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The Application of Next-generation Sequencing in Pharmacogenomics Research

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Abstract

Clinical and research facilities are increasingly using next-generation sequencing (NGS) techniques. It is commonly acknowledged that genetic variation at pharmacogenetic loci has a significant role in determining phenotypic variations in drug response and may have therapeutic implications. Nonetheless, new research indicates that a sizable number of unique, uncommon pharmacogene variations probably account for a large amount of the observed inter-individual variability, which is still unknown. As a quick and reasonably priced large-scale DNA sequencing technology, next-generation sequencing (NGS) holds promise as a comprehensive pharmacogenetic genotyping platform for identifying genetic variations linked to medication therapy. The clinical application of NGS-based test results is currently beset by a number of issues, including functional interpretation, technological difficulties, and strict diagnostic testing requirements. High-throughput screening techniques, sophisticated computational analysis, and the creation of shared resources, such as cell-based and clinical data, may make it easier to incorporate NGS data into prospective genotyping strategies in order to enhance patient drug phenotype predictions in the future.

Keywords: Next-generation Sequencing (NGS) Techniques, Pharmacogenetic, DNA.

1 INTRODUCTION

Interindividual variability in drug response and toxicity during clinical therapy is a frequent occurrence that puts a significant strain on the medical system. Unfavourable treatment outcomes can be caused by a variety of causes, including drug interactions, physiological and pathophysiological factors, environmental impacts, treatment adherence, and genetic variances, which are thought to account for 20–30% of this variability (Vaidhya et al., 2024). The majority of genetic polymorphisms affecting drug response and the probability of adverse drug reactions (ADRs) are located in genes encoding pharmacological targets or in locations associated with the absorption, distribution, metabolism, and excretion (ADME) of pharmaceuticals (Horgan et al., 2024). Further elucidating these pharmacogenomic interactions would be advantageous, as genetic variants in these genes that exhibit a strong correlation with efficacy and/or safety could potentially function as biomarkers for customised medication therapy. Currently, 180 medications have the proper pharmacogenomic labelling approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Notably, more than 90% of people carry at least one gene linked to efficacy (Halman et al., 2024). Pharmacogenomic and a cogenetic guidance is provided for at least one medicine prescribed to 58% of adult patients. This percentage rises

to 89% among individuals who are 70 years of age or older. However, only a small number of associations are frequently employed in clinical treatment outside of targeted oncology, and pharmacogenomic biomarkers are not widely used in clinical practice.

At the moment, genotyping preselected variations within a panel of pharmacogenes is the main method used to do pharmacogenomic testing. The human pharmacogenomic genome, however, has tens of thousands of distinct variations, according to developments in sequencing technology over the past 20 years. More than 90% of all documented pharmacogenomic mutations are rare, with minor allele frequencies (MAFs) of less than 1% (Wang et al., 2024). In particular, population-scale sequencing research have demonstrated that each individual harbours a huge number of rare functional mutations in drug target genes and ADME. The current standard procedure for doing pharmacogenomic testing involves genotyping of preselected mutations within a collection of pharmacogenes (Amanat & Singh, 2024). The genetically encoded functional variation in pharmacogenes is thought to be mostly caused by uncommon coding and noncoding variation, which together account for about 30% and 20% of the variation. These estimates imply that individual predictions of pharmacological phenotypes could be much enhanced by taking into account extensive sequencing data, including such uncommon mutations. It is a difficult but exciting step to incorporate next-generation genomic sequencing into PGx practice. In order to scan entire panels of genes involved in drug absorption, distribution, metabolism, and excretion (ADME), the PGx field is currently moving from testing the responsiveness of individual genes to the application of various types of next-generation genotyping and sequencing (NGS) platforms (preemptive genotyping) (Qahwaji et al., 2024).

