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Considerations for rare variants in drug metabolism genes and the clinical implications

Britt I Drögemöller, Galen EB Wright & Louise Warnich†
†*Stellenbosch University, Genetics, Stellenbosch, South Africa*

Introduction: Large-scale whole genome and exome resequencing studies have revealed that humans have a high level of deleterious rare variation, which has important implications for the design of future pharmacogenetics studies.

Areas covered: Current pharmacogenetic guidelines focus on the implementation of common variation into dosing guidelines. However, it is becoming apparent that rare variation may also play an important role in differential drug response. Current sequencing technologies offer the opportunity to examine rare variation, but there are many challenges associated with such analyses. Nonetheless, if a comprehensive picture of the role that genetic variants play in treatment outcomes is to be obtained, it will be necessary to include the entire spectrum of variation, including rare variants, into pharmacogenetic research.

Expert opinion: In order to implement pharmacogenetics in the clinic, patients should be genotyped for clinically actionable pharmacogenetic variants and patients responding unfavourably to treatment after pharmacogenetics-based dosing should be identified and resequenced to identify additional functionally relevant variants, including rare variants. All derived information should be added to a central database to allow for the updating of existing dosing guidelines. By routinely implementing such strategies, pharmacogenetics-based treatment guidelines will continue to improve.

Keywords: CYP2C9, CYP2C19, CYP2D6, dosing guidelines, DPYD, drug metabolising enzymes, genome sequencing, pharmacogenetics, rare variants, TPMT, UGT1A1, VKORC1

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1. Introduction

Next-generation sequencing (NGS) technologies have enabled the characterisation of human genetic variation on a scale that has not been possible until recently. These NGS-based methodologies for calling genetic variants are flexible with regards to the portions of the genome that can be targeted for genotyping and can vary from whole genome sequencing, to exome sequencing (i.e., the protein coding regions of the genome), to smaller targeted candidate regions of interest. Studies utilising these technologies have not only characterised the vast majority of common single-nucleotide variants (SNVs) [minor allele frequency (MAF) > 5%], but they have also revealed that rare SNVs (MAF < 0.5%) comprise the largest proportion of human genetic variation [1-6]. This configuration of human genetic variation is very much a function of population history and can probably be ascribed to recent accelerated population growth and the subsequent limited time for selection to eliminate deleterious variants, except those with severe damaging effects [3]. Fu and colleagues [6] estimated that > 86% of potentially functional SNVs in protein coding regions are only between 5000 and 10,000 years old and are thus

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Article highlights.

- Whole genome and exome sequencing studies have revealed that humans contain a large amount of rare variation, including rare deleterious variants.
- Current pharmacogenetic-based dosing guidelines focus on the examination of common variation.
- There is a large amount of rare functional variation present in important drug metabolising enzyme genes.
- Rare variation in pharmacogenes can be detected through the use of sequencing technologies; however, there are many challenges associated with these analyses.
- The examination of rare variation can be facilitated by resequencing the relevant pharmacogenes of genotype–phenotype discordant individuals.

This box summarises the key points contained in the article.

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geographically confined. The population-specific nature of the majority of rare variants was also eloquently illustrated by the 1000 Genomes Project, which showed that 17% of low-frequency variants (MAF between 0.5 and 5%) and 53% of rare variants were observed in a single population [1]. Consequently, population-specific rare variant profiles need to be established for different populations from the major global ancestry groups [4], including closely related populations that have been shaped by unique local demographic events [7].

One of the distinctive characteristics of rare variants is their enrichment for deleterious functional effects [1,3,5,8-10]. The 1000 Genomes Project Consortium [1] reported that rare variants comprised the bulk of nonsynonymous (82%) and stop-gain and splice site mutations (> 90%) in highly conserved coding regions, and comprised only 65% of synonymous variants, while Tennessen *et al*. [5] found that rare variants are more likely to be functional compared with variants with a MAF > 0.5% (odds ratio: 4.2, 95% CI: 4.0 – 4.3, $p < 10^{-15}$). Genome-wide sequence data also showed that regulatory regions, such as transcription factor binding motifs, contain a high proportion of rare variation, which is predicted to have a moderate effect on the function of these binding motifs [1]. The abundance of rare variants in modern human populations may thus have a major impact on genetically determined phenotypic variation. This holds important implications for study designs that aim at identifying variants associated with disease and, importantly in the context of this paper, drug response traits [6].

2. Previous approaches to pharmacogenetic studies

Prior to the introduction of NGS and the corresponding ability to sequence entire pharmacogenes (or whole genomes) at affordable prices, past pharmacogenetic studies have been reliant on examining candidate genes [11-14]. The candidate genes were usually selected from genes encoding drug metabolising enzymes (DMEs), transporters and targets [13], and there are numerous examples of the successful utility of this candidate gene approach, with particular reference to candidate genes relating to DMEs. Variants in candidate genes were mostly identified by means of Sanger sequencing, and many studies placed their focus on the identification of common functional variation, for example, the identification of the genetic variants in *CYP2D6* causing the poor metaboliser (PM) phenotype in 5 – 10% of individuals of European descent [15,16]. These variants were occasionally further examined in larger cohorts and other populations using alternate affordable genotyping strategies [17-22]. Subsequent detection of novel alleles often followed the discovery of novel allelic haplotypes for candidate genes or discordant phenotype/genotype profiles [23]. Nonetheless, the majority of past pharmacogenomic research has focused on common variation [24] and rare variants have remained largely neglected.

The validity of examining variation in DME genes to optimise various therapeutic strategies is highlighted by the fact that there are several groups that have provided pharmacogenetic guidelines for these genes, such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) [25] and the Dutch Pharmacogenetics Working Group [26], to name just two examples. For the purpose of this paper, the information provided by the CPIC, which is updated every 2 years [25], has been chosen to serve as an example of such guidelines. These CPIC guidelines are provided for robust drug–gene pairs, some of the most convincing examples of which include the genotyping of: i) *CYP2C19* alleles to optimise clopidogrel treatments [27,28]; ii) *CYP2D6* alleles to optimise codeine treatments [29]; iii) *CYP2D6* and *CYP2C19* alleles to optimise tricyclic antidepressant treatments [30]; and iv) *CYP2C9* and *VKORC1* variants to optimise warfarin treatments [31].

Dosing algorithms for these drug–gene pairs are available, and one of the best examples of these dosing algorithms is the one provided for *CYP2C9* and *VKORC1* variants to optimise warfarin treatments. This algorithm takes other important factors such as age, gender and weight into account, and it has been estimated that the common variants included in this algorithm account for approximately 50% of the variability that is observed with regards to the doses of warfarin that are required [31]. Nonetheless, it has been shown that rare variants in both *CYP2C9* and *VKORC1* influence warfarin response outcomes and the inclusion of these variants in dosing algorithms may improve their performance [31,32]. However, as the CPIC guidelines are designed to guide the interpretation of results, they focus only on clinically actionable variants. In order for the CPIC to consider a variant clinically actionable and therefore of high relevance to pharmacogenetics, a strict protocol is implemented whereby consistent results from well-designed studies are required [33]. Due to the low frequency of rare variants, it will be difficult to

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achieve this level of evidence [31], as cost restrictions may prohibit many studies from examining the entire spectrum of variation in enough samples to detect these rare variants in additional studies for replication purposes. It is for this reason that rare variants are not frequently included in current pharmacogenetic guidelines, even though this variation plays an important role in modern day human populations.

The lack of consistent results obtained for rare variants may in part be attributed to insufficient technological advancements, which in the past have prevented the examination of rare variants. However, with the constantly decreasing costs associated with NGS technologies, as well as improvements in bioinformatic algorithms to asses variant functionality, the information pertaining to the role that rare variants play in pharmacogenomics may increase. NGS technologies may facilitate the ability to obtain a comprehensive picture of the variation contributing to drug response outcomes, which is likely to include rare variants. The need to examine such variants in DME genes is further highlighted by the fact that genes such as *CYP2A6*, *CYP2B6* and *CYP2D6* are included in the 110 genes in the human genome that have unusually high proportions of rare variants [5], and this abundance of rare variants has also been reported for other drug target genes [4]. It should however be noted that some of these genes, particularly *CYP2A6* and *CYP2D6*, may not be ideally suited to NGS technologies due to the sequence complexities associated with these genes and thus variants called in these areas may be false positives [34] (refer to Section 4.2 for more details). Nonetheless, the need to examine rare variants in DMEs remains important and may be of particular relevance to certain populations, such as those originating from Africa [20]. Strategies such as NGS-directed approaches may be especially relevant in these populations due to the frequent occurrence of low-frequency variants [1] and the fact that previous pharmacogenetic and genomic studies have been largely restricted to European and Asian populations [35], potentially limiting the applicability of current pharmacogenetic tests for African individuals. Furthermore, the 'missing heritability' of complex traits such as variable drug response has been ascribed to the fact that the role of rare variants has not been explored widely to date [5]. Thus, future pharmacogenetic research should include the examination of rare variants in many diverse individuals.

3. Rare variants in drug metabolising genes

3.1 Databases and strategies to examine the genetic variation present in DME genes

As highlighted in the section above, DME pharmacogenetic research has predominantly focused on examining the allele frequency differences that are observed with regards to common variants. However, with the more frequent use of sequencing technologies, studies have also begun to shift their focus towards the large-scale resequencing of these genes [36-38]. Research that has utilised resequencing strategies, particularly strategies that employ the resequencing of genotype–phenotype discordant cases [39], has consistently identified novel variants. This is reflected by the large number of alleles that are recorded on the various allele nomenclature websites for DME genes [40-44] (accessed 21 January 2014) and specifically highlighted in the studies by Dai *et al.* [36] and Qian *et al.* [37]. Allele nomenclature sites [43-45] are valuable resources that provide information regarding the alleles that have been identified to date, including the predicted effects of the variants and links to supporting evidence. Thus, these databases provide a resource whereby researchers can record rare variants and their associated effects, which can subsequently be utilised in future research. For *CYP* alleles, a novel allele will only be assigned if a haplotype is identified that contains at least one variant (the allele-defining variant) that has a functional impact on the corresponding gene [45]. Furthermore, there remain a large number of variants that are not yet recorded on the *CYP* allele nomenclature site and not all haplotypes associated with the allele defining variants are documented. It should also be noted that in the case of variants that are recorded as *CYP* alleles, it is not always known what the effect of these alleles on pharmacogenetic traits will be. Therefore, although these databases provide valuable resources for assigning function to DME genes based on the variants detected, including rare variants, the exact effect that these variants have on the function of the protein is in many cases unknown, limiting the clinical utility of these data.

In order to critically examine the variation present in genes coding for DMEs, the seven DME genes that have dosing algorithms available in the CPIC guidelines (*CYP2C9*, *CYP2C19*, *CYP2D6*, *TPMT*, *UGT1A1*, *DPYD* and *VKORC1*) [25] were focused on in this paper. When examining the available allele nomenclature websites for these DME genes, it was found that not all of the genes have allele nomenclature databases. In addition, although there are several alleles recorded on these websites, only a few of these variants are included in the CPIC dosing algorithms. This is either due to the fact that these variants are rare or the effect that the variants exert on the corresponding protein product is not well characterised [31]. **Table 1** provides a summary of the seven DME genes that are present on the CPIC gene–drug pairs website [25] (accessed 21 January 2014), including information regarding the alleles that are included in these dosing guidelines, as well as which of these genes have active allele nomenclature websites and how many alleles are recorded on these websites. As can be seen from this table, not only are there no active nomenclature sites for two of the genes (*DPYD* and *VKORC1*), but only a small percentage of the recorded alleles are currently included in the dosing guidelines. This highlights two key points: i) databases recording the alleles present in these genes need to be regularly updated and maintained; and ii) the effects of these variants need to be determined so that they can be included in dosing algorithms.

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Table 1. The drug metabolising enzyme genes and the alleles included in the CPIC dosing algorithms.

Gene	Allele nomenclature website	Number of alleles recorded (accessed 21 January 2014)	Alleles/variants included in CPIC dosing algorithm (accessed 21 January 2014)	Percentage alleles included in CPIC dosing algorithm
CYP2C9	*CYP2C9* allele nomenclature [40]	58	**1 – 3*	5.17
CYP2C19	*CYP2C19* allele nomenclature [41]	34	**1 – 8*, **17*	26.47
CYP2D6	*CYP2D6* allele nomenclature [42]	105	**1 – 21*, **27*, **29*, **31*, **33*, **35*, **36*, **38 – 42*, **44 – 51*, **53 – 57*, **59*, **62*, **69*, **72*, gene duplications	46.67
TPMT	*TPMT* allele nomenclature [43]	36	**1 – 4*	11.11
UGT1A1	*UGT1A1* and common exons allele nomenclature [44]	113	**1*, **28*	1.77
DPYD	No active site	NA	**1*, **2A*, **13*, rs67376798	NA
VKORC1	No active site	NA	-1639 G>A	NA

CPIC: Clinical Pharmacogenetics Implementation Consortium; NA: Not applicable.

One of the genes for which numerous alleles are included in the CPIC dosing algorithm is *CYP2D6*. There are three likely reasons for this: i) the gene is highly polymorphic with numerous alleles, including gene deletions, duplications and hybrid genes [46,47] (refer to Section 4.2 and Drögemöller *et al*. [34] for potential issues that these sequence complexities may cause with reference to NGS); ii) the gene has been the focus of a large number of studies, with 1536 articles recorded on PubMed with *CYP2D6* in their title [48]; and iii) the gene is small enough (~ 5 kb, including the exons, introns, 5′-upstream and 3′-downstream regions) [49] to allow for complete resequencing (including Sanger sequencing) to detect rare alleles, which further highlights the value of resequencing studies for other DME genes. Due to the large amount of variation that has been detected in this gene [27], individuals are grouped according to the predicted function of the *CYP2D6* alleles present with the use of the activity score system as described by Gaedigk *et al*. [50]. These activity scores can then be used to place individuals into metaboliser classes [PMs, intermediate metabolisers, extensive metabolisers or ultrarapid metabolisers (UMs)], and this information can subsequently be incorporated into dosing algorithms. This system may be a useful template for other DME genes. If the functionality of rare variants can be predicted, the genes can be given an activity score and the prescription recommendations can subsequently be determined based on these scores. A lack of allele nomenclature websites and the inaccurate predictive capabilities of current prediction algorithms will, however, be confounders to this process.

