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Single Nucleotide Polymorphism: *A Review On Use Of These Biological Markers In Personalised Therapy*

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ABSTRACT:

Pharmacogenomics is transforming personalized medicine by tailoring medication regimens to a patient's genetic composition, with a special emphasis on single nucleotide polymorphisms. This paper explores the two primary approaches utilized in SNP-based research: LD mapping, which searches the whole genome for SNP-related connections, and the candidate gene method, which looks at particular genes known to affect medication reactions. Applications of these approaches in diseases such as rheumatoid arthritis serve as examples. In this case, genetic variations like mutations in the HLA-DRB1 gene are important in determining treatment response and vulnerability. Even if a lot has been accomplished, there are still obstacles to overcome in order to address genetic diversity and turn these findings into useful therapeutic applications.

Keywords: SNPs, Rheumatoid Arthritis, Pharmacogenomics

1. INTRODUCTION

SNP (Single Nucleotide Polymorphism) is a variation at a single position in a DNA sequence among individuals of a species. These are the most common type of the genetic variation that plays very important role in the diversity of humans genetically and their evolution. SNPs are like tiny changes in one of the nucleotide of the genetic material of the body as the DNA has 4 types of nucleotide viz. A (Adenine), T (Thymine), C (Cytosine), G (Guanine) sometimes at some specific spot, one can have different sequence of the nucleotides then the usual or common one. For example if maximum number of peoples have genetic sequence (AGTC) but if one has sequence of (AGTT) then that T is the marker of the variation or SNP this is the reason SNPs are also called as biological markers or genetic markers which can influence the body triats like response to drugs, variation in the phenotype, how genes are expressed and the body's susceptability to diseases. SNPs can be used to understand the molecular mechanisms of sequence evolution, they also helps scientists to locate the disease causing genes, used to predict the body's response to particular drugs, they also play crucial part in gene mapping and plays key role in identifying the potential risk of diseases.

SNPs are highly universal in the human DNA polymorphisms, with a frequency of about 1 in 1000, and they have an abundance of 1% or more at the lowest frequency in the human population.[2] this review is aiming on providing overview on the information and the mechanism of how SNPs work in detection of various autoimmune diseases and pharmacogenomics which is the study of, how SNPs are responsible for the personalised drug therapy for the verious diseases and how SNPs are identified in an indivisual to understand the genetic function and health of an indivisual .

Pharmacogenomics examines how a person's genetic makeup influences their response to medical treatments. It has the potential to transform healthcare by personalizing treatments using advanced diagnostic tools. This emerging field aims to minimize the trial-and-error process in selecting therapies, reducing patient's exposure to ineffective or harmful drugs. Single Nucleotide Polymorphisms (SNPs) play a crucial role in identifying an individual's susceptibility to certain diseases and predicting their response to medications.

1.1 Introduction to Autoimmune Diseases

Autoimmune diseases are quite common, impacting more than 5% of people worldwide. These conditions occur when the immune system mistakenly targets the body's own tissues, and genetics play a role in their development [21].

When we talk about the autoimmune diseases, the gene named Interleukin-27 (IL-27) plays a crucial role in regulating T helper (Th) cell responses and is associated in the development of certain autoimmune diseases. IL-27 is part of the IL-12/IL-6 family and is made up of two components: the Epstein-Barr virus-induced gene 3 and the IL-27 p28 subunit. It's found on chromosome 16p11 and plays a key role in T cell development and differentiation. IL-27 is part of the IL-12/IL-6 family and is made up of two components: the Epstein-Barr virus-induced gene 3 and the IL-27 p28 subunit. It's found on chromosome 16p11 and plays a key role in T cell development and differentiation [3]. Some of the most studied autoimmune diseases include rheumatoid arthritis (RA), multiple sclerosis (MS), systemic lupus erythematosus (SLE), type 1 diabetes (T1D), and Crohn's disease (CD). Studies reveal that genetic susceptibility, much of which is due to SNPs, plays a significant role in the development and progression of these disease.

Even after giving the traditional treatment of drug therapy for any of the disease, it can still have loophole of several adverse drug reactions associated with the drug, different strength of drug metabolising enzymes in each person. as, certain genetical polymorphisms can affect the metabolism of drug. Also, they can be associated with the appearance of adverse drug reaction of drug. These genetic differences mean that the same drug dose can be therapeutic for some individuals but toxic or ineffective for others. To address these issues, studying SNPs is critical for developing personalized drug therapy, allowing clinicians to optimize dosing and select drugs based on a patient's genetic profile. This approach enhances drug efficacy, minimizes ADRs, and advances the safety and precision of medical treatments.

