LADOKE AKINTOLA UNIVERSITY OF TECHNOLOGY

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INAUGURAL LECTURE SERIES 12

Titled: **FROM DNA TO MOLECULAR DIAGNOSIS:**

The Future of the Past in the Nigerian Contemporary Medical Practice

By

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From DNA to Molecular Diagnosis: The Future of the Past in the Contemporary Medical Practice.

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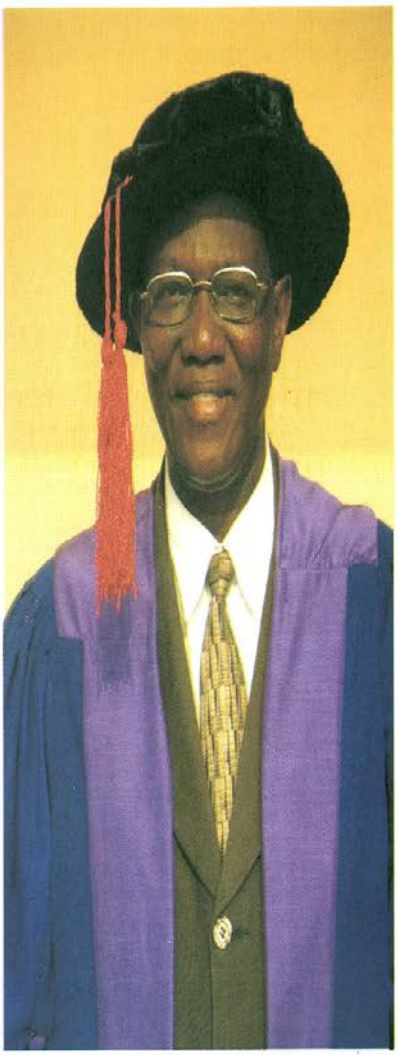

TAIWO ADEGBOYEGA ADEWOLE

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Directors of Programmes and Units
My Wife, Core and Extended Family Members
My Pastors, Deacons and all members of New Heritage Baptist Church, Lagos.
Eminent invited Guests and Friends
The Great LADOKITES
Gentlemen of the Print and Electronic Media
Distinguished Ladies and Gentlemen.

I feel extremely humbled to stand before such an august audience to perform a very important academic exercise which is to deliver an inaugural lecture. That great Scientist, Louis Pasteur, once remarked: "*Chance favours the prepared mind*". The chance to be appointed as the first "indigenous" Professor in the College of Health Sciences and the Faculty of Basic Medical Sciences was due to a mind that was prepared from the Nigerian Institute of Medical Research, Yaba, Lagos. That preparation is the major subject of this lecture. Because of the unusual mode of my appointment, I had considered that delivering an Inaugural Lecture is a debt I owe this University. Today therefore affords me a chance to pay that debt. I am grateful to God Almighty for

this is the day He has made, and I shall rejoice in it. I wish to thank the Vice-Chancellor most sincerely for your encouragement for I never thought this lecture will come again, having been appointed a professor ten years ago.

This lecture is important in many respects. First, it is the first to be delivered since your substantive appointment as the Vice-Chancellor of this great University. So, in a way, this lecture "inaugurates" you to the exalted seat of the Vice-Chancellor, and I wish to assure you publicly of my cooperation with you at all times. In the second place, this lecture is the first to be delivered on behalf of the Faculty of Basic Medical Sciences, indeed by the College of Health Science. Thirdly, this lecture is the first to be given by a Medical doctor, indeed a pathologist in a University of Technology in Nigeria. I am aware of the importance and responsibilities attached to inaugural lectures as it affords an opportunity for a new Professor to render an account to the town and gown about what he professes, and address any major issue of national concern in a way that will be understood by the audience. I hope to stick to that tradition in this lecture.

Mr. Vice-Chancellor Sir, before I address this audience on my subject matter, permits me to single out two people who made me among of several others who influence my academic life. One is Prof. Afonja that I will mention again in the course of this lecture. The other is Professor Adeyinka Afolayan, one of the finest gentlemen and astute Biochemist in this country. He supervised my PhD thesis; I was his first PhD Biochemistry student at Obafemi Awolowo University. Professor Afolayan taught me the rudiments of science and laboratory ethics – he taught me how to hold pipettes so that people will not laugh at me when I go

5taught me hard work, being a workaholic himself. He gave me solid background in Protein Chemistry and Enzymology. Vice-Chancellor Sir, kindly permit me to share a story with this audience to illustrate how Prof. Afolayan made me who I am today. At the conclusion of my experimental work for PhD. Thesis, and about to start writing, sometime around 1980, I told him I have gained admission to read Medicine at the University of Calabar and I am going. Initially, he was shocked that I am leaving without completing the PhD for which I have labored so hard. But he was excited that I wanted to read Medicine. He cited the case of one Jim Chou, a PhD student in Molecular Biology in his laboratory at St. Louis, Missouri, USA who after graduation went on to study Medicine at Columbia University, New York. That was the turning point in my life as it provided the necessary encouragement that I needed. He ensured that I completed the writing and obtained the PhD before my first professional examination in the Medical School. Here I stand today, Mr. Vice Chancellor delivering an Inaugural lecture as a Professor of Chemical Pathology – a melting point where science and clinical practice meet. To Professor Afolayan, I say, today I pay my debt to you for all your labour of love. Thank you Sir.

I wish to conclude this preliminary remark by saying that after the medical degree, I settled at the Nigerian Institute of Medical Research (NIMR) for a career. In the course of time, I went for a Postgraduate (Residency) training in Laboratory Medicine, specifically Chemical Pathology at the University of Lagos Teaching Hospital. While at NIMR, I interacted extensively with the University of Lagos and Lagos State University as Associate lecturer and I had opportunity to supervise B.Sc., M.Sc. and PhD students.

INTRODUCTION

My major research question in the past thirty years or so is to search for DNA that plays a role in diseases affecting man. Why am I searching for DNA? That question will be answered in the course of this lecture. In a way the art of searching for DNA is called **Molecular Diagnosis** or in some parlance, Molecular Diagnostics. Molecular Diagnosis is now a major subject in Chemical Pathology and other disciplines of pathological sciences. Indeed, most recent textbooks of Chemical Pathology contain chapters on Molecular Diagnosis or DNA-based Diagnosis. I have therefore chosen for the lecture, the title *"From DNA to Molecular Diagnosis – The future of the Past in the Nigerian Contemporary Medical Practice"* to express my view on the direction in which medical practice is going, to arouse the curiosity of this audience and to advocate for the preparation of medical students in that direction.

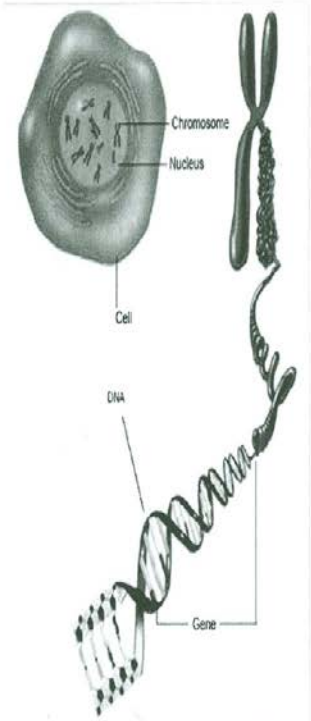
I appreciate the mixed audience that I am addressing and for that reason I will like to start by explaining the basic concepts: DNA and Molecular Diagnosis and its tools. I apologize if this is too elementary for some in this hall. I will then proceed to describe my modest contribution to the art and science of molecular diagnosis of diseases that I have investigated and in other areas of scientific endeavours. I will then discuss the reliance of molecular diagnosis in disease management now and in the future in Nigeria. But first, the basics.

I. THE BASICS

DNA is a popular acronym for **D**eoxyribo**n**ucleic **A**cid. It is a double-stranded filamentous structure (molecule) found in the nucleus of every cell of higher organisms including man. It is

ubiquitous, being present in all forms of life, from viruses to bacteria, fungi, plants etc. In man it exists in its natural state tightly coiled around nucleo-proteins to form chromatin and chromosomes (Figures 1 and 2).

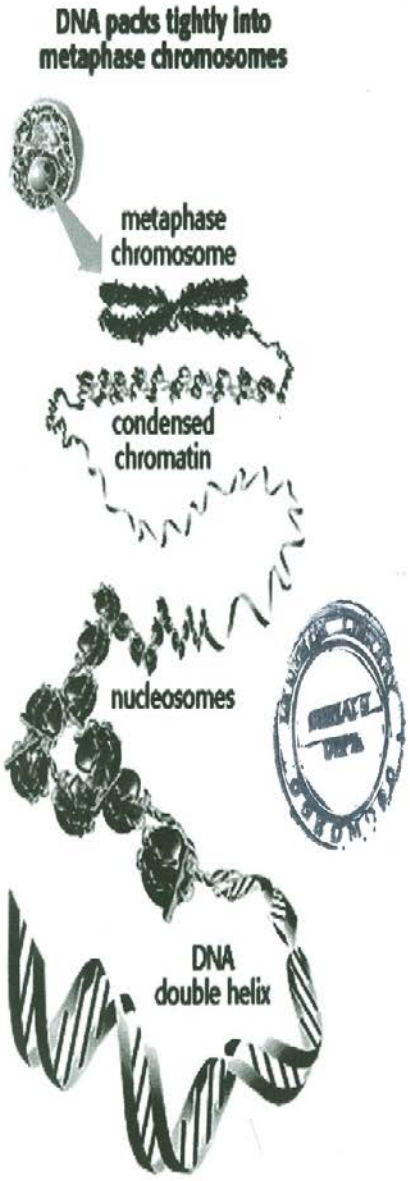
Figure 1



(http://www.bristol.k12.ct.us/page.cfm?p=7097)