3.2 The patterns of DME gene variation present in the 1000 Genomes Project individuals

To examine the number of low-frequency (MAF between 0.5 and 5%) or rare variants (MAF < 0.5%) in the seven DME genes that were selected for examination in this paper, the 1000 Genomes Project data (accessed 21 January 2014) were utilised. However, due to the fact that it has previously been reported that the use of whole genome sequence data to identify variants in *CYP2D6* may not be reliable [34], *CYP2D6* was excluded from the downstream analyses. To examine the variation in the remaining six genes, the variant call format (vcf) files were obtained from the 1000 Genomes Browser using the default gene region for all 14 populations. The variants present in these files were then separated into: i) those with MAF ≥ 5%; ii) those with MAF between 0.5 and 5%; and iii) those with MAF < 0.5%, employing the Genome Analysis Toolkit's (GATKs) SelectVariants utility [51]. These vcf files were then submitted to SeattleSeqAnnotation137 [52], and the number of functional variants (missense, splice site, stop/start and frameshift variants) occurring in the genes of interest was determined. When the allele frequencies of the variants were examined, it was observed that there were a far greater number of potentially functional variants with MAF < 0.5% compared with the potentially functional variants with MAF ≥ 0.5% (**Figure 1**). Once again, a note of caution should be made here with reference to the fact that these variants have been detected from the NGS short read data generated by the 1000 Genomes Project and confirmatory genotyping has not been performed. Further studies will therefore be required to determine whether these are true variants or sequencing artefacts, as well as to determine the haplotypes associated with these variants. Nonetheless, as the 1000 Genomes Project whole exome sequence data have been shown to exhibit a 90% accuracy compared with *HLA*-typing [53], the presence of rare variants should not be ignored and strategies to account for the potential effects of rare variants on drug metabolism need to be developed. It should, however, be noted that future studies should assess the functionality of the missense variants as < 45% of rare missense variants are expected to be deleterious [10]. These analyses will unfortunately not be trivial due to the scarcity of the variants [54].

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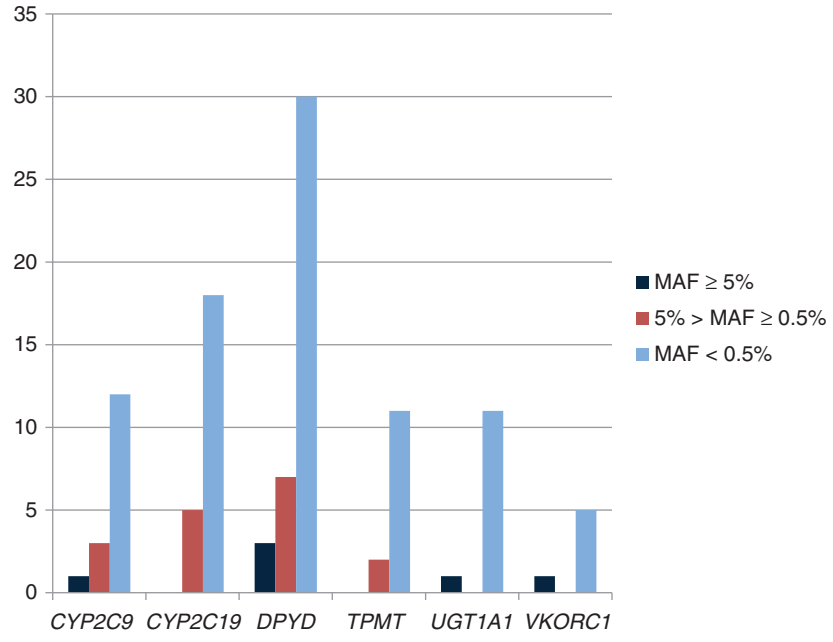

Figure 1. The number of variants predicted to have functional consequences in each of the examined DME genes, stratified according to allele frequencies.
MAF: Minor allele frequency.

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4. Strategies to detect rare variation in drug metabolism genes

4.1 NGS of drug metabolism genes

As mentioned in the Section 1, recent large-scale resequencing studies in human populations have harnessed the power of NGS to determine the spectrum of rare variation in genomes more accurately than previously possible [1] and these technologies may be useful for pharmacogenetic research. However, compared with precision medicine research in cancer, where studies examine somatic mutations [55], pharmacogenomic research of germline variation has been slower to adopt NGS technologies. Nonetheless, as a proof of principle, Ashley *et al.* [56] performed a clinical assessment of a patient's genome and generated a pharmacogenomic profile from the genome sequence. These analyses showed that the patient had DME genetic mutations contributing towards impaired warfarin metabolism (i.e., *VKORC1* and *CYP4F2*) and clopidogrel (i.e., *CYP2C19*), therefore highlighting the power of these NGS-based analyses for downstream pharmacogenomic applications. Furthermore, NGS has also proven to outperform traditional genotyping methods in certain pharmacogenomic studies [57], and it has been suggested that targeted resequencing strategies will improve pharmacogenomic research [58]. A development that offers the potential to accelerate the use of NGS-based technologies in pharmacogenomics is the PGRNSeq Test that the National Institutes of Health Pharmacogenomics Research Network is developing. This genotyping assay utilises a targeted pharmacogenomic resequencing strategy, which includes the capture of 84 important pharmacogenes [59].

4.2 Considerations for the NGS of drug metabolism genes

Although NGS technologies are likely to improve the predictive capabilities of pharmacogenomic tests, an additional consideration relates to the fact that the sheer volume of data that are generated with these technologies increases the risk of incidental findings (i.e., those unrelated to the original research hypothesis) [60]. The role that incidental findings play in pharmacogenomics has been reviewed in Brothers *et al.* [61], and researchers and clinicians should have contingency plans for dealing with such results when performing NGS studies focusing on rare variants. Additionally, pharmacogenes can display pleiotropy, which further complicates the interpretation of the results of a pharmacogenomic test [61]. Deciding which results to return to research participants or patients is context dependent, but incidental findings that are believed to have clinical relevance and are medically actionable are the most important to consider in this regard [62].