1.2 History

SNPs, or Single Nucleotide Polymorphisms, were first discovered in 1980, when restriction endonuclease assays were employed to detect the presence or absence of DNA cleavage sites. These genetic variations account for approximately 90% of the human genome. A significant number of SNPs were thoroughly investigated during the Human Genome Project, which played a key role in advancing our understanding of genetic diversity and its impact on health and disease. This research laid the groundwork for future studies in personalized medicine and pharmacogenomics, where SNPs are now recognized as critical in determining individual susceptibility to diseases and responses to medications [2]

Here, in this review, first we will get to know about the role of SNPs in pharmacodynamics. Then we will study about the personalised therapy of Rheumetoid Arthritis.

2. Use of Single Nucleotide Polymorphisms in Pharmacogenomics

Pharmacogenomics is the study of how an individual's genetic makeup influences their response to drugs. It combines the fields of pharmacology (the study of drugs) and genomics (the study of genes and their functions) to understand how genetic variations affect drug efficacy, dosage, metabolism, and potential side effects. The goal of pharmacogenomics is to develop personalized medicine, where treatments and medications are tailored to each person's genetic profile, optimizing therapeutic outcomes and minimizing adverse effects.

There are two approaches in imploying the SNPs into pharmacogenomics studies

1) Candidate Gene Studies
2) Whole Genome Linkage Disequilibrium Mapping

2.1 Candidate Gene Studies

Candidate gene :

The candidate gene approach involves using experimental methods or prior knowledge of the drug's pathway, metabolism, or disease mechanisms to identify genes potentially related to drug response. Single nucleotide polymorphisms (SNPs) found in these genes are then evaluated in groups of patients treated with the drug, and tested for statistical associations or correlations with their drug response. If a connection is found, these "susceptibility genes" are believed to directly affect an individual's probability of responding to the drug. Candidate genes for pharmacogenomic analysis may involve drug targets, pathway-related genes, drug-metabolizing enzymes, or disease-associated genes. The selection of these genes is guided by knowledge of the disease's underlying mechanisms and the drug's mode of action. To achieve this, experimental methods can be used during the preclinical phases of drug development to identify genes involved in drug metabolism, as well as the drug's primary and secondary targets. Biological models for identifying markers of drug efficacy or toxicity may include comparative gene expression profiling of tissues or cell lines exposed to therapeutic compounds in vitro or in vivo. There are numerous examples of pharmacogenomic markers discovered through the candidate gene approach. [5]

2.1.1 Disease Genes

Advances in genomic technologies, like transcriptional profiling, are helping classify diseases at the molecular level. Conditions once thought to be the same disease will be divided into subtypes using a new genomic system. Recent discoveries in the molecular pathology of cancer have highlighted important and clinically significant differences in the gene expression patterns of a variety of tumors.[5]

An example of a disease gene where SNPs have been linked to therapy response is the apolipoprotein E (APOE) gene, associated with Alzheimer's disease. Individuals carrying the E4 allele, a known risk factor for Alzheimer's, have been shown to respond differently to certain cholinesterase inhibitors compared to non-carriers. Although these findings have not been definitively confirmed, they illustrate a valid hypothesis that different molecular classifications of a disease may impact how patients respond to treatment [5]. Another one example would be Deep vein thrombosis, Deep vein thrombosis is affected by the Gly20210Ala polymorphism in the prothrombin gene. This example highlights the complexity of defining the role of SNPs, as more than one SNP may contribute to a specific phenotype [6].

2.1.2 Drug targets and pathway

Present-day drug therapies are designed around approximate number of targets (600-1500) [7] primarily focusing on cell membrane receptors and enzymes. Furthermore, genomic strategies are being applied to identify novel gene targets for drug discovery. As these targets interact with therapeutic compounds to influence disease, they are considered strong candidates for pharmacogenomic studies. Identifying SNPs in drug targets could offer a valuable set of pharmacogenomic markers to investigate for their correlation with responses to current therapies.

A well-documented example of a drug target where SNPs have been associated with therapeutic response is 5-lipoxygenase (ALOX5). Research has shown that genetic variations in the ALOX5 gene can influence how patients with asthma respond to treatments that inhibit 5-lipoxygenase. Specifically, some asthma patients who carry certain SNPs in the ALOX5 gene were found to have a reduced or absent response to these inhibitors. This highlights the role of
genetic variability in determining the effectiveness of targeted therapies and emphasizes the importance of considering individual genetic profiles in treatment planning [5].