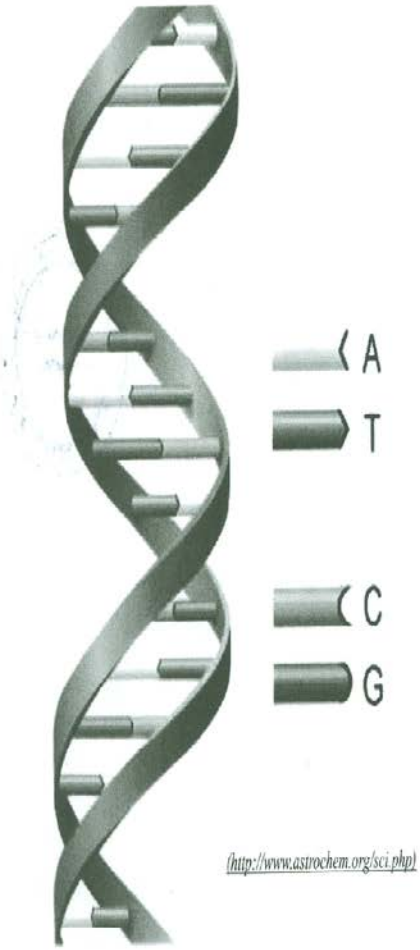
A few of them, typically mitochondria DNA are found outside the nucleus but within the cell where they perform important functions that will not be discussed further. Studies by Watson and Crick and others in the 1950s showed that DNA is also a polymer, consisting of millions of repeating monomers called nucleotides. Each nucleotide joined the next in a specific way to form the linear polymer or strand. Another polymer, constructed in a similar way is attracted to the other polymer through a special bond but in opposite direction to form a double-stranded structure. The nucleotides are well known chemical molecule.

Figure 2



http://www.biology.arizona.edu/cell_bio/tutorials/cell_cycle/cells1.html

Four types of nucleotides are found in DNA, named according to their aromatic bases: Adenine (A), Cytosine (C), Thymine (T) and Guanine (G). The attraction of one strand to the other is due to attraction of a nucleotide in one strand to another nucleotide in the other strand to form a complementary pair such that Adenine of one strand always pair Thymine of the other strand and Guanine of one strand pairs with Cytosine of the other strand. The whole structure gives the appearance of a twisted ladder – this time, a molecular ladder (Fig 3).



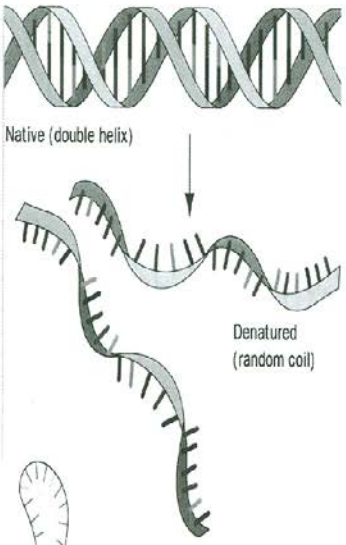
(http://www.astrochem.org/sci.php)

Each step of the ladder represents one nucleotide base pair.

DNA obeys all the laws of chemistry in vivo (that is, in the body) and under appropriate conditions, in vitro (in the laboratory). It can be extracted and purified from cells and tissues. It can be quantified and characterized. It can be heated and denatured in a test tube. At 92-94°C, the strands dissociate and when cooled to between 50-35°C, the strands re-associate in a specific manner following A–T and G–C pairing rules (Figures 4, 5).

Figure 4: schematic representation of the strand separation in duplex DNA resulting from heat denaturation

Slide 25. FCH 532. Lecture 03.Chris Nomura



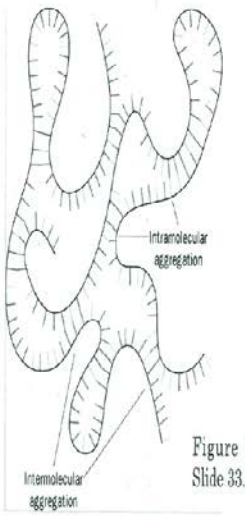


Figure 5: Partially renatured DNA
Slide 33. FCH 532. Lecture 03.Chris Nomura

Another important property of DNA, especially which makes it a versatile tool in the hand of a chemist is that DNA can be cut into several fragment by a special technique. It can also be joined together. Therefore a foreign DNA can be inserted to a native one to form a chimeric molecule referred to as "Recombinant DNA".

The size of DNA varies with increasing complexity along the phylogeneric scale (Table 1).

Comparative Genome Sizes (DNA content) of Some Organisms

Class	Organism	Number of base pairs (kb)	DNA length (mm)	Number of Chromosones (haploid values)
Viruses	λ phage	48.6	0.017	
	T2, T4, T6 Bacteriophage	166	0.055	
Bacteria	Escherichia coli	4,600	1.6	1
	Haemophillum influenza			1
Eukaryotes	Yeast (Saccharamyces cerevisine)	12,000	4.1	16
	Fruitfly (Drosophila melanogaster)	180,000	61.0	4
	Human	3,900,000	990	23

Thus while DNA of a bacteria, Escherichia coli is about 1.6mm and contain about 4,600 kilobases, that of human is about 1,100mm (1.1cm) long and has 3.2 million kilobase-pairs.

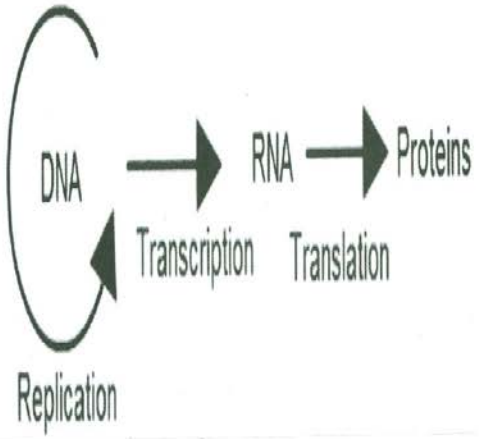
Sequence homology studies show that over 99% of human DNA is similar in nucleotide sequence and other characteristics.

It is the less than one percent that makes us who we are as an individual. Furthermore, the DNA of organisms is similar in nucleotide sequence arrangement for that organism. Some sequences are found in almost all organisms. The total DNA content of an organism is the genome of the organism.

DNA – The Molecule of Life

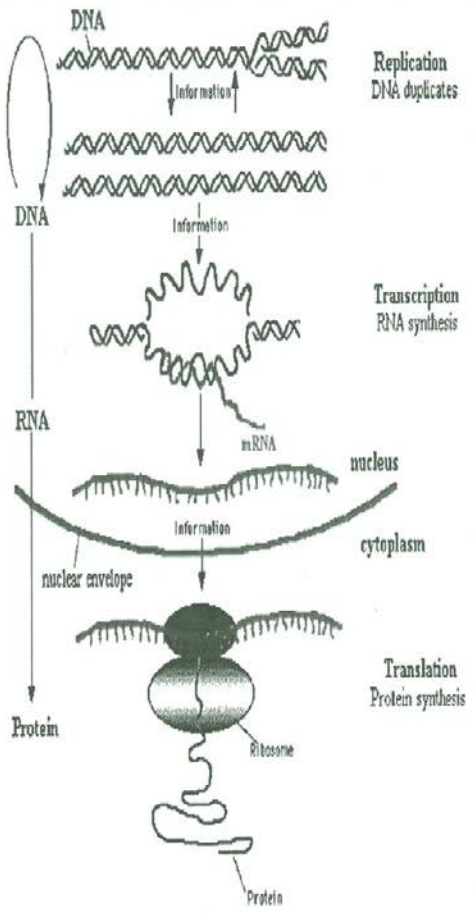
The structure of DNA confers some biological function on it that is relevant to this lecture. All forms of life – from viruses, bacteria, fungi, plants to man – owe its existence to DNA. What we inherit from our parents and what is passed to offspring depends on our DNA composition. Inheritable characters, called genes are now known to be segmental portions of DNA. DNA dictates what proteins are to be made through a process called "transcription" and "translation". This important principle is called "Central Dogma of Molecular Biology" depicted in Figures 6 and 7.

Figure 6



(http://biology.tutorvista.com/cell/central-dogma.html)

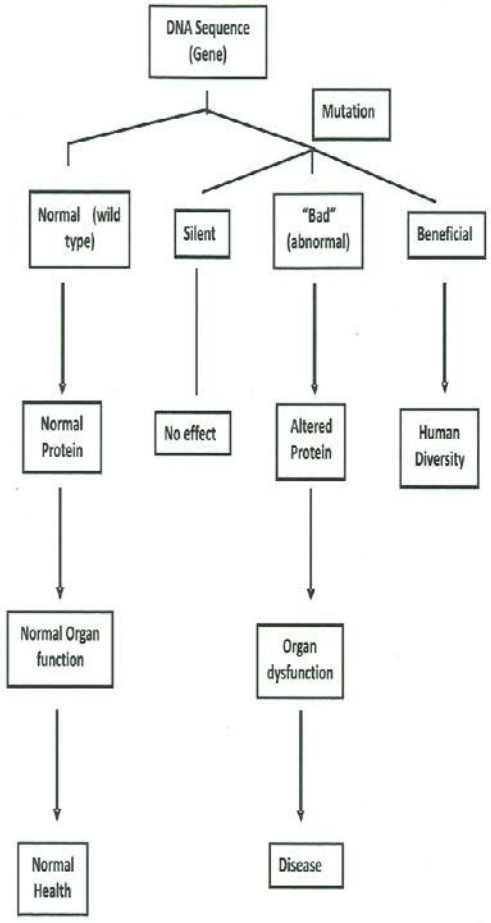
Figure 7
(http://nuweb.neu.edu/bbarbiellini/CBIO3580/DOGMA/DNA_CenDog.html)



The Central Dogma of Molecular Biology

Here lies the importance of DNA and why it is often referred to as the molecule of life. DNA can replicate itself, that is, produce a faithful copy of itself as happens during normal cell division. DNA directs the type of proteins to be made in the body. Proteins, the product of gene expression serves as major structural components of the body (e.g. muscles, bone, hair), serves as enzymes and hormones and regulators of cellular processes that enable life processes to go on.