Furthermore, despite the numerous advantages of NGS methodologies, they remain imperfect technologies and certain regions of the genome remain refractory to this kind of sequence analysis. The 1000 Genomes Project estimated that 94% of the genome was accessible for their analyses and released the coordinates of these regions in

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the form of mask files [1]. It is essential to keep such issues in mind as, even though rare variants are four times more likely to confer a functional consequence on the resulting protein product than common variants [5], rare and deleterious variants are also more prone to be false positives [63] and the reliable detection of these variants also depends on the sequence coverage and technology that is used [1]. These considerations are highly relevant to pharmacogenomic analyses (including those involving studies of DMEs) as accurate genotyping of many pharmacogenes, such as those belonging to the *CYP* and *HLA* families, is hampered by high sequence similarity to pseudogenes as well as other isoforms [34]. For example, of the DMEs discussed in previous sections, < 70% of the genomic regions of *CYP2C9*, *CYP2C19*, *CYP2D6*, *TPMT* and *VKCOR1* are accessible to current sequencing technologies, according to the 1000 Genomes Project mask files. Furthermore, with regards to the complexity of the variation present in genes such as *CYP2D6*, it seems likely that variants such as gene deletions, duplications and hybrid genes may not be reliably detected with the use of whole genome/exome sequencing (WGES) [34]. Fortunately, some of these issues are likely to improve as sequencing technologies become more accurate and read lengths become longer.

In order to achieve robust results with regards to the variants examined in pharmacogenes, stringent quality control needs to be performed, especially when working with NGS data. The current gold standard with regards to NGS analysis is the GATK's 'Best Practices For Calling Variants', which includes numerous steps to ensure high-quality genotypes are produced [64]. Nonetheless, there are certain complications that need to be considered in terms of the detection of SNVs and these issues are highlighted when examining the variation present in the seven DME genes that were focused on in this paper (**Figure 1**). The first issue relates to the presence of regulatory variants, such as *CYP2C19*17* and *VKORC1* -1639 G>A. Although both of these variants are included in the CPIC dosing guidelines, even with the vast amount of regulatory information that has been provided by the Encyclopedia of DNA Elements project [65] and corresponding prediction programs [66], the significance of noncoding variants is difficult to interpret. Thus, without functional evidence it is currently not feasible to include rare regulatory variants in dosing algorithms. The second issue relates to the fact that although *CYP2C19*2* is known to result in a nonfunctional protein [67], the annotation program predicted that this variant would result in a synonymous amino acid change with no functional consequences. This incorrect assignment of function is related to the fact that this variant does not occur within a known splice site, but instead creates a new splice site, the effects of which are more complicated to predict. With the large amounts of data that are generated by WGES studies, it is difficult to extensively assess the effects of individual variants.

4.3 Alternative strategies to detect rare variation in drug metabolism genes

In addition to NGS, there are alternative approaches to detect rare variation in DMEs. Recently introduced exome chips form an intermediate between WGES approaches and genotyping arrays employed in genome-wide association studies as rare variants that have been identified through large-scale resequencing studies are included. However, these arrays remain unable to detect novel SNVs [68,69]. Genotyping arrays that have been designed for pharmacogenomic applications can genotype multiple alleles, but do not cover rare variants comprehensively [70]. Additionally, such arrays may not be representative of understudied and genetically diverse populations, such as those found in Africa, and should therefore be implemented with caution when analysing cohorts including these individuals. Finally, traditional Sanger-based sequencing, although low throughput, may be more accurate for certain genes encoding DMEs, such as *CYP2D6* [17,23,35,37,71-74], until NGS technologies mature sufficiently to allow for accurate genotyping of these challenging regions.

5. Computational and statistical considerations for the analysis of rare variation in drug metabolism genes

Once rare variants in genes encoding DMEs have been identified following quality control, the functionality of these variants needs to be evaluated if no prior information regarding impact on the drug metabolism phenotype is available. Currently, *in silico* approaches provide an efficient manner to assess the deleteriousness of variants, especially because the experimental validation of rare variants is not always feasible. Numerous prediction algorithms are available for this task, but a comprehensive analysis of computational methods to predict amino acid function is beyond the scope of this review as more comprehensive descriptions are available elsewhere [75-77].

The majority of current computational approaches to assess the impact of genetic variants rely on evolutionary information to facilitate prediction. Additionally, algorithms designed to assess the impact of missense variants can also make use of the biochemical (i.e., characteristics of the amino acid that is changed) and structural properties of the position of these variants [76]. Examples of popular protein-based predictors are the 'Sorting Intolerant From Tolerant' [78] and PolyPhen [79] algorithms, whereas an example of a nucleotide-based predictor is the 'Genomic Evolutionary Rate Profiling' (GERP) algorithm [80]. A previous study showed that the correct prediction of damaging effect for studies of polymorphisms in genes encoding DMEs is around 68% [81]. Various genes encoding for DMEs, such as the *CYP* genes, have many paralogues that may affect the predictive power of certain algorithms. Thus, nucleotide constraint score-based approaches, such as GERP, may be more appropriate for these genes in certain situations [76].

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In addition to the difficulties associated with the detection of rare variation and the assignment of function to these variants, statistical methods to determine whether these variants are associated with treatment response outcomes are hampered by a lack of statistical power [82]. Thus, in order to detect associations with rare variants, large cohorts of patients are required. However, obtaining large cohorts of patients that are treated with the same medication and/or have comparable phenotypes for pharmacogenetic studies can be challenging. In addition, specific adverse drug reactions (ADRs) and treatment response outcomes are often rare and thus it is difficult to collect a large number of individuals displaying the trait of interest for association analyses [54,83]. Therefore, to improve the power of statistical analyses, instead of considering rare variants as separate entities, deleterious variants occurring in the same genes may be examined in combination with one another using statistical strategies such as those described by Panoutsopoulou *et al.* [82]. Novel bioinformatics tools are also available to combine rare variants for further analysis, based on various levels of biological knowledge obtained from public databases [84].

Although there are many challenges related to the examination of rare variation, which include the difficulty in detecting, recording and accurately assigning function to these variants, the development of cheaper and more accurate NGS data may aid in overcoming these obstacles. Together with these data, it may be necessary to collect large cohorts of samples to increase the likelihood of detecting rare variants for discovery and replication purposes, as well as to improve the power of such studies [85]. Furthermore, due to the infancy of strategies that examine rare variants, it may be necessary to initially focus only on variants that abolish the function of the corresponding protein, the effect of which can be determined in a more reliable and accurate manner [85]. If these variants can be examined as a proof of concept, statistical methods and prediction algorithms can be improved so that rare variants with less clear effects may also be included in the future. As strategies that focus on rare variants are improved, the understanding of the role that these variants play in pharmacogenomics can be improved.

This will allow for the ability to include rare variants in dosing algorithms, the importance of which is highlighted by the fact that previous research has demonstrated the predictive utility of including rare variants in dosing guidelines. For example, Sagrieya *et al.* [32] demonstrated that including pooled rare *CYP2C9* variants of known functional effect on a warfarin dosing algorithm increased the capacity to estimate drug dose. Furthermore, Ramirez *et al.* [86] highlighted the increased frequency of rare deleterious variants in patients experiencing QT interval and ventricular tachycardia ADRs compared with controls. Thus, ideally, the effects of both rare and common variation in DME genes should be examined simultaneously to improve prediction of the drug metabolism phenotype.