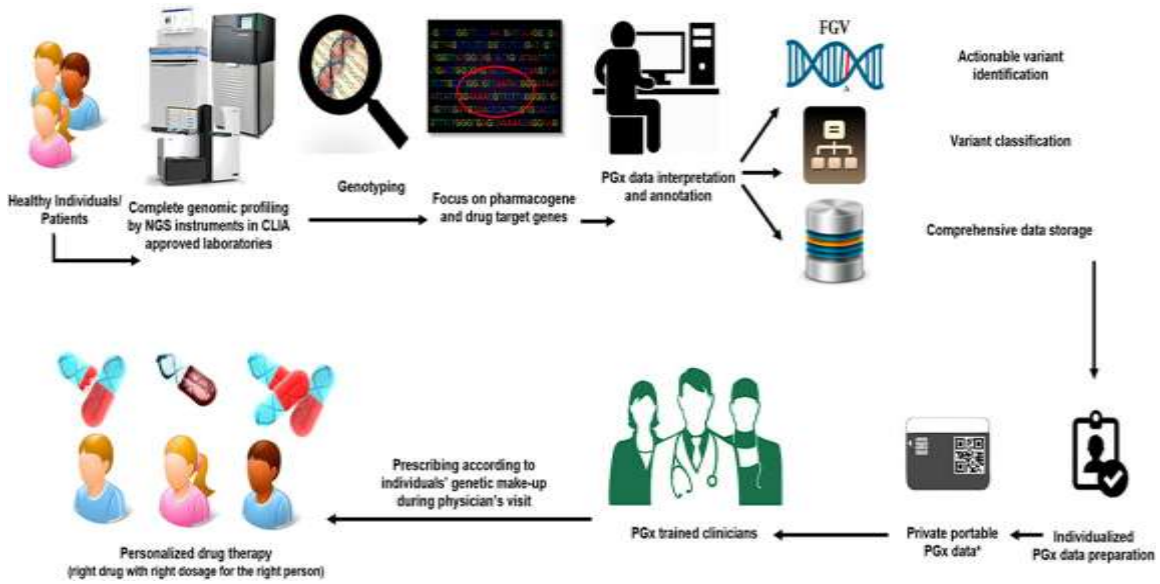

Figure 1: An Outlook on the Application of Pharmacogenomics in Contemporary Medicine. All patients, whether they are unwell or well, will get a thorough genetic test prior to visiting a doctor or clinic

All PGx-related genetic variants detected in the genome are included in the results, which are utilised to recommend prescription dosages based on the anticipated phenotype ascertained during the

sequencing process Figure 1. Although difficult, integrating next-generation genomic sequencing into PGx practice is an intriguing and promising move. With the application of various next-generation genotyping sequencing (NGS) platforms, the PGx field is currently transitioning from single-gene reactivity testing to scanning entire panels of genes involved in the absorption, distribution, metabolism, and excretion (ADME) of a drug before prescribing (preemptive genotyping). Based on the expected phenotype identified in the sequencing assay, the results, which comprise all PGx-related genetic variants in the genome, are used to propose prescription dosages Figure 1. All PGx-related genetic variants detected in the genome are included in the results, which are utilised to recommend prescription dosages based on the anticipated phenotype ascertained during the sequencing process Figure 1. Although difficult, integrating next-generation genomic sequencing into PGx practice is an intriguing and promising move. With the application of various next-generation genotyping sequencing (NGS) platforms, the PGx field is currently transitioning from single-gene reactivity testing to scanning entire panels of genes involved in the absorption, distribution, metabolism, and excretion (ADME) of a drug before prescribing (preemptive genotyping). Based on the expected phenotype identified in the sequencing assay, the results, which comprise all PGx-related genetic variants in the genome, are used to propose prescription dosages Figure 1. The difficulties in identifying particular kinds of variations in PGx and interpreting the resulting data in clinical practice are covered in this article. Some helpful tables that offer further details on NGS-PGx are also included, along with solutions for configuring and maintaining NGS equipment in clinical practice. Some helpful tables that offer further details on NGS-PGx are also included, along with solutions for configuring and maintaining NGS equipment in clinical practice.

