homozygotes and in heterozygotes than in patients with the wild-type allele (18.81 mg/week ± 7.98, 29.15 mg/week ± 11.79, and 37.80 mg/week ± 13.37). It was also noted that the mean warfarin dose was 17% and 32% lower for CYP2C9*2 and *3 polymorphisms verses the wild type [14]. **Figure 2** below combines the results from this study along with the data collected by Chan et al. [13] showing the varying mean dose (mg/week) of warfarin in relation to different genetic variants.

In addition, the study by Arici and Ozhan [15] highlights the importance of genotyping CYP2C9 variants with regards to warfarin

therapy. Patients carrying CYP2C9*3 receiving warfarin for over 30 days had a 2 fold risk of major bleeding. Carriers of the *2 and *3 variant have an increased risk of bleeding events as well as 10 fold lower S-warfarin clearance contrary to *11 and *33 [15].

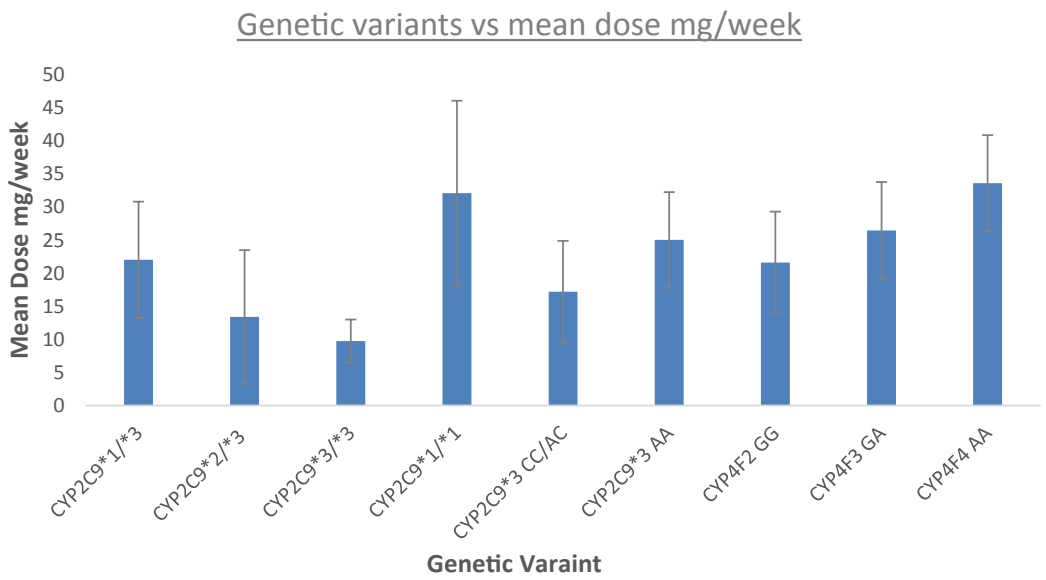

Figure 2. Graph showing differing warfarin doses (mg/week) across various genetic variants [13,14].

The articles by Tao et al. [16] and Song et al. [17] both focus on cyclosporine. The data collected by Tao et al. [16] relates to 56 healthy Chinese subjects who received 5 mg/kg^{-1} of the drug orally with genotyping 30 h post dose. This study combined gender alongside genetic variants when reporting it's outcomes. A significant finding (p=0.023) found that the C_{max} (peak drug concentration in the body) of the drug was higher in CYP3A4*1/*1 carriers over CYP3A4*1/*18B in the male group only. Conversely, C_{max} was significantly (p=0.01) lower in CYP3A5*1/*3 compared to CYP3A5*3/*3 in the male group also. It was concluded that polymorphisms in CYP3A5*3 and CYP3A4*18B significantly affect cyclosporine pharmacokinetics. Song et al. [17] reported with high significance (p=<0.0001) that the clearance was lower in carriers of CYP3A5*3/*3 compared to CYP3A5*1. This data was from 69 patients post liver donor renal transplantation collected for up to 400 postoperative days. Differences in drug clearance due to CYP3A5 significantly affect the pharmacokinetics of the drug, alongside factors such as postoperative days and gender [17].

Berno et al. [18] analysed 20 Caucasian HIV infected patients treated with LPV/r monotherapy. It was found that CYP3A5*3 was not accredited with decreased drug efficacy whereas, CYP3A4*1B was associated with low level TDM (therapeutic drug monitoring) and virological rebound. The data suggested SNP's of CYP3A4 in particular CYP3A4*1B may play a role in reducing LPV/r efficacy [18]. Mas et al. [19] is based upon risperidone dosing in 151 participants from Spain with a strong correlation demonstrated between CYP2D6 status and drug doses. However, although there was an observed trend with ultra-rapid metabolizers (UM) receiving a higher drug dose it was not statistically significant. The same was calculated for CYP3A5 where patients with higher metabolic activity polymorphisms received higher doses but again it was of non-statistical significance (Kruskal-Wallis test p=0.42). ABCB1 polymorphisms also required higher doses with higher efflux pump activity, but again this was not significant (Kruskal-Wallis test p=0.24) [19].

Uckun et al. [20] relates to demethylcitalopram (DCIT) metabolism, with the study based on CYP2C19. 50 patients were analysed with the following reported: mean plasma concentrations were significantly higher (p=<0.05) in CYP2C19*1/*1 carriers over *1/*2 and *2/*2. This study found that the genotype CYP2C19*17 did not have a significant effect on drug metabolism, whereas, the *2 variant is a likely contender for inter-individual variability in drug metabolism at therapeutic doses. Furthermore Scott et al. [21] also study the CYP2C19 genotype but with an emphasis on clopidogrel response. The blood samples for this were obtained from 250 individuals undertaking routine screening for Jewish genetic diseases in New York. It was reported that there was an increased risk for drug non responsiveness for individuals carrying a CYP2C19 loss-of-function allele and/or members with the ABCB1 c.3435T/T genotype.

Fonseca et al. [22] observed the effects of ALDH5A1 variability with regards to methadone treatment. The study recruited 132 opioid dependent patients attending methadone maintenance treatment (MMT) in an outpatient centre in Spain. A significant finding (p=0.0024) amongst patients carrying the T variant allele showed that they had a high risk of being non responders to methadone treatment, hence being likely to drop out or relapse.

Finally Yelensky et al. [23] studied ADRB2 polymorphisms and indacterol response in 626 patients enrolled in two double blind clinical trials. Common to both trials was the use of 150 or 300 mcg of indacterol and a placebo, with the third drug being either

tiotropium 18 mcg or salmeterol 50 mcg. Genotyping took place however overall analysis found little evidence for a significant association between ADRB2 genetic variants and subsequent indacterol response in moderate to severe COPD patients. Yelensky et al. [23] reported the response of genetic variants to indacterol in COPD, with none yielding a significant p value.

DISCUSSION

Pharmacogenomic studies have estimated that genomic differences between individuals occur every 300-1000 nucleotides with over 14 million single nucleotide polymorphisms (SNPs) [24]. Hence, the identification of genetic variants which are responsible for differing drug response are a core concept of modern day genetic science, with the major variants discussed below. Due to this within a large patient population treatment will be successful for some whereas, in others it may fail to elicit even an adequate response. It is further noted that most drugs are effective in only 25-60% of patients, making it difficult to optimize a 'one size fits all' dosage regime [24].

CYP3A4/CYP3A5

The major enzyme group responsible for the oxidative biotransformation of most drugs and other lipophilic xenobiotics are the CYP450 superfamily. The CYP-families 1-3 are of major concern with regards to drug metabolism. It has been demonstrated that genetic variation within these enzymes influences drug biotransformation to varying degrees. The most abundant enzyme in the liver is CYP3A4 with over 50% of clinically-administered drugs metabolized by it, and around 26 variants currently known. Ethnicity has established effects on expression levels with the *2 and *3 variants seen in Caucasians, whilst **18 is more prevalent in Chinese people. To contribute to the complexity of CYP3A4; CYP3A5 has the same metabolizing capacity with a few exceptions. In vivo studies have shown that the metabolic rates for drugs metabolised by 3A4 and 3A5 are the sum of the activities of both [24]. Tao et al. [16] and Song et al. [17] both concluded a significant effect of CYP3A4/3A5 on cyclosporine activity, which is used as an immunosuppressant to prevent allograft rejection. Therefore, when accounting for its narrow therapeutic index it is fundamental that drug doses are within the required range to avoid sub therapeutic and toxic outcomes. In particular this effect was seen with CYP3A4*18B and CYP3A5*3; the latter allele produces premature stop codon, leading to reduced CYP3A5 protein expression hence the lower clearance values.