Concerning DNA, we can safely conclude that if DNA is "good", then the protein, its product, will be normal; but if the DNA is "bad" then its protein will be defective (Fig 8)



and may lead to diseases. Some DNA mutations may be beneficial, leading to diversity in human trait and characters. Diseases that are collectively called Genetic Disorders are due to inheritance of "bad" DNA. "Bad" or mutated DNA may not necessarily result in diseases. They may remain silent only to surface in the next generation.

II. MOLECULAR DIAGNOSIS

Mr. Vice-Chancellor, Sir, let me try and answer the question I asked earlier; why am I looking for DNA in diseases? The science of using DNA to identify diseases is Molecular Diagnosis and I am in this field for at least two reasons.

First, scientific evidence increasingly reveals that a lot of diseases are known to be caused by disorders in the human DNA composition and structures (Cotton and Scriver, 1998; Rivenbark, 2010). A deviation from normal DNA may be that the normal sequence of nucleotide is altered as when one nucleotide replaces the other. This is called point mutation: The altered or "bad" DNA, as mentioned earlier, will result in altered or "bad" protein whose function will also be altered.

In the story of creation, the Bible records: *"And God saw everything he has made, and, behold, it was good"* (Holy Bible, KJV Chapter 1, verse 31). That creation story, I believe, started with making perfect, very good DNA. And that is why all creations, including man, in the beginning were perfect and very good. Mr. Vice-Chancellor, Sir, my postulation is that mutation (alteration in the structure of DNA) is what is responsible for diseases and abnormal behaviours in man. Yes, abnormal behaviours because modern scientific evidence now link some cases of manic tendencies, rapism, depression and other psychiatric diseases to DNA abnormalities.

Classical example of point mutation in our environment is sickle cell disease where altered haemoglobin (a protein) is inherited from both parents. The search for the altered or mutated DNA is an example of molecular diagnosis. A list of some genetic diseases due to altered DNA is presented in Table 2.

EXAMPLE OF DISEASES DUE TO GENE SUBSSTITUTION (A) OR DELETION (B).

A: GENE SUBSTITUTION

DISEASE	GENE LOCATION	MOLECULAR PATHOLOGY	RESULT
1 Sickle Cell anaemia	Chr. 11 β - Globin gene	GAG -> GTG in exon 1	Glu^{6s} -> Val
2 Haemoglobin C disease	Chr. 11 β - Globin gene	GAG -> AAG in exon 1	Glu^{6s} -> Lys
3 α_1 - Antitrypsin deficiency		M -> Z mutation in exon V (GAG -> AAG)	Glu -> Lys
4 Haemophilia A	Chr. Xq Factor VIII gene	TCG -> TAG (a termination codon)	Incomplete Factor VIII molecule
5 G6PD A deficiency	Chr. Xq28	ΔAT^{376} -> GAT (exon 5) GTG^{202} -> ATG (exon 4)	Asn^{126} -> Asp Val^{68} -> Met
B: GENE DELETION			
1 α - Thalassaemia	Chr. 16 α-Globin gene	3.7kb deletion 4.2kb deletion 3.8kb deletion in Bam H1 fragment	α^+ - Thal.[1] α^+ - Thal.[2] ↓ production -> Growth retardation
2 Familial Growth hormone deficiency	Chr. 17 cluster. GH-N gene		
3 Cystic fibrosis (CF)	Chr. 7p	3 bp deletion in exon 10	Loss of Phe^{508} -> lethal.
4 Duchene muscular dystrophy (DMD)	Chr. Xp21 Dystropin gene	Large deletion	↑muscle wasting. Lethal

Chr = Chromosome; G6PD = Glucose-6-phosphate dehydrogenase

1 - $\alpha^{3.7}$ is common among Africans, Mediterraneans and Asians
2 - $\alpha^{4.2}$ is common among Asians

Apart from point mutation, other mutations may be that a segment of DNA is deleted or added to another segment, resulting in diseases. Examples of such diseases include the thalassemias, Familial Growth hormone deficiency, Cystic fibrosis and Duchene muscular dystrophy.

There are a large group of other diseases which although have been linked to one gene or the other but which cannot be associated with a single gene (DNA) disorder. They are thought to arise from the combined interaction of many genes, to environmental factors and to personal risk behaviors. Examples include:

Heart diseases, typically coronary heart disease
Cancers
Diabetes, especially Type I diabetes mellitus
Asthma
Obesity
Dementia
Depression

For these diseases, molecular diagnosis proved challenging and elucidation of the roles played by genes (DNA) in their inheritance is an active and intense field of research. Certainly such efforts hold promises of better management in the future.

The second reason why I am looking for DNA in diseases is to diagnose or detect disease–causing micro-organisms (or pathogens) in man. The basis for this area of molecular diagnosis is that these pathogens in the blood or other tissues of man have DNA of specific sequences that are lacking in human. Fragments

of DNA containing the pathogen-specific sequences can be identified from extracted DNA from blood and other tissues to confirm their presence. Examples where this has found application include detection of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HBC), *Chlamydia trachomatis*, *Mycobacterium tuberculosis* and *Neisseria gonorrhea*. Today, more than 80% of molecular tests performed are on infection disease detection and management. Molecular diagnosis is especially useful in identifying resistant strains of parasites of medical importance. This has found application in *Plasmodium falciparum* malaria.

The Tools of Molecular Diagnosis

The starting point for the conventional molecular diagnosis is the extraction of DNA from blood or other tissues. This is usually not a problem since the DNA molecule is a chemical structure. Such procedures usually employ the physico-chemical properties of DNA.

Further treatment of DNA relies on four techniques commonly used in molecular biology laboratories. They are briefly described and illustrated in the diagram below.

1. Use of Restriction Enzymes (RE's)

These are bacterial enzymes, which evolved as a bacteria defence mechanism against invasion by foreign DNA such as viruses. Their important property is that they cut double-stranded DNA once they see a specific DNA sequence of four or more

ıucleotides. Such recognition sequences are also present in the ıuman DNA. Thus by using various RE's, it is possible to cut the ong strands of DNA into fragments of reproducible sizes.

2. Molecular cloning

Molecular cloning allows for rapid reproduction of genes in a oreign host.

)NA molecule can be cut by a restriction enzyme as described ıbove. The fragments can be joined by DNA ligase, an enzyme hat seals double-stranded nicks (holes) between adjacent ıucleotides in a duplex DNA chain.

'ragments of DNA (termed *foreign* DNA) can be inserted into a *ost* DNA (e.g. bacteria or plasmid DNA). This is done so that a ıarticular property or trait in the foreign DNA may be ransferred to the bacteria. The product (called chimeric or *ecombinant* DNA) can be put back into the bacteria and taken ıp by the cells. This is called transformation. Since bacteria eproduce rapidly, the original foreign DNA is also reproduced, nd its properties expressed more rapidly than it would in its atural host. Thus clones of the original ("foreign") DNA can be eproduced in a large scale. This large-scale production allows or the gene to be studied by sequencing or other means.

3. Hybridization & Southern Blotting:

'his was first described by E.M. Southern in 1975.

: allows a scientist to transfer or blot fragments of DNA eparated in a gel medium to a special fibre-paper and thereby nhance better identification and manipulation of the ·agments.

A human diploid gene (of about $7x10^9$ bp) may be cleaved by RE into about 1,000,000 discrete fragment of 100 – 100,000 bp in length. These fragments can be separated by electrophoresis in agarose gel by size. The product is transferred from the gel to a fibre-paper by Southern blotting. The DNA fragments, after being fixed to the filter are hybridized to a gene probe. The probe is in fact small pieces of single stranded DNA, usually in the order of 14-30 base-pairs which is labeled either radioactively or non-radioactively. The probes – called ASO (Allele-specific oligonucleotide) probes anneal (hybridize) to fragments on the fibre-paper to which it has corresponding base sequence and therefore enable that paper-bound fragment to be identified.

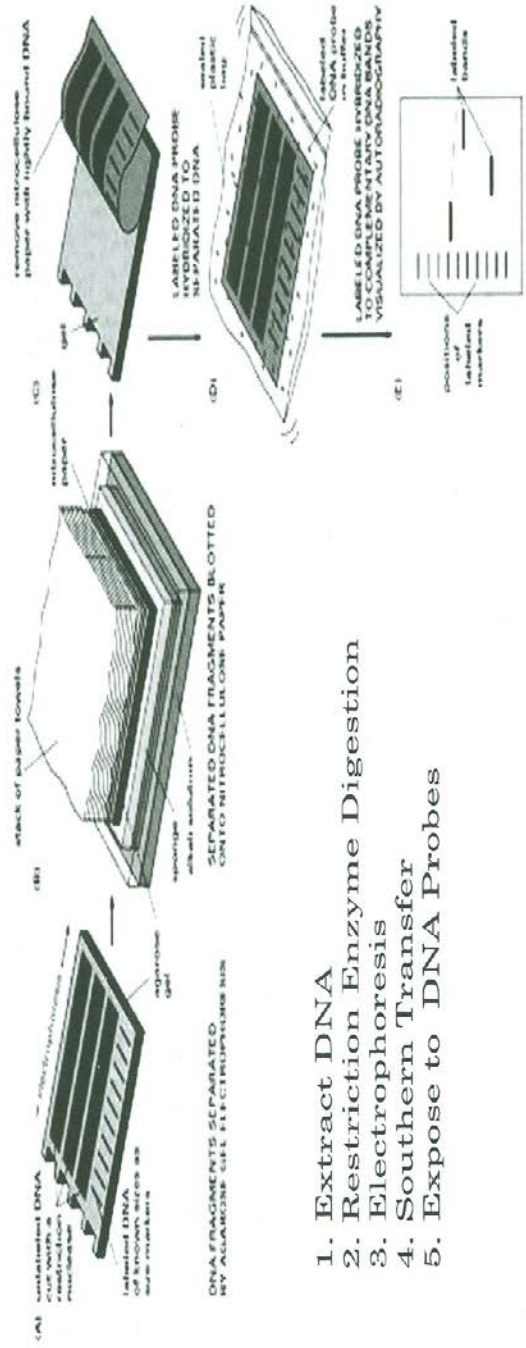
The basis for this technique is that DNA can, under appropriate condition hybridize to complementary sequences on the gene by hydrogen bonding.