Readers are referred to reviews in this area 5, 13, for specifics on the platforms themselves, while this review concentrates on the many applications of NGS to drug discovery and development (Phanthunane et al., 2024). NGS technology and their current uses are described in the first part. The application of the technology to various stages of the drug research and discovery process is then examined. We conclude by talking about the difficulties in using NGS, followed by a summary and some thoughts for the future.

2 LITRATURE SURVEY

In recent times, NGS has been employed to do extensive profiling of pharmacological genes associated with PD and drug pharmacology. Early findings imply that this approach may provide a reliable and effective tool to discover both common and unusual genetic variations in these genes (Balogun et al., 2024). The frequency and possible functional significance of uncommon and primarily novel SNVs in comparison to common known variations in numerous pharmacological genes have been demonstrated by a thorough analysis of large population sequencing datasets that are currently accessible (Ji & Shaaban, 2024). A recent analysis, combining SNV data from the 1000 Genomes Project (1092 individuals) and the ESP (6503 individuals), examined genetic variants in phase I and II metabolic enzymes, drug transporters, and nuclear receptors in particular. It found that all variants in coding regions were either very rare (>83%, MAF < 0.01%) or rare (>93%, MAF < 1%) and that the majority were nonsynonymous (56%–65%). It is

believed that these uncommon mutations account for between 30% and 40% of functional variation (Goljan et al., 2024). Similarly, more than 95% of the variants detected in a resequencing study of 202 drug-targeted genes in 14,002 individuals were rare, with a marginal allele frequency (MAF) of less than 0.5%, and 90% of these variants had not been reported before. A recent analysis, combining SNV data from the 1000 Genomes Project (1092 individuals) and the ESP (6503 individuals), examined genetic variants in phase I and II metabolic enzymes, drug transporters, and nuclear receptors in particular. It found that all variants in coding regions were either very rare (>83%, MAF < 0.01%) or rare (>93%, MAF < 1%) and that the majority were nonsynonymous (56%–65%). It is believed that these uncommon mutations account for between 30% and 40% of functional variation (Goljan et al., 2024). Similarly, more than 95% of the variants detected in a resequencing study of 202 drug-targeted genes in 14,002 individuals were rare, with a marginal allele frequency (MAF) of less than 0.5%, and 90% of these variants had not been reported before.

Genetic variation in genes encoding drug targets or enzymes and transporters involved in drug disposition have long been considered as promising biomarkers to predict toxicity and identify patients that will benefit most from the therapy in question. Germline variations, i.e. inherited variants that are passed on to offspring, are mainly used to predict drug pharmacokinetics whereas somatic mutations, i.e. variants that change the DNA sequence of a somatic cell but are not inherited and not passed on to offspring, guide therapy selection in oncology (Zhou & Lauschke, 2024; Fukunaga et al., 2021; Achour et al., 2021). The testing of somatic variations has become increasingly common in routine clinical care, often in the form of companion diagnostics; in contrast, the clinical implementation of most germline biomarkers lags behind and <10% of patients who are prescribed a medication that contains germline pharmacogenomic labeling receive preemptive testing (Echeverría et al., 2024). So far, only one variant allele requires preemptive testing (HLA-B*57:01 for abacavir), while screening for a few additional variants is mandated only for certain ethnogeographic groups (e.g. HLA-B*15:02 for carbamazepine in patients of South East Asian descent). Furthermore, certain variants with mounting evidence of their clinical utility and cost-effectiveness might soon be incorporated into routine testing prior to initiation of therapy, including reduced function alleles in DPYD and TPMT for fluoropyrimidine and thiopurine toxicity, respectively (Crisà et al., 2020).