2.1.3 Drug metabolising enzymes

One of the earliest and most extensively studied categories of pharmacogenomic markers are drug-metabolizing enzymes. These consist of genetic variants in genes that encode proteins responsible for the absorption, distribution, metabolism, and excretion of drugs. Since these processes can vary significantly between individuals, they are considered strong candidates for pharmacogenomic research. Variants in several drug-metabolizing enzymes have been associated with adverse drug reactions.

An example of a drug-metabolizing enzyme is thiopurine methyltransferase (TPMT), which breaks down thiopurines. These drugs, including mercaptopurine, azathioprine, and thioguanine, are commonly used to treat cancers, autoimmune disorders, and in transplant patients. Individuals with reduced TPMT activity metabolize thiopurines poorly, leading to the accumulation of toxic 6-thioguanine nucleotides at significantly higher levels. The genetic basis for TPMT deficiency is well understood, and genotyping to identify poor

metabolizers before starting thiopurine therapy can help determine those who require much lower doses, preventing toxicity and treatment failure [5,8]

Examples of Notable SNPs in DMEs:

CYP2D6: Over 100 variants of CYP2D6 affect drug metabolism, with some resulting in poor metabolism (e.g., CYP2D6,3,4,

5) and others in ultra-rapid metabolism due to gene duplications [2, 10].

CYP2C19: SNPs like CYP2C19 2 and 3 reduce enzyme activity, leading to impaired metabolism of drugs like clopidogrel [11].

UGT1A1: The UGT1A1 28 variant is associated with reduced glucuronidation capacity, impacting drugs like irinotecan and increasing toxicity risk [12].

TPMT (Thiopurine S-Methyltransferase): Polymorphisms such as TPMT 3A, 2, and 3C significantly reduce TPMT activity, affecting thiopurine metabolism and increasing toxicity risk [13].

2.2 Whole Genome Linkage Disequilibrium Mapping Studies

This is an alternative to the Candidate gene approach. This method depends on linkage disequilibrium (LD), which refers to the non-random association of nearby SNPs. It requires the identification and mapping of tens to hundreds of thousands of anonymous SNPs within the genome. Whole-genome linkage disequilibrium (LD) mapping studies are a powerful tool in genetic research aimed at identifying the genetic variants associated with specific traits or diseases. These studies leverage patterns of linkage disequilibrium the non-random association of alleles at different loci in a population to map genes that contribute to complex phenotype.

2.2.1. Introduction to Linkage Disequilibrium Mapping

Linkage Disequilibrium (LD) refers to the non-random association between alleles at two or more loci. If alleles are in LD, it means their combination is more frequent than expected by chance. In whole-genome LD mapping, researchers use this principle to identify loci associated with traits of interest (such as diseases, drug response, or physical traits) by studying the correlation between genetic variants (often single nucleotide polymorphisms, or SNPs) across the entire genome. The effectiveness of linkage disequilibrium (LD) mapping relies on the allele frequencies of SNPs and the degree of LD between them. Genome-wide LD mapping assumes the presence of LD between SNPs, which is influenced by factors like population admixture, genetic drift, mutation, and natural selection. LD typically decreases as the distance between SNPs increases, especially over 10-100 kb. However, it can vary significantly between different genomic regions, with some regions showing extensive LD over several thousand kilobases and others, particularly around single genes, showing much less LD.

Understanding the average extent of LD is essential for estimating the number of markers needed for an SNP map and the strength of their associations. Theoretical estimates of LD in the human genome vary, ranging from less than 3 kb to less than 100 kb. Recent studies show that LD tends to be stronger between nearby markers, though the genomic location of markers is more important than their physical distance. LD also varies across populations, with stronger LD generally observed in Asians compared to Europeans and Africans.

The LD measure, denoted as D', ranges from 0 (no disequilibrium) to ±1 (complete disequilibrium), and several studies report average values of D' close to 0.70 for markers less than 500 kb apart. However, estimates of how much useful LD exists for markers within 50 kb differ widely. While some studies suggest that half of the markers within this range are useful, others suggest that markers need to be much closer, within 5 kb, to be informative.

Further research is needed to map the patterns of LD across the genome and among different ethnic groups to create effective SNP maps. Regions with weak LD will require dense SNP maps, while regions with extensive LD will require fewer SNPs. Although LD mapping has been used successfully in genetic studies of families with monogenic diseases, it has not yet been widely successful in studying unrelated individuals for diseases or drug responses. Improved analytical methods may make this approach more feasible in the future.