Fig. 9 is a hypothetical flow chart of a Diagnostic Laboratory.

4. The polymerase chain reaction (PCR) Technology:

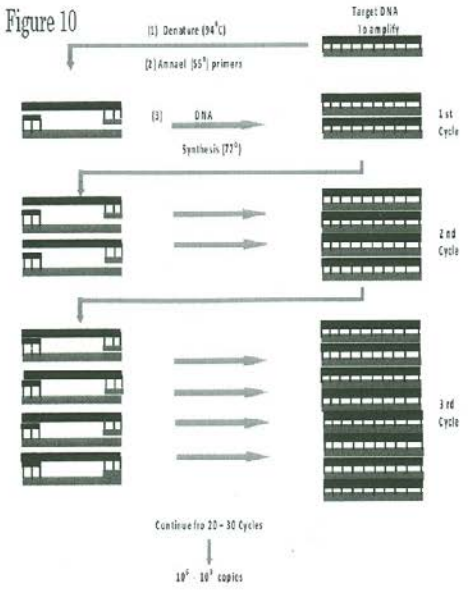
The polymerase chain reaction, PCR is a technique by which large amounts of a specific DNA fragment are produced from small amounts of many simple starting materials. It is an in vitro method for the enzymatic synthesis of specific DNA sequences of a predetermined length in the test tube. The length is predetermined by using two oligonucleotide primers that hybridize to opposite strands and flank the region of interest in the target DNA (Fig. 10). The synthesis is carried out by DNA polymerase.

Fig. 9 is an hypothetical flow chart of a Diagnostic Laboratory



1. Extract DNA
2. Restriction Enzyme Digestion
3. Electrophoresis
4. Southern Transfer
5. Expose to DNA Probes

Figure 10



A SCHEMATIC REPRESENTATION OF PCR AMPLIFICATION
Showing complementary genomic strands to be amplified (Long Lines) and their corresponding primers (short lines)

In its simplest form, the technology can be considered primarily a method for producing copies of a specific sequence of DNA usually containing the gene of interest. PCR, by producing enough (million-fold) copies of the target sequence of a gene allows simple, rapid and robust methods for identifying that gene. The gene so amplified is thus available for sequencing. PCR was originally devised about 1985 by Kary Mullis and his colleagues (Ref 4) in Henry Erlich's laboratory at the Cetus Corporation in Emeryville, California and has found numerous applications for research and diagnostic purposes. The first application was for the diagnosis of sickle cell anemia by Saiki and co-workers (1985).

Before the invention of PCR, researcher who wanted to produce sufficient quantities of a specific gene had to clone the gene in a vector (e.g a plasmid) by recombinant DNA technology described arlier. With PCR, DNA in a single cell is sufficient for

amplification. A further advantage of PCR is that it produces sufficiently large amounts of DNA so that its detection can be accomplished by simple stains like ethidium bromide thereby eliminating the need for using radioactive probes with its attendant problems.

The starting materials for the basic PCR include:

- The nucleotides, in their triphosphate forms, in equimolar proportions to serve as monomers,
- The template (genomic) DNA containing the segments of DNA to be amplified,
- Oligonucleotide primers, two of them, designed to define the length of the fragment to be amplified,
- A DNA polymerase (Taq polymerase) to catalyze the synthesis of the fragment
- A buffer tailored to optimize the synthesis.

The reaction occurs in a three step cycling process consisting of:

- Denaturation of double stranded DNA typically at 94°C or 95°C
- Annealing of primers to the DNA at between 35 to 55°C, depending on the purpose, and
- Primer extension or synthesis of a new strand at 72°C

Each cycle of PCR results in doubling of the DNA fragment being amplified. Therefore a twenty-cycle PCR can theoretically amplify DNA by a factor of 1 million (2^n where n is the number of cycles), while 30 cycles can amplify it by about a billion-fold. The enzyme used in the early PCR experiments was the Klenow fragment (*Pol I kf*) of *Escherichia coli* DNA polymerase I. This enzyme is however heat-labile and had to be added in each subsequent cycle because the enzyme was inactivated during

each denaturation step. Subsequently, the Klenow fragment was replaced with a thermostable *Taq* DNA polymerase isolated from the thermophillic bacterium, *Thermus aquaticus* YT1. This modification renders the process amenable to automation since the enzyme will not have to be added after each cycle. Furthermore, by allowing synthesis to proceed at 72°C, rather than 37°C with Klenow fragment, synthesis of non-specific product is avoided thereby increasing the yield and specificity of the reaction.

The reaction of Taq polymerase is quite specific. Analysis of the rate of nucleotide misincorporation in a particular study of ß-globin synthesis was shown to be in the order of 1/400 and the error rate calculated to be 2×10^{-4} nucleotide/cycle.

PCR has found numerous applications in clinical medicine and research. Some of the early applications include:

- Prenatal diagnosis, neonatal screening and carrier testing
- Characterization of a gene defect.
- Analysis of biological evidence and other forensic application.
- Detection of oncogenes.
- Detection of human infections.
- HLA typing.

PCR is the gold-standard tool in molecular diagnostic laboratories. PCR today has undergone several modifications to optimize the process and improve its fidelity. We now hear of "nested PCR", "Hot start PCR", "Reverse Transcriptase PCR" etc. The serious revolution in molecular biology (Table 3)

TABLE 3 Advances in Molecular Biology Tools that Influenced Molecular Diagnosis

Date	Molecular Biology Tools	Associated Techniques	Major Application
1960s	Hybridization Techniques	In-situ Hybridization	Gene transfer
1970	Discovery of Restriction enzymes		Molecular cloning
1975	Southern Blotting	Gel electrophoresis Probes Visualization techniques	Genetic mapping of large deletions or re-arrangement. Linkage studies
1977	DNA sequencing	Improved detection methods	Gold standard for gene identification
1985	Restriction fragment length polymorphism (RFLP) analysis	Allele-specific oligonucleotide (ASO) probes	Detection of single nucleotide polymorphism (SNPs)
1985	Invention of PCR	Gel electrophoresis	Detection of single nucleotide polymorphism (SNPs)
1986	Fluorescent *in situ* hybridization (FISH)		Detection of chromosomal translocation
1988	Automation of PCR due to use of thermo-stable (Taq) DNA polymerase	Ligase chain Reaction	
1992 - 1996	Real time PCR (qPCR)	Fluorescent assay with fluorochrome	Qualitative and quatitative analysis of PCR process
		Capillary Electrophoresis DNA microarray assays	Pathogen detection Resistance testing Identification of multiple gene expression simultaneously
2001			Disease screening
2003	Completion of Human Genome Project		Personalized Medicine
2005	High throughput sequencing technology		
2009	Microfluidic Technology Solid-phase Technology	Laboratory on microelectronic chip	Point-of-care Testing

and increased demand for molecular diagnosis led to the development of high throughput PCR systems. In the mid 1990s, Real-Time PCR (qPCR), described by Holland *et al*, (1991), Heid *et al*(1996) and Gibson *et al*(1996) was developed to produce not only qualitative but also quantitative DNA and RNA in real time. Real-Time PCR is quite fast (40 minutes), with improved sensitivity and specificity. It is applicable to rapid screening for diseases. Another state-of –the-art development is the microarray assay which is used to screen several mutations at the same time. Microarray assay has found application in screening for disease markers such as cancer.

III: CONTRIBUTION TO MOLECULAR DIAGNOSIS

(i) In the Beginning...

When I was in the Medical School, one of my mentors, Professor Edward B. Attah impressed on me that the future of medicine lies in the molecular pathology of diseases. The term *molecular diseases*, first coined by Pauling in 1949 was due to his discovery that sickle-cell diseases, characterized by recurrent episodes of crisis, acute pain and severe lysis of blood was due to a single change of an amino acid of ß-globin chain of haemoglobin from non-sicklers. The opportunity to study molecular diseases came when I took appointment at the Nigerian Institute of Medical Research after completing my medical degree. Meanwhile, at the Institute, a research project titled "Pre-natal Diagnosis of Sickle Cell Anaemia", conceived by the then Director, Professor Fola Esan was lying there and became attractive to me. My first task was to learn how to extract DNA from tissues in pure state, quantify it and perform some simple experiments. The

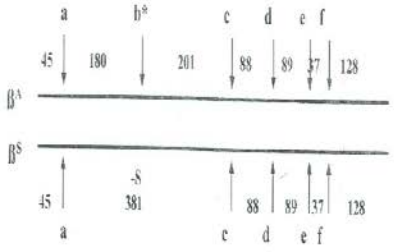
laboratory of Dr. D.K. Olukoya became readily available. Dr Olukoya, is an astute, first-class molecular biologist, who by divine call is now a Pastor and the General Overseer of the Mountain of Fire and Miracle Ministry. He allowed me the benefit of his experience. Although Dr. Olukoya has left the laboratory, he left a virile laboratory which today has produced great genetic engineers and molecular biologists of repute. I must point out that not many DNA laboratories were in existence in Nigeria in the 1980's. Therefore our first task, as reported by Adewole, Olukoya and Afonja(1997) was to carry out a validation experiment by comparing genotyping of leucocytes DNA by PCR with the conventional genotyping of haemoglobin in the blood of the same person. Our data showed that genotype can be predicted correctly by the DNA-based tests. Whatever rudiment I learnt was perfected when, through the DAAD Fellowship grant, I was trained in Germany in the technology of PCR and its use in the diagnosis of Sickle Cell anaemia prenatally. I came back to Nigeria with a lot of gifts from my German trainers in order to establish the procedure in Nigeria. Disappointingly, the project could not take off because we were unable to purchase a PCR machine for my laboratory. Determined not to fail, I joined a team of sickle cell physicians and gynaecologists at the Lagos University Teaching Hospital, who were also thinking of the same project but could not find a trained molecular analyst until I got to the scene. We got the very first PCR machine for my research work as a donation by the British Council.

