REVIEW ARTICLE

Molecular Biology and Medicine: A Review of Developments

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ABSTRACT

Background: *The human genome-sequencing project was completed in April 2003, in the same year that marks the 50th anniversary of the discovery of the double helical structure of DNA. Much of the knowledge derived from the huge number of discoveries in molecular biology research over the past five decades and the genome project has the potential to be of immense benefit world-wide. Such knowledge is already of widespread application in the developed world and much more potential in the future.*

Methods & Results: *This article briefly reviews the literature of research works on molecular biology in English language and discusses some of the findings and highlights recent developments and future trends.*

Conclusions: *The advancement in molecular biology presents huge opportunities and potentials for improved health services worldwide. The article also proposes an approach whereby benefits could be reaped from the application of molecular biology techniques and the information derived from the human genome project in sub-Saharan Africa.*

KEYWORDS: *DNA; Gene; Human genome project; Mutation; PCR; Polymorphism; Genetic screening.*

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INTRODUCTION

In the present era of genomic and molecular medicine, scientific thinking on the pathogenesis of disease has shifted from traditional dogma on human tissue and organ function in health and disease to a reductionistic model where human body function is studied predominantly at the cellular and molecular level. The discovery of the double-helical structure of DNA in 1953 was equated by Watson and Crick to the discovery of the 'secret of life'[1]. Subsequently, significant effort has been devoted to determining how genetic make-up influences cellular function in health and disease. The rapid pace of progress in understanding genetic function up till the early 1990s led to the setting up of the human genome sequencing consortium in 1992, funded primarily by governments and charity organisations in the developed world[2]. Its target was to sequence and annotate the entire human genetic make-up, which is approximately 99.7% identical between all human beings to a high degree of precision. It was thought that this project would permit identification of the genes that exist in humans for further delineation of their structure and function. The massive effort and investment culminated in the release of a draft sequence of the human genome in February 2001 and the final draft in April, 2003[3]. Leaders of all the major developed nations celebrated the final achievement worldwide as a giant step in human progress comparable with man landing on the moon. However, we reckon that this project may be of greater potential and immediate benefit to mankind than that of man landing on the moon. This article is a brief review of the relevance of molecular biology and the findings of the human genome project to medicine at the present time.

Genetic function and human disease

The human genome consists of approximately 3 x 10^8 bases. However, only a small fraction of the genome actually encodes proteins and the size of this fraction is debatable. A decade ago humans were estimated to have as many as 100,000 genes but upon completion of the human genome project, it is now thought that humans actually have only about 30 000 genes[4].

A comprehensive discussion of the current concepts of genetic function and regulation is available in the literature[5] and is beyond the scope of this article. It suffices to say that polymorphisms or mutations in genes themselves or regulatory elements such as promoters, enhancers or transcription factors could be important in the pathogenesis of disease. Mutations could be in the form of substitutions, translocations, insertions, deletions or amplifications of DNA sequences. More recently, interest has arisen in telomeres, which are non-coding genetic sequences that occur at the end of chromosomes, and help to maintain genomic integrity by limiting the number of cell divisions. Telomere length variations have been shown to be important in the pathogenesis of diseases such as cancer[6].

The genetic and molecular mechanisms underlying many diseases even though complex are now relatively well defined and knowledge is increasing at a fast rate. Future advances in

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molecular medicine will be fuelled by the availability of genome project resources. While the socio-economic situation in developing countries, particularly in Sub-Saharan Africa (SSA), may not permit significant contribution to advances in molecular medicine at this stage of their development, much of the accumulating knowledge is of potential application and immense benefit if effectively understood and applied.

At the present time most of the application of molecular biology in medicine is in screening for diseases and diagnosis, usually involving amplification or hybridisation assays[7]. Amplification assays involve producing multiple copies of a specific DNA sequence from test samples often by polymerase chain reaction (PCR)-based techniques; while hybridisation assays usually involve binding of specific DNA probes with unique complementary sequences in test samples.

Molecular diagnosis of diseases is the mainstay of the application of molecular biology in medicine. Other areas of potential and important usage are gene therapy, disease prevention and control. A review of the application of molecular biology in medicine is undertaken below, with illustrations drawn from specific diseases that are of significance to SSA, without discussing complicated molecular techniques and procedures.