2.2.2 Key SNPs in Pharmacogenomics: Relevance, and Genomic Locations

The human genome, consisting of 3.2 billion base pairs, is estimated to contain around 11 million SNPs with a minor allele frequency of at least 1%. These SNPs arose from mutations that occurred at a rate of approximately 1 nucleotide per 1,331 base pairs. Efforts like the Human Genome Project and the SNP Consortium, led by the Wellcome Trust (a company), have focused on identifying and mapping these SNPs. As a result, public databases are now filled with SNPs spread across the genome, including within and around genes. To determine which SNPs are valuable for pharmacogenomic studies, it's essential to understand what makes a SNP useful [5].

Most SNPs (single nucleotide polymorphisms) in the genome are located within non-coding regions, which make up about 99% of the genome, and may not have a direct role in disease or drug response. However, these SNPs can still be valuable as markers for linkage disequilibrium (LD) mapping studies. SNP maps should be designed with genomic organization in mind, as genes are not randomly distributed. SNPs located in gene-rich regions are likely to be more useful than those in gene-poor areas. Genotyping SNPs in repetitive elements, which make up about 43% of the genome, can be challenging. SNP density in mapping studies should also be adjusted based on patterns of LD, with denser maps needed in regions of low LD and sparser maps in areas of high LD.

For candidate gene studies, the most relevant SNPs are found within genes, either in the coding region, where they can alter protein structure, or in regulatory regions, where they can affect gene expression. The International SNP Map Working Group (ISMWG) identified an average of two coding SNPs (cSNPs) per gene, while more focused studies found up to four. If approximately 10% of the 30,000 genes in the human genome are involved in drug response, around 12,000 candidate cSNPs may exist, with about 4,800 of these likely to alter amino acids.

SNPs in regulatory regions upstream or downstream of genes may also influence gene expression by affecting regulatory factor binding. Although the exact location of most regulatory elements is unknown, they can sometimes be identified through computational methods or genome-wide expression studies. When regulatory regions are not well defined, it
may be necessary to examine several thousand base pairs around the gene. However, this approach might miss regulatory regions located further away.

SNPs in intronic regions should also be considered, as they can influence gene splicing of expression. SNPs near intron-exon boundaries are particularly good candidates for affecting splicing, though splice sites can also be found deeper within introns. For example, splice site variants in the SURF1 gene are associated with Leigh syndrome due to loss of the SURF1 protein. Intronic sequences can also play regulatory roles, as demonstrated by a regulatory intronic sequence in the BRCA1 gene that acts as a transcription repressor.

2.2.3 Number of SNPs Required for LD Mapping Studies

The size or density of a useful SNP map is partly influenced by the patterns and strength of linkage disequilibrium (LD) across the genome. LD mapping depends on the ability to detect a susceptibility allele using a marker located within the interval provided by the SNP map's density. Considering the human genome is around 3 billion base pairs, at least 30,000 evenly spaced SNP markers would be necessary to place a marker every 100 kilobases (kb), which corresponds to the maximum estimated average extent of LD. Since this extent represents an ideal scenario, 30,000 markers are the minimum required. As the LD patterns in the human genome are empirically defined, the optimal number and distribution of SNPs for whole genome LD mapping will become clearer. A more efficient SNP selection could be achieved by first identifying blocks of LD [5].

2.2.4 Concept of Haplotypes

Haplotypes are combinations of SNPs inherited together on a chromosome [5]. They are groups of closely linked polymorphisms on a chromosome that are inherited together due to strong linkage disequilibrium (LD). Although haplotypes themselves may not affect drug response, they can act as markers for underlaying cause of the drug response and nearby causative SNPs. This allows associations between genes and drug response to be identified without needing to pinpoint the specific variant first. An example is the identification of the HFE gene in hereditary hemochromatosis through LD with MHC genotypes. The MHC is located on chromosome 6 which is associated with the genes involved in immune regulation. In some cases, specific haplotypes can directly influence drug response, as shown in a study where variations in haplotypes of the β2-adrenergic receptor gene significantly impacted responses to albuterol, unlike the individual SNPs involved. The authors discovered that the average responses differed by more than 2 times for different combinations of genetic variations (haplotypes). While the haplotypes were strongly linked to

the response, the individual genetic markers (SNPs) making up the haplotypes were not. These specific haplotype combinations were later found to be associated with the levels of both the mRNA and protein of the β2-adrenergic receptor in lab studies, giving support to the biological relevance of the findings [5].