(ii) **Pre-natal Diagnosis**

Why Pre-natal Diagnosis? Sickle cell disease constitutes a major public health problem in Nigeria as it is in most Blacks in Africa. Studies by Fleming and co-workers(1979) and Adewuyi and co-workers showed that about 20 per 1,000 new born are homozygous (HbSS) for the disease. In Nigeria alone, 25% of populations carry sickle cell trait (Hb AS) and these are at risk of producing offspring with sickle cell anaemia. The increased awareness of the populace led to the search for the diagnosis of the disease *in utero* by couples at risk. As far back as 1987, a WHO informal consultative Group on Hereditary Diseases Programme presented a proposal for a feasibility study on the control of sickle cell diseases in Africa recommending pre-natal diagnosis (PND) of sickle cell anaemia by chorionic villi sampling (CVS) and DNA analysis of the villi. The chorion, which is to develop to placenta later, carries the same genetic information as the baby. Programmes for PND could not however take off in Nigeria because of the dearth of gynaecologists skilled in foetal CVS and because of the logistics of setting up a molecular diagnosis laboratory. To overcome the first problem, two of our collaborators (gynaecologists) were trained locally in safe chorionic villi sampling.

We embarked on a pilot study of Prenatal Diagnosis of sickle cell anaemia with 50 pregnant women with gestation age of between 10 – 18 weeks who desired to know the genotype of their foetuses, recruited on the basis that the foetus of each of them was "at risk" for sickle cell anaemia. Chorionic villi sampling was performed on each of the pregnant women by the gynaecologist in our team. My job in the theatre during the procedure was to examine the

chorionic villi tissue under low power microscope and carefully dissect out any maternal tissue that may contaminate the specimen and lead to wrong diagnosis. Back to the laboratory with the tissue, the diagnostic strategy was to extract the DNA from tissue, amplify a fragment of the ß-globin gene containing the sickle gene by polymerase chain reaction and restriction enzyme analysis. The latter procedure is to cut the amplified gene such that the diagnostic segment may be readily identified (Fig. 11).



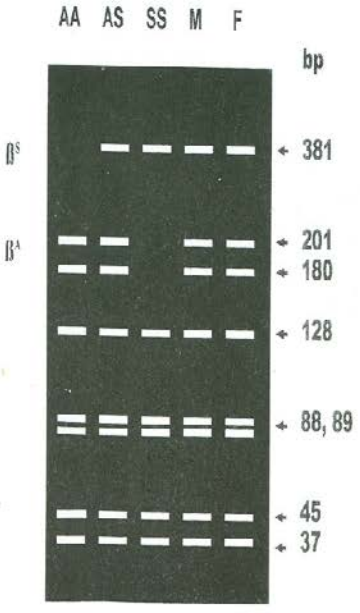


Table 4: Analysis of chorionic villi samples for sickle cell genotype by DNA/PCR technology: Summary of result.

	Result	Number	Percent
1.	AA	13	26
2.	AS	25	50
3.	SS	11	22
4.	Indeterminate	1	2
	Total	50	100

*For a CVS sample, no clear-cut result emerged from analysis by both methods.

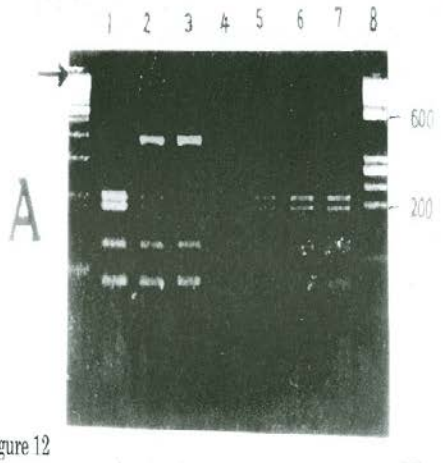
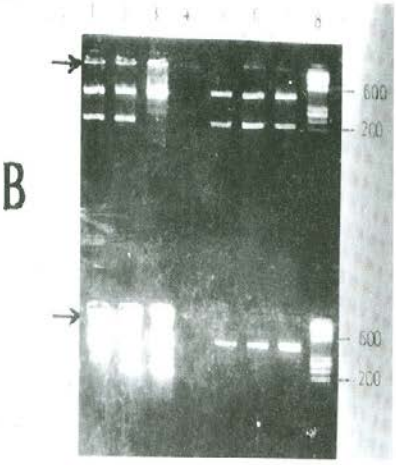


Figure 12



PCR was performed either by amplifying the genomic DNA followed by restriction enzyme analysis (PCR-Dde 1, Fig 2a) or by amplifying DNA with allele-specific primers (PCR-ARMS, Fig. 2b). Lanes 1 (AA), 2(AS) and 3(SS) are standards (Positive control). lane 4 is the experiment without DNA (Negative control). lanes 5-7 are diagnostic samples. Lane 8 is the DNA size marker (100 bp ladder).

Figure 12 shows a typical PCR analysis. Our result, summarized in Table 4 show that of the 50 samples from the foetuses, 13 were AA (normal), 25 were AS (Trait or carrier) and 11 were SS (affected). One was indeterminate as no clear-cut result emerged from the analysis(Adewole *et al*, 1999). A post-natal genotype screening on the product of conception (a baby or the abortions in cases of miscarriage) showed that in each of the 42 cases (84%) that reported, the genotype was correctly predicted prenatally. Mr. Vice-Chancellor Sir, please permit me to report that to the best of our knowledge, this was the first prenatal diagnosis of sickle cell anaemia not only in Nigeria but in Africa from the South of Sahara to North of Zambesi river.

After this initial effort, we have documented our experience, logistic problems and how they were solved so as to guide other centres (Adewole *et. al*, 1999, Akinyanju *et al*, 1999).

Vice-Chancellor Sir, the greatest derivative for me is the joy I have in helping our populace through the transfer of this technology. The major role of PND as a service is to provide information so that parents can have a choice or at least they are better enlightened. The initiative to establish PND as a service delivery was taken up by Sickle Cell Club of Nigeria, a non-governmental organization that carries out this test till today at a relatively small fee.

I wish to conclude this discourse on Prenatal diagnosis of sickle cell anaemia by quoting the conclusion of one of our papers:

> *Prenatal diagnosis of sickle cell anemia is very relevant in Africa because of the high prevalence of the gene. In view of our experience, and the other Third World countries with successful programs, we suggest the following guidelines for a successful PND program:*
>
> 1. *There should be a centralized testing centre, (Fig. 13) which should be well equipped. This centre should collaborate with other centers abroad for the purpose of quality control and manpower development.*
> 2. *Counselling and health education should go hand-in – hand with prenatal diagnosis.*
> 3. *Multidisciplinary research involving physicians, scientist and social scientists should be established to evolve the best method of delivery of the service.*
> 4. *Effective programs for 'training of trainers' who will initiate other centers should commence early.*
> 5. *The political will to make the program succeed must be present. The service must be equitable and funding must be actively pursued.*
>
> *In the absence of any specific therapy, prevention of inherited disorders is the best option. Control of genetic diseases has come of age in many countries, except in Africa where the tendency is still to concentrate on infectious diseases to the neglect of other diseases. Nigeria in particular with the largest population of patients with sickle cell disorder, relatively higher levels of trained personnel and more available funding, must take the lead in Africa.* (Adewole and Akinde, 2001).

An important lesson I have learnt is that adaptation of foreign technology needs a lot of ingenuity on the part of the players. What took only few months to learn took some 2 – 3 years to establish as a routine procedure.

2. Malaria: Genotyping for Polymorphic forms and Resistance strain

Malaria caused by infection with *Plasmodium* species, is by far the world's most important tropical parasitic disease. The World Health Organization reported the worldwide prevalence of the disease to be in the order of 300 – 500 million clinical cases and killing over a million each year (WHO, 1999). Infection with *Plasmodium falciparum*, the predominant strain in Nigeria, according to Salako (1997) is associated with severe mortality and morbidity. The asexual blood state is responsible for the manifestation of the clinical symptoms and involves the release of merozoites, the young form of the parasites, from red blood cells when they rupture and re-infect new ones in a cyclic manner.

My main research on malaria is to study the distribution of the genes of a specific surface protein called Merozoite Surface Protein (MSP) on the surface of the merozoites. The protein codenamed MSP-1, MSP-2, MSP–3, EBA-175 are important because they are good targets for the development of vaccine against malaria. They elicit the production of inhibitory antibodies in the bloodstream. While extensive work had been carried out on MSP-1, we were not aware of any previous studies in Nigeria before the project. Our study (Adewole et al, 2002) was designed to obtain information on the genetic polymorphism of MSP–1. This is considered a critical first step in designing effective vaccine for the population for whom the vaccine is intended.

The diagnostic strategy involved DNA extraction from blood of 51 subjects, carrying out "nested" PCR, first to amplify MSP-1 gene and then MSP-1 alleles codenamed "MAD 20" and "WELLCOME". Our results showed that MAD 20 is more predominant of the two dimorphic types of MSP-1 as it was present in 92% of samples alone and 7.8% in combination with the WELLCOME strain. The predominance of MAD 20 strain in the study area would suggest that a vaccine based on this fragment would probably be more useful.