6. Conclusion

NGS technologies have played a large role in characterising human variation and have highlighted the abundance of rare variants, particularly rare deleterious variation, found in human populations and the role that this class of variant may play in human disease and treatment response outcomes. Although the pharmacogenetic role of DMEs in treatment outcomes has been established and guidelines for the inclusion of this genetic information into dosing algorithms are available [25], rare variation has largely been neglected to date. Despite the fact that the inclusion of these types of variation into pharmacogenetics research offers many challenges, this article has demonstrated the prevalence of this variation in DME genes and has also highlighted that inclusion of these variants into dosing guidelines may improve therapeutic outcomes in the future.

7. Expert opinion

Previous research has shown that DMEs play a large role in drug response outcomes [87], which has led the CPIC and other groups to develop guidelines that facilitate the utilisation of this pharmacogenetic information to optimise treatment regimes [25]. These guidelines are user-friendly and offer the potential to act as a predictive tool to optimise treatment prior to dosing and reduce the socioeconomic burdens associated with nonoptimal treatments. However, unfortunately, the variants included in these guidelines are predominantly from the common frequency spectrum [25]. Therefore, variants that: i) have not been well characterised; ii) are rare; and iii) occur in understudied populations are usually not accounted for. These shortcomings need to be addressed due to the role of rare variants in human populations, which has recently been described, and more specifically, this paper has highlighted the large number of rare functional variants that are present in DMEs (**Figure 1**).

It is therefore important that pharmacogenomic strategies include as many functionally relevant variants in dosing guidelines as possible, including those from the rare frequency spectrum. In order to detect these variants, sequencing strategies and analyses pipelines will be required. Due to the fact that the CPIC have reported that none of the seven DME genes examined in this paper have consistently been linked to any diseases to date [27,29-31,88], incidental findings in these genes should not be of ethical concern. It is, however, important to note that there are several challenges that need to be considered with regards to the implementation of sequencing-based pharmacogenetic strategies. These include the fact that: i) not all of the genes have well-currated allele nomenclature websites that can guide decision-making processes; ii) it is not always trivial to predict the functional consequences of novel, uncharacterised or regulatory variants; iii) NGS technologies remain imperfect and thus variants may

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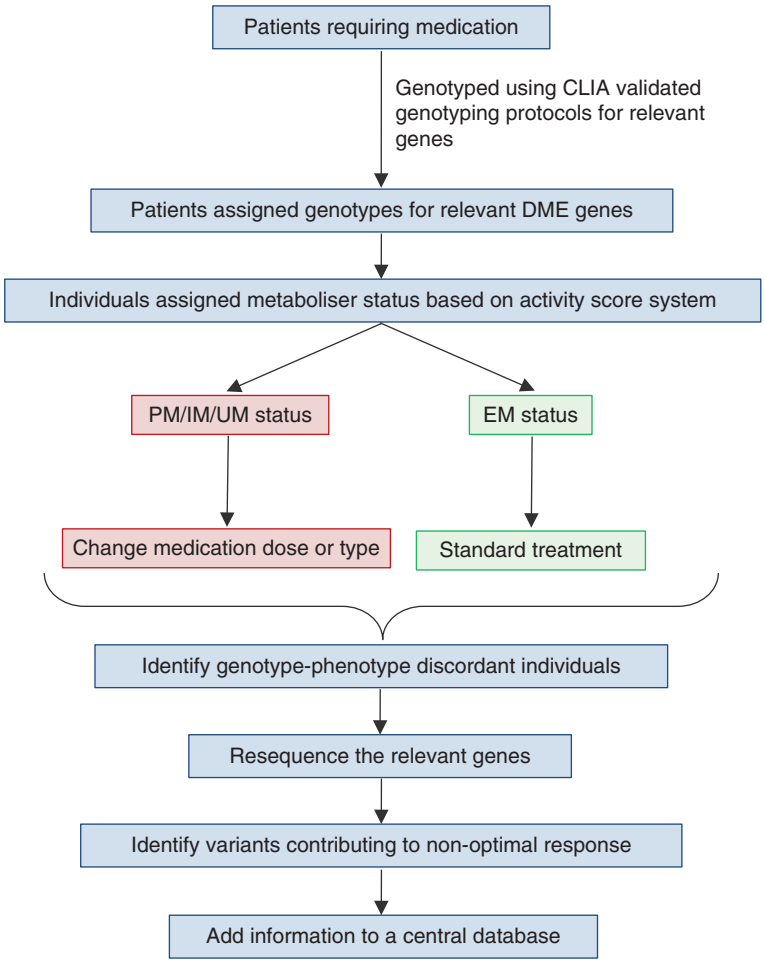

Figure 2. Dosing guidelines for implementation of pharmacogenetic information, including rare variants into the clinic.
CLIA: Clinical Laboratory Improvement Amendments; DME: Drug metabolising enzyme; EM: Extensive metaboliser; IM: Intermediate metaboliser; PM: Poor metaboliser; UM: Ultrarapid metaboliser.

be called as a result of sequencing artefacts; and iv) the cost-effectiveness of these pharmacogenetic tests needs to be assessed [89]. Therefore, future research should focus on: i) examining the cost-effectiveness of sequencing-based pharmacogenomic tests; ii) creating well-currated and regularly updated databases for pharmacogenes; iii) improving the capabilities of prediction algorithms for novel and regulatory variants; iv) enhancing the reliability of sequencing technologies by, for example, increasing the sequence read lengths; and v) procuring the required funding and resources for the development of databases, prediction algorithms and sequencing technologies.

Furthermore, in addition to the information pertaining to genetic variants, it will be important to include data relating to gene–gene interactions, drug–drug interactions and other nongenetic factors in future research [90], as well as considering information obtained from other 'omics' disciplines (e.g., transcriptomics, epigenomics, proteomics and metabolomics) [91]. A review of these aspects of pharmacogenomic research is beyond the scope of this article; however, other more extensive reviews on these topics are available [92-96]. The data obtained from these studies should be utilised in combination with genetic data to better predict treatment outcomes in the future.