Infectious diseases and molecular biology

Infectious diseases still constitute the highest disease burden in SSA, with malaria, HIV/AIDS, and tuberculosis as some of the leading health challenges. In a multi-centre study spanning over six centres, PCR based techniques on sputum for the diagnosis of Tuberculosis (TB), such as for the DNA gyrase β subunit common to Mycobacteria which have a specificity of approximately 100%, have been observed to have a similar degree of sensitivity to culture techniques (85-90%)[8]. Hybridisation techniques for the diagnosis of TB that have a very high degree of sensitivity and specificity have also been developed[9]. Whereas a PCR-based test that identifies *Myocabacterium tuberculosis* specific proteins could be carried out within a day, culture for *Myocabacterium tuberculosis* could last 6-8 weeks. Application of such molecular diagnostic tests therefore, could permit more rapid diagnosis and reduce inadvertent treatment for tuberculosis based on non-specific clinical or X-ray findings alone. More recently, similar molecular tests for cytokine expression by T-cells obtained from bronchioalveolar lavage have been developed to distinguish active from latent Tb[10]. Application of knowledge of this nature, where necessary, could influence the clinical management of TB.

Highly sensitive and specific molecular techniques have also been developed for the detection of other important pathogens such as *Neisseria meningitides, Neisseria gonorrhoeae and Staphylococcus aureus* based on the identification of DNA for proteins specific to these organisms[11-13]. Such techniques could permit rapid identification of these common pathogens, avoiding the delays of routine culture techniques, thus permitting prompt treatment of infected individuals. Furthermore in some instances, genes encoding antibiotic resistance factors have been detected by molecular analysis of *S. aureus* specimens[14]. Application of such techniques may ensure more judicious use of antibiotics.

Besides the sensitivity, specificity and rapidity of molecular diagnostic tests, another major advantage of such tests is the speed with which they can be developed and validated. A PCR-based test for detection of the Corona virus responsible for Severe Acute Respiratory Syndrome (SARS), for example, was developed within 11 days of worldwide research activity in response to WHO initiation of a global-response to the epidemic[15]. This implies that similar tests could be rapidly developed and validated to detect other disease conditions caused by species or strains limited to the local setting.

A homozygous mutation that develops in the basic core promoter region of hepatitis B virus (HBV) is associated with progressive liver damage and the development of cirrhosis, whereas mutations in the pre-core antigen region of the HBV genome are associated with chronic liver disease without cirrhosis[16]. It has been recently reported that the mutation in the HBV promoter gene also predicts the risk of subsequent hepatocellular carcinoma development in infected individuals[17]. Therefore, detection of such a mutation in HBV infected individuals could influence subsequent management of such patients, which would involve regular surveillance for the detection of cirrhosis and/or cancer. PCR-based techniques have long been in existence for the diagnosis of HCV RNA[18]. At the present time, blood screening for HCV is not as common as for HBV in West Africa, despite the fact that both viruses cause a similar spectrum of liver diseases in the long term[19].

HIV Infection and Blood screening

The development of molecular techniques that assess human immunodeficiency virus (HIV) viral

load and acquisition of genotypic resistance have revolutionized the treatment of HIV infection[20]. Commercially available viral load assays use a number of different approaches such as reverse-transcriptase PCR. Tests have been developed to detect different sub-types of the virus including drug resistant sub-types[21]. More and even better molecular tests are being developed as more information is being acquired about the genetics of different forms of the virus. With reference to blood screening for transfusion, multiplex-PCR screening of blood samples (which detect HBV, HCV and HIV all in one PCR) with very low failure rates of approximately 0.2% and false-positive rates of 0.3% is being developed[22]. It should be noted that multiplex-PCR screening is actually more sensitive than the antibody tests presently in use, because it detects infection present in the window period prior to the appearance of anti-viral antibodies. Furthermore, DNA amplification is more specific than antibody binding. The preliminary reports on this technique have been from a drug company, and it will be useful to follow up on on-going evaluation of the test by the Japanese Red Cross.