We have also applied molecular diagnosis to investigate the acquisition of chloroquine resistance in acute, uncomplicated malaria. During the time of our study, chloroquine was the drug of choice for treating malaria. However resistance to chloroquine, first reported from the Eastern part of Nigeria in 1986, ultimately spread across the whole country. Failure to control malaria with chloroquine was due to a combination of factors, including adulteration of the drug, self medication and treatment with sub-therapeutic levels of chloroquine especially in children. The resistance to chloroquine had, in previous studies to ours, been attributed to a point mutation (Adenine to Cytosine) in the *Pfcrt* gene of the parasite(Happi et al, 2004). That change favours severity and multiplicity of malaria infection. Our study (Olukosi *et al*, 2005) was a two-year molecular surveillance of the acquisition and spread of *Pfcrt* (K76→T mutation) in malaria-infected children in Ijede (a rural community near Ikorodu) and in Lagos metropolis (Massey Street children hospital). Again, our diagnostic strategy was to extract DNA from blood of the children, amplify genomic DNA of the parasite by PCR and identify the diagnostic fragment through electrophoresis techniques (Fig. 14). We were able to show, over a period of two years, that the children were infected

with parasites that harbor this gene with increasing frequencies of 48.7% and 73.8% (Table 5a). Furthermore children ≤ 5 years were found to be infected with parasites that have acquired the gene more than the older children.

Mr. Vice-Chancellor Sir, before I leave the subject of malaria, I wish to inform you that I have collaborated with other investigators to carry out studies on other aspects of malaria research: on diagnosis, chemotherapy and Phase IV clinical trials as summarized in Table 6.

Figure 13. PCR-RFLP of secondary pfcrt amplicon (134 bp) of *P. falciparum* strains recovered from the pretreatment blood samples of children with acute uncomplicated malaria. Lanes 1 and 15 = 100 bp ladder DNA markers. Lanes 2 – 11: Pretreatment *P. falciparum* infected blood samples. Lane 12: DD2 *P. falciparum* (wild pfcrt K76 control). Lane 13: HB3 *P. falciparum* (mutant pfcrt T76 control). Lane 14: uninfected blood sample (-ve control).

Table 5a. Parasitaemia profile and incidence of pfcrt T76 allele acquisition in children with acute uncomplicated *Plasmodium falciparum* malaria in rural and urban Lagos, Nigeria.

	Study site					
	Ijede (Rural) R		Massey Hospital (Urban)			
Period	N	n(%)pfcrtT76 (+ve)[@]	N	n(%)pfcrtT76 (+)[@]	χ^2	P
Year 1	39	19(48.7)	42	31(73.8)	4.4	0.04
Year 2	52	33 (67.3)*	63	47 (74.6)	1.7	0.2
Total	91	52 (57.1)	105	78 (74.3)	6.4	0.1

N = Pre-treatment sample size. Parasitaemia = 2080 – 24,420 asexual forms /µl of blood; Geometric mean = 2138 /µl. [@] Data represent number and percentage (in parenthesis) of cases positive by PCR-RFLP for pfcrt T76 in both monoclonal (pfcrt T76 band only) and polyclonal (pfcrtK76/T76 bands seen) parasitaemia. Differences in proportions between and within groups on yearly basis were analyzed by chi-square test with Mantel -Haenszel modification. * $P < 0.05$ versus year 1 rural. Allelic frequency rates R = 1.38 /year in Ijede (rural) and 1.01 / year in for Massey Children Hospital (urban).

Table 5b. Gender variation in the acquisition of pfcrtT76 allele in rural and urban Lagos, Nigeria.

Rural	Sex			
Period	M	F	χ^2	P
Year 1	11(57.9)	8(42.1)	0.92	0.3
Year 2	18(54.5)	15(45.5)	0.54	0.5
Total	29(55.8)	23(44.2)	1.37	0.2
Urban				
Year 1	18(58.1)	13(41.9)	1.59	0.2
Year 2	26(55.3)	15(44.7)	0.54	0.5
Total	44(56.4)	34(43.6)	1.37	0.2

Figures in parenthesis represent percentages of pfcrt T76 positive cases in the pre-treatment samples analyzed by PCR-RFLP.

Table 6: Other Studies on Malaria

Area of Interest	Research Study	Major Outcome	References
Diagnosis	Measurement of the optical density of urine as a non-invasive method for the diagnosis of malaria.	Can the optical density of urine serve as non-invasive method for diagnosis of malaria? The study suggests a strong positive correlation between malaria parasitaemia and optical density of malaria. Method can be useful, if combined with clinical signs and symptoms.	Afolabi *et al* (2000)
	Investigation of the efficacy of two rapid assessment techniques (Optimal 1 and SD-Bioline) for the diagnosis of malaria in rural areas of Nigeria	Efficacy of three new kits for rapid diagnosis of malaria was studied. Sensitivity, Specificity, positive and negative predictive values for the three blood tests suggest that Optimal 1 was more useful as it could also detect *P. malariae*. The other two were not recommended for use in Nigeria.	Agomo *et al* (2003)
Management	A Study of Intercurrent Infections during Episode of Severe Falciparum Malaria attack in Nigerian Children	Children diagnosed with cerebral malaria have co-existing infections: Staphylococcus aureus (32.5%), Klebsiella Corynebacterium (6.25%) and Hepatitis B (18.75%) of patients. The paper calls attention of physicians to this infection in the overall management of the patient	Afolabi *et al* (2002)
Basic Biochemistry (Kinetics)	Comparative Kinetics of Dipsin Sensitive (NaK) ATPASE and Ca^{++} ATPASE in Symptomatic and Asymptomatic Patients with Falciparum Malaria	The paper is a contribution to the understanding of the functional changes in red cell membrane pump system due to pathophysiological challenges Plasmodium falciparum invasion of the red cell by Plasmodium falciparum. There is disparity in the kinetics of erythrocyte membrane pumps in symptomatic and asymptomatic malaria episodes.	Iwalokun *et al* (2002)
Vector Studies	Investigation of Seasonal and Temporal Variation in the Biting Activities and Habit of Mosquitoes on the Atlantic Coast of Lagos, Nigeria	Malaria was studied in coastal area of Ibeshe Island, Lagos. 81.2% of Anopheles mosquitoes were caught indoors and 37.2% were indoor. Human biting rate - 2/hr. Sporozoite rate - 3%. Study useful for integrated management for control of malaria.	Afolabi *et al* (2006)
	Larval habitats of mosquito fauna in Otopko metropolis, South Western Nigeria	Identified six species of mosquitoes whose occurrence and distribution in discarded tyres was significantly higher than other habitats encountered. Paper calls for sustained environmental sanitation.	Adeleke *et al* (2013)
Multicentric Drug Trial	Clinical Trial of Co-Artesn in the treatment of acute Plasmodium falciparum malaria. (Novartis Pharmaceutical Company)	Parasitaemia was cleared in 12 - 24 hours. Clinical symptoms and signs abated in 12 - 36hours.	Adewole *et al* (Technical Report)
	Comparative Clinical Evaluation of Asumax against Fansidar in Nigerian children with uncomplicated malaria (South-Synthelabo Pharmaceutical Company)	Parasitaemia was cleared in 12 - 36 hours. Clinical symptoms and signs abated in 24 - 36hours. Fansidar was still effective. Parasitaemia cleared in 24 - 48 hours, but the clinical signs cleared and stated more slowly in 2 - 3 days.	Adewole *et al* (Technical Report)

3. HIV/AIDS: Research and Clinical Care

Mr. Vice-chancellor Sir, please permit me to prove my worth as a physician-in-scientific research, who had to combine clinical care with laboratory practice. I wish to refer to my modest contribution in the management of HIV/AIDS.

AIDS (Acquired Immunodeficiency Syndrome) is caused by infection with HIV (Human Immunodeficiency Virus). AIDS was, and is still a major public health problem as over 5% of the population was afflicted with the disease. It was estimated that about 4.9 million Nigerians were HIV positive. About year 2000, there arose the need to create a Department of Clinical Research at the Nigerian Institute of Medical Research. Again chance divinely favored me to be appointed as the first (foundation) Head of that department. Our main focus was to provide clinical care to HIV / AIDS patients. It must be noted that in the early 1990s, there were no drugs that could attack the virus directly, only palliative care.

The drugs that were being developed abroad were too expensive and beyond the reach of our population. Fortunately, in January 2002, the Federal Government of Nigeria initiated a national anti-retroviral (ARV) treatment programme as part of an expanded response to care and support for people living with HIV/AIDS. The programme commenced in February 2002 in 25 treatment centers and our center (NIMR) was one of them, with my humble self as the Principal Investigator of our centre. Our paper (Idigbe et al, 2003, 2005) documented our experience with the first 50 patients who were enrolled after meeting the inclusion criteria for the programme. Each of the 50 patients was placed on a combination of three drugs – a treatment referred to

as Highly Active Antiretroviral Therapy (HAART). Data were generated for each patient at baseline, and at 12, 24, 36 and 48 weeks of treatment. Our data showed that median Viral load (assessed by PCR-based Amplicor HIV-1 monitor system) and expressed as viral copies per ml of plasma) decreased from 3.64 $\log_{10}$ at baseline to 2.30 $\log_{10}$ at week 24, reflecting a median decrease of 1.23 $\log_{10}$ copies per ml. The percentage of patients with viral load values below detectable levels increased from 2.6% at baseline to 84.8% by week 24. During the treatment period, the patient's ability to carry out normal activities increased remarkably, median body weight increased and the proportion of the patients who presented with opportunistic infections decreased to 23.9% at week 12, 19.7% at week 24 and 6.8% at week 48. The frequency of opportunistic infection decreased in a similar fashion.