In terms of implementing pharmacogenetic data in current dosing algorithms, the following hypothetical standardised protocol (**Figure 2**) has been recommended. If an individual undergoes Clinical Laboratory Improvement Amendments (or equivalent) validated WGES or alternate genotyping, the variation detected should be recorded in a central storage database to allow for access to these data in the future. However, due to the immense amount of data generated by WGES and other genotyping strategies, a prioritisation strategy should be implemented. The strategy could utilise the pipeline described by St. Jude's PG4KDS, which is a study that focuses on the clinical implementation of pharmacogenomics (more details are given in Hicks *et al.* [97]), as a guideline. Genes that are considered robust in terms of pharmacogenetic

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applications can be prioritised for integration into patients' electronic medical records (EMRs), if such records are available. The variation detected in these genes can be further prioritised by only including variants that have been deemed clinically actionable. WGES may be of particular value to this system due to the fact that as more information becomes available regarding other variants that are considered clinically actionable, as well as additional genes that are found to be involved in treatment response outcomes, the relevant data will already be available for inclusion in the EMRs. To simplify the EMR updating system, it may be linked to the CPIC guideline updates. Using the information recorded in the EMR, the individual can be assigned an activity score based on the alleles present in the gene(s) of interest, which will be used to determine the metaboliser status of the individual. This information should be linked to an easily interpretable program, which will guide the dosing of the patient (e.g., a change in dose or medication type for PM and UM individuals). Thereafter, the patient should be monitored and all individuals showing an unfavourable response to the medication should be identified. If no explanation can be found for this unfavourable response, the genes relevant to the drug of interest should be resequenced (or if WGES data are available, the relevant genes should be examined) to identify novel or rare variants that may contribute to the nonoptimal treatment response that was observed [71,98]. All variants identified from these resequencing studies should be added to a central database (e.g., the *CYP* allele, CPIC dosing guideline and NCBI's ClinVar websites [25,45,99]) to facilitate the dosing of future patients.

If such strategies can be routinely implemented, more information can be incorporated into dosing algorithms. In so doing, our knowledge regarding the effects of variants will be improved and the implementation of pharmacogenetic data, including information pertaining to rare variants, can be streamlined. Utilisation of this strategy should provide comprehensive personalised pharmacogenetic data on an individualised level across populations [100], and this information should be more effective in predicting the treatment outcomes of patients.

Declaration of interest

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. The 1000 Genomes Project Consortium. An integrated map of genetic variation from 1,092 human genomes. Nature 2012;491(7422):56-65

•• **Important paper describing the prevalence of rare variants.**

2. Abecasis GR, Altshuler D, Auton A, et al. A map of human genome variation from population-scale sequencing. Nature 2010;467(7319):1061-73

3. Coventry A, Bull-Otterson LM, Liu X, et al. Deep resequencing reveals excess rare recent variants consistent with explosive population growth. Nat Commun 2010;1:131

4. Nelson MR, Wegmann D, Ehm MG, et al. An abundance of rare functional variants in 202 drug target genes sequenced in 14,002 people. Science 2012;337(6090):100-4

•• **Important paper describing the prevalence of rare variants particularly with regards to drug target genes.**

5. Tennessen JA, Bigham AW, O'Connor TD, et al. Evolution and functional impact of rare coding variation from deep sequencing of human exomes. Science 2012;337(6090):64-9

•• **Important paper describing the prevalence of rare variants.**

6. Fu W, O'Connor TD, Jun G, et al. Analysis of 6,515 exomes reveals the recent origin of most human protein-coding variants. Nature 2013;493(7431):216-20

7. Casals F, Hodgkinson A, Hussin J, et al. Whole-exome sequencing reveals a rapid change in the frequency of rare functional variants in a founding population of humans. PLoS Genet 2013;9(9):e1003815

8. Li Y, Vinckenbosch N, Tian G, et al. Resequencing of 200 human exomes identifies an excess of low-frequency non-synonymous coding variants. Nat Genet 2010;42(11):969-72

9. Marth GT, Yu F, Indap AR, et al. The functional spectrum of low-frequency coding variation. Genome Biol 2011;12(9):R84

10. Agarwala V, Flannick J, Sunyaev S, GoT2D Consortium, Altshuler D. Evaluating empirical bounds on complex disease genetic architecture. Nat Genet 2013;45(12):1418-27

11. Madian AG, Wheeler HE, Jones RB, Dolan ME. Relating human genetic variation to variation in drug responses. Trends Genet 2012;28(10):487-95

12. Evrard A, Mbatchi L. Genetic polymorphisms of drug metabolizing enzymes and transporters: the long way from bench to bedside. Curr Top Med Chem 2012;12(15):1720-9

13. Wei C-Y, Lee M-TM, Chen Y-T. Pharmacogenomics of adverse drug reactions: implementing personalized medicine. Hum Mol Genet 2012;21(R1):R58-65

14. Ni X, Zhang W, Huang RS. Pharmacogenomics discovery and implementation in genome-wide association studies era. Wiley Interdiscip Rev Syst Biol Med 2013;5(1):1-9

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15. Gough AC, Miles JS, Spurr NK, et al. Identification of the primary gene defect at the cytochrome P450 CYP2D locus. Nature 1990;347(6295):773-6

16. Kagimoto M, Heim M, Kagimoto K, et al. Multiple mutations of the human cytochrome P450IID6 gene (CYP2D6) in poor metabolizers of debrisoquine. Study of the functional significance of individual mutations by expression of chimeric genes. J Biol Chem 1990;265(28):17209-14

17. Wright GEB, Niehaus DJH, Drögemöller BI, et al. Elucidation of CYP2D6 genetic diversity in a unique African population: implications for the future application of pharmacogenetics in the Xhosa population. Ann Hum Genet 2010;74(4):340-50

18. Drögemöller BI, Wright GEB, Niehaus DJH, et al. Characterization of the genetic profile of CYP2C19 in two South African populations. Pharmacogenomics 2010;11(8):1095-103

19. Drögemöller B, Plummer M, Korkie L, et al. Characterization of the genetic variation present in CYP3A4 in three South African populations. Front Genet 2013;4:17

20. Matimba A, Del-Favero J, Van Broeckhoven C, Masimirembwa C. Novel variants of major drug-metabolising enzyme genes in diverse African populations and their predicted functional effects. Hum Genomics 2009;3(2):169-90

21. Gaedigk A, Gotschall RR, Forbes NS, et al. Optimization of cytochrome P4502D6 (CYP2D6) phenotype assignment using a genotyping algorithm based on allele frequency data. Pharmacogenetics 1999;9(6):669-82

22. De Leon J, Susce MT, Murray-Carmichael E. The AmpliChip CYP450 genotyping test: integrating a new clinical tool. Mol Diagn Ther 2006;10(3):135-51

23. Gaedigk A, Bhathena A, Ndjountché L, et al. Identification and characterization of novel sequence variations in the cytochrome P4502D6 (CYP2D6) gene in African Americans. Pharmacogenomics J 2005;5(3):173-82

24. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138(1):103-41

25. CPIC Gene-Drug Pairs [PharmGKB]. Available from: https://www.pharmgkb.org/page/cpicGeneDrugPairs [Last accessed 21 January 2014]

•• **A valuable site that guides the implementation of pharmacogenetic tests.**

26. DPWG: Dutch Pharmacogenetics Working Group [PharmGKB]. Available from: https://www.pharmgkb.org/page/dpwg [Last accessed 21 January 2014]

27. Scott SA, Sangkuhl K, Stein CM, et al. Clinical pharmacogenetics implementation consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. Clin Pharmacol Ther 2013;94(3):317-23