Endocrinology and Metabolic Medicine

Diabetes mellitus (DM) is considered to be a multi-factorial disorder, with some forms of the disease having a strong genetic component. Mutations in genes encoding insulin, its receptor and several other proteins in the pathway of glucose/lipid metabolism could predict the risk of occurrence of DM or some of its complications[23]. Several mutations of the lipoprotein lipase gene have been found to be associated with hypertriglyceridaemia and insulin resistance[24]. The relatively rare Type-1 DM (IDDM) has a genetic basis while the commoner form of Type-2 DM (NIDDM) is a polygenic disorder. However, in a rare monogenetic form of NIDDM known as maturity onset diabetes of the young (MODY), genetic studies have revealed mutations in at least eight different genes that could be associated with different forms of the disease[25]. Such mutations are detectable by direct DNA sequencing, PCR or PCR restriction fragment analysis (variations in PCR fragment lengths amplified or obtained when such amplified fragments are digested with appropriate enzymes). Application of such screening techniques could influence the management of individuals at risk. Population wide screening for MODY may not be practicable, but would be desirable in families at risk so that lifestyle could be modified accordingly.

Furthermore, polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene that could be detected by PCR-restriction fragment length polymorphisms are associated with an increased risk of development of diabetic micro-angiopathic complications[26]. Polymorphisms in a homeobox gene (encoding a transcription factor that plays a role in tissue differentiation and development) that could be detected by PCR-restriction fragment length polymorphisms could be associated with an increased risk of development of DM[27].

Automated methods of genetic screening for familial hypercholesterolaemia by means of DNA sequencing are available[28]. Results suggest that screening for such a relatively rare genetic disorder is cost effective when carried out within families at risk[29]. Potentially, individuals at risk of other rare genetic disorders such as disorders of thyroid metabolism or multiple endocrine neoplasias could also be screened early in life.

Maternal and Child Health

Pre-natal screening for chromosomal and genetic disorders such as Down's syndrome by means of chorionic villus sampling and amniocentesis is an already well-established routine procedure in some centres[30]. Sickle cell anaemia (SCA) is one of the most important genetic diseases in SSA. Widespread screening for the HbS mutation in children and adolescents, coupled with effective counselling services can markedly reduce the national incidence of SCA. The point mutation in HbS is amenable to detection by molecular techniques such as allele-specific oligonucleotide probes or the amplification refractory mutation system-PCR (ARMS-PCR)[31] on small numbers of cells from any body tissue. Such techniques could give more specific diagnosis than routine haemoglobin electrophoresis and the diagnosis could be made as early as possible in life, even while foetal haemoglobin predominates in neonatal blood. Furthermore, panels of tests could be developed to screen for less common genetic disorders of haemoglobin which are largely ignored at present and other genetic disorders which could be deleterious in infancy such as glucose-6-phosphate dehydrogenase deficiency.

Mutations in the gene encoding a clotting factor (Factor V Leiden) have been found to pre-dispose to some forms of pre-eclampsia[32]. Since the mutation could also pre-dispose to deep venous thrombosis, screening for such a mutation could influence the management of pregnant women and those wishing to commence oestrogen-based oral contraceptives.

Drug Therapy

Genetic factors in individuals could influence the efficacy of drug therapy and the adverse effect profiles of drugs. While routine screening for genetic factors which may predict response to drug therapy may not be practicable for the treatment of acute disorders, such screening may be beneficial when considering long-term therapy for diseases such as epilepsy, cancer or TB. Polymorphisms in drug metabolizing enzymes or drug transporters have been linked with resistance to anti-epileptic drugs. Differential responses to phenytoin for example are related to individual genetic differences in the metabolic enzyme CYP2C9, and to a lesser extent, CYP2C19[33]. Furthermore, polymorphisms in drug efflux transporters such as the ATP-binding cassette sub-family B member 1 (ABCB1) have been linked with multi-drug resistant epilepsy[34]. In the treatment of TB, mutations in katG315 have been associated with resistance to isoniazid[35], while mutations in rpoB531 have been associated with resistance to rifampicin.

Anti-malarial induced pruritus has been linked to decreased rates of metabolism of chloroquine to its desethylchloroquine metabolite[37]. Enzymes of the cytochrome p450 metabolising pathway are involved in chloroquine metabolism[38]. Polymorphisms in the genes encoding such enzymes could be linked to differences in the rate of chloroquine metabolism. If such polymorphisms could be detected, chloroquine doses could possibly be reduced for people with slower rates of metabolism of the drug thereby avoiding pruritus while maintaining eradication of parasitaemia.

The proton pump inhibitors are important in the management of peptic ulcer disease. Proton pump inhibitors are metabolised by an enzyme known as S-mephenytoin 4'-hydroxylase (CYP2C19) in the liver. Polymorphisms or mutations in the gene encoding this enzyme could enhance or delay eradication of drugs belonging to this class either leading to their ineffectiveness or toxic effects[39].

Malignancies

Genetic alterations predict the occurrence and biological behaviour of cancers. Using breast cancer as a prototype, the presence of mutations in the BRCA-1 and BRCA-2 genes predict the occurrence of aggressive forms of breast cancer (in females or males) at earlier ages, even though less than 10% of individuals with breast cancer actually have BRCA-mutations[40]. Rapid immunoassays to detect truncated BRCA proteins in cells from the oral mucosa have been developed[41] since over 90% of BRCA mutations result in truncated proteins. Genetic testing and subsequent genetic counselling could be particularly beneficial to families at risk of breast cancer. Over-expression or amplification of the proto-oncogene Her-2/neu (c-erb-B2) is associated with a poor prognosis in breast cancer[42]. The drug Herceptin (a humanized monoclonal antibody to Her-2) has recently been approved for the treatment of cancers in which Her-2/neu is over-expressed[43]. Consequently, molecular screening for over-expression of this oncogene will be beneficial in planning adjunctive therapy for breast cancer.

The development of metastasis is often the principal event that renders cancers incurable and accounts for the high rates of mortality from the disease. The genetic expression profile of metastatic tumours has been found to be similar to those of their primary tumours[44]. Consequently, genetic expression profiling of primary tumours could predict the probability and mechanism of metastasis. Such knowledge from screening carried out on primary tumours may be beneficial in the future to the application of decoy molecules to antagonise the tumour metastatic machinery[45]. A very wide spectrum of genetic alterations could occur in different tumours and a comprehensive discussion of screening for such alterations is beyond the scope of this article. Nevertheless, sometime in the future, screening for genetic alterations is likely to become routine in the prevention and management of cancer.

Gene Therapy

Gene therapy involves the replacement of a mutant DNA sequence in tissues by the appropriate sequence. Even though the application of gene therapy has potential benefits, the prospect remains highly controversial because the overall effect of insertion of new DNA sequences in individuals cannot be predicted. The technical difficulty and irreversibility of the process are further important complications of gene therapy. At the present time, clinical trials of gene therapy have been mainly for the severe combined immunodeficiency disorders. The results of gene therapy trials for different genetic forms of the disease have given conflicting results, largely with a low success rate[46]. However, gene therapy trials for adenosine deaminase deficiency, haemophilia and leukaemia have shown great deal of promise[47,48].

CONCLUSION

The advancement in molecular biology, as discussed above, presents opportunities and

potentials for improved health services. To take advantage of this advancement, there is a need to develop appropriate laboratory infrastructures in SSA, preferably specialised molecular biology laboratories. The development of such laboratories should preferably be carried out in close links with established molecular medicine units in developed countries, since many laboratories in developed countries already carry out these tests on a routine basis. Such centres will be quite expensive to set up and are probably best done regionally in the first instance. Laboratory equipment has to come from abroad initially, but several, such as thermocyclers (PCR machines), can be produced locally in the short term. Many of the reagents/organic materials required including PCR primers can be produced locally and this will permit expansion of the biotechnological industry in sub-Saharan Africa. The abundant pool of science graduates in the continent particularly in fields such as biochemistry and medical laboratory science will ensure the availability of individuals who can be easily trained in molecular techniques. Well utilised and managed centres should at least cover running costs in the medium term and expand their services in the long term.

The volume of research publications emerging yearly in molecular medicine is expanding at an exponential rate. Mechanisms have to be put in place to ensure awareness of fresh knowledge and recent developments. Critical review and appraisal of emergent literature by experts is also necessary, to define investigations that are potentially useful, worthwhile, cost effective and that can be established locally. Perhaps initially, molecular laboratories should be set up to carry out only a few investigations which could be of benefit in diagnosing the most important problems such as SCA and TB with close links to existing pathology laboratories. Realistically, it will probably take a while to incorporate screening for diseases in which the underlying genetic factors are complex and diverse, such as cancer or DM. Standardization of diagnostic tests and quality control will also be very important in the safe and proper application of molecular technology.

On a final note, in the present genomic era, it is not a matter of if but when Sub-Saharan Africa will join the molecular revolution in medicine. We are strongly of the opinion that the time should be now.

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