It has been estimated that a common effect on PK or drug response may be caused by a variety of genetic variants that have been discovered over the past few decades and that could function as germline pharmacogenomic biomarkers in 18% of all outpatient prescriptions (Gulilat et al., 2020). Eighty percent of phase I drug metabolism is carried out by the cytochrome P450 (CYP) drug-metabolizing enzymes, which are encoded by genes that contain several of these biomarkers. Examples that are well-known include duplications of the functional CYP2D6 gene linked to toxicity from codeine, decreased function of the CYP2C9*2 and *3 alleles linked to the requirement for adjusting the dose of warfarin, and loss of function of the CYP2C19*2 allele resulting in decreased clopidogrel bioactivity. Poorer cardiovascular outcomes are experienced by patients following percutaneous coronary intervention. Moreover,

correlations that are clinically significant have been discovered between CYP2C19 genotypes and exposure to and rates of treatment failure for the antidepressants escitalopram and sertraline. Moreover, correlations that are clinically significant have been discovered between CYP2C19 genotypes and exposure to and rates of treatment failure for the antidepressants escitalopram and sertraline.

3 MATERIALS AND METHODS

It can be difficult to identify possible treatment targets and validate their applicability without using a variety of experimental platforms. Like more conventional methods like microarrays, NGS can be used to deliver detailed genomic data from very early target identification stages. Genes and signalling pathways that may be significant in the pathophysiology of the disease can be found using RNA-Seq differential gene expression studies between sick and normal tissues. Using NGS, a cutting-edge technique, clinically important genetic variants can be analysed to modify medication dosage and strike a balance between the likelihood of side effects and medication efficacy. Given that polymorphic enzymes metabolise up to 80% of medications, genetic polymorphisms in drug-metabolizing enzymes may be the cause of severe drug responses, extremely fast drug metabolism, and decreased drug efficacy Table 3. Drug metabolism involves many genes, but there are numerous places where the system can malfunction.

Sample Collection and DNA Extraction/Data Acquisition and Interpretation

PGx phenotype predictions generated from NGS data annotation is a highly specialised endeavour requiring both molecular and clinical expertise. Accurate validation techniques and a significant amount of time and effort are needed to extract actionable, putative, or likely pathogenic variations from huge and complicated raw data sets. The study included patients who met specific haematological requirements (HbA level <15%, absolute neutrophil count ≥2,000 µl, platelet count ≥100,000/µl, haemoglobin level ≥5.0 g/dl, and absolute reticulocyte count ≥100,000/µl) and who were 5 years of age or older and had never received hydroxyurea treatment before. Patients received care at Temeke Regional Hospital, Amana, and Muhimbili National Hospital (MNH). Participants provided 4 ml peripheral blood samples at baseline (before to the commencement of HU administration) and at every follow-up appointment. The manufacturfer's buffers (AL, AW1, AW2, EB, and Proteinase K) and the QIAamp Blood Mini Kit (QIAGEN, Germantown, USA) were used for the manual and column-based DNA extraction process. The Qubit DNA High Sensitivity (HS) Assay Kit and a Qubit 2.0 Fluorometer were used to assess the quantity of extracted DNA, and a NanoDropTM 2000/2000c Spectrophotometer (ThermoFisher Scientific, Waltham, USA) was used to measure the quality of the DNA. The manufacturer's buffers (AL, AW1, AW2, EB, and Proteinase K) and the QIAamp Blood Mini Kit (QIAGEN, Germantown, USA) were used for the manual and column-based DNA extraction process. The Qubit DNA High Sensitivity (HS) Assay Kit and a Qubit 2.0 Fluorometer were used to assess the quantity of extracted DNA, and a NanoDropTM 2000/2000c Spectrophotometer (ThermoFisher Scientific, Waltham, USA) was used to measure the quality of the DNA.

NGS in Pharmacogenomics

Investigating novel uses for pharmaceuticals that are already on the market is known as drug repositioning. By using this tactic, the expense of creating new medications is decreased and patients with incurable illnesses can receive new medicines more easily. Whether a medicine is being developed or has already received approval, genomics can play a significant role in establishing new indications. One of the main topics of discussion during the Institute of Medicine panel on applying genome-based research for health was innovative medication repositioning. Drugs, both new and old, can find new targets by using genetic information about humans. Repurposing to further drug research and development is made possible by high-throughput genomics and bioinformatics technologies. It is being developed to create comprehensive databases with integrated genetic, phenotypic, and clinical data.