28. Scott SA, Sangkuhl K, Gardner EE, et al. Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 2011;90(2):328-32

29. Crews KR, Gaedigk A, Dunnenberger HM, et al. Clinical pharmacogenetics implementation consortium guidelines for cytochrome P450 2D6 genotype and codeine therapy: 2014 update. Clin Pharmacol Ther 2014. [Epub ahead of print]

30. Hicks JK, Swen JJ, Thorn CF, et al. Clinical pharmacogenetics implementation consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. Clin Pharmacol Ther 2013;93(5):402-8

31. Johnson JA, Gong L, Whirl-Carrillo M, et al. Clinical pharmacogenetics implementation consortium guidelines for CYP2C9 and VKORC1 genotypes and warfarin dosing. Clin Pharmacol Ther 2011;90(4):625-9

32. Sagreiya H, Sagrieya H, Berube C, et al. Extending and evaluating a warfarin dosing algorithm that includes CYP4F2 and pooled rare variants of CYP2C9. Pharmacogenet Genomics 2010;20(7):407-13

33. Relling MV, Klein TE. CPIC: clinical pharmacogenetics implementation consortium of the pharmacogenomics research network. Clin Pharmacol Ther 2011;89(3):464-7

34. Drögemöller BI, Wright GEB, Niehaus DJH, et al. Next-generation sequencing of pharmacogenes: a critical analysis focusing on schizophrenia treatment. Pharmacogenet Genomics 2013;23(12):666-74

35. Drögemöller BI, Wright GEB, Niehaus DJH, et al. Whole-genome resequencing in pharmacogenomics: moving away from past disparities to globally representative applications. Pharmacogenomics 2011;12(12):1717-28

36. Dai D-P, Xu R-A, Hu L-M, et al. CYP2C9 polymorphism analysis in Han Chinese populations: building the largest allele frequency database. Pharmacogenomics J 2014;14(1):85-92

37. Qian J-C, Xu X-M, Hu G-X, et al. Genetic variations of human CYP2D6 in the Chinese Han population. Pharmacogenomics 2013;14(14):1731-43

38. Jin T-B, Ma L-F, Zhang J-Y, et al. Polymorphisms and phenotypic analysis of cytochrome P450 2D6 in the Tibetan population. Gene 2013;527(1):360-5

39. Gaedigk A, Bradford LD, Marcucci KA, Leeder JS. Unique CYP2D6 activity distribution and genotype-phenotype discordance in black Americans. Clin Pharmacol Ther 2002;72(1):76-89

40. CYP2C9. Available from: http://www.cypalleles.ki.se/cyp2c9.htm [Last accessed 21 January 2014]

41. CYP2C19. Available from: http://www.cypalleles.ki.se/cyp2c19.htm [Last accessed 21 January 2014]

42. CYP2D6. Available from: http://www.cypalleles.ki.se/cyp2d6.htm [Last accessed 21 January 2014]

43. Table of TPMT alleles. Available from: http://www.imh.liu.se/tpmtalleles/tabell-over-tpmt-alleler2?l=en [Last accessed 21 January 2014]

44. UGT1A1 allele nomenclature. Available from: http://www.pharmacogenomics.pha.ulaval.ca/files/content/sites/pharmacogenomics/files/Nomenclature/UGT1A/UGT1A1.htm [Last accessed 21 January 2014]

45. Human Cytochrome P450 (CYP) Allele Nomenclature Committee. Available from: http://www.cypalleles.ki.se/ [Last accessed 21 January 2014]

46. Gaedigk A. Complexities of CYP2D6 gene analysis and interpretation. Int Rev Psychiatry 2013;25(5):534-53

47. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: overview and update on pharmacology,

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Expert Opin. Drug Metab. Toxicol. Downloaded from informahealthcare.com by University of Stellenbosch on 03/28/14
For personal use only.

genetics, biochemistry. Naunyn Schmiedebergs Arch Pharmacol 2004;369(1):23-37

48. CYP2D6[Title] - PubMed - NCBI. Available from: http://www.ncbi.nlm.nih.gov/pubmed?term=CYP2D6%5BTitle%5D [Last accessed 21 January 2014]
49. Ensembl genome browser 72: homo sapiens - Gene summary - Gene: CYP2D6 (ENSG00000100197). Available from: http://www.ensembl.org/Homo_sapiens/Gene/Summary?g=ENSG00000100197;r=22:42522501-42526908 [Last accessed 21 January 2014]
50. Gaedigk A, Simon SD, Pearce RE, et al. The CYP2D6 activity score: translating genotype information into a qualitative measure of phenotype. Clin Pharmacol Ther 2008;83(2):234-42
51. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2001;43(5):491-8
52. SeattleSeq Variation Annotation [Internet]. Available from: http://snp.gs.washington.edu/SeattleSeqAnnotation137/ [Last accessed 21 January 2014]
53. Major E, Rigó K, Hague T, et al. HLA typing from 1000 genomes whole genome and whole exome illumina data. PloS One 2013;8(11):e78410
54. Yeo KR, Kenny JR, Rostami-Hodjegan A. Application of in vitro-in vivo extrapolation (IVIVE) and physiologically based pharmacokinetic (PBPK) modelling to investigate the impact of the CYP2C8 polymorphism on rosiglitazone exposure. Eur J Clin Pharmacol 2013;69(6):1311-20
55. Watson IR, Takahashi K, Futreal PA, Chin L. Emerging patterns of somatic mutations in cancer. Nat Rev Genet 2013;14(10):703-18
56. Ashley EA, Butte AJ, Wheeler MT, et al. Clinical assessment incorporating a personal genome. Lancet 2010;375(9725):1525-35
57. Altimari A, de Biase D, De Maglio G, et al. 454 next generation-sequencing outperforms allele-specific PCR, Sanger sequencing, and pyrosequencing for routine KRAS mutation analysis of formalin-fixed, paraffin-embedded samples. OncoTargets Ther 2013;6:1057-64
58. Oetjens MT, Denny JC, Ritchie MD, et al. Assessment of a pharmacogenomic marker panel in a polypharmacy population identified from electronic medical records. Pharmacogenomics 2013;14(7):735-44
59. PGRN: Network-wide Projects. Available from: http://pgrn.org/display/pgrnwebsite/Network-wide+Projects [Last accessed 21 January 2014]
60. Tabor HK, Berkman BE, Hull SC, Bamshad MJ. Genomics really gets personal: how exome and whole genome sequencing challenge the ethical framework of human genetics research. Am J Med Genet A 2011;55A(12):2916-24
61. Brothers KB, Langanke M, Erdmann P. Implications of the incidentalome for clinical pharmacogenomics. Pharmacogenomics 2013;14(11):1353-62
62. Wright GE, Koornhof PG, Adeyemo AA, Tiffin N. Ethical and legal implications of whole genome and whole exome sequencing in African populations. BMC Med Ethics 2013;14(1):21
63. Pelleymounter LL, Moon I, Johnson JA, et al. A novel application of pattern recognition for accurate SNP and indel discovery from high-throughput data: targeted resequencing of the glucocorticoid receptor co-chaperone FKBP5 in a Caucasian population. Mol Genet Metab 2011;104(4):457-69
64. GATK | Best Practices. Available from: http://www.broadinstitute.org/gatk/guide/topic?name=best-practices [Last accessed 21 January 2014]
65. ENCODE Project Consortium. A user's guide to the encyclopedia of DNA elements (ENCODE). PLoS Biol 2011;9(4):e1001046
66. Boyle AP, Hong EL, Hariharan M, et al. Annotation of functional variation in personal genomes using RegulomeDB. Genome Res 2012;22(9):1790-7
67. De Morais SM, Wilkinson GR, Blaisdell J, et al. The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. J Biol Chem 1994;269(22):15419-22
68. Axiom® Exome Genotyping Arrays | Affymetrix. Available from: http://www.affymetrix.com/estore/browse/products.jsp?productId=prod570018#1_1 [Last accessed 21 January 2014]
69. Infinium HumanExome BeadChip Kit. Available from: http://www.illumina.com/products/infinium_humanexome_beadchip_kit.ilmn?scid=2011017PR2 [Last accessed 21 January 2014]
70. Warnich L, Drögemöller BI, Pepper MS, et al. Pharmacogenomic Research in South Africa: Lessons Learned and Future Opportunities in the Rainbow Nation. Curr Pharmacogenomics Pers Med 2011;9(3):191-207
71. Montané Jaime LK, Lalla A, Steimer W, Gaedigk A. Characterization of the CYP2D6 gene locus and metabolic activity in Indo- and Afro-Trinidadians: discovery of novel allelic variants. Pharmacogenomics 2013;14(3):261-76
72. Fohner A, Muzquiz LI, Austin MA, et al. Pharmacogenetics in American Indian populations: analysis of CYP2D6, CYP3A4, CYP3A5, and CYP2C9 in the Confederated Salish and Kootenai Tribes. Pharmacogenet Genomics 2013;23(8):403-14
73. Raimundo S, Toscano C, Klein K, et al. A novel intronic mutation, 2988G>A, with high predictivity for impaired function of cytochrome P450 2D6 in white subjects. Clin Pharmacol Ther 2004;76(2):128-38
74. Contreras AV, Monge-Cazares T, Alfaro-Ruiz L, et al. Resequencing, haplotype construction and identification of novel variants of CYP2D6 in Mexican Mestizos. Pharmacogenomics 2011;12(5):745-56
75. Fernald GH, Capriotti E, Daneshjou R, et al. Bioinformatics challenges for personalized medicine. Bioinformatics 2011;27(13):1741-8
76. Cooper GM, Shendure J. Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. Nat Rev Genet 2011;12(9):628-40
77. Patnala R, Clements J, Batra J. Candidate gene association studies: a comprehensive guide to useful in silico tools. BMC Genet 2013;14(1):39
78. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc 2009;4(7):1073

