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# Genetic Polymorphism Studies and their Application on livestock Breeding and Health

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**Abstract:** This review investigated the genetic polymorphism applications on the breeding and the health of farm animals. Genetic polymorphism is the existence of at least two variants with respect to gene sequences, chromosome structure, or a phenotype (gene sequences and chromosomal variants are seen at the frequency of 1% or higher). Polymorphisms occur within gene sequences and also found outside of genes, in the vast quantity of DNA that does not code for protein. SNPs are the most simple form and most common source of genetic polymorphism in the human genome (90% of all human DNA polymorphisms). SNPs mostly are located in noncoding regions of the genome and have mostly no direct known impact on the phenotype of an individual but their role till now remains elusive. Indels are DNA variation type in which a specific nucleotide sequence of different lengths ranging from one to several 100 base pairs is inserted or deleted. VNTRs are repeated units of two or more nucleotides that based on the size of each repeat unit, satellite repeats can be further divided into microsatellites, minisatellites and microsatellites. Another frequent source of genome variability is structural and copy number variations (CNVs). DNA-based molecular markers of various forms can be tracked using a variety of techniques. These techniques include restriction fragment length polymorphisms (RFLPs) with Southern blots and polymerase chain reactions (PCRs), real time PCR, genomic array technology and Sequencing. Molecular markers have many applications on health and breeding; Gene mapping and mapping of QTL by linkage, gender Identification, pre and post natal diagnosis of diseases, anthropological and molecular evolution studies and in animal breeding such as marker assisted selection.

**Key words:** Genetic Polymorphism · Molecular Markers · Restriction Fragment Length Polymorphisms · Single Nucleotide Polymorphism

## INTRODUCTION

Genetic polymorphism is the existence of at least two variants with respect to gene sequences, chromosome structure, or a phenotype (gene sequences and chromosomal variants are seen at the frequency of 1% or higher), typical of a polymorphism, rather than the focus being on rare variants [1].

At deoxyribonucleoside triphosphate **(**DNA) level genomic variability exists in the nucleus or mitochondria. There are two major sources for genomic variability the first one is mutations that may result as chance processes or have been induced by external agents such as radiation and another one is recombination. Once formed, it can be inherited, allowing its inheritance to be tracked from parent to child [2].

Polymorphisms occur within gene sequences and also found outside of genes, in the vast quantity of DNA that does not code for protein. In fact, regions of DNA that dis not code for proteins likely to have more polymorphism. This is because a change in the DNA sequences that encode proteins may have a damaging effect on the individual who carries it. Synonymous polymorphisms those that cause no amino acid change in the protein produced and do not have any effect on the organism as the selectively neutral. This is also called a silent mutation [3].

Non-synonymous substitutions are those form of polymorphisms which results in an alteration of the encoded amino acid. A missense mutation changes the protein by causing a change in the codon. A nonsense mutation results in a misplaced termination codon.

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One half of all coding sequence SNPs result in nonsynonymous codon changes [2].

On equivalent chromosome of any two individuals when the genomic DNA sequences are compared, there is ample variation in the sequence at many points throughout the genome. Many forms of these genetic variations are available [4]. The simplest type results from a single base mutation substitute's one nucleotide for another and is known as single nucleotide polymorphism (SNP). The most common insertion/deletion events occur in repetitive sequences elements, where the repeated nucleotide patterns or variable number tandem repeat polymorphisms (VNTRs) expands or contacts as a result of insertion or deletion [5].

Since understanding the applications of various forms of molecular diagnostic techniques is very crucial in improving the production and productivity of livestock, this seminar was aimed: to review the recent available literatures on genetic polymorphism studies and their application on breeding and health.

**Types of Dna Polymorphisms**

**Single Nucleotide Polymorphisms:** Single nucleotide polymorphism (also referred to as "snip") is the most recent contribution to studying DNA sequence variation. SNPs are the most simple form and most common source of genetic polymorphism in the human genome (90% of all human DNA polymorphisms) [6]. It is the most common type of mutational change in which a nucleotide changes from one purine base to another (e.g. adenine to guanine), which is called a transition, or from a purine to a pyrimidine base (e.g. guanine to cytosine, or vice versa), which is called a transversion. There are an estimated 3-10 million SNP variants in the human genome with a frequency higher than 1% [7].