Routine testing using NGS technology must meet the requirements for analytical quality for clinical laboratory tests approved by the US Clinical Laboratory Improvement Amendments (CLIA) or other national certification authorities in order to ensure both patient safety and analytical quality. NGS sequencing must be performed by trained personnel in facilities that have been accredited. Clinical laboratories can also develop and validate tests in-house and offer them for sale as testing services; however, these tests must follow the general regulatory requirements set forth by the certifying authority. A CLIA or other organisation licence is required for laboratories that offer services in order to perform very complex tests. Standardised preanalytical, analytical, and postanalytical processes are required for NGS tests on clinically orientated NGS platforms in order to produce high-quality clinical testing with enough precision and accuracy.

Target Identification and Validation

Finding potential therapeutic drug targets and demonstrating their usefulness can be challenging processes involving a variety of experimental platforms. In the very early stages of target identification, NGS can be used in the same way as traditional methods. B. Microarrays provide detailed genomic data. RNA-Seq can be used to perform differential gene expression analysis in both healthy and diseased tissues. Through the identification of genes and pathways that may be important in the pathophysiology of disease, this could help select novel targets for intervention. discovery of genetic variants underlying rare Mendelian illnesses has been effectively accomplished with whole exome NGS data, which may also aid in target discovery. Moreover, the resolution of genetic linkage studies made possible by the application of NGS may enable the identification of novel therapeutic targets from intricate trait genetic investigations. Additionally, NGS has shown to be a helpful method for describing therapeutically significant mutations in mice and can frequently yield crucial data for target validation. Without the need for drawn-out genetic mapping investigations, underlying mutations can be quickly isolated using capture sequencing of the exome or target region.

Next-Generation Sequencing to Identify Variation Related to Disease and Drug Therapy

Large-scale NGS methods have been employed recently to find and describe putative functional variants in human genes in an effort to better understand the genetic component of disease and how medication treatments work, the latter being particularly important for the developing field of personalised or precision medicine. The necessity of gathering data is especially crucial. Vast international or multi-institutional research projects have already yielded sizable population datasets including DNA sequence variations for tens of thousands to thousands of people; in certain instances, these databases have been connected to clinical information that is accessible to the scientific community.

The Exome Sequencing Project (ESP) of the National Heart, Lung, and Blood Institute (NHLBI) currently has information on protein-coding variants from data from more than 6,500 clinically well-characterized patients. Similarly, the 1000 Genomes Project has mapped and catalogued genetic variants in 1,092 human genomes from diverse populations thus far. An even more comprehensive resource is the Exome Aggregation Consortium (ExAC), which offers exome sequence data from 60,706 individuals along with their predicted pathogenicity. Furthermore, functional variations in 50,726 whole-exome sequences linked to electronic health record data were assessed for possible clinical significance as part of the Discov EHR project.

Genetic Variation

Thus far, a multitude of genetic correlations between drug response and small-to-medium-sized candidate gene studies as well as genome-wide scans using single nucleotide polymorphism (SNP) genotyping have been documented. According to a recent study, patients with bipolar illness who have the XBP1-116C/G genotype with the DRD4 gene may benefit more from smoking cessation treatments like valproate. One of the Newly Developed Third-generation Platforms is Shown Alongside the Three Most Popular Platforms in Green shown in Figure 2.