A review by Klein and Zanger [25] supports the importance of CYP3A4, with twin studies showing that this variant accounted for 66% to 88% of inter individual variation. Affected agents include lipophilic or bulky compounds: e.g. cyclosporine A, erythromycin, tamoxifen, benzodiazepines, statins and antidepressants. Further examples from clinical studies have shown that the CYP3A4*22 allele corresponds to a decreased AUC ratio in atorvastatin patients or a 1.7 to 5 fold reduction in statin dose for T allele carriers vs. non T carriers with the goal of optimal lipid control. There are external influences on the CYP3A4 phenotype from outside the CYP3A locus including influence from: networks of nuclear receptors, transcription factors, hormonal and inflammatory pathways, heme and protein synthesis and degradation pathways.

CYP2C19

CYP2C19 genotyping categorizes patients into 4 metabolizer phenotypes: ultrarapid (UM) (*1/*17 or *17/*17), extensive (EM) (*1/*1), intermediate (IM) (deficient allele heterozygote) and poor (PM) (deficient allele compound heterozygote or homozygote). There are over 35 CYP2C19 variants and approximately 2000 SNPs identified. This genotype is related to the metabolism of drugs such as: anxiolytics, proton pump inhibitors, anticonvulsants and antimalarial biguanides. The reduced activity allele CYP2C19*2 is caused by a single nucleotide alteration in exon 5 resulting in an abnormal splicing site. It is seen at a high frequency of 30% in South Indians and lowest at 2.9% in the Faroeses. Conversely, another reduced activity variant CYP2C19*3 is found at its higher frequencies of 13% in Japanese and at 0 among populations such as Italians, South Africans, Greeks and European-Americans. Patients with either of these two variants are known as PMs [24]. Uckun et al. [20] found that the reduced enzyme activity with CYP2C19*2 has a role in the variation of citalopram metabolism, with better treatment outcomes but a higher risk of ADR's. Citalopram is used worldwide to treat depressive disorders, therefore tight regulation on dosage is required in a vulnerable patient group [20]. There is a growing body of literature which addresses these losses of function alleles in relation to clopidogrel response. Scott et al. [21] reported that there was an increased risk of clopidogrel non response amongst individuals carrying a CYP2C19 loss of function allele. This is of significance as clopidogrel is utilized for its antiplatelet properties hence, it is vital in the prevention of adverse cardiovascular events.

The United States Food and Drug Administration has implemented specific warnings for clopidogrel for CYP2C19 carriers especially in PM's, this warning is also prevalent on other drugs strongly affected by CYP2C19 genotypes [21]. Further clinical applications of CYP2C19 activity has shown that for Omeprazole, which is used for indications such as peptic ulcers; a single 20 mg dose resulted in intragastric pH values 4.5, 3.3 and 2.1, respectively for PM's, heterozygous EM's and EM's. It is evident that both CYP2C19*2 and CYP2C19*3 are associated with inactive enzyme production, which then effects the various drugs metabolized by them.

CYP2C9

Pharmacogenomic studies have identified over 60 variant alleles for the CYP2C9 gene, as well as recognizing it is responsible

for the metabolism of around 25% of drugs in clinical use. These drugs include: anti-inflammatory agents, hypoglycaemic agents, the anticoagulant S-warfarin and anticonvulsant phenytoin. Among the alleles discovered CYP2C9*1 is the wild type version whereas, *2 and *3 are the common variants accountable to the reduced CYP2C9 enzymatic activity. The frequencies of heterozygous CYP2C9*1/*2, homozygous CYP2C9*2 or CYP2C9*3 was lower (0.1%-1%) in Chinese and Japanese populations vs. Caucasians and Iranians. Furthermore, Chinese, Vietnamese, Korean, Bolivian and Malaysian populations have a CYP2C9*1 frequency of >90%, whereas CYP2C9*2 was undetected in Chinese, Vietnamese, Korean populations but at 1% in the Japanese [24]. Research by Chan et al. [13] and Mazzaccara et al. [14] and Arici and Ozhan [15], surrounds warfarin therapy which is utilized for its prevention of thromboembolic disorders; however, it is subject to frequent international normalised ratio (INR) monitoring in light of its narrow therapeutic index. Despite newer anticoagulant medication coming into the clinic warfarin still remains a popular choice, especially for mechanical heart valve patients and those intolerant to the newer medication.