Since the initial publication, we had other publications documenting our experience on limitation and access to the use of antiretroviral drugs(Adeneye et al, 2006), perception of the benefits and affordability by the patients(Ogunro et al, 2006) and willingness of pregnant women to seek voluntary HIV counseling(Adeneye et al, 2007). These had contributed to the huge success of ART programmes in Nigeria.

Running HIV/AIDS clinic gives me joy for the disease which was considered fatal before is now controllable, although not curative. Before leaving that programme, I saw several patients, who had no hope before, marrying and having children who are free from the disease. The joy of our patients became our joy.

IV. MOLECULAR DIAGNOSIS: The Present and the Future.

In the last 10-15 years, molecular diagnosis has undergone revolutionary advances occasioned by the sequencing of the whole human genome (Venter *et al*, 2001; Collins *et al*, 2001). The Human Genome Project (HPG), estimated to cost about 3 billion dollars and which took about 18 years (1985 – 2003) to complete, was to determine linear arrangement of nucleotides on the genome (total DNA composition) of man. This effort allowed for identification of portions of the gene that were important for the development of diseases and those that could be used as markers for diseases(Rivenbark, 2010). Through information available through HGP, molecular diagnosis has not only helped in predicting whether a patient will be at risk for some diseases, but had led to other areas of health care (Fig 15).

Today, we now know that molecular diagnosis plays a key role in the management of patient and disease in the following areas:

(i) Increased screening for pathogens responsible for diseases, inherited diseases and screening for changes in the genetic make-up of an individual that is responsible for cancer, for example breast, prostate and colo-rectal cancers.

(ii) In genetic testing to predict the right dosage of the right drug that can be administered to patient (Personalized medicine).

(iii) In rational design of drugs and drug discovery, through identification of target genes for new therapies through DNA.

Figure 14

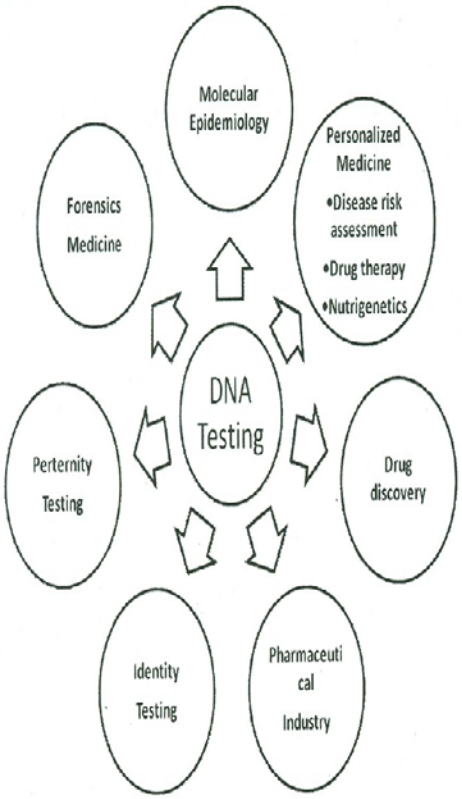


Fig. 14: DNA Revolution
Influence of DNA Testing / Molecular Diagnostics on Medicine and Health Care

(iv) In forensic science, where profiling of a person individuals enables identify testing from DNA extracted from a minute amount of biological specimen such as hair, blood, semen found in cases of homicide, rape, sexual assault and others.

(v) In paternity testing where DNA of the offspring (baby) can be correctly and rapidly be associated with the parents;

Innovation and advances in methodology and detection techniques have also impacted on molecular diagnosis resulting in high speed, high sensitivity and specificity. For instance, when

we were using PCR for our work, we can only work in one single-gene disorder. Today more than 20 genetic loci can be amplified and identified through micro-array technology, high fidelity PCR, label-improved probe technologies. The implication is that diseases which have multi-genetic origin (diabetes) can be studied better. In future a single test will be able to detect multiple targets.

Furthermore, development in micro-fluid technology and nanotechnology are already impacting on genetic testing, leading to miniaturization of the whole process(Jain, 2003; Bhandari et al, 2011; Robinson and Dittrich, 2013). Kits for molecular diagnostics are being developed. It is expected that in a decade or so, people would have their own genes sequenced, for a relatively small fee and carry a smart card providing access to his or her genetic information.

Every technological advances has its own limitations. The issue of patents of developed mutation detection platform limits access, especially in the third world. Some of the patented discoveries cost hundreds to thousand of dollars. Skilled manpower, quality control and correct interpretation of tests require training and retraining. Legal consideration and ethical concern are hurdles that had to be overcome in coming years.

Ferns and co-workers (2003) in their paper: Molecular Diagnostics in routine practice: Quality issues and Application to complex diseases noted:

> *"Although high-throughput molecular biology testing within hospital laboratories is an exciting prospect, a great deal needs to be done to ensure quality of analysis and their interpretation. A first step would be to introduce EQA (External Quality Assurance) schemes for tests that are currently, or are becoming widely used"*

Medical Training for Disease Management of Tomorrow

Mr. Vice-Chancellor Sir, disease diagnosis and management of patients is going molecular and the end does not appear to be in sight. It is against this background that I wish to discuss the current curriculum for medical training. Certainly, the curriculum cannot meet the challenges of medical practice of the future. First, I agree with the current clamor for integrated teaching where medical students will be exposed to the clinicals very early in their training. But it is my view that the content of molecular biology and medical genetics and their applications should be increased. I know that the National University Commission is planning towards this trend, but the proposed curriculum is foot-dragging and is yet to be accepted by most medical schools.

MOLECULAR DIAGNOSIS: Relevance in Chemical Pathology and other Basic Medical Sciences.

I am aware that I am giving this lecture on behalf of the Faculty of Basic Medical Sciences. Fortunately all the Departments of Pathological Sciences have something to do with molecular diagnosis. Having carried out research related to some of these Departments, I wish to address what we should be doing that is relevant to the management of diseases in Nigeria. First, I wish to observe, on a happy note, that this University has invested in the development of molecular biology laboratories, although much still needs to be done. For example the Faculty of Basic

Medical Sciences has two functional laboratories; one in the Department of Medical Laboratory Science, which I initiated when I was the Provost. The others are molecular laboratories in the Departments of Medical Microbiology and Chemical Pathology. There are two functional PCR machines and other basic equipment. We have scientists actively working in these laboratories for research and training post-graduate students. Therefore, the future is bright.

(i) **Metabolic and Inherited disease**

Sickle Cell Disease. Prenatal diagnosis should occupy the front burner where the Chemical Pathologists, Haematologists and Physicians should establish viable programs. Genetic counseling by trained counselors should go hand-in hand with prenatal diagnosis. Beyond PND, genetic studies should be embarked upon to investigate the well known variations in the clinical manifestation of the diseases. It is known that the severity of illness of sickle-cell patients vary from mild to severe, Although this had been explained in terms of persistent foetal haemoglobin, environmental and social factors, it is my postulation that other genes, yet unknown, may modify the phenotypic expression.

Neonatal screening of treatable inherited diseases, for example phenylketonuria, should also be attractive to Chemical Pathologists. I am not aware that neonatal screening programs, which is well established in developed countries is available in Nigeria.

(ii) **Malaria**

Some centres in Nigeria (e.g UCH and NIMR) are actively involved in study of resistance to malaria chemotherapy. Few are

involved in malaria vector studies. Such studies, involving molecular typing and profiling should be well coordinated nationally and data made available. Active molecular surveillance centres should be set up.

(iii) **Bacteria and Viral Infection**

More than 80% of molecular test performed today is directed to pathogens in humans, surveillance of resistance species, genotyping and to a lesser extent, drug sensitivity. Viral and bacteria infections are major causes of morbidity and mortality in Nigeria. Many laboratories, including our own Medical Microbiology are actively involved in such studies. The trend should continue. Our own experience on shigellosis, a causative agent of diarrhoea and dysentery and had been documented (Iwalokun et al, 2001a, 2001b, 2002a, 2002b, 2002c, 2002d, 2002e and 2003). The work was the major focus of my first PhD student. The advantage of molecular diagnosis of pathogens is it's rapidity, specificity, and sensitivity compared to the convention culture methods.

(iv) **Cancer**

Efforts world-wide had been directed towards the cause of cancer (malignant proliferation of cells) in humans. Advances in molecular biology have contributed to our understanding of the pathogenesis of cancers. This, in my opinion, is where our histopathologists can make their own modest contributions. Cancer of the breast, prostate, heamatological cancer and bone tumours constitute more than 60% of cancers affecting man. There is paucity of data on molecular studies on cancer, yet there is a lot of funding for anybody interested in cancer research. My humble submission is that if we are unable to carry out the high-

tech research as it is being done elsewhere, we can at least settle for drug- receptor studies, so as to provide data that will assist the physicians in the management of their patients.

(v) Forensic Analysis and Paternity Testing

There is a dire need for DNA laboratory in this country. This view is underscored by two recent presentations. Obafunwa, in his inaugural lecture said:

> *"A Forensic Laboratory is an essential tool of the Forensic Pathologist. Sadly, this lecturer is not aware of the existence of any in the country... The DNA laboratory will not only help to solve problem of paternity identification, but also crime with the analysis of blood stain, body fluids, saliva, tissue and organs"*

His view was collaborated by Olusegun Abimbola, a lawyer, in his recent lecture, "Law and Medicine: A meeting point" stated:

> *"Forensic analysis of hair follicles, saliva, semen, and blood samples have become admissible in law as expert evidence to ground convictions in rape cases and if properly demonstrated before Nigerian courts, will assist immensely in prosecution of rape cases. Nevertheless, a good number of cases go unprosecuted because the available science, already perfected in other jurisdictions, remains largely unused in Nigeria. The marriage of medicine and the law in forensic analysis has been established over a long list of cases, but the limited or non-availability of the science and technology in Nigeria continues to frustrate its application in Nigeria. We do hope that the Colleges of Medicine and our governments at all levels will deepen this knowledge that is already in the public domain and no longer exclusive and make its application to legal practice in Nigeria to facilitate the administration of justice"*

I hope this will stimulate our University to take up the challenge. Paternity testing through molecular technologies had been of interest to me since I encountered a parent who approached me some years ago, desperate for this service. I was unable to help.