Single base pair change guides to single nucleotide variant, presumably accounting for many genetic conditions caused by single gene or multiple genes [8]. These markers are abundant and found in both coding and non-coding regions of the genome [9]. SNPs mostly are located in noncoding regions of the genome and have mostly no direct known impact on the phenotype of an individual but their role till now remains elusive and depending on where SNPs occurs, it might have different consequences at the phenotypic level [2]. SNPs within a coding region cause genetic diseases including sickle cell anemia. SNPs responsible for a disease can also occur in any genetic region that can eventually affect the expression activity of genes, for example, in promoter regions [10].

**Insertion/deletion Polymorphisms (Indels):** Indels are DNA variation type in which a specific nucleotide sequence of different lengths ranging from one to several 100 base pairs (bp) is inserted or deleted. They are widely spread throughout the genome. Some scholars are considering one base pair as SNPs or repeat insertion/deletion as indels [10].

**Tandem Repeat Polymorphisms:** Variable number tandem repeats (VNTRs) are repeated units of two or more nucleotides that occur throughout an organism's genome [11]. By far they are common throughout the genomes of a wide range of species, including humans and are often highly conserved, suggesting that they have an important function [12]. Indeed, centromeres and telomeres largely comprise tandem repeats [11].

VNTRs are organized in a head-to-tail orientation; based on the size of each repeat unit, satellite repeats can be further divided into macrosatellites, minisatellites and microsatellites [13]. Some of these repeats are described as follows: macrosatellites, with sequence repeats longer than 100 bp, are the largest of the tandem DNA repeats and located on one or multiple chromosomes [14], minisatellites, stretches of DNA, are characterized by moderate length patterns, 10-100 bp usually less than 50 bp [10] and microsatellites also known as short tandem repeats (STRs) repeat units of less than 10 bp [2].

Microsatellites are short tandem repeats (STRs), repeat units, or motifs of less than 10 bp; because of high variability, microsatellite loci are often used in forensics, population genetics and genetic genealogy. Significant associations were demonstrated between microsatellite variants and many diseases [15].

**Structural and Copy Number Variations:** Another frequent source of genome variability is structural and copy number variations (CNVs) [16]. A CNV is a variation in which a segment of DNA can be found in different copy numbers in the genomes of different individuals. CNVs range in size from a few hundred nucleotides to several megabases. Compared with SNPs, CNVs affect a more significant fraction of the genome and arise more frequently. Hence, CNVs significantly contribute to human evolution, genetic diversity and an increasing number of phenotypic traits [17].

**Major Technique for Dna-based Molecular Marker Detection:** DNA-based molecular markers of various forms can be tracked using a variety of techniques. Some of these techniques include Restriction fragment

length polymorphisms (RFLPs) with Southern blots and polymerase chain reactions (PCRs). Sophisticated advances in methodology for DNA polymorphisms detection are using real time PCR, hybridization techniques using DNA microarray chips and genome sequencing each of the technique has its own advantage and disadvantage [18].

**Restriction Fragment Length Polymorphism (pcr-rflp):** This technique relies on the amplification of variable regions of the target genome, with the amplicon then being digested with one or more sequence-specific restriction enzymes [10]. The DNA fragments of different lengths are then subjected to electrophoresis and fragments migrate according to their weights, the smaller fragments faster and the larger fragments more slowly [18].

Thus, RFLP generally refers to the differences in banding patterns obtained from DNA fragments, after sequence-specific cleavage with restriction enzymes. Before the advent of PCR, RFLP analyses typically entailed restriction enzyme (RE) digestion of chromosomal DNA, followed by electrophoretic separation, membrane blotting and hybridization with a labeled probe, usually radioactive [18]. The method of hybridization of DNA with probes is called Southern blotting, after the name of the inventor, Southern [19].

Although reliable, RFLP process is requires relatively large amounts of DNA. Hence, it cannot be performed with the samples degraded by environmental factors and also takes longer time to get. PCR-RFLP is, now replaced and therefore, probably the simplest PCR procedure for comparisons of sequence polymorphism [20].

**Polymerase Chain Reaction:** Amplification of particular DNA sequences in vitro with the help of specifically designed primers and DNA polymerase enzyme is done. The amplified fragments are separated by gel electrophoresis and detected by different staining methods. Real-time PCR useful modification of PCR can detect polymorphisms by various methodologies using real-time PCR chemistries, for example, TaqMan assay or molecular beacons [10].