The three sources mentioned above come to a joint consensus regarding the *2 or *3 variant, with the association of a reduced warfarin dose, due to the reduced clearance putting individuals at risk of major bleeding events. The US Food and Drug Administration, Centre for Drug Evaluation and Research has acted upon this by issuing guidelines for the genotype testing of patients for CYP2C9 and VKORC1 prior to commencing warfarin therapy. This further encouraged specific research in the area with the development of genetic based dosing algorithms [14]. The warfarin that is used is a racemic mix of S and R enantiomers, with the S-isomer having 5 fold higher anticoagulant activity. The inactivation of this S isomer is almost completely dictated by CYP2C9 activity; as a result, patients with the wild type or CYP2C9*1 clear S-warfarin normally from the body. PM`s carrying CYP2C9*2, *3 or both require lower warfarin doses as they metabolize S-warfarin at an impaired rate. It was also ascertained that despite genes encoding blood-clotting factors also having an effect on warfarin, the CYP2C9 polymorphisms have the larger impact [24].

Drug Transporters

The pharmacokinetic variability of a drug is subdivided into ADME (absorption, distribution, metabolism and excretion). Drug transporters are responsible for regulating the movement of active and inactive metabolites alike, into and out of cells. Therefore, polymorphisms in the genes that regulate these transporters have the ability to influence the ADME of a drug substance. An extensively studied transporter is P-glycoprotein which is situated at the blood brain barrier (BBB) and it is responsible for heavily limiting access of foreign matter into the brain area, this is advantageous in the case of toxins however, it poses a barrier to the efficacy of CNS related therapy [3]. In addition, the ABC and solute carrier (SLC) transporters are two super-families of membrane bound transport proteins. ABC transporters require the energy source ATP in order to transport substances against a concentration gradient, with 49 genes in 7 families. ABCB1 encodes a P-glycoprotein which effluxes: chemotherapeutic agents, physiological metabolites and carcinogens alike. There are many allelic variants, with the location being the surface of epithelial cells preventing: absorption to the intestine, protection for the brain and fetus from xenobiotics and modulating renal and hepatobiliary excretions [24]. The data collected by Scott et al. [21] did find an increased risk of clopidogrel non responsiveness in individuals carrying the ABCB1 c.3435T/T genotype, which is of significance as there appears to be ethnicity dependent distribution of its allelic variants. The SNP 3435C>T has a frequency of 60%-72% in Asians vs. 34%-42% in Caucasians. Despite this observed trend with regards to digoxin therapy, studies with the mutant allele (3425C>T) have reported conflicting higher and lower serum levels. This is indicative that there may yet be further mutations which have not yet been identified or it may be due to the complex pathways of drug disposition itself [24].

Pharmacogenomic Research

In order to evaluate the relationship between genetic variants and therapeutic outcomes, there are two research approaches which are commonly adopted. The first is the candidate gene approach, where the genes and polymorphisms for a therapeutic phenotype are pre-selected based upon a known pathway or previous study. A successful application of this is seen with the association of polymorphisms in CYP2C9 and VKORC1 with warfarin use. The development of next generation sequencing technology allows the identification of genetic variants as well as MRNA transcripts mapped against the phenotypes they contribute to. The second method is genome-wide association (GWA) studies, which uses genotyping arrays containing SNPs to screen for genetic variants associated with the phenotype. This technique has recently become common practice when measuring the genetic influence in a treatment effect. Examples for this cover a plethora of indications: from flucloxacillin induced liver injury, glycemic response for metformin in type 2 diabetes to glucocorticoid response in asthmatics. A phenotype can be understood via pharmacokinetic end points, e.g. drug plasma concentrations and with symptom control, e.g. blood pressure, therefore GWA study results need to be viewed with that context [26].

LIMITATIONS OF PHARMACOGENOMIC RESEARCH

A study carried out by Gamazon et al. [27] evaluated the high-throughput genotyping technologies for their ability to assay variation in key pharmacogenes. Among the genes covered in this study there were: drug metabolizing enzymes, transporters, receptors and drug targets. The results upon analysis showed that for the 253 pharmacogenes studied, no platform gave over 85% coverage of these genes. Platforms used included data from the Axom Genomic Database, Omni 2.5M and the DMET chip. It also highlighted that for public genotype databases such as The HapMap Project (containing 4 million SNPs); the ability to re-sequence full data has not been assessed. This is relevant for the previously discussed CYP450 genes which reside in highly

homologous regions which can obscure their interrogation by high throughput methods. In addition, the reduced coverage of rarer gene variants can reduce the power for detecting association in pharmacogenetic studies. It was confidently concluded that GWA studies alone are unlikely to completely ascertain a gene`s contributions to drug response. The genes of interest will be indicated with further re sequencing to find additional variants. If the gene of interest is poorly integrated in the gene platform then an association may not even be detected [27].

On the other hand, when taking into account the candidate gene approach, there still may be unknown genes or variants that may also affect the phenotype of interest. This is apparent for many drug responses as known genes do not explain them in their entirety, suggesting that there may be other genes or pathways that are also exerting an influence [26].

Furthermore, it is evident that amongst the individual studies carried out regarding genetic polymorphisms and drug variability there are limitations. Firstly, although the influence of genetics on medication is well established this may not always be applicable, nor will every correlation yield statistically significant results. Yelensky et al. [23] found little evidence for the association of ADRB2 variants and indacterol response for COPD patients. It also brings to light pervious inconsistent findings across studies with the ADRB2 gene and beta-agonist therapy for asthma. The interaction of the ADRB2 with beta agonist drug therapy has long been hypothesized however, despite the measurement of various clinical parameters such as exacerbations and the use of large populations, no such relationship could be justified. This further questions previous statistically underpowered smaller studies which did claim interactions between long acting beta agonists and a worse response post treatment in Arg16 homozygotes (polymorphism of ADRB2) [23].

The variability in drug response cannot always be accredited to genetic variation exclusively. Tao et al reports the differences in the C_{max} of cyclosporine were affected by CYP3A4/5 polymorphisms alongside gender. This multifactorial range of factors influencing medication is also noted by Song et al. [17] with regards to the clearance of cyclosporine in renal transplant patients as the gastrointestinal tract function is impaired immediately after transplantation hence, a higher initial clearance of the drug which stabilizes after. Concurrent drug administration also has an impact, with the coining of the term 'polypharmacy' which is the norm for most of the elderly population who have more than one comorbidity. Corticosteroids inhibit the CYP3A enzyme which increases cyclosporine concentrations [17]. Other factors that affect medication outcomes include a patient's age, body mass index, liver or kidney function, diet, medication compliance and smoking status. In addition, the quality and reproducibility of a clinical trial also needs to be taken into account, as it is inventible that there will be some degree of heterogeneity amongst the participants enrolled. For this reason a larger population size is preferable where possible; Berno et al. [18] gathers its findings about HIV drugs based on a population size of only 20 patients. Tao et al. [16], specifically states that the study was limited due to the numbers of patients representing each genotype especially CYP3A4*18B/*18B as well as a lack of clinical data.

INDIVIDUALIZED MEDICINE AND FUTURE DIRECTIONS

Considering the range of scientific data available regarding pharmacogenomics and drug variability, many envision that an era of routine individualized medicine is nearly a reality. Individualized medication attempts to tailor medication choices to suit the pharmacogenetic makeup of an individual patient, with the aims of increasing drug efficacy and reducing the incidence of adverse events. As established in the previous studies mentioned, genotyping for certain CYP450 enzymes can aid drug choice. This can be relevant in the clinic, e.g. using the work by Chan et al. [13] and Mazzaccara et al. [14] and Arici and Ozhan [15], whom collectively agree that carriers of the CYP2C9*2/*3 variant require lower warfarin doses. An aspect of individualized medication has already been implemented in UK healthcare, as NICE (National Institute for Health and Care Excellence) focusses its recommendations towards patient groups that will benefit from a medication. The first pharmacogenetic test approved by the FDA was the AmpliChip which tests for CYP2D6 and CYP2C19 genotypes based on a whole blood sample. Although it was rejected for assistance in antipsychotic prescribing in the UK due to a lack of clinical evidence, other countries have decided to reimburse it [28].

Another strategy heavily anticipated in mainstream healthcare is the ability to sequence a patient's entire genome. This information can be used to an individual's advantage especially if this is undertaken shortly after birth, as this will be before the onset of any potential disease. The patient then has the options to consider potential treatments, preventative healthcare measures, as well as informing healthcare providers to ensure they get the support that they require. This is of particular desirability in cancer genomics as it allows specific understanding of a patients carcinogenesis therefore, it can inform the best possible treatment option. This measure is further supported by the genomic data available in the public domain such as the 1000 genomes project; the analysis of public gene expression provides an additional affordable avenue compared to traditional drug discovery routes. Routine use of pharmacogenetic testing in practice has been criticized for its long turnaround making it impractical however; for antiplatelet therapy a quick buccal swab test can highlight patients for whom clopidogrel is not suited post percutaneous coronary intervention. Preemptive pharmacogenomic testing involves the inclusion of a range of genes covering "high risk" drugs at a low cost. This information can be made available to doctors and healthcare professionals alike, to help guide prescribing and dispensing decisions for the individual patient. For example, a CYP2D6 genetic test result will affect the prescribing of: tramadol, tricyclic antidepressants and selective serotonin reuptake inhibitors [29].

