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Pharmacogenomics: Applications in Drug Discovery and Pharmacotherapy

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Abstract: Pharmacogenomics is the scientific study which explains individual variability of drug targets and to explore the genetic basis for such changes. With the completion of human genomic study, clear relation could now be established between the drug response in relation to a person's genome. Pharmacogenomics, also known as personalized medicine, uses the person's genome to determine the dose and dosage regimen, so that therapy could be optimized. As with the techniques like DNA microarray technologies person's response to a therapy can be predicted and new therapies could be assigned. In the present review, the current technologies, and past significance has been discussed.

Keywords: Pharmacogenomics, personalized medicine, DNA Microarray.

1. INTRODUCTION

Pharmacogenomics, deals with the relationship between heredity and response to drugs. It is the scientific field that attempts to explain individual variability of drug targets and helps individuals to explore the genetic basis of such changes. The individual variation in drug metabolism rate has been known for several years (William *et al*., 2001). Earlier, the study of pharmacogenetics was only of academic interest, but now it helps the pharmaceutical industry in the documentation of metabolism of a new drug development before registration. The information of drug metabolism and enzymes involved for the process will help to identify the drug-drug interactions and also the rate at which individual patients may metabolize a specific drug. Pharmacogenomics shows development of a successful research by applying genomic techniques to accelerate the identification of new drug reaction-markers and to confirm whether those markers act at the level of drug metabolism, target, or disease pathway.

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The continue analysis of the human genetic code will give the scientific support on which it will be possible to identify new possible drug targets, the genetic factors that can affect drug metabolism and toxicity, lead to variability in pharmacological responses. The utilization of genetic techniques like microarray technology helps the scientists to develop a link between gene sequence and function. Currently, the new approaches are integrated in various aspects of drug discovery process. The genetic polymorphism analysis has been applied to target validation, pharmacokinetics, toxicology, and clinical pharmacogenomics. (Marton, M. J. *et al*., 1998).

In the year 2001 the results of human genome project were published in the Nature (Eric *et al*, 2001) and Science (Craig *et al*, 2001) initiating the fields of pharmacogenetics and pharmacogenomics. But this work originated from the early coining of term pharmacogenetics by the scientist F. Vogel in 1959 (Vogel, 1959). In the past century scientists are trying to connect the response of drug on patient with its different genes. The concept of personalised medicine is first involved in checking the patient genome such that the drug dose and regimen could be designed personally for the patient. Pharmacogenomics can be defined as the study of variation in DNA sequence and its relation to drug response (Nebert, 1999). The single-nucleotide polymorphisms (SNPs), genetic insertions and deletions, and genetic copy-number variations (CNVs) are all the part of genetic variations. The pharmacogenetics basically targets the study of genetic variations in enzymes that metabolizes the drug, the receptors and targets and how these genetics variations in them affect the response of drugs. It also helps to identify the cause behind the variations from individual to individual. Many drugs work according to the targeting to genetic mutations. The personalised medicines include the scanning of one's genome so that any genetic difference or defect could be reported (Cheok *et al*, 2009). As a result it will minimise the toxic effects of drugs. If the mutations occur in the genes abnormal proteins may appear which will ultimately affect the other functions of enzyme functions.

1.1 Benefits of Pharmacogenomics

Pharmacogenomics has the potential to provide modified drug treatment based on genetic alternation in efficacy and side effects.

1.1.1 New potent molecules

Using pharmacogenomics, the Pharmaceutical industries will be able to generate new potent molecules with maximal remedial effects with least side effects.

1.1.2 New methods to determine correct dose of drug –

The methods are based on a person's genetics that how the body processes the drug and the time it takes to metabolize.

As it has already been proved that age, lifestyle and individual health also affect the response of patient towards drugs but understanding the genetic makeup of patient can improve the response and techniques such as DNA analysis have added new dimension to pharmacogenomics. Thus, pharmacogenomics can play a major role in the treatment of patient, with minimised side effects. The original concept of one drug for all approach which has been in use for past century, is based on statistical approach in which the patients are classified in broader classes, mainy as per age. But the pharmacogenomics classifies the patients into smaller groups based on the phenotypic and genotypic content.

1.2 Need For Pharmacogenomics

Pharmacogenetics is a broad area of research. The effects of polymorphism of genes for enzymes like cytochrome P450, can be applied in drug delivery, development and the clinical application of drugs. The cost effective methods of genotyping can be used by the physicians in patient treatment. Currently, genotyping is not a clinically established technique, but now its status is likely to be changed. Although pharmacogenomics and tailored treatment shows increased efficacy and safety of treatment, it also includes ethical, social and race as important issues which need to be analyzed, in line with FDA guidelines on pharmacogenomics. With the use of new high-throughput screening methods and data-mining approaches, pharmacogenomics results can be improved.

1.3 Techniques in pharmacogenomics

1.3.1 Single nucleotide polymorphism (SNP)

The genetic dissimilarity in the human genome may be SNPs, which is due to the effect of point mutations that create single base-pair changes in chromosome sequences. Many laboratories and computational approaches are used to find out single nucleotide polymorphisms (SNPs) within a genome. The identification of single nucleotide polymorphism can be based on expressed sequence tags (ESTs), which is generated by single run sequencing of cDNAs obtained from different individuals. The non-coding SNPs can be classified according to whether they are found in gene regulating segments of the genome. Many complex diseases may arise from quantitative, rather than qualitative differences in gene products. Coding SNPs can be classified

as to whether they alter the sequence of the protein encoded by the altered gene (Chakravarti A. 2001). This Pharmacogenetic testing could be used in the patients as a powerful tool. With the help of this we can define a patient with the individualized medicine dosage regimen and will give the highest therapeutic effect. The important advancements such as availability of large number of single nucleotide polymorphisms (SNPs), the Haplotype map (Hap Map) and Roche microchip for microarray, have taken us one step ahead to the genomic medicines. Now with this we can analyse the drug metabolizing and drug-transporting enzymes which were found to be responsible for the genomic variations and with more defined evidence we can say that whether the variation in the person is chromosomal or is there any other reason behind it (Barkur, 2006). Many applications of SNP's have been done in biological studies and drug development.

1.3.2 DNA-microarray technology

A DNA microarray is also known as gene /genome chip or DNA chip. It is a compilation of microscopic DNA which is attached to a solid support mainly made up of glass, plastic, or silicon. The genome chip technology has originated from Southern-blot technique which is based on detecting exact sequences along with DNA fragments which are removed by gel electrophoresis (Southern, 2000). By using this method, the Southern blot was developed as first array. The next steps included screening of filter-based clone libraries and the gridded libraries were stored in microtiter plates and then immobilized with filter membranes. It was innovated by Pat Brown and colleagues and it concerns the use of non-porous solid bodies, such as glass slides, as activated surface, on which the molecules of interest are positioned and immobilized. The immobilized molecules are then reacted with labelled probe molecules. Fluorescent dyes are used for labelling. With this method, 10,000 molecule groups per slide are marked instinctively. The benefit of these methods involves the flexilibility, i.e. the array can be produced in molecular- biology laboratories. The cost of production is very less. (Saluz, H.P. *et al*., (2002).

Several scientists tried to investigate the gene expression by the hybridization of mRNA to cDNA on nylon base. The two key advancements in the technology of DNA array were the trend of using the nonporous solid base (glass) which was further developed to include fluorescence- hybridization detection (Lockhart *et al*, 1996; Schena, 1995, 1996). The second important improvement was the high density spatial synthesis of oligonucleotides, which has got the capacity to simultaneously analyse thousands of genes. As there is a problem that DNA cannot be directly attached to the surface of glass and so the surface is first treated silane to covalently attach reactive amine, aldehyde, or

epoxies groups so that stable bond could be formed. The SNP's can be detected by short oligonucleotides. High and strong signals are produced by the PCR-amplified cDNAs (DeRisi *et al*, 1996). The cDNAs can be derived from the cDNA library. The long nucleotides produce good signals with good specificity. Thus the cells or tissues are exposed to the drugs and the expression is checked by collecting the mRNA and converting them to the cDNA hybridising it with the DNA array and staining them with the fluorescent dye. Then the activity is checked by suitable fluorometer. The results helped in compiling biological information and different patterns, which are counted for their activity.

1.3.2.1 ROCHE Amplichip

The Amplichip CYP450, developed by Roche, is the first microarray to be approved for the clinical use. It has ability to check the genetic changes for CYP2D6 and CYP2C19 genes.The test includes five steps, and the analysis time is 8 hour. Firstly, Polymerase chain reaction amplification is carried out to amplify the genes of interest with the help of gene-specific primers. The next step involves fragmentation and biotin labelling of the amplicons at the 3 termini with the enzyme terminal transferase (TdT). The biotin-labelled amplicon is then hybridized to the AmpliChip DNA microarray (Rebsamen, M. C., 2009).