3. Single Nucleotide Polymorphisms associated with the Rheumatoid Arthritis, an autoimmune disease

3.1Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease in which there is joint inflammation, synovial proliferation and destruction of articular cartilage. Immune complexes composed of IgM activate complement and release cytokines (mainly TNFα and IL-1) which are chemotactic for neutrophils. These inflammatory cells secrete lysosomal enzymes which damage cartilage and erode bone, while PGs produced in the process cause vasodilatation and pain. RA is a chronic progressive, crippling disorder with a waxing and waning course. NSAIDs are the first line drugs and afford symptomatic relief in pain, swelling, morning stiffness, immobility, but do not arrest the disease process [22].

3.2 Genetic Association of RA

Unlike single-gene disorders, rheumatoid arthritis (RA) is thought to involve multiple genes and how they interact with each other. The strongest genetic link is found in the HLA-DRB1 region (on chromosome 6p21), which accounts for about 30% of the genetic influence on RA. Beyond the HLA region, other genes, such as PTPN22 and PADI4, have also been identified as contributors to the risk of developing RA [23].

Genetic differences don't just affect a person's risk of developing rheumatoid arthritis (RA); they also influence things like whether they test positive for rheumatoid factor (RF), when the disease starts, how well they respond to medications, and their chances of developing heart problems.

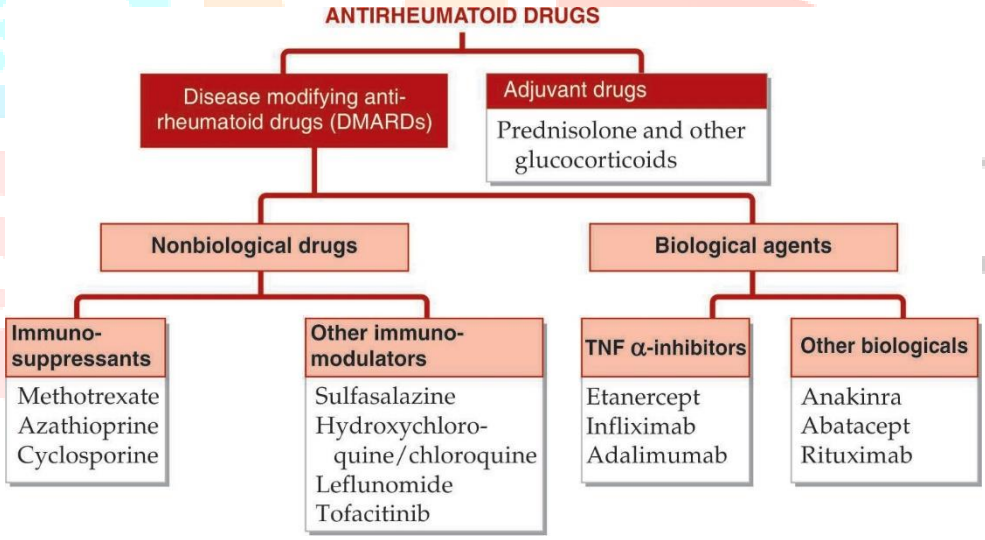

fig.no.2 classification of antirheumatoid drugs

3.3 Role of HLA Genes in Rheumatoid Arthritis Development

The most important genes so far identified to confer susceptibility to RA lie within the MHC region of the genome. This region on chromosome 6p21.3 spans around 4 million base pairs and contains about 250 genes that assist the immune system to recognize antigens. It is estimated that as many as 60% of the genetic variants associated with RA come from this region of the MHC.

One such gene in this region is the HLA-DRB1, which encodes variants known as the shared epitope; it's of great importance in T cell presentation. Approximately 18% of the heritable components in ACPA-positive RA and 2.4% in ACPA-negative RA are due to these HLA-DRB1 alleles. Roughly between 64% to 70% of RA patients and 55% of their healthy relatives bear these predisposing HLA-DRB1 SE alleles, whereas the prevalence was only 35% within the general population.

Some amino acids are critical in facilitating intracellular trafficking of HLA-DR molecules. Citrulline residues are well fitted into the positively charged pocket of the DRB1 whereas they do not fit the negatively charged pocket due to the protective allele. The studies indicate that positions 13 and 71 are crucial for the amino acids for the binding to citrulline. These positions are therefore essential in the development of RA.The variations at the unique positions of the amino acids in HLA-DRB1 as well as the positions 9 for HLA-B and HLA-DPB1 define the risk for RA development.the amino acid at position 13 is an important factor for RA development in any population.The relationships between HLA variants and RA are present in ACPA-positive as well as in ACPA-negative cases. aspartic acid at position 9 have also been linked to ACPA-negative RA in other populations. This, therefore brings out the genetic heterogeneity of the disease. Thus, it means, the amino acid positions of the HLA molecules associated with RA are located within the centre of the binding clefts.