On the other hand, there are challenges to the widespread use of individualized therapy in everyday practice. Firstly, with

regards to genome sequencing the clinical knowledge required to interpret and use this information is too vast for one clinician to readily apply, with the genomic information not entirely understood for the more complex diseases. There is also the ethical implications with regards to diseases that an individual is found to be susceptible to as a potential diagnosis will affect all aspects of their life, especially with regards to consent to this for children. There is a suspicion that drug companies will possibly play to a 'genetic lottery', where they may focus their resources towards developing drugs for which there is a larger sub patient population that will benefit them financially in return. Surveys in the US have shown that pharmacists and physicians feel inadequately trained in pharmacogenetics, with a lack of guidance on how to implement findings. This is observed as drugs are widely prescribed to patients regardless of their genotype, e.g. Tramadol in CYP2D6 PM's is not likely to have an analgesic effect whereas, it can be toxic in UM's. A clear reason for this is the rising costs and healthcare personal that would be required to drive such a system; with the average genome sequencing costing around $3000 per person being too high for health authorities and insurers. For countries like the UK with a nationally funded healthcare system, this is not yet a viable option to be implemented for routine use. Further afield towards lower income countries; an efficient healthcare infrastructure still remains a great challenge [29].

CONCLUSION

In conclusion, the majority of studies cited in this systematic review have all found a statistically significant effect for the influence of genomics on measured aspects of drug variability. There has been a tremendous amount of work done in deciphering the activity of the CYP450 enzyme superfamily and its perceived effect on drug behaviour; from CYP2C9 and warfarin to CYP3A5 and cyclosporine. The role of ethnicity in determining individual genetic composition is very pronounced with certain variants being more common to a particular group. Zanger et al. [30] orders the genomic markers in order of confirmed effect: CYP2D6>CYP2C19-CYP2A6>CYP2B6>CYP2C9>CYP3A4/5. The studies used span a wide range of geographical locations hence, covering a bountiful range of different alleles responsible for altering drug behaviour. For example Chan et al. [13] focuses on diverse sub groups in the Asian population whereas Scott et al. [21] recruited members from the Jewish population. Looking beyond this, variation in drug transport systems such has the highly anticipated p glycoprotein and even specific markers, e.g. human epidermal growth factor receptor (HER2) all have the potential to influence drug choice. Research has made it clear that when understanding drug behaviour a scientist must look at all the confounding factors. This was highlighted across all the literature as no one drug or disease state is influenced solely by a single genetic moiety, e.g. Mas et al. [19] studies the impact on risperidone dosage from CYP450 enzymes and drug transporters alike. When considering an individual drug other factors alongside genes need to be taken into account such as patient age, health, nutritional status and medication compliance levels [30].

Although the genotyping of common variants is providing ample data to apply in drug studies, the contribution of rare variants should not be overlooked especially with regards to a complex phenotype. The pooling of genetic data across databases and projects provides a direction for this, as is seen with the 1000-genomes project. This opens an avenue towards the concept of individualized medicine, which in a sense is any therapy that is guided by an individual`s genetic makeup. Indeed in order for this to become a reality several challenges will need to be addressed as discussed above cost, pharmacogenomic education alongside legislation addressing legal and ethical implications. However, over the next decade promising advances will be made as already it is possible to simultaneously analyse genomic data for the >300 genes that encode proteins involved throughout the ADME of drugs. Finally, the opportunities that individualized therapy can bring are of particular desirability as fundamentally it will: improve patient care and compliance, reduce the occurrence of ADRs hence hospital bills, improve economic productivity, improve medication safety as well as increasing the knowledgebase of patients and professionals alike [31].

The potential effect of genomics on drug variability is clearer than ever, but the implementation into the forefront of practice is in its infancy; the question now is if whether we have the resources and infrastructure to develop a robust 21st century pharmacogenomic healthcare system.

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