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Review

The importance of genetics in the diagnosis of animal diseases - A review

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Genetic diseases have always been present in the animal population but their significance has increased in recent decades. The wealth of knowledge on genomic information, systems biology and mechanisms of diseases provide great opportunities to elucidate the genetic bases of diseases. The use of recombinant DNA techniques in conjunction with conventional genetic methods have led to a rapid increase in knowledge of the genetic map. Many animal genes have been mapped to chromosomes. A detailed genetic map has become of great value in the diagnosis of genetic diseases and in the development of potential cures through gene transfer therapy. In view of the emerging animal diseases like avian influenza, swine influenza among others with serious health implications for humans, this review aims at highlighting the association between diseases and genes in animals. The information derived could assist in the prevention and management of emerging animal diseases and in future drug discovery processes.

Key words: Genetics, diagnosis, animal diseases.

INTRODUCTION

The development in gene technology is based on the discoveries in molecular biology by Watson and Crick (1953), with the discovery of the double helical structure of DNA and the breaking of the genetic code. The model for the DNA molecule described by Watson and Crick explains how DNA molecules are duplicated, that is, the process of DNA replication. Deoxyribonucleic acid (DNA) is the most important molecule in living cells. It carries within its structure the hereditary information which determines the structures of proteins to be synthesized (Bewaji, 2003). The instructions for cell division, growth and differentiation are also encoded in DNA. DNA is usually located in the cell nucleus and mitochondria on coiled ladder-like structures called chromosomes. Cells which contain a nucleus are referred to as eukaryotic cells, while those without clearly defined nucleus, such as bacteria and blue-green algae, are known as prokaryotic cells. The number of chromosomes per cell varies with species. Bacterial cells usually contain between one and twenty extrachromosomal, small, circular, duplex DNA molecules called plasmids. These can be replicated along with the chromosomes. Some of them, called episomes, have the ability to move on and off the main chromosome.

Methods have been devised for isolating plasmids from bacteria, incorporating other DNA molecules into them and then re-introducing the new (hybrid) plasmid into the bacteria. The newly incorporated DNA will then replicate along with the rest of the plasmid.

It is now becoming increasingly clear that genes and their products interact in complex biological networks and distortions of these networks contribute to disease states (Sakharkar and Sakharkar, 2008). The network of genes and diseases demonstrates how the aberrant network gene/protein nodes contribute to disease(s) and defines the multiple protein nodes that can be targeted to restore the network. This provides an example of integrating molecular/biochemical and physiological information needed to capture the diverse characteristics of complex disorders.

Veterinary genetics encompasses those aspects of genetics that are relevant to animal diseases and to animal production (Nicholas, 1993). The importance of genetic diseases in animal population has increased in

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recent decades. This is because vaccination programmes, antibiotics and improved sanitations have reduced the incidence or prevalence of infectious diseases of animals. This results in a greater population of animals now dying from a disease that has genetic component, especially the late onset diseases. Veterinary and genetic research has been successfully used in diagnosis and control of many infectious diseases of animals. There are many reasons why identifying the gene responsible for a disease is important. These include:

1. All diseases have genetic bases (Murray et al., 2000).
2. Gene identification may provide an indication of the biochemical basis to the disease, thus enabling therapies to be designed (Brown, 1998).
3. Identification of the defective gene is a prerequisite for gene therapy.
4. Identification of the mutation present in a defective gene can be used to devise a screening programme so that the mutant gene can be identified in individuals who are carriers or who have not yet developed the disease (Brown, 1998).

Early identification of individual animals that have not yet developed the disease allows appropriate precautions to be taken to reduce the risk of the disease becoming expressed. During the last few years, a number of genes responsible for inherited diseases have been identified. Once a gene associated with a single gene disorder has been isolated and cloned, it is usually easy to develop a gene-based diagnostic test. This can be used to confirm the diagnosis of an existing condition, as a prognostic indicator that an individual animal will eventually develop a disease, or may be at increased risk of developing disease conditions or to reveal information on a healthy individual animal's carrier status. The growing number of pathogen sequencing programmes in which scientists are deciphering genomes of microbes of medical and veterinary importance is also beginning to yield new, less controversial diagnostic tools.

GENETICS AND ANIMAL DISEASES

The application of genetics to the diagnosis of animal diseases has been mentioned by Hauge, (1989). The availability of DNA clones or the knowledge of primer sequences from humans and experimental animals has enhanced this development. Examples of the use of genetics for the diagnosis of disease mutations include:

(i) Deficiency of uridine monophosphate synthase (DUMPS) in bovine is an autosomal recessive disorder in Holstein and Red Holstein cattle, that results in early embryonic death of homozygous offspring (Hauge, 1989). Heterozygotes are phenotypically normal, but show about one-half the normal enzyme level and elevated levels of orotic acid. Heterozygotes may have a higher genetic advantage in milk and protein production. With accurate DNA-based genotyping, it would be possible to preserve the mutation in the population while avoiding matings between carriers. Schober et al. (1992) have also isolated and sequenced wild type bovine UMPS cDNA using a human UMPS-specific cDNA to screen the library. Genetic studies were carried out which showed that complete concurrence between low levels of UMPS, presence of the point mutation was found in a large Holstein pedigree, and this confirmed that this mutation was the basic defect in DUMPS cattle.
(ii) Cittrullinemia in cattle: Cittrullinemia is widespread in Australian Friesian cattle and is the first disease to benefit from a DNA test (Hauge, 1989). Normal bovine cDNA for the enzyme (argininosuccinate synthetase) was isolated with a rat probe and sequenced by Dennis et al. (1989).
(iii) Marple syrup urine disease (MSUD) in bovine results from a deficiency of the branched chain α-ketoacid dehydrogenase, a mitochondrial multisubunit complex of enzymes and the E1 component catalyses the oxidative decarboxylation (Hauge, 1989). MSUD has been identified both in humans and in cattle and is relatively common in the Polled Hereford breed of cattle in Australia. Studies on MSUD have also been reported by Hu et al. (1988); Zhang et al. (1990); Healy and Dennis (1994).
(iv) Hyperkalemic periodic paralysis (HYPP) in Quarter Horses, a genetic disease observed among quarter horses (Hauge, 1989). The disease causes attacks of paralysis, inducible by ingestion of potassium. In humans, hyperkalemic periodic paralysis is caused by a single base substitution within the skeletal muscle sodium channel gene (Hauge, 1992). Studies on HYPP have also been reported by Rudolph et al. (1992a and b); Orita et al. (1989) and Neibergs et al. (1993).

Others examples of the use of genetics for the diagnosis of disease mutations are Leukocyte adhesion deficiency in Cattle and malignant hyperthermia in pigs.

Genetics and diagnosis of disease susceptibility

The relationship between diseases and alleles of genes in the major histocompatibility complex (MHC) is also known for domestic animals (Hauge, 1989). This has been reported for Marek's disease in chickens, which is caused by a herpes virus (Hanson et al., 1967; Hepkema et al., 1993); resistance to bovine leukosis, which is caused by BLV retrovirus, Lewin (1994) and Mejdell et al. (1994), reported an influence of the bovine MHC on resistance to mastitis. Andersson et al. (1986), Juul-Madsen et al. (1993) and Park et al. (1993) have also reported the association between diseases and alleles of genes in the major histocompatibility complex. More informative studies are now being conducted based on sequencing of the MHC polymorphic exons (Hauge, 1989)

and such data have been presented for cattle (Andersson et al., 1991), sheep (Fabb et al., 1993), chickens (Moon Sung et al., 1993), horses (Szalai et al., 1993) and pigs (Vage et al., 1994).

One major challenge in livestock improvement and productivity is the question of sustainability especially when positive changes, which have, genetic basis have been accidentally achieved through mere phenotypic analysis. Only the detection and full exploitation of such genetic contents can sustain such changes and put disease incidence, a significant component of productivity, on a permanent check.

Genetics and drug formulations for animal health

Drug development strategies have been influenced a lot by the potential targets offered by genome projects. Also, advances in genomics (Peltonen and McKusick, 2001; Janssens and Duijn, 2008), proteomics (Chung and Jordan, 2008) and understanding of the molecular mechanisms of diseases (Zhan, 2007) enable the search for new targets and facilitate the study of existing targets for finding clues to new target identification (Sakharkar et al., 2007; Sakharkar et al., 2007). These advances also help in probing the molecular mechanisms of drug actions, adverse drug reactions and the pharmacogenetic implications of variations in gene sequences and the profiles of gene expression and post-transcriptional processing (Cotsarelis and Millar, 2001, Nicholls, 2003; Roth et al., 2004; Morphy and Rankovic, 2005; Roden et al., 2006).

As knowledge about the biology of diseases like cancer is ever increasing, the chances of developing better ways to prevent and cure such diseases have been improving rapidly by the use of genetic engineering. Individuals at high risk of developing genetic diseases could be monitored carefully through out their lives for early signs of the diseases, thus assuring early diagnosis and maximizing chances for successful therapeutic intervention. Molecular biology has helped to advance the management of cancers in animals with the discovery and knowledge of P-glycoprotein. Considerable evidence has accumulated indicating that the multidrug transporter or P-glycoprotein plays a role in the development of simultaneous resistance to multiple cytotoxic drugs in cancer cells (Ambudkar et al., 1999). In recent years, various approaches such as mutational analysis and biochemical and pharmacological characterization had yielded significant information about the relationship of structure and function of P-glycoprotein. However, there is still considerable controversy about the mechanism of action of this afflux pump and its normal physiological role (Ambudkar et al., 1999). The final resolutions of the controversies surrounding P-glycoprotein and the ability to suppress its action could be a major breakthrough in the scientific world and a source of great relief to animal health care providers. In the area of drug metabolism, the gene cloning and molecular biology approaches to the structure, function and regulation of cytochrome P_{450} gene super family has enabled considerable progress in the characterization of cytochrome $P_{450.}$ A better understanding of the molecular mechanisms by which antimicrobial resistance emerges and spreads should enable us in the future to design intervention strategies to reduce its progression (Chen et al., 2008). Aminov et al. (2001) reported the molecular ecology of tetracycline resistance: Development and validation of primers for detection of tetracycline resistance genes encoding ribosomal protection proteins. In their study, based on phylogenetic analysis, a set of polymerase chain reaction (PCR) primers for detection, retrieval and sequence analysis of corresponding gene fragments from a variety of bacterial and environmental sources were developed and characterized. A pair of degenerate primers targeted at all tetracycline resistance genes were used to detect the circulation of these genes in the rumen of cows, in swine feed and faeces and in swine faecal streptococci. The report by Aminov et al. (2001) was the first demonstration of the applicability of molecular ecology techniques to estimate the gene pool and the flux of antibiotic resistance genes in production. They reported that development of genotyping pools for detection and tracking of antibiotic resistance genes in a variety of commensal and pathogenic bacteria, as well as in the environment, is essential for understanding the ecology of antibiotic resistance. Since their introduction in the 1950s, tetracyclines have been widely used in human and veterinary medicine, as growth promoters in animal industry and for prophylaxis in plant agriculture and aquaculture. At present, resistance to tetracyclines has spread to almost all bacteria genera and this situation is perhaps the consequence of previous overuse (Aminov et al., 2001). Their findings demand that there be further research targeted at development of genotyping tools for tracking the movement of antibiotic resistance genes in the environment.

The goal of drug discovery is to create a single chemical substance (drug) that interacts specifically with a single molecular target to reinstate healthy state biochemistry (Sakharkar and Sakharkar, 2008). However, it is observed that some of the most specific drugs are directed towards a target that is not central to the pathophysiology of the disease and simply produce improvements in a limited number of symptoms (Zheng et al., 2006; Yildirim et al., 2007). Also, many drugs designed to interact with a single target have unexpected effects on ‘‘off target’’ biochemical mechanisms (Greef and McBurney, 2005). Drug target networks are of general importance for the understanding of drug action (Sakharkar and Sakharkar, 2008).

Genetics and plant toxicity in animals

Fu et al. (2002) reported the genotoxic pyrrolizidine alkaloids-mechanisms leading to DNA adduct formation

and tumorigenicity. In their report, they noted that plants that contain pyrolizidine alkaloids are widely distributed in nature and that although pyrrolizidine alkaloids have been shown to be genotoxic and tumorigenic in experimental animals, their mechanisms of actions have not been fully understood. They conducted mechanistic studies which suggested that pyrrolizidine alkaloids induce tumours via a genotoxic mechanism mediated by 6, 7-dihydro-7-hydroxy-1-hydroxymethyl 5H-pyrrolizine (DHP)-derived DNA adduct formation. They reported that this mechanism may be general to most carcinogenic pyrrolizidine alkaloids. Livestock are poisoned by grazing on plants containing pyrrolizidine alkaloids, causing livestock loss due to liver and pulmonary lesions (Smith, 1981; Woo et al., 1988; Roeder, 1995; Stegelmeier et al., 1991; Roeder, 2000). It is now well recognized that a large variety of animal species are susceptible to pyrrolizidine alkaloid toxicity (Mattocks, 1968; 1986; IARC, 1976). Pyrrolizidine alkaloids also contaminate sources of human food such as milk, wheat, honey, herbal medicines and herbal teas and this may potentially cause human health problems worldwide (Hirono et al., 1976; Huxtable, 1980; Winship, 1991; Betz et al., 1994 and Prakash et al., 1999).

Genetics and ageing in mammals

Molecular biology has also brought more light into the principle and causes of ageing in mammals. Since molecular structure and function underlie all other levels of biological organization and since there are strong, precise research tools at this level, investigation of the molecular basis of ageing offers the prospect for both understanding and control of ageing (Brash et al., 1979).

Diagnosis and treatment of genetic diseases

Recombinant DNA technology has immensely contributed to the diagnosis of genetic diseases in fetuses and is also used in the treatment of some genetic disorders in animals. The basic approach to diagnosis of disease in foetus is to obtain foetal cells by amniocentesis (that is, a sample of amniotic fluid taken by inserting a needle into the amniotic cavity) and then test for biochemical defects. However, genetic defects may not be recognizable in the amniotic fluid cells obtained by amniocentesis through routine biochemical methods only (Daini, 2000). In the case of lethal recessive mutations, gene cloning provides the potential for direct diagnosis. After a normal gene has been cloned, the nucleotide sequence and restriction endonuclease map has been determined, test can then be devised to detect the mutations responsible for that particular defects. Alternatively, the normal gene can be used as a probe to isolate or locate the corresponding gene from the DNA of the foetal cells (Daini, 2000).

The restriction fragment length polymorphism (RFLP) is now being used as a tool in clinical diagnosis of human and animal diseases. This involves prenatal diagnosis using amniotic fluid, chorionic villus, northern and southern blotting, electrophoresis and hybridization. In developed countries, the use of RFLP in the diagnosis of diseases reduced the stress of gene disorders since a series of test will diagnose the abnormality and if it cannot be treated, then termination of such species may be necessary before birth (Correttee et al., 1998; Mosleni et al., 1998). Diagnosis and management of haemoglobinopathies is now possible through the use of RFLP analysis, indirect and direct analysis, using gene probe, gene expressions and other recombinant DNA techniques (Embury, 1995; Morti et al., 1997). In cases where the clinical phenotype is the result of more than the genetic factor interacting with environmental factor, recombinant DNA technology may be used to identify those individuals in the population most at risk (Singuret and Andreux, 1997). Genetic engineering techniques are the most sensitive and reliable human DNA fingerprinting for use in forensic analysis (Daini, 2000). These techniques could be maximized for the diagnosis and management of animal diseases (Soetan, 2009). The use of recombinant DNA techniques in conjunction with conventional genetic methods have led to a rapid increase in knowledge of the genetic map. Many animal genes have been mapped to chromosomes. A detailed genetic map is of great value in the diagnosis of genetic diseases and in the development of potential cures by gene therapy.

APPLICATIONS OF GENE PROBES

1. Restriction fragment length polymorphisms (RFLP). This exploits the fact that there is considerable phenotypically neutral sequence variation between individuals created by single base changes in the DNA sequence or by variation in the length of certain stretches of DNA due to insertion/deletion events (Daini, 2000). RFLP makes use of restriction endonucleases to determine if two or more DNA segments are similar. The segments being compared are cleaved or cut into discrete fragments with a restriction enzyme and separated according to size on an agarose gel. The gel is stained with ethidium bromide, visualized under ultraviolet (UV) light to reveal the fragments as bands on the gel, which can be photographed by instant polaroid camera. The resulting fragments, represented as bands on the photograph can be used to directly compare the different DNA segments. RFLP can be combined with Southern blotting and nucleic acid probes (Kwaga and Kabir, 1999). RFLP is also used to demonstrate the genetic basis of disease susceptibility due to the presence of unique polymorphism or sequences (Neibergs et al., 1994) or to determine whether a disease is caused by mutation. Changes in the virulence of certain infectious agents have been

demonstrated to be due to the acquisition of genetic elements (DNA or RNA) that code for the traits. Extra-chromosomal DNA (plasmids) that are known to be responsible for toxin production, antibiotic resistance and host adaptability have been shown in bacteria by the use of RFLP and other techniques (Farrar, 1983; Finlay, 1992).

RFLP is used predictively in antenatal diagnosis by determining which RFLP pattern a fetus at risk has inherited (Daini, 2000). This approach has been used in the clinical diagnosis of many monogenic disorders in animals.

2. Gene probe is used for the direct analysis of gene mutations using gene-specific probes. The understanding of the molecular pathology of animal diseases is most detailed for some genetic diseases which show considerable diversity in the molecular mechanisms of their aetiology for example haemoglobinopathies.

3. Gene Mapping: Genetic maps are essential for diagnostic and therapeutic purposes and for understanding of gene expression and regulation. Gene mapping can be used for identifying a gene of interest known to be near another gene for which a clone is available (Daini, 2000).

4. Indirect analysis using DNA probes: Recombinant DNA probes may also be used when the biochemical defect underlying a monogenic disorder is unknown. This again exploits linkage of a disease with RFLPs, but in this case the RFLPs are characterized by random cloned segments of DNA. The RFLPs may be localized several million base pairs away from the defective locus. RFLPs may be used as markers for mapping animal diseases. The RFLPs is used in the localization of genetic diseases where neither the biochemical defect nor the chromosome localization, has been particularly successful in the diagnosis of such diseases.

5. Gene probes are used for the diagnosis of disease states or the presence of infectious micro-organisms in body fluids. The earlier methods used for the detection of micro-organisms are relatively slow and involve culture and this can take several days. In contrast, DNA probe technique can be done within minutes or hours and also need little expertise. The major biological tool used is monoclonal antibody, an innovation that has brought major advances on biology and medical sciences in the area of disease diagnosis.

Gene probe is also used in the detection of sexually transmitted diseases and have advantages over the present methods used which are inadequate and expensive. Gene probe is also used to detect canine distemper virus in canine species and salmonella strains that affects dairy products. Also probes for the *Escherichia coli* strain that causes scours, a form of pig diarrhoea that results in high pig mortality have been produced (Daini, 2000).

6. Gene probes are also used in tissue typing. It involves cloning of the series encoding the histocompatibility antigens (HLA) which are the proteins that define immunological specificity of an individual. Through these, the immune system distinguishes between self and non-self (foreign antigens). The HLA system is used in tissue typing for organ transplants, blood transfusions etc. Instead of using antibodies to detect proteins, it is now possible to use DNA probes to detect the genes that code for them.

MARKER ASSISTED SELECTION (MAS)

This is the process of the selection for a particular trait using genetic markers. MAS can accelerate the rate of genetic progress by increasing accuracy of selection and by reducing the generation interval. The advantages of MAS are that it is greatest for traits with low heritability. It facilitates increased rate of genetic gain by allowing measurement in young stock thereby reducing generation interval. MAS could also enhance future prospects for breeding for such traits as tolerance or resistance to environmental stresses, including diseases. Identification of carriers of genes for resistance and introduction of such genes into a particular animal could be used for resistance against diseases. An example is the gene responsible for trypanotolerance in cattle. A genetic marker for a trait is a DNA segment which is associated with and hence segregates in a predictable pattern as the trait. Markers are also employed to determine the genetic relatedness in animals (Grunder et al., 1994; Medjugorac et al., 1994) using RFLP and other DNA fingerprinting methods, for purposes of trade, litigation or phylogenetic studies. DNA fingerprinting could also be used to accurately trace offsprings to parents or genetic source.

Results from studies involving allozyme analysis have also become useful in assisting selection as markers for making genetic improvement by reducing cost of drugs in management of livestock. Protein electrophoresis which separate alleles on fractionating gels such as polyacrylamide, cellulose acetate or even starch gel have been used to study association between helminth resistance and hemoglobin genetic fractions. Increase in selection pressure on the ‘a’ allele of the erythrocyte protein has been recommended for sheep because of the positive association between the allele and susceptibility to the infestation. Other gene products such as carbonic anhydrase, transferrin, albumin and glucose-6-phosphate dehydrogenase are being investigated so that other diseases could be traced to specific gene products in the plasma.

CONCLUSION

It is clear from the review that comprehensive solutions to a wide range of biological problems of humans and animals require adequate and in-depth knowledge of the structure of the gene and the regulatory sequences governing biological phenomenons. All diseases have

genetic bases. Mutations could affect the production of a given gene product in many different ways (Hauge, 1989). Studies of haemoglobin synthesis in humans are examples of these possibilities. Replacement of a single base with another base as revealed in the study of micro satellites can produce the effect that an amino acid is replaced by another amino acid and one hundred and eighty-nine of such structural variants are known for the human ß-globin chain (Little, 1981). Genetics have been successfully used in the diagnosis of human diseases and gene therapy has been successfully utilized to prevent or manage such diseases. There is an urgent need to maximize the critical role that genetics play in the diagnosis of diseases and this should be extended also to the area of veterinary sciences, especially in view of the recent outbreaks of animal diseases globally having adverse effects on human health for example avian influenza, swine influenza etc.

Application of genetics to the diagnosis of animal diseases can put veterinary scientists, public health experts, the policy makers and other stake holders a step ahead for the prevention and or quick management of infectious animal diseases which have economic and health consequences. Therefore, collaborative researches between veterinary scientists and experts in the field of genetics are imperative and critical to the achievement of sustainable livestock improvement and productivity.

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