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79. Adzhubei IA, Schmidt S, Peshkin L, et al. A method and server for predicting damaging missense mutations. Nat Methods 2010;7(4):248-9

80. Cooper GM, Stone EA, Asimenos G, et al. Distribution and intensity of constraint in mammalian genomic sequence. Genome Res 2005;15(7):901-13

81. Hao D, Xiao P, Chen S. Phenotype prediction of nonsynonymous single nucleotide polymorphisms in human phase II drug/xenobiotic metabolizing enzymes: perspectives on molecular evolution. Sci China Life Sci 2010;53(10):1252-62

82. Panoutsopoulou K, Tachmazidou I, Zeggini E. In search of low-frequency and rare variants affecting complex traits. Hum Mol Genet 2013;22(R1):R16-21

•• **An important article describing analyses utilising rare variants.**

83. Daly AK. Genome-wide association studies in pharmacogenomics. Nat Rev Genet 2010;11(4):241-6

84. Moore CB, Wallace JR, Frase AT, et al. BioBin: a bioinformatics tool for automating the binning of rare variants using publicly available biological knowledge. BMC Med Genomics 2013;6(Suppl 2):S6

85. Zuk O, Schaffner SF, Samocha K, et al. Searching for missing heritability: designing rare variant association studies. Proc Natl Acad Sci USA 2014;111(4):E455-64

86. Ramirez AH, Shaffer CM, Delaney JT, et al. Novel rare variants in congenital cardiac arrhythmia genes are frequent in drug-induced torsades de pointes. Pharmacogenomics J 2013;13(4):325-9

87. Zhou S-F, Di YM, Chan E, et al. Clinical pharmacogenetics and potential application in personalized medicine. Curr Drug Metab 2008;9(8):738-84

88. Relling MV, Gardner EE, Sandborn WJ, et al. Clinical pharmacogenetics implementation consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther 2013;93(4):324-5

89. Korf BR, Rehm HL. New approaches to molecular diagnosis. JAMA 2013;309(14):1511-21

90. Altman RB, Whirl-Carrillo M, Klein TE. Challenges in the pharmacogenomic annotation of whole genomes. Clin Pharmacol Ther 2013;94(2):211-13

91. Zhou K, Pearson ER. Insights from genome-wide association studies of drug response. Annu Rev Pharmacol Toxicol 2013;53:299-310

92. Shah RR, Shah DR. Personalized medicine: is it a pharmacogenetic mirage? Br J Clin Pharmacol 2012;74(4):698-721

93. Howland RH. Future prospects for pharmacogenetics in the quest for personalized medicine. J Psychosoc Nurs Ment Health Serv 2012;50(12):13-16

94. Ivanov M, Kacevska M, Ingelman-Sundberg M. Epigenomics and interindividual differences in drug response. Clin Pharmacol Ther 2012;92(6):727-36

95. Russell C, Rahman A, Mohammed AR. Application of genomics, proteomics and metabolomics in drug discovery, development and clinic. Ther Deliv 2013;4(3):395-413

96. Wang L. Pharmacogenomics: a systems approach. Wiley Interdiscip Rev Syst Biol Med 2010;2(1):3-22

97. Hicks JK, Crews KR, Hoffman JM, et al. A clinician-driven automated system for integration of pharmacogenetic interpretations into an electronic medical record. Clin Pharmacol Ther 2012;92(5):563-6

98. Rotger M, Tegude H, Colombo S, et al. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. Clin Pharmacol Ther 2007;81(4):557-66

99. Introduction - ClinVar - NCBI. Available from: http://www.ncbi.nlm.nih.gov/clinvar/intro/ [Last accessed 21 January 2014]

100. Urban TJ. Race, ethnicity, ancestry, and pharmacogenetics. Mt Sinai J Med 2010;77(2):133-9

Affiliation

Britt I Drögemöller[1], Galen EB Wright[2] & Louise Warnich[†1]
[†]Author for correspondence
[1]Stellenbosch University, Genetics, Stellenbosch, South Africa
E-mail: lw@sun.ac.za
[2]University of the Western Cape, South African National Bioinformatics Institute/Medical Research Council of South Africa Bioinformatics Unit, Bellville, South Africa

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