I am not advocating that we can embark on such a highly specialized discipline now. But we can prepare for the future by training younger colleagues abroad after exposure to basic technologies here. There is a commercial reward for such ventures, but more importantly, I look forward to when this University will be able to award a Master's degree in Forensic Science or related fields.

(vi) Non-communicable Multi-factorial Diseases

Genetic predisposition to some non-communicable diseases mentioned earlier in this lecture is also for the future but simple preliminary molecular epidemiology studies can begin now.

V. ETHICAL, LEGAL, SOCIAL AND LOGISTIC ISSUES.

There are hurdles to cross for a successful practice of molecular diagnosis and use of DNA – based technologies. Medical practice respects the right of an individual over his or her body. The DNA of a person contains all the information of the blue print of life. His or her DNA is a potential tool in the hand of a molecular diagnostician which can be used for intended purpose or misused for other purposes. Remember that DNA can be cloned! This and

other ethical issues such as inappropriate genetic testing raised serious concerns. The public may have fear and mistrust in the molecular testing. For instance, who has the right to *information*? How should the information be used? Will there be discrimination in employment and insurance policy due to genetic make-up of the individual? What happens to incorrect or inappropriate ordering of tests? What is the legal protection or the right of the clients when test results are mis-interpreted? Suffice to say that there is no regulation on genetic test or Standard Operation Procedure (SOP) and Quality Control of the laboratories. In developed countries, there are serious regulations guiding the use of DNA (Masui & Takada, 2003).

What tests are to be performed? Obviously the level of tests must depend on the sophistication of the laboratory and the appropriateness of the training of manpower. A major issue is the cost of tests. Who bares the cost of say a large scale genetic screening programmes?

A major logistic problem, apart from power supply and quality of water, is the problem of procurement of reagents and getting it to the laboratory at the right temperature and the right condition. I had a bad experience some years ago when reagents I ordered from Germany that should be stored frozen at -20^{0}C was seized by the Custom Officers and not released for 3 weeks!

But these problems and issues raised are not un-surmountable if there is political will, appropriate regulations and funding. The truth is that the benefits of molecular testing as a service and as a research tool are too enormous in disease management. The demand is compelling.

RECOMMENDATION

Against the background of the views expressed above, I recommend as follows:

1. DNA laboratories and genetic testing (molecular diagnosis) must be established in the relevant University Departments and Teaching Hospitals as Centres of Excellence. Funds from TETFUND can be justifiably invested in such projects. This is common place in developed countries but we cannot always depend on carrying samples abroad – at great cost.
2. There should be urgent National Policy on DNA-based Diagnosis both for service and for research. For services, the policy should address which laboratories will be licenced to carry out which tests. As a research, the policy should specify how DNA should be handled, what type of research to carry out, who should handle what. The question of how to dispose wastes, some of them toxic or carcinogenic, must also be addressed. Such policy is urgent because we are beginning to hear of "DNA labs" in the country, most of them publicly soliciting for clients. The policy should also address issues of confidentiality and privacy.
3. Government should seriously consider a special tariff for reagents and equipment for DNA laboratories. This is to encourage rapid advancement in this technology.
4. Government should open a direct corridor to purchase reagents and equipment. This is where inter-governmental cooperation becomes important. Today, most reagents, manufactured in USA and Europe are

purchased by Nigerian scientists from South Africa.

5. For the diagnosis of human diseases, genetic tests must go hand-in-hand with genetic counseling with appropriately trained staff.
6. Any Laboratory involved in genetic tests must, by law have policy for training and re-training of staff.
7. The content of molecular biology and medical genetics teaching and their applications must increase in the current curriculum for medical training in order to meet the challenges of medical practice of the future.

CONCLUDING STATEMENT

When men began to study diseases, it was to observe symptoms and signs. Later people began to study diseases through observation of organ and tissue dysfunction at macroscopic and microscopic levels. Then came the analysis of body fluids and their components. Today, diseases are gradually being understood at molecular (DNA) level.

Every disease is about DNA; DNA is the key to the diagnosis, prognosis (disease outcome) and treatment of diseases.

This is in my submission.

I am most grateful to Baba God, The Almighty, The Omnipotent, The Omniscience for putting me in this field. He has taken me through the winding path, like David and Moses, two great characters in the Bible. But at last He planted my feet on higher ground. He gave me the insight to see DNA research as the key to disease management of the future.

All the way my Saviour leads me
What have I to ask beside?

Can I doubt His tender mercy
Who thro' life has been my guide?
Heav'nly peace, divinest comfort,
Here by faith in Him to dwell!
For I know what'er befall me
Jesus doeth all things well
(Fanny J. Crosby, 1820–1915)

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God has used many human agents to shape my life and it is impossible to mention all of them. In a special way, I wish to thank all who are in this auditorium; our great LAUTECH community, and all who travelled from far and near.

I wish to thank the Vice-Chancellor and the Council and Management of this University, first for granting the request of Osun State Government on my behalf, to serve at Osun State University ('UNIOSUN') as Foundation Provost of the College of Health Sciences for two terms. Therefore, the story of UNIOSUN cannot be completed without LAUTECH. Secondly, LAUTECH was magnanimous to grant the request of Bowen University to serve as the Provost of the College of Health Sciences and Acting Chief Medical Director of Bowen University Teaching Hospital following the sudden death of Prof. Wole Adebo (R.I.P.).

I cannot but express my unalloyed gratitude to the very important personalities here: Kabiyesis, I salute you. To the political figures and their representatives, I say thank you for sparing time out of your busy schedules to be here.

I must recognize in a special way, my great mentor, adviser and

encourager, Prof. Adebayo Afonja. Prof. Afonja is a Teacher of Teachers. He has produced not only Professors, but two Vice Chancellors as of the last count! He was so excited about the lecture and created time to be here. He supervised my Postgraduate Fellowship Diploma and graciously granted my training abroad. Thank you very much Sir. To my other mentors, Professors Fola Esan and Akin Aboderin, I say thank you.

I have had the unique opportunity of working with several Vice-Chancellors in different Universities. I have learnt a lot from them. Prof. Olusegun Oke, Prof. Salau (during whom's tenure I was appointed), Prof. Adeleke, Prof. Sola Akinrinade, Prof. Timothy Olagbemiro and Prof. Matthew Ojo have been a source of inspiration. To them: I say thank you.

The Director-General and all members of the Nigerian Institute of Medical Research are hereby recognized with appreciation. The Director-General, Prof. Ujah is a friend and great supporter. He is well appreciated. I wish to express my unalloyed gratitude to the former Director-General of NIMR, Prof. Oni Idigbe for being such a great unbiased and productive leader. He has also contributed to my success in no small way.

To all members of the College of Health Sciences, the Provost, Prof. Adesokan encouraged me to give this lecture. I thank you for your understanding. To the Dean, Prof. S. S. Taiwo, not only shall we say "The Young Shall Grow"; indeed the Young Has Grown and is doing well. You shall go places.

I must say a big thank you to my Molecular Biology Group both at LAUTECH and UNIOSUN. Drs. Ojurongbe, Olowe, Adeleke and others in the team. You make me proud. Thank you for carrying

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If I am here today and doing fine, it is because God has assigned to me great spiritual leaders. My pastors, led by Rev. Julius Omomola, virtually moved the whole church to Ogbomoso. To all the Pastors, Deacons and beloved members of New Heritage Baptist Church and the New Estate Baptist Church family; I say thank you for your prayers and your presence. In the same vein, I wish to thank our Baptist Convention leaders, especially the former General Secretary: Rev. Dr. Ademola Ishola and the current President, Rev. Dr. Supo Ayokunle, for appointing 'one of their own' to serve at Bowen University and Teaching Hospital.

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I come from an extended family, ably led by my Sister and our Matriach – Mrs. Abiola Ajao, and my very own Funmilayo Adewole, my special sibling. Your love and understanding is appreciated. You have been so supportive; your encouragement has contributed to the success of today. God will keep us forever. Amen. I thank God for being blessed with such a family. We relate with each other as one big family, following the legacy of our parents.

Words cannot express my gratitude and respect for my family from Ondo. You have taken me as a son, and a precious one for that matter! You always treat me as such. True to your usual character, you are here in a large number. God will keep us forever.

To my children, Adedokun and Adeoye, I thank God for giving you to me as special gifts. Thank you for your love and understanding. I am always proud of you. You are my pillar of success.

To my wife, Morounkeji Ajike, who my family nicknamed "Ruth", that great character in the Bible, I say with my whole heart: "THANK YOU FOR BEING THERE FOR ME ALL THE TIME". Your love is exemplary; your support is colossal. She contributed to who I am today. She arranged for all the "Item 7" for this lecture. Even when I am sleeping, she is busy working to make sure I rest well.

I want to say a special thank you to my late parents, who incidentally were both teachers. My father, Pa Samuel Ladeji Adewole (Papa "SLA") was a very strict disciplinarian who instilled Christian virtues in me.

Ladies and gentlemen, please permit me to single out my mother, Mama Lydia Oladuntan Adewole, who trained me at home and at school; but did not wait to see me become a professor. Maa'mi, you are fondly remembered and appreciated.

Awero Opo
Opo mo ja, Alekan
Omo Balogun ni'le Awe
Awero r'ogun, Awero duro
Awero r'ogun, Awero dide
Awero ja'gun Omo
O gba yi, o gbe ye
Sun re o. Maa 'mi.

It is in appreciation of the role my mother played in my life that I dedicate this Inaugural lecture to her memory.

Mr. Vice-Chancellor Sir, Ladies and Gentlemen, the great audience, I thank you for your attention.

I am done!

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