1.3.2.2 DNA Microarrays Applications In Drug Discovery and Development

The natural product research is based on ethnobotanical information and its aim includes use of the pharmacological effects of medicinal plants which were traditionally used in treatment of various diseases (Heinrich M. 2003; Vuorela P *et al*. 2004). Plants are regarded as a source of new therapeutic agents due to their higher structural variations as compared to synthetic molecules.

The DNA microarrays lead to the discovery of novel diagnostic indicators and biomarkers of therapeutic response; mechanism of action of a herb, its formulations or its phytochemical constituents. The detection and validation of novel molecular targets is also possible with DNA microarray technology. It is also used for the prediction of side-effects of herbal drugs during preclinical studies. It gives the true botanical identification of crude plant materials.

1.3.2.3 Pure Components

The triterpenoid compounds isolated from the medicinal plant *Centella asiatica* when studied with gene microarrays showed that *Centella* triterpenes give gene-expression response in treatment of connective tissue disorders such as wound healing and microangiopathy (Iizuka, N. *et al*. 2003). The antiproliferative activity of *Coptidis rhizoma*, and its major constituent berberine was investigated in human pancreatic cancer cell lines. The gene

expression patterns were analyzed with oligonucleotide arrays that contains 11,000 genes. It was possible to detect similiar and different genes in relation to anti-proliferative activities of purified berberine and *Coptidis rhizome*.

1.3.2.4 DNA Microarrays In Pharmacogenomics

The DNA microarrays will lead to the development of optimized drugs which are based on differential gene expression patterns.The genetic polymorphism studies can be done to classify individuals according to the drug metabolizing capacities or their response to different diseases. Oligonucleotide-based DNA chip technology can be used to analyze gene expression profiles that are induced or repressed by xenobiotics (Wang Z *et al*. 2004). The microarray genotype system can be used for multiplex analysis of single nucleotide polymorphisms (SNPs) in genes encoding proteins involved in blood pressure regulation. The system explains the feasibility of anti-hypertensive drug response in pharmacogenomics (Freeman *et al*. 2001; Liljedahl *et al*. 2003).

In latest studies, (Na L, *et al*. 2014) it is concluded that metagenomic DNA from fecal samples of various age groups showed antibiotic resistance genes in human gut microbiota by using microarray technique. The study indicated different number of gene types in different age groups and it was also reported that antibiotic resistance genes become more complex with age.

1.3.2.5 DNA Microarray In Study Of Herbal Drugs

The use of genuine herbal materials is the first step to ensure quality, safety and purity of herbal medicines. For the identification of herbal drugs, DNA polymorphism-based arrays have been developed (Warude, D *et al*. 2003). In this method, small fragments of DNA are amplified by the polymerase chain reaction and their products are analyzed by using gel electrophoresis and hybridized with species-specific probes. In recent studies, microarrays have been applied for the DNA sequence-based detection of medicinal plants (Trau *et al*. 2002; Tsoi *et al*. 2003).The microarray analysis of gene expression can be used for the detection of molecular mechanisms and pharmacological activities of herbal extracts. The gene expression patterns at many stages during treatment process will help in the identification of biomarkers with undesirable or possible effects. It will also help in detection of new therapeutic applications of a herbal drug. The microarrays have been used in drug discovery process in selection of biological targets and lead compounds.

Phytomics technology provides a platform for the characterization of herbal compositions. Now, Herbal Bio Response Arrays (HBR Arrays) are used to determine bioactive components and pharmacological activities of herbal drugs has been developed and patented (Ventura C; 2005). DNA microarrays

involved in different phases of herbal drug discovery and development. This includes quality control, standardization, identification of herbal drugs and validation of new targets(Tonisson *et al*. 2002).