In addition to knowing about the polymorphisms that predispose to RA, the polymorphisms associated with the prognosis of RA and the severity of the disease are of interest. For example, the HLA-DRB108, HLA-DRB111, and HLA-DPB102:01 alleles seem to be associated with oligoarticular RA (that occurs in up to four joints), while polyarticular RA-which affects five or more joints-is associated with the HLA-DRB111:01 and HLA-DRB111:04 alleles [24,25]. In a meta-analysis of 2,775 cases of RA, the HLA-DRB104:01 polymorphism seems to have been associated with the degree of joint damage. To conclude, although there is a particular genetic contribution to the risk of developing RA from certain alleles of HLA genes, several other genes encoding enzymes, receptors, and supportive proteins modulate the clinical symptoms of disease severity [25].

3.4Therapy of Rheumatoid Arthritis with Antirheumatic Drugs

The primary goal in treating rheumatoid arthritis (RA) is to achieve clinical remission or maintain low disease activity. The treatment involves the use of disease-modifying antirheumatic drugs (DMARDs). There are two major groups of DMARDs: synthetic DMARDs and biological DMARDs.

1) Synthetic DMARDs:

a) Conventional synthetic DMARDs (csDMARDs): These include methotrexate (MTX), leflunomide, sulfasalazine, and hydroxychloroquine. Methotrexate is the most commonly used csDMARD and can be used alone or in combination with others. If methotrexate cannot be tolerated, leflunomide or sulfasalazine may be prescribed.

b) Targeted synthetic DMARDs (tsDMARDs): These include baricitinib, tofacitinib, and upadacitinib, which are specifically designed to target certain pathways involved in RA.

2. Biological DMARDs (bDMARDs):

These drugs target specific molecules involved in the inflammatory process and are divided into several classes:

A) TNF inhibitors: (adalimumab, infliximab)

B) IL6 pathway inhibitors: (sarilumab, tocilizumab) could be used as a treatment of choice

C) B-cell inhibitors: targeting CD20 (rituximab)

D) T-cell costimulatory inhibitors: (abatacept)

According to the European League Against Rheumatism (EULAR) guidelines (2019), methotrexate should be initiated as soon as RA is diagnosed. It can be used alone or combined with other csDMARDs, tsDMARDs, or bDMARDs. If methotrexate is ineffective after six months, the second line of therapy is used, which may include adding another csDMARD or switching to a bDMARD or JAK inhibitor (like tsDMARDs). If there are factors suggesting a poor prognosis (such as high levels of rheumatoid factor or anticitrullinated protein antibodies, high disease activity, or early joint damage), a bDMARD or JAK inhibitor may be started earlier. If remission is achieved, DMARD doses can be reduced and the patient's condition closely monitored.

3.5 Genetic Variants Affecting DMARD Effectiveness

Several genetic polymorphisms have been associated with how patients respond to DMARDs, particularly methotrexate. Some of the key genetic variations influencing methotrexate response include:

1) ABCB1 gene: The T-allele is linked to a better response to methotrexate. ABCB1 gene is the gene that codes for P – glycoprotein. P- glycoprotein is the glycoprotein which helps in removal of toxic molecules as well as xenobiotics from the cells. Hence, expression of this gene in indivisual can impact the response of patient to the drug like MTX.

2) TYMS gene: These patients require higher doses of methotrexate. The gene TYMS encodes an enzyme that is essential for the synthesis of Thymidine.

3) ATIC gene (C > G substitution at position 347): This variant is linked to methotrexate toxicity.
Other genes associated with methotrexate response and toxicity include AMPD1 (34C/T), ATIC (675T/C), and SLC19A1 (80G/A). In particular, the MTHFR gene (C677T and A1298C variants) has been associated with increased side effects.
Resistance to methotrexate is linked to certain gene polymorphisms, such as 3435C/T in ABCB1 and 28-bp 2R/3R in TYMS.

Some studies also point to genetic markers affecting the response to glucocorticoids, which are sometimes used as transitional therapy before DMARDs take full effect. Polymorphisms in the GLCCI1 gene have been associated with reduced glucocorticoid sensitivity in men [25].
These genetic insights suggest that in the future, personalized treatment plans based on a patient’s genetic makeup may optimize DMARD therapy and reduce the risk of side effects.