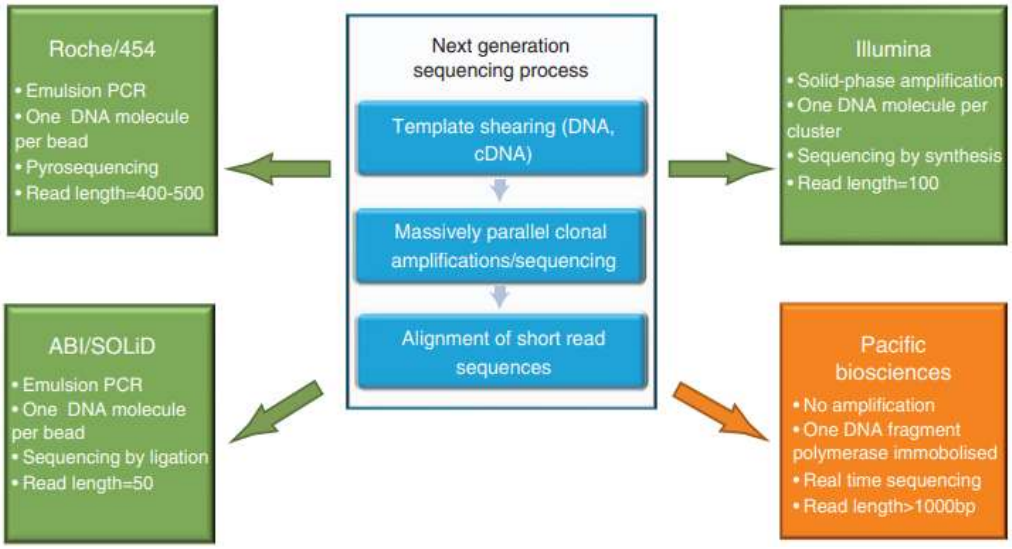

Figure 2: One of the Newly Developed Third-generation Platforms is Shown Alongside the Three Most Popular Platforms in Green

Based on their genetic profiles, pharmacogenetics is frequently used to divide patient populations in therapeutic trials into responders and non-responders. This technique aids in lowering the quantity and price of subsequent clinical research. Some do note that pharmacogenetics has not yet been extensively applied in medication discovery, clinical practice, or research. By identifying all common and uncommon genetic variants in the human population, NGS promises to boost research in this area, and the 1000 Genomes Project has undoubtedly made significant strides towards this aim thus far. Scientists can carry out more in-depth research to determine the genetic variants underlying a patient's reaction to a medication by utilising NGS to generate a comprehensive genomic map of all human variants. The use of nucleotide substitution analysis (NGS) has shown to be beneficial in the genomic, transcriptome, and epigenetic characterisation of tumour cells. It can identify low-level somatic mutations in a germline background and is very sensitive. This method's characteristics make it perfect for researching tumour cells, which naturally harbour a high number of these mutational events. In a matter of weeks, researchers can now catalogue all mutations, copy number abnormalities, and somatic rearrangements in a cancer genome at base pair resolution. Several tumour types, such as breast and colon cancer, have already had their entire genome sequences published, along with potential biomarkers. By locating and classifying mutations within tumours, targeted medicines can be developed based on the discovery of tumor-specific mutations. In addition, this procedure can aid in the discovery of novel therapeutic targets and genes and signalling pathways implicated in the growth of tumours.

4 CONCLUSION

Pharmacogenomics currently has limited practical utility outside of oncology, where somatic and some germline indicators are routinely employed to direct cancer treatment. The field of pharmacogenomic research is predicted to grow from genotyping for cryptic mutations in small case-control studies to extensive population-scale discovery investigations as a result of advancements in genomic technology that make genome-wide studies feasible and affordable. A multitude of uncommon pharmacogenomic alterations, many with unknown functional implications, have been discovered as a result of this shift in viewpoint. The incorporation of pharmacogenomic knowledge into clinical practice has been sluggish, despite these advancements. Despite the fact that numerous studies have demonstrated that a sizable fraction of people carry significant pharmacogenetic alterations, most health systems do not frequently employ point-of-care testing, and such data is rarely created proactively. Rough calculations suggest that 20–40% of the total genetically encoded inter-individual variability in medication pharmacokinetics, action, and toxicity may be attributed to uncommon mutations. NGS-based mutation profiling and pharmacogenomic techniques are challenging to use in clinical settings due to the ongoing challenges in translating personalised mutation profiles into practical applications. Efforts are presently being made in Europe, the US, and Asia to measure the benefits of pharmacogenomically regulated treatment. These will offer crucial details about how pharmacogenomics affects patient outcomes and the economical use of genes, medications, and medical facilities.

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