1.4 Pharmacogenomics and drug development

The drug development is too costly and takes years to fructify. Even after 10-15 years of study, out of 5000 New Chemical Entities (NCE) only 1 or 2 makes their way to the market. But if we join pharmacogenomics with the drug development process, we can greatly reduce this failure rate and can get the potential NCE in shorter time. The pharmaceutical companies should also try to study the pharmacogenetics factors in the patients with the drugs in which high inter individuality difference is observed such as in patients with warfarin (Ewans *et al*., 2001). The information of drug metabolism and enzymes involved in metabolism process helps to detect drug-drug interactions. Now, the information is required to be registered by the U.S. Food and Drug Administration (FDA) and other similar authorities (Salerno, L, 2004). The main step is to include pharmacogenomics data in regular clinical practice. It is important to know the molecular mechanisms of drug response i.e. pharmacological and side effects. The personal variations in response to a drug may result from different reasons, for example, genetic changes and expression levels of drug-targeted molecules, including membrane, nuclear receptors, signal transduction components, and drug-metabolizing enzymes and drug transporters (Evans *et al*., 2001). Now, genome wide association studies (GWAS) have been applied to different variants that affect drug toxicity and efficacy and risk of diseases. The GWAS approach has been used for the identification of genetic contributions to variation in drug response (Kamatani *et al*., 2010, Schuldiner *et al*., 2009). It increases our knowledge to study the mechanisms of drug action and genetic determinants for variable responses to many drugs like warfarin, tomoxifen, and clopidogrel.

1.5 Challenges for the future

The use of pharmacogenetic data in health system faces many challenges. The selection of drug dosage becomes more complex with the involvement of genetic data. Mostly patients receive more than one drug for a single disease and use of many combined therapies make the task more complicated. The drug dose is adjusted based on clinical parameters like liver and renal function tests. However, clinicians hesitate to adjust the dose on the basis of genetic data of individual and they prefer to accept the clinical parameters. (Relling, M.V. *et al*., 2005).

1.6 Pharmacogenomics and the drug metabolism

The technologies such as proteomics and genomics could as the only base for dose adjustments provide potential to identify novel proteins as the targets, different mechanism of action and may provide the different specificity to the drugs. In humans, there are many metabolizing organs but main organ of interest is the liver. In the liver, the metabolism occurs in three phases. In phase I, the compound is turned to more polar nature. Groups such as hydroxyl, N- or 0- may be added to the drugs to make them more polar. This process involves the reduction of cytochrome-bound oxygen and formation of highly-reactive oxyferryl species (Guengerich, 2001; Schlichting *et al*. 2000). The moieties obtained after the phase I then undergo phase II reaction. In phase II, mainly the conjugation occurs due to glutathione S-transferases. In phase III, the conjugated drugs, if needed could be processed and finally pumped out (Akagah, 2008). The clinical trials are classified into four phases. The drug to be approved for the human use must pass through phases I, II and III. But with pharmacogenetics study during phase I if we study only individual with known genotypes, we can reduce the number of dropouts from the study in the phase III.

1.7 Pharmacogenomics in therapeutic application

1.7.1 Warfarin therapy

Warfarin is a powerful anticoagulant used in the treatment of thromboembolic disorders. It is given to the patients with atrial fibrillation and is given on prophylactic basis to the patients before major orthopaedic surgery. But it has got narrow therapeutic window due to which it is a bit difficult to adjust its dose. If higher dose is administered it may result in bleeding and there is inter individual variation in the patient response. So it is the main problem for the doctors to correctly administer its dose. SNPs are the sequences which lead to the generation of various alleles of a gene. The CYP 2C9*2 (or 430 C>T) and CYP 2C9*3 (1075A>C) are the alleles discovered in the 30 CYP 2C9 and they are found to be responsible for over anticoagulation (Zhu *et al*, 2007; Rettie, 2006). Vitamin K epoxide reductase regulates the renewal of reduced vitamin K (KH2) cofactor which is responsible in formation of clotting factors. Warfarin blocks the whole network working by blocking the VKORC1 non competitively. The main aim behind the testing of individual's CYP2C9 and VKORC1 is to help the doctor to administer a lower warfarin dose which will result in lower bleeding effects and lower risk to the patients (Schwarz, 2008).

1.7.2 Codeine

Breast feeding mothers have high capacity to metabolize codeine having multiple copies of the gene for cytochrome P450 2D6 which is responsible for the conversion of codeine into morphine which is its active metabolite. As a result, feeding mothers may have high amount of morphine and codeine in their milk (Gage, 2007).Thus, codeine administration to lactating mothers could be monitored using pharmacogenomics relating to plasma morphine level.