**Genomic Array Technology:** Genomic array technology is a type of hybridization analysis allowing simultaneous study of large numbers of targets or samples in a very small area. In which automated depositing systems (arrayers) can place thousands of spots on glass substrate of the size of a microscope slide (chip) with spotting representative sequences of each gene in triplicate, simultaneous screening of the entire human genome on a single chip. The technique helps bring about the process of identifying specific homozygous and heterozygous alleles, by comparing the disparity of hybridization of the target DNA with each redundant probe. Microarray is also used to determine the characteristics of genetic diversity and drug responses, to identify new drug targets and to assess the toxicological properties of chemicals and pharmaceuticals [10].

**Sequencing:** That technologies for quick DNA sequencing have become available they are now to great degree used. There is a large scale progression for the detection of single nucleotide variants (SNVs) by direct sequencing, but intermediate-sized (from 50 bp to 50 kb) structural variants (SVs) remain a challenge. Such variants are too small to detect with cytogenetic methods but too large to reliably discover with short-read DNA sequencing [21].

Recent high-quality genome assemblies using long-read sequencing have showed that each human genome has approximately 20,000 structural variants, spanning 10 million base pairs, more than twice the number of bases affected by SNVs. New long-read sequencing approaches are needed to meet this challenge, as short-read sequencing technologies only detect 20% of the SVs present in the human genome [22].

Next-generation sequencing technologies, such as pyro sequencing, sequence less than 250 bases in a read, which limits their ability to sequence whole genomes. However, their ability to generate results in real-time and their potential to be massively scaled up makes them a viable option for sequencing small regions to perform SNP genotyping. Compared with other SNP genotyping methods, sequencing is particularly suited to identifying multiple SNPs in a small region, such as the highly polymorphic major histocompatibility complex region of the genome [23].

**Applications of Dna Markers on Health and Breeding:** DNA markers have a potential application over a relatively broad field in animal breeding and genetics. The technique has direct practical application for livestock breeders, DNA markers have a potential application over a relatively broad field in animal breeding and genetics. The technique has direct practical application for livestock breeders, for example for parentage verification, individual identification and

identification of certain genetic disorders. A very exciting and fast developing application of genetic markers is in the mapping of the various animal genomes. Conservationists also use various genetic markers in evolutionary and genetic biodiversity studies [24]. Molecular markers can easily be used as reference points in transgenic breeding and to identify the animals having the specific transgenes. Thus, the overall improvement in livestock species is greatly aided by the use of molecular markers [25].

**Genome Mapping:** Genome mapping is almost synonymous with the Human Genome Project which was established during 1990 as a 15 year project to map the estimated 30 000 genes in the human genome (www.ornl.gov/hgmis). This project had far reaching implications for genetic research including the quest to map genomes of other species [26].

Livestock genome mapping is a complex and time-consuming effort but, once completed, holds promise for finding functional genes, Quantitative Trait Loci (QTL) and genes associated with disease resistance. Advantages include the identification of conserved regions between species, important contributions to the search for QTL and the provision of valuable information for gene expression studies [27].

**Marker Assisted Selection:** Marker Assisted Selection (MAS) is a process in which a marker is used for indirect selection of a genetic determinant or determinants of a trait of interest, i.e., abiotic stress tolerance, disease resistance, productivity and/or quality [28]. The idea behind marker assisted selection is that there may be genes with significant effects that may be targeted specifically in selection. Some traits are controlled by single genes but most traits of economic importance are quantitative traits that most likely are controlled by a fairly large number of genes. However, some of these genes might have a larger effect. Such genes can be called major genes located at QTL [29].

In order to identify a QTL for a specific trait; many animals have genotyped for a large number of markers on different chromosomes. Phenotypic data for the trait is also required [30]. MAS programs usually require the identification of the QTL for the trait, evaluation of the QTL (where the markers are tested in target populations) and implementation, where the animals can be genotyped and genetic and phenotypic information be combined to predict genetic merit [18].

**Biodiversity Studies:** It is inevitable that selection, inbreeding and various crossbreeding systems may lead to the loss of genetic variation within breeds and that the breed itself may become extinct. The need to conserve genetic diversity is widely accepted for biological, economic and cultural reasons. A main reason is that an abundant resource of genetic diversity within each livestock species is the prerequisite of coping with putative future changes in livestock farming conditions [31].

The aim of studying genetic diversity is to understand the extent of differentiation of populations within species. Population-specific genetic markers (alleles) can be generated using a range of methods available for detection of polymorphic loci. A variety of different molecular techniques are being used in various laboratories for the study of inter-and intra-specific genetic variation at the DNA level [32].