4. Determination of the Single Nucleotide Polymorphisms

DNA biosensors have become a popular tool for detecting genetic variations, including single nucleotide polymorphisms (SNPs), due to their high sensitivity, quick response times, ease of use, and affordability. In recent years, a variety of biosensors have been developed for this purpose, categorized based on the type of signal conversion element they use. The most common types include fluorescent biosensors and electrochemical biosensors, which are frequently employed for disease diagnosis and analyzing genetic variations.

4.1 Fluorescent Biosensors

Fluorescent biosensors are the most common type of optical biosensor and are widely used for detecting SNPs due to their ease of use and high sensitivity. In these biosensors, fluorescent probes bind to the target substance, and a transducer converts the interaction into a fluorescent signal, allowing for the precise measurement of a specific target.
Xiao and colleagues developed a novel branch-migration molecular (BM) probe that can identify both known and unknown single-base variations, even in regions with high GC content, with a sensitivity of 0.3–1%. This innovation used strand exchange and displacement reactions in oligodeoxynucleotide (ODN) molecular probes to greatly enhance detection specificity and accuracy. Additionally, Yu and colleagues leveraged toehold exchange (TE) technology to create a fluorescent assay that uses strand displacement and nuclease-mediated digestion to detect point mutations. This method is highly effective, with a 50–1000-fold distinction between different single-nucleotide mutations and a detection limit as low as 200 Pm
Compared to organic dyes and fluorescent proteins, nano-fluorescent materials offer significant advantages such as excellent photostability, higher fluorescence output, and unique optical properties that depend on

their size. These nanomaterials also have a large surface area, which makes it easier to bind various biorecognition molecules. When single-nucleotide polymorphisms (SNPs) are integrated with fluorescent nanomaterials, they create highly sensitive biosensors.

Silver nanoclusters (AgNCs) stand out as excellent fluorescent materials due to their high fluorescence efficiency, good biocompatibility, and strong photostability. Liu and colleagues developed a new AgNC-based biosensor for SNP detection. They used AgNCs to create nanocluster dimers (NCDs). When SNP interactions happened at different positions, the spacing between the two AgNCs increased, which enhanced the fluorescence intensity. As the mismatch in the target DNA moved, the fluorescence intensity gradually decreased. This system allows for precise SNP location detection in a sensitive, low-cost, and enzyme-free manner.

Guo and his team also created a DNA probe with a cytosine loop scaffold that generates fluorescent AgNCs. This method was highly dependent on the DNA sequence and could accurately identify single-nucleotide mutations, including those linked to sickle cell anemia.

Furthering this technology, Martinez and colleagues developed a fluorescent molecular probe called a nanocluster beacon (NCB), which emitted different colors when binding to SNP targets. By recognizing specific DNA sequences through AgNCs, the color of the fluorescence changed dramatically. This method has been used to detect three synthetic DNA targets and six SNPs associated with diseases.

4.2 Electrochemical Biosensors

Electrochemical biosensing methods have gained popularity because they are sensitive, selective, quick, and easy to miniaturize. A variety of approaches have been developed to improve how well these sensors recognize targets and transmit signals. Gao and colleagues created a reusable DNA sensor for detecting SNPs (single nucleotide polymorphisms) using enzyme-free, LNA-integrated, and toehold-mediated strand displacement reaction (SDR) techniques. This sensor not only provides precise SNP detection but also works effectively in complex, contaminated samples, such as human urine, soil, saliva, and even beer.

Zhao and colleagues created an ultra-sensitive electrochemical method to detect point mutations in the K-ras gene. They achieved this by combining streptavidin horseradish peroxidase (streptavidin-HRP)-modified SiO2 nanoparticles with DNA polymerase in a sandwich design. In this setup, the streptavidin-HRP-SiO2 nanoparticles amplified the signal. The HRP enzyme catalyzed a reaction with 3,3’,5,5’-tetramethylbenzidine (TMB), generating an electrochemical signal. This method demonstrated a wide linear detection range of 0.001 to 100 pM and a detection limit of 0.42 fM. Its simplicity and cost-effectiveness make it a strong alternative to PCR-based assays.