1.7.3 The human leukocyte antigen

The human leukocyte antigen (HLA) allele HLA-B*1502, has been found to be genetic marker in patients with Stevens-Johnson syndrome (SJS) and Toxic Epidermal Necrolysis (TEN) after treatment with antiepileptic drugs. This gene is generally found according to the geographical area diversity in the patients residing in the South Asia. The patients with SJS/TEN carried high mortality and morbidity and many survived patients have showed long term complications like ocular damage, renal failure etc. So it is important to perform genotyping of HLA-B*1502 before carbamazepine prescription for high risk Southeast Asians in whom HLA-B*1502 allele frequency is more to avoid life threatening conditions. (Alfirevic et. al., 2006; Hung *et al*., 2006; Lonjou *et al*., 2006; and Man *et al.*, 2007).

1.8.4 Targeting of drugs

The pharmacogenomics can lead to better targetting of anticancer drugs. Many anticancer drugs are being studied for such targetting:

- Imatinib (Gleevec) for targeting bcr-abl tyrosine kinase in several tumor types
- Cetuximab (Erbitux) for targeting epidermal growth factor receptor (EGFR) in head, neck and colorectal cancer
- Trastuzumab (Herceptin) for targeting variants in the Her2 receptor in breast cancer.

Targetting in pharmacogenomics is important because it protects other normal cells from the effects of anticancer drugs. The targeting could be characterized by the interaction of drug with the protein molecule receptor present on the surface of cell (Paolo, D. A., 2004).

1.7.5 HIV therapy

The HIV is one of the major problem in the today's world and its detection is also difficult. As a result, it may be possible that with the help of traditional therapies the patient may become resistant to all the drugs and the virus could

not be prevented any more. So more rational drugs use must be done and more combination therapies must be used to eradicate the virus. The TRUGENE HIV-1 Genotype Kit is now commercially available to detect the HIV (Pirmohamed, M. , & Back, D. J. 2001).

1.7. 6 Pharmacogenetic testing and health care

The Pharmacogenetic testing helps to determine the genotypic and phenotypic differences involved in the pharmacodynamics and pharmacokinetics of drug metabolism. The pharmacogenetics means genetic variations which can effect in drug metabolism and response both interms of therapeutic action and adverse effects. The pharmacogenomics refers that how the genetic composition effects an individuals response to drugs. Molecular alterations in enzymes involved in metabolism lead to the genetic variability in drug response. (Ensom, M. H. *et al*; 2001). Drug–drug interactions (DDIs) have shown serious effects like adverse drug reactions (ADRs), and in extreme cases lead to death. DDIs have become a major problem particularly in the care of aged patients, as they are often prescribed with broad variety of medications (Routledge, P.A., 2004). Recently, ADRs are considered as the fourth leading cause of death in the United States, resulting in 106,000 deaths per year, and the fifth leading cause of illness. Presently, approx. 28% of adults and 17% of children have drug-related ADRs. The pharmacogenetic techniques will become an essential part for the drug monitoring and health management of patients. By using pharmacogenetic methods the patient genotyping can be carried out before drug treatment and helps in minimizing the unfavourable effects (Lundkvist, J., 2004).

1.8 International Declaration on Human Genetic Data

In the year 1990, the member states of UNESCO requested to have some system which will lay down some guidelines and ethical standards for the human genome project. So, on the recommendations from the member states the International Bioethics Committee (IBC) having 36 experts as its members was made. The IBC laid down the Universal Declaration on the Human Genome and Human Rights. In this declaration there were the strict guidelines related to the collection, processing, use and storage of human genetic data. This was on whole adopted in the year 2003. But the latest guidelines known as Universal Declaration on Bioethics and Human Rights adopted in the year 2005. These guidelines are followed by each government of the member states. In the late 90's the era of globalization started as a result there were not only advances in the scientific and technological department but also on bioethical level

there were advances too. The medical science is the international technology having various facets which can be welcomed or banned by the certain country according to its personal bioethics. Example includes the rules for the organ transplantation which is different of different countries. (Shawn, H., 2005).

1.9 CONCLUSION

Pharmacogenomics play an important role in drug discovery and development. During the past decade there is greater emphasised research going on the genetic polymorphisms which is related to the pharmacokinetics and pharmacodynamics of the drug. And in the future decade the aim of research would be to have more individualised drugs for the therapy. The science of pharmacogenomics is greatly widespread not only in the USA or other western countries but also in the Asian countries too. The various health departments are setting up the different data guidelines for the proper monitoring of the pharmacogenomics. In the year 2003, the UNESCO had set up the "International Declaration on Human Genetic Data". The various countries like China has now conform to the basic principles of the declaration. These types of centers help in development and the phase IV study of drugs as well as helpful in performing clinical studies.

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