The most widely used techniques are restriction fragment length polymorphism of nuclear DNA and mitochondrial DNA, minisatellites, randomly amplified polymorphic DNA, microsatellite, amplified fragment length polymorphism and sequencing of mitochondrial DNA [32]. Among these, microsatellites have quickly become the favorite agents for population genetic studies as they offer advantages which are particularly appropriate in conservation projects [33].

**Molecular Approaches to Disease Resistance:** Genetic resistance to infectious diseases has been a subject of many controversies. One of the applications of genetic improvement is to select animals resistant to diseases, especially those difficult and expensive to eradicate, in order to obtain healthy animals in which endogenous potentiality is optimized and therapeutic events reduced [34]. The linkage disequilibrium, genome scan approach using anonymous molecular markers is one of the major strategies used to identify QTL affecting economic traits. Many studies have mapped QTL affecting several economic important traits in farm animals [35].

**Parentage Determination:** The effectiveness of DNA fingerprints for parentage identification derives from the fact that over an entire population, each minisatellite locus exhibits a wide range of alleles, differing in their fragment lengths. As a result, over the population as a whole, numerous bands, differing in fragment length, can be identified, but only a few of these bands will be present in any one individual. There is thus only a small probability

that two randomly chosen individuals will share all, or even a large proportion of the bands in their respective DNA fingerprints [36].

Breeding programs have been of significant importance to improve productivity in the animal industry. In dairy cattle, progeny testing is the method of choice; however, this method presents two drawbacks limiting its use: high cost and increased generation intervals. These obstacles can be overcome when large numbers of progenies are obtained by artificial insemination [37].

DNA testing is highly accurate and reliable genetic analysis available for parentage testing. Since we utilize the most advanced genetic testing procedure, we are able to achieve at least specificity of 99.9%. In most cases, DNA testing will result in specificities of 99.99% or greater. If DNA patterns between the child and the alleged father do not match on three or more genetic markers, then the alleged father is excluded with 100% certainty. Parentage testing using molecular markers yields much higher exclusion probability (> 90%) than the testing with blood groups (70-90%) or other biochemical markers (40-60%) [35].

**Pharmacogenetics and its Applications:** The response of an individual to a drug is governed by many factors such as genetics, age, sex, environment and disease. The influence of genetic factors on the response of a drug is a known fact. Polymorphic STRs, together with SNPs and CNVs, can explain variability in response to pharmacotherapy because of their prevalence in the human genome and their functional role as regulators of gene expression and its applications. Pharmacogenetics is the study of the influence of genetics factors on drug response and metabolism. The science of pharmacogenetics when applied can be used to evade adverse drug reactions, predict toxicity and therapeutic failure and refine therapeutic efficiency and improve clinical outcomes [10].

## CONCLUSIONS

Livestock genome mapping is a complex and time-consuming effort but, once completed, holds promise for finding functional genes, Quantitative Trait Loci (QTL) and genes associated with disease resistance. Genetic polymorphisms are the different DNA sequences among individuals, groups, or populations. Genetic variation includes a wide range of variations from single base pair change, many base pairs and repeated sequences. Sources include single nucleotide polymorphisms (SNPs), sequence repeats, insertions, deletions and recombination. Genetic polymorphisms may be the result of chance processes or may have been induced by external agents such as viruses or radiation. If a difference in DNA sequence among individuals has been shown to be associated with disease, it will usually be called a genetic mutation.

Molecular markers are based on the nucleotide sequence mutations within the individual's genome they are the most reliable markers available. Today, researchers use molecular markers to find genes of interest that control how animals perform. Molecular marker that reveal polymorphism at gene level along with traditional selection method are now playing crucial role in selection of animals for improvement of milk and meat production at an early and in both sexes. Molecular markers have many applications on health and breeding; Gene mapping and mapping of QTL by linkage, gender Identification, pre and post natal diagnosis of diseases, anthropological and molecular evolution studies and in animal breeding.

Therefore, in light with above concluding remarks, the following recommendations are suggested:

- Possible types of genetic polymorphisms should be scientifically studied to ensure its applications in livestock improvements.
- Sound research should be conducted using model systems to test the consistency, efficiency and reproducibility of various forms of techniques

**Abbrevations:**

CNVs: Structural and copy number variations
DNA: Deoxyribonucleoside triphosphate
Indel: Insertion/deletion polymorphisms
MAS: Marker Assisted Selection
PCR: Polymerase chain reactions
QTL: Quantitative Trait Loci
RFLP: Restriction fragment length polymorphisms
SNPs: Single nucleotide polymorphisms
STRs: Short tandem repeats
VNTRs: variable number tandem repeats

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