DNA sequencing and DNA microarrays are the most popular methods for identifying single nucleotide polymorphisms (SNPs) because of their high throughput and cost effectiveness. However to get highly specific SNP detection nanotechnology based approaches can provide a solution. Nanotechnology enhanced electrochemical sensors have shown great promise in detecting mismatched base pairs in DNA. One of the widely used tool for SNP detection is DNA stabilized gold nanoparticles (AuNPs). For example Han et al. developed a simple and ultrasensitive DNA biosensor using urchin-like carbon nanotube–AuNP-conjugated (CNT-AuNP) nanocluster signal amplification. In their design a dopamine modified gold electrode was attached to DNA probes, DNA functionalized AuNPs bind to the sensor through complementary DNA base pairing. Then carbon nanotubes (CNTs) with end modified DNA linked up with AuNPs, forming 3D radial nanoclusters that generated significant electrochemical signals. Due to the large surface area and excellent electronic conductivity of CNT-AuNP clusters this 3D nanostructure showed ultra sensitive detection, high selectivity and strong stability and regeneration ability for DNA detection with a detection limit of 5.2 fM. Nanotechnology enhanced electrochemical biosensors have also shown great potential to distinguish single base mutations. Integration of graphene (GR) into electrochemical biosensors has gained much attention due to its 2D structure and outstanding properties such as large surface area, efficient electron transport and good biocompatibility. Khoshfetrat and Mehrgardi developed a graphene–gold nanoparticle (GR-AuNPs) nanocomposite based biosensor using triple amplification strategy for SNP detection. This innovative design showed excellent sensitivity and specificity for G-T and A-C mismatch targets with detection limits of 2 pM and 10 pM respectively. This graphene based assay can play a significant role in detecting SNPs related to various diseases[2].

Hwang and his team just created an unlabeled SNP sensing platform. To make it more effective, they combined DNA nanotweezer probes with graphene field-effect transistor chips. This way, real-time wireless signal transmission is possible when SNPs are detected. In their design, the DNA nanotweezers were attached to the surface of the graphene and SNP genotyping was done using the GR field-effect transistor sensor. And wow, this is 1,000 times more sensitive than the old way. Big progress in SNP genotyping technology[2].

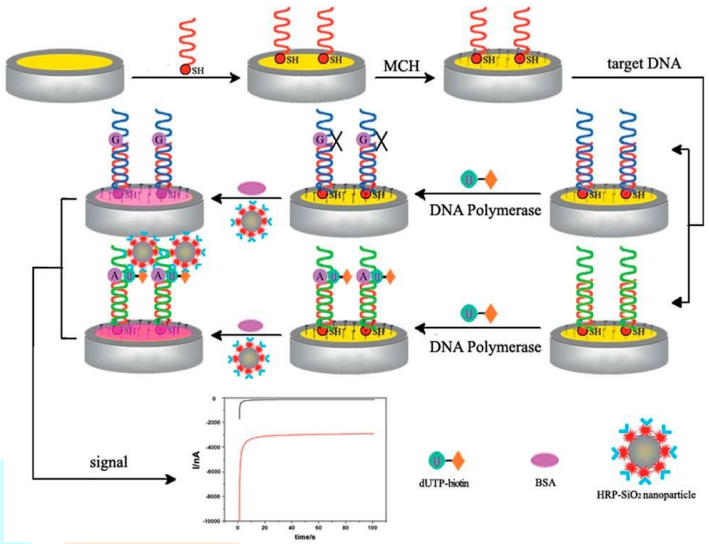

fig.no.4 electrochemical biosensor for detecting snps in the k-ras gene [2].

5. CONCLUSION

From the above review paper we learnt that the SNPs are important factor to be considered for the treatment guideline of the diseases which are associated with the genes in body. Firstly, we saw the approaches regarding the pharmacogenomic studies of SNPs, the use of SNPs in pharmacogenomics marks a major step toward personalized medicine, helping us understand how genetic differences affect drug responses, effectiveness, and safety. The candidate gene approach offers a focused view by examining genes already known to play a role in disease, drug targets, and metabolism, allowing researchers to identify genetic markers linked to specific drug responses. Meanwhile, whole-genome linkage mapping provides a broader search for SNPs across the entire genome, uncovering new genetic factors that impact drug reactions. Together, these methods leverage genetic variation to improve drug choices, dosing, and safety, with pharmacogenomics holding strong potential to enhance treatment success and reduce side effects. then the SNPs which are associated with the Autoimmune disease, Rheumatoid Arthritis. Lastly we saw some biosensors used for the detection of these biological markers.

Here we learnt that, by merging genetic approach we can think about the novel techniques or the approaches for right line of treatment to the right patient, disease treatment, drug resistance and it's response to the treatment. Although they still faces challenges on which the future research must be done but the continue efforts will show the impressive and widespread genetic testing in clinical applications

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