USE OF VNTR LOCUS D1S80 VARIABILITY IN ASSESSING GENETIC RELATIONSHIPS OF INDIVIDUALS FROM SELECTED FAMILIES IN THE KUMASI METROPOLIS

BY

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DECLARATION

I hereby declare that this thesis is my own work and that to the best of my knowledge, it contains no material previously published by another person nor materials which has been accepted for the award of any other degree of the University, except where due acknowledgement has been made in text.

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DEDICATION

This work is dedicated to my precious daughter Nyametease Ama Serwaa Ayisi.

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ABSTRACT

Several challenges bordering on paternity has been of much interest in our society today due to increased infidelity in marriages, rape cases, inheritance of estates of deceased parents and immigration issues. There is, therefore, the need for a more reliable technique for establishing the paternity of children in such families. The aim of this study was to assess genetic relationship involving the use of Variable Number of Tandem Repeats D1S80 DNA fingerprinting marker among individuals from 13 selected families of Anwomaso Township in the Kumasi Metropolis. Whole blood samples were collected from the study subjects. A part of the samples was used for ABO blood typing and the remaining used to extract genomic DNA. The DNA samples served as template in the subsequent polymerase chain reaction involving primers for the VNTR DIS80 marker. The PCR product representing the amplified VNTR regions were resolved on 2.5% agarose gel. The allelic bands scored were analyzed with NTSYSpc version 2.1 for similarity among members of the families. The ABO blood typing results for all the families involved in the study agreed 100% with the measured ABO phenotypes of the children although definitive paternity could not be established. The DNA finger print confirmed paternity of 24 children (82.7%) while five failed the paternity test. Two children in two different families, F2 and F6, were neither related to the supposed father nor mother and thus suggesting adoption of the children by the families. In one of the thirteen families, none of the two children had genetic relationship with the supposed father. It is concluded from this study that the use of VNTR DIS80 locus for DNA fingerprinting is more robust and definitive in refining paternity determination among families compared to the ABO system.

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CHAPTER ONE

1.0 INTRODUCTION

The issue of establishing parentage is ubiquitous in the natural world and it has fascinated human beings since the beginning of recorded history. It has been an age old challenge to human beings, dating back to biblical times when King Solomon was confronted with a case pertaining to the parentage of a boy (I Kings 3:25). Among such cases, the ones involving paternity is perhaps the most significant and thus presents a significant challenge to the society, both scientists and potential parents alike.

Paternity determination is important for both the child and the parents. A child deserves the comfort and the sense of belonging that come from knowing both parents. Determining paternity is important to give children access to family medical histories, access to enjoy benefits of social security, inheritance and medical insurance available through the father (Draper, 2007).

In legal terms, paternity determination helps prevent misattributed paternity and also makes the father have complete right over the child to participate in major decisions about the child such as medical treatment, education or religious training (Draper, 2007).

The technique employed in determining paternity has evolved over time. During the first half of the twentieth century, researchers used the ABO phenotypes of people to establish paternity. However, the ABO blood grouping system provided information that could only be used to exclude potential fathers rather than confirm the existence of a parental relationship (Adams, 2008). As a result, additional markers such as the Rhesus (Rh)

antigen, MN antigen and Human Leucocyte Antigen (HLA) have been considered to increase the reliability of paternity testing (Adams, 2008). However, significant test errors still exist, for instance, non-blood cells such as sperms do not have HLA and this renders testing less useful.

Advances in molecular genetics, such as DNA analysis and genome sequencing, provided an alternative molecular approach to resolving paternity of individuals by comparing DNA of offspring and parents (Adams, 2008). The technique was first introduced in the early 1980s. Unfortunately, this method is not easily accessible, because of the cost and lack of personnel with such expertise. This molecular method is also known as DNA fingerprinting (Kirby, 1992).

Human-DNA fingerprinting involves the use of DNA probes synthesized from regions of the human genome that exhibit great variability between individuals (Jeffreys *et al.*, 1991). Over the years, genome investigations have revealed a number of repeated nucleotide sequences in tandem with high polymorphism in the population (Levy *et al.*, 2007). These sequences may be put under 4 classes, Variable Number of Tandem Repeats (VNTRs), Short Tandem Repeat (STR), Single Nucleotide Polymorphism (SNP) and Copy Number Variation (CNV) (Levy *et al.*, 2007).

Tandem repeat sequences are common in higher eukaryotes and account for a larger proportion of the human genome. The large size coupled with exclusive variability makes it suitable for DNA fingerprinting (Levy *et al.*, 2007).

VNTRs are common hyper-variable regions of DNA used for genetic identity and relationship testing because of their high polymorphism (Clark, 2005).

At present, highly polymorphic loci of the human genome, whose alleles are the results of VNTRs, are the most genetic markers for genetic characterization (Budowle *et al.,* 1991). DNA profiling, using multilocus core probes (probes capable of detecting simultaneously several Variable Number of Tandem Repeat loci), has been shown to be a powerful tool for identity and paternity testing of humans (Jeffreys *et al.,* 1991). VNTR loci are very similar between closely related humans, but so variable that unrelated individuals are extremely unlikely to have the same VNTRs (Destro-Bisol *et al.*, 2000). VNTR loci for paternity testing include D2S44, D4S139, D14S13 and D16S85, and they provide exclusion power of about 99.93% which is very effective, but relatively lower than VNTR locus D1S80 which has 99.99% power of exclusion (Bloom, 1994).

This research sought to use DNA fingerprinting technique that employs the power of Polymerase Chain Reaction (PCR) and Gel Electrophoresis to establish paternity of individuals from a selected population with respect to polymorphism in VNTR locus D1S80 located on chromosome 1 of the human genome. In this study, DNA fingerprints of children were compared with their fathers (and mothers) to establish evidence of inheritance and biological relationship.

1.1 Problem Statement

In Ghana, there have been several questions raised on the paternity of children due to infidelity in marriages, increased rape cases, immigration issues and inheritance of estates of deceased parents. The Ghana Immigration Service recorded 354,000 men who took paternity tests from 2003 to 2006, of which 30% were not the biological fathers of children claimed (www.ghanaweb.com, 2007). Also, a report of activities of the year 2009 and 2010 from the Department of Social Welfare, Domestic Violence and Victim Support Unit (DOVVSU) of the Kumasi Central Police Station recorded 65 and 61 cases of paternity respectively. The reported cases were pregnancy and child disputes (DOVVSU, 2009). These examples and many more challenges bordering on paternity support the need to provide an accessible and affordable paternity service in the community.

1.2 Main Objective

The main objective of this research was to assess genetic relationship of individuals from selected human families in Anwomaso in the Kumasi Metropolis through the use of ABO blood typing and analyses of PCR amplification products of VNTR DIS80 locus.

1.3 Specific objectives

Determine ABO blood group status of family members and to use the information to establish genetic relationship among them.

Determine the allelic variation at the VNTR D1S80 locus among members of families.

1.4 Justification

As far as I know, there is no DNA fingerprinting study involving the use of VNTR D1S80 locus in the Ghanaian population. The outcome of this study will serve as a baseline for further genetic characterization of individuals in the population to aid resolutions of legal matters pertaining to paternity considering the 99.9% exclusivity of the technique.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 History, evolution and current trends in paternity testing

Paternity tests in humans are conducted to establish genetically whether a man is indeed the biological father of a said child. The issue of establishing paternity has been part of social discussions since the beginning of recorded history, because there has always been the need for association through family relations, ethnic groups, royalty and the like (Draper, 2007). Thus identifying and determining blood lines of people has always been of special interest to man.

Though genetic markers are the most reliable methods for establishing paternity now, older methods of paternity testing employed the use of markers which were congenital traits, such as facial appearance, cleft chin, color of the eye, skin color or attached ear lobes. These methods were convincing but not reliable, because ability to detect strong resemblance between two people is relative (Horn *et al.*, 1989).

The Chinese also employed unique blood tests in solving genealogic problems by dripping the blood of a relative onto the skeleton of a deceased to determine if they were related (Silver, 1989). Alternatively, blood from two living individuals were dripped into a bucket of water to see whether they mixed or not (Silver, 1989). In the first method, relation was confirmed when the skeleton absorbed the blood and in the second method, when the blood mixed and flowed together. Unknowingly, they were using the technique of blood groups and agglutination of the blood to identify human relations which became

scientifically proven in the early 20th century. In 1900, an Austrian physician, Karl Landsteiner, discovered the first genetic marker used for paternity testing, the ABO blood groupings (Silver, 1989). Surface proteins located on the red blood cells called ABO antigens determine the blood groups or types labelled as A, B, AB or O (Silver, 1989). The ABO trait is hereditary, thus parents' blood types are genetically inherited by their children. This method for paternity determination, however, is weak and inconclusive. It has 30% power of exclusion (Silver, 1989).

The weakness in the ABO blood grouping system for determination of paternity led to discovery of more powerful genetic markers. These approaches included for example, improvement of the ABO system by adding Rhesus (Rh), Kell or Duffy blood groups in the 1940s. However, these methods were also weak considering their power of exclusion of only 40%.

In 1960s, the MN blood grouping system was discovered. This involved 17 blood groups and the most useful group among them was the Human Leucocytes Antigen (HLA). HLA was accepted in the 1970s as a reliable tool for paternity determination with a powerful exclusion of nearly 80% (www.dnacenter.org; 2011).

During the 1980s, advances in genetics involving DNA markers and genome sequencing brought with it an alternative for scientists to approach the issues concerning paternity testing. Generally, the technique involves comparison of the DNA markers and inheritance among individuals involved (Adams, 2008).

In the early 1980s, the first DNA fingerprints, Restriction Fragment Length Polymorphism (RFLP), were employed to establish paternity of children (Butler, 2008).

RFLP has 99.99% power of exclusion which therefore became the most reliable method for paternity testing in the history of genetic variability studies (Butler, 2008).

A major drawback for using RFLP is that it is time consuming and requires large volume of blood sample (Budowle *et al*., 1991). Also, RFLP usually requires the use of radioactive isotopes to detect very small quantities of DNA (Budowle *et al*., 1991). This negative environmental impact results in strict regulations and thereby makes the technique relatively unpopular. In the late 1990s and the early 2000s, Variable Number of Tandem Repeats (VNTR) became the new DNA fingerprinting technique for determining paternity. VNTR polymorphism uses DNA extracted from biological samples and its variability at specific loci amplified by PCR is the dominant marker and rapidly used in paternity (Budowle *et al*., 1991). PCR is currently used by most laboratories worldwide, because of its accuracy and requires nanograms of samples for amplification to produce millions of copies for profiling.

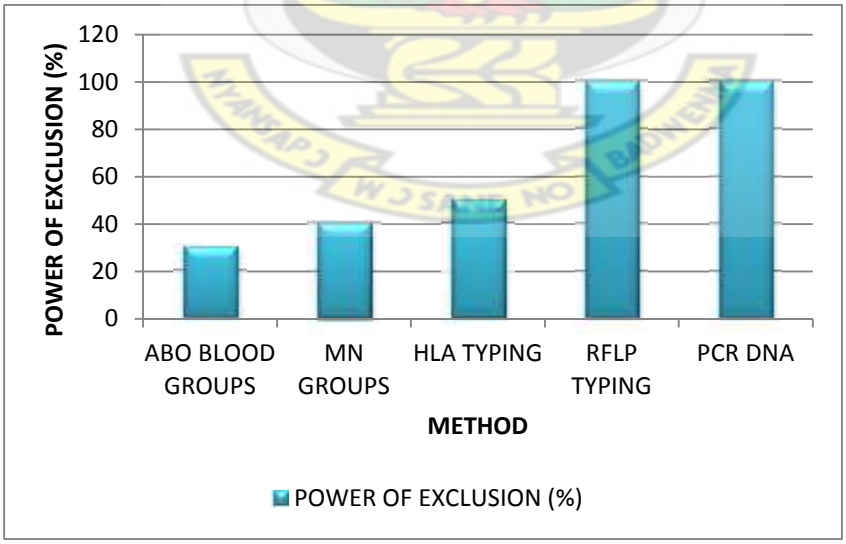


Fig. 1 Progression in the power of exclusion methods used for paternity testing as new and more improved methods was discovered and used (Chromosomal Laboratories Inc., 2005).

2.2 Social and legal implications of paternity

The Department of Social Welfare, Domestic Violence and Victim Support Unit (DOVVSU) report for 2009 and 2010 showed 65 and 61 paternity disputes, respectively, in the city of Kumasi alone.

In 2009, 61.54% of the reported cases were pregnancy disputes, while 38.46% concerned child-parental disputes. The pregnancy cases constituting 57.5%, which were referred to Family Tribunals Courts for legal actions, whiles 42.5% of the respondents agreed to maintain the pregnancy until delivery. All the 25 child disputes (38.46%) were referred to Family Tribunal (DOVVSU, 2009). Of this figure, 70.5% of the cases in 2010 were pregnancy disputes with nearly half of them (48.8%) referred to Family Tribunals for necessary legal actions. The remaining 19.50% of the cases involved child paternity claims and all were referred to the Family Tribunal court (DOVVSU, 2010). Forbes (1951) argues that paternity can be determined when the child is born in wedlock. However, this method is difficult and involves high cost to test the paternity of children. This creates a hindrance in determining paternity and, therefore, forcing innocent adult males to accept pregnancy responsibilities in the DOVVSU Unit.

In law, a child born to the wife during marriage is the husband's child under the "presumption of legitimacy" and the husband is assigned complete rights, duties and obligations of the child. The presumption of legitimacy is a common law rule which supports the evidence that a child born within the subsistence of a marriage is deemed to be the child of the husband.

The presumption of legitimacy is not always true due to infidelity of some couples. Paternity testing is, therefore, an effective test for genetic relatedness and infidelity within marriages (Draper, 2007).

2.3. Variations in human chromosomes form the basis of paternity testing

Humans have always been fascinated by the variation among individuals. That a certain trait was hereditary and has been understood in many different cultural traditions for a long time. However, understanding the genetics and how those traits are inherited only began about a century ago through the works of Gregor Mendel (Garrod, 1992).

The genome is the full DNA complement of an organism or cell. Genomes are stored on one or more chromosomes. The region of the chromosome at which a particular gene is located is called its locus. A chromosome consists of a single, very long DNA double helix on which thousands of genes are encoded (Braig and Schmitt, 2006).

Our characteristics, traits and physical features are determined by the specific arrangement of DNA base-pair sequences in the cell. It is this distinct arrangement of the nucleotide bases in DNA molecules that regulates the production of specific proteins and enzymes in an individual and this bringing about phenotypic variations (Brenhaupt, 2003).

There are certain loci of the chromosomes called the heterochromatin which are highly variable (Destro-Bisol *et al.,* 2000). These loci contain repeats of nucleotides which vary in number and distribution among all individuals (Destro-Bisol *et al.,* 2000). These loci are very similar between closely related individuals, but so variable that unrelated individuals are extremely unlikely to have the same number of repeats (Destro-Bisol *et al.,* 2000).

It is the variation in the number and sizes of these repeats, which lie in tandem to each other on the chromosome that forms the basis for paternity testing using Variable Number of Tandem Repeats (VNTR) (Alford *et al.*, 1994).

2.4 Chromosomal and DNA structural basis for paternity determination in human

There are 23 pairs of chromosomes in the human somatic cells. Each pair of chromosome contains different sets of genes. If a particular gene of an individual is expressed on both chromosomes then it is a homozygote dominant gene for that individual (Clark, 2005). If that gene is expressed on just one chromosome then it is a heterozygote dominant gene (Clark, 2005). However, the gene will be recessive if it is expressed on neither of the two chromosomes (Clark, 2005).

Chromosomes are named by numbers from one to twenty-three. The largest chromosome is chromosome one and it follows that order down to the smallest which is chromosome 23. Chromosome one contains the D1S80 locus which is used in paternity testing (Clark, 2005).

2.4.1 DNA structure and markers

Some regions of DNA contain useful genetic information, and other regions do not (noncoding regions). Non-coding DNA accounts for the majority of the DNA found in most plants and animals and their functions are not well understood.

Any segment of the DNA, whether coding or not, can be referred to as locus (plural, loci); that is, a location on the chromosome (Clark, 2005). The locus, which is a stretch of DNA, is what is analyzed for variability using different methods of testing. The portion of the genome where there is a lot of diversity among individuals is called a polymorphic

region. The polymorphic regions used for forensics are the non-coding regions, because these regions of the DNA do not code for proteins and they make-up 95% of our genetic DNA (Clark, 2005). These regions are therefore called the "junk" portion of the genome. Although these "junk" regions do not generate proteins, they can regulate gene expression, they also aid in the reading of other genes that do formulate the proteins, and they make up a large portion of the chromosome structure (Clark, 2005).

2.4.2 DNA Complementarity in Molecular Marker - Assisted Techniques

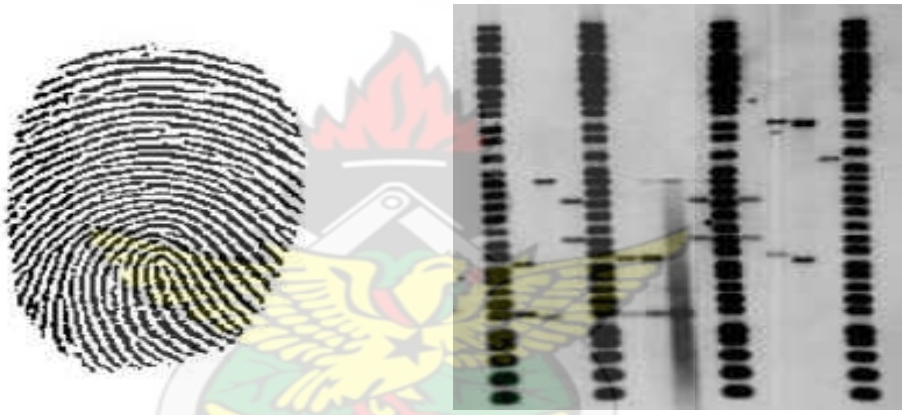
Base pairing is a crucial property of DNA in many of the molecular hybridization techniques. Complementarity in DNA has been exploited to develop molecular research techniques. A classic example is the technique of the polymerase chain reaction (PCR) which imitates the process of DNA manufacture *in vitro* to produce more DNA molecules (amplify) in a relatively short time. The PCR is no doubt an important technique used in paternity determination.

2.4.3 Uniqueness of DNA fingerprint among individuals

A classical fingerprint is the mark left by a person's finger on touched surfaces. Fingerprints are somehow unique to each person, just as in the case of DNA fingerprints. Traditional fingerprints involving thumbprints are divided into three separate categories: plastic prints, latent prints, and visible prints (Geberth, 2006). Plastic prints occur when the finger touches or presses against a soft pliable surface such as putty, gum, newly painted area, wax, flour, thick dust, soap, grease, tar, resin, or clay (Geberth, 2006). Latent prints occur from natural skin secretions such as perspiration. Example is when grease or dirt is mixed with the natural secretions; a stable print may be deposited on the

surface (Geberth, 2006). Visible prints occur when the finger, palms, or feet, which have been contaminated with a foreign substance, come into contact with a clean surface and are pressed onto the surface, leaving a print (Geberth, 2006).

Although the traditional fingerprinting involving thumbprints (fig.2.A) and other fingers was also used for identification, it could not be used to determine the relationship between two individuals.



(A) (B)

Figure 2 Comparison of classical fingerprinting **(A)** and DNA fingerprinting **(B)** (Lach and Pastis, 2006)

The normal or traditional fingerprint and DNA fingerprint are both unique to each individual (fig.2.A). However, the traditional fingerprint could only be obtained from the fingers when marks are left behind and each finger may have a unique pattern of arrangement (Lach and Pastis, 2006). With the exception of identical twins developed from the same egg, each individual DNA fingerprint is unique. The uniqueness of DNA fingerprints is due to the arrangement of some nucleotides in the non-coding regions of

the DNA sequence. This uniqueness is what makes the DNA fingerprint ideal for paternity testing. Unlike traditional fingerprinting, DNA fingerprinting provides information that allows genetic relatedness to be established (Lach and Pastis, 2006).

2.5 The Polymerase Chain Reaction (PCR) as an essential tool for paternity determination

Many inventions and discoveries in science are unexpected. The invention of the Polymerase Chain Reaction (PCR) is by no means a happy accident. The idea and concept of the PCR was built on already existing knowledge in science (Bartlett and Stirling, 2008). The PCR was invented by Kary Mullins in the early 1980s.

The PCR imitates the process of DNA replication *in vitro*. Target sequences in DNA samples are amplified (many copies are made) using a thermocycler within a short time period. The process requires two primers, a short single stranded nucleotide sequence that is complementary to the opposite ends of the target sequence DNA, specifically designed from flanking sequences and added to PCR reaction tube.

The success of the PCR is closely tied to the timely discovery of the Taq Polymerase (Bartlett and Stirling, 2008), a thermostable DNA polymerase enzyme that was isolated from the bacteria *Thermophilus aquaticus.* Originally, PCR employed the use of DNA polymerase isolated from *E. coli*. The use of this enzyme from *E. coli* in PCR had one major drawback. The enzyme was sensitive to the temperature that was used to denature DNA during PCR and caused irreversible enzyme denaturation. Thus, fresh supply of the enzyme was required at the beginning of every cycle. So the discovery and isolation of the Taq polymerase helped overcome this limitation (Kirby, 1992).

The PCR technique can be used to amplify few copies of DNA (in nanogram quantity) into millions of copies within a very short time. The synthesis of DNA *in vitro* using PCR requires a template DNA (target sequence), two primers specific for the template, free nucleotides (dATP, dGTP, dCTP and dTTP) and the DNA polymerase (Taq polymerase).

During the replication process, template DNA is heated to 94 to separate it into single strands. The temperature is then lowered to 60 to allow the primers pair with the complementary bases on the single stranded template DNA. The temperature when raised to 72 allows DNA polymerase to synthesize complementary strands by extending the primers. The steps could be repeated for several times with each new DNA serving as a template in the next cycle of replication. The application is done exponentially with the formula 2^nX where n is the number of cycles and X representing starting amount of template DNA.

The PCR has become a major tool for analysis in molecular biology since its discovery. A simple survey conducted on Pubmed by searching for articles containing the phrase 'Polymerase Chain Reaction' and plotting a graph of the results showed an exponential rise in the usage of PCR from the late 1980s to 2001 and the graph was still rising (Bartlett and Stirling, 2008).

2.6 Application of Gel Electrophoresis in DNA fingerprinting

Electrophoresis is the motion of charged particles in a colloid under the influence of an electric field. Particles with a positive charge go to the cathode and particles with a negative charge go to the anode.

Electrophoretic technique is used commonly to separate DNA, RNA and protein molecules on agarose or polyacylamide gel (Andrews, 1986). The gel refers to the matrix used to hold and separate the molecules. In most cases, the gel is a cross-linked polymer whose composition and porosity is chosen based on the specific weight and composition of the molecules being separated (Andrews, 1986). When separating proteins, small nucleic acids or oligonucleotides, different concentrations of *acrylamide* and a *cross-linker* molecule are used to produce different sized mesh networks of polyacrylamide. When separating larger nucleic acids, usually greater than a few 100 bases, the preferred matrix is purified agarose (Griffiths *et al.*, 2004).

2.7 Methods Used In Paternity Determination

2.7.1 ABO blood grouping as a method for paternity determination

2.7.1.1 Genetics and inheritance of the ABO blood type

During sexual reproduction, two haploid gametes (egg and sperm) combine to generate a diploid cell in which each chromosome pair consists of maternal and paternal chromosome. Because the somatic cell has 46 chromosomes (23 pairs), each half is inherited from one of the parents to form the required diploid number (2n) for the offspring (Lehninger *et al.*, 2000). The surface of human erythrocytes contains genetically determined sets of molecules which then is divided into blood groups or blood types depending on which sets of molecules are present (Murray *et al.*, 2003).

About 32 human blood group systems are recognized. The best known among them include ABO, Rh (Rhesus), and MN systems (International Society of Blood Transfusion, 2008). The genes responsible for production of the ABO blood groups are present on the long arm of chromosome 9 which shows that there is only one locus for the genes

determining the blood groups in a single pair of chromosomes (Forbes, 1951; Murray *et al.*, 2003). In 1910 Von Dungern and Hirszfeld established the Mendelian inheritance of the ABO blood groups, but its exact manner of inheritance was worked out by Bernstein in 1924 who postulated the three allelic genes; A, B, and O (Forbes, 1951).

The first observation of agglutination of human red cells by serum belonging to the same species was made by Landsteiner in 1900 (Forbes, 1951). Because these sets of molecules are genetically determined, a person's blood type is inherited and thus making them important markers in paternity testing (Johnson, 1983). The term "blood group" applies to a defined system of red blood cell antigens (blood group substances) controlled by a genetic locus having a variable number of alleles (Murray *et al.*, 2003). There are three alleles, two of which are co-dominant (A and B) and the other (O) recessive. These ultimately determine the four ABO phenotypes namely A, B, AB, and O (Murray *et al.*, 2003).

2.7.1.2 The biochemistry of the ABO blood grouping system

Carbohydrates are attached to glycoproteins and glycolipids on the surfaces of red blood cells and these carbohydrates act as antigens (Koolman and Roehm, 2005). Each of the blood group types has in common an oligosaccharide foundation called the O (or sometimes H) antigen (Stryer *et al.*, 2002).

Specific glycosyltransferases add the extra monosaccharide to the O antigen and each person inherits the gene for one glycosyltransferase of this type from each parent (Stryer *et al.*, 2002). Individuals with blood group A means the specific glycosyltransferase add *N*-acetylgalactosamine specifically to the oligosaccharide foundation, whereas galactose

is added to those with blood group B (fig.3). The glycosyltransferases that add the monosaccharides are identical in all positions of their amino acid sequence except 4 of the 354 positions (Stryer *et al.*, 2002).

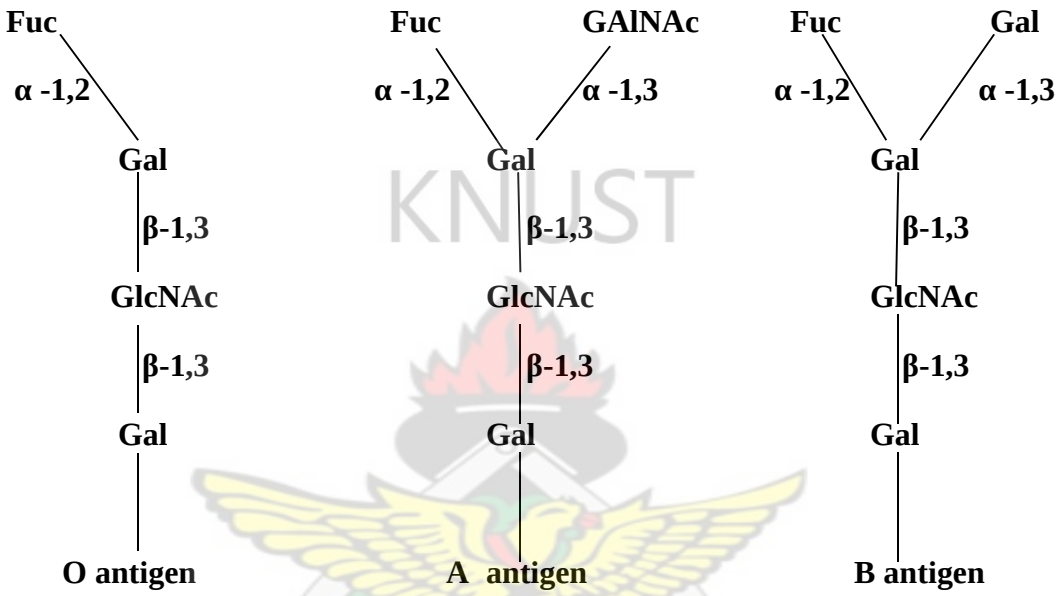


Figure 3: Structures of A, B and O oligosaccharides antigens

The O phenotype is formed as a result of mutation that leads to a premature termination of translation and, hence, to the production of no active glycosyltransferase (Koolman and Roehm, 2005; Stryer *et al.*, 2002). In all, there are four blood groups from the ABO system, A, B, AB and O (tab.1.0).

Table 1.0 Blood group and their corresponding genotypes, antigens and antibodies

Blood group	**A**	**B**	**AB**	**O**
Genotype(s)	I^AI^A , $I^A I^O$	$I^B I^B$, $I^B I^O$	$I^A I^B$	$I^O I^O$
Antigens	A	B	A and B	None
Antibodies	B	A	None	a and b

Specific glycosyltransferase genes are genetically inherited by off-springs from their respective parents. Thus, it is possible to predict blood types a child may have if the parents' blood types are known.

Table 2.0 List of possible genotypes that a child could inherit based on the parents phenotype

Mother's blood type (Phenotype)	Father's blood type (Phenotype)				Child's blood type
		A	**B**	**AB**	**O**	
	A	I^AI^A , I^OI^O I^AI^O	I^AI^O, I^BI^O $I^A I^B$, I^OI^O	I^AI^A , $I^A I^B$ I^AI^O, I^BI^O	I^AI^O, I^OI^O	
	B	I^AI^O, I^BI^O, $I^A I^B$, I^OI^O	I^BI^B , I^OI^O	I^AI^O , I^BI^B, $I^B I^O$	I^BI^B , I^OI^O	
	AB	$I^A I^A$, I^AI^B , $I^A I^B$, I^BI^O	I^AI^O , I^BI^B $I^B I^O$, $I^A I^B$	I^AI^A , I^BI^B, $I^A I^B$	I^AI^O , I^BI^O	
	O	I^AI^O , I^OI^O	I^BI^B , I^OI^O	I^AI^O, I^BI^O	I^OI^O	

From the principle of human inheritance, each child inherits one allele from the father and the mother therefore, the alleles of the children are donated by their parents. If a blood sample of a child is type O and the alleged father is AB, then the suspect could not be the father of the child.

2.7.2 Molecular Marker Techniques Used For Paternity Testing

A molecular marker is any site (locus) in the genome of an organism at which the DNA base sequence varies among different individuals of a population (Oxford dictionary of Science, 2010)

Molecular marker tools are relatively newer technologies used for paternity testing. These techniques promise the value of enhancing our understanding of the distribution and extent of genetic variation within and between individuals (Mondini *et al.,* 2009). Almost two decades and in recent times there has been a considerable rise in the application of these techniques in paternity determination. This rise in the application of the technologies could be attributed to the enormous exclusion power (99.99%) that is obtained from the use of these techniques (Karp *et al.,* 1997). The techniques are DNA-based and they highlight differences (polymorphism) in DNA sequences between individuals (Mondini *et al.,* 2009).

The molecular techniques for testing paternity are based on the use of either restriction enzymes, or PCR or both. Several classes of molecular markers for determining paternity have been identified, these include the non-PCR-based restriction fragment length polymorphisms (RFLPs), and the PCR-based variable number of tandem repeats (VNTRs), microsatellite DNA, and single nucleotide polymorphisms (SNPs) (Cayres and Rosario, 2003).

2.7.2.1 Use of Non-PCR-based technique

This molecular marker employs restriction and hybridization techniques to assess variation. The restriction digestion of DNA is performed using restriction endonucleases.

In this technique, genomic DNA is digested with restriction enzymes and the resultant DNA fragments are separated by gel electrophoresis. An extension to this technique is the use of Southern Blotting techniques, where restricted DNA fragments are transferred onto nitrocellulose paper and radio-labelled probes are used to detect fragments on filter through the process of hybridization.

RFLPs are a codominant marker (Mondini et al., 2009) that is, they can distinguish between heterozygotes (fig.4.). They also give highly reproducible profiles thus the assays could be repeated in the different laboratories under different conditions and the same results will be obtained (Karp *et al.*, 1997; Mondini *et al.*, 2009). This technique has certain limitations which have contributed to a decline of its use in diversity assessments. Since probes are required to hybridize the restricted DNA fragments during Southern Blotting, a good supply of probes is required, so in case these probes are not available, the technique may not be applicable. Also probe construction will require that sequence information for target DNA fragment be known before hand and this renders the technique a bit complex. Again another limitation observed in the use of this technique is the difficulties that are presented by the blotting and hybridization steps which are time consuming and cumbersome. More so, relatively higher quantity and good quality DNA is required and this may be a problem when limited amount of specimen is available.

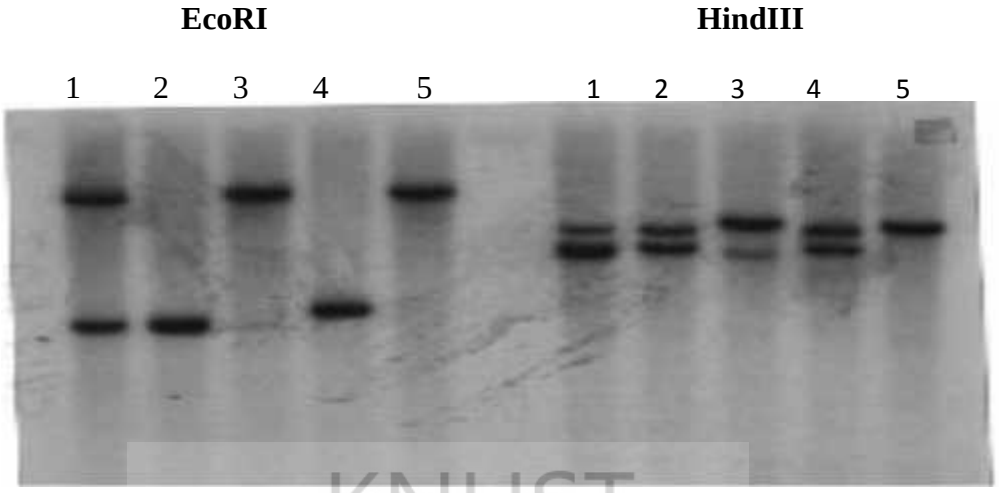


Figure 4. Autoradiogram for RFLP analysis of five different and unrelated inbred lines of maize digested with EcoRI (five tracks on the left) and HindIII (five tracks on the right) (Karp *et al.*, 1996)

These limitations triggered the development of more advanced techniques which effectively overcome these limitations. The advent of the Polymerase Chain Reaction also contributed enormously to the development of a range of new technologies which overcame the many observed limitations of RFLPs (Karp *et al.,* 1996: Karp *et al.,* 1997).

2.7.2.2 Polymerase Chain Reaction-based techniques

2.7.2.2.1 Short Tandem Repeats (STRs)

Short Tandem Repeat Technology is one of the most useful methods for detecting individuals, especially in cases of crime, where samples for DNA profiling are few (Butler, 2007). This method does not need many samples as compared to the use of RFLP and is a highly informative marker for individual identification. About 3% of the human genome is made up of short tandem repeats and, averagely, occurs once in every 10,000 nucleotides (Stanciu *et al.,* 2009). They can also occur once in every 300-500 kb. The

Federal Bureau of Investigation (FBI) uses a standard set of 13 specific STR regions for the combined DNA index system (CODIS).

The probability of two individuals having these variable regions of STR using 13-loci DNA profile is about one in a million individuals. STRs have the ability to use degraded samples since smaller fragments of DNA can be analyzed and also the rapid process of completion of the analysis in a day or two makes this method preferable over other methods (Kirby, 1992). Also the availability of test kits makes it less expensive because of the use of multiplexes inexpensive silver stain as compared to the initial usage of expensive dyes (Kirby, 1992; Geberth, 2006).

This method has low heterozygosity rate and is expensive in using all these 13-loci for identification. STR markers are more mutable than the conventional serological markers; therefore, there is high possibility of encountering mutations when using STRs in resolving paternity disputes (Alford *et al.*, 1994).

2.7.2.2.2 Random Amplified Polymorphic DNA (RAPD)

Among the techniques described under the arbitrary primed techniques, the Random Amplified Polymorphic DNA (RAPD) (Williams *et al.,* 1990) and the Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.,* 1995) are the most commonly employed techniques in assessing genetic diversity.

RAPDs are the first molecular markers to be developed based on PCR (Mondini *et al.,* 2009). The RAPD markers are created by the amplification of DNA using random primers. The amplification products are then separated by agarose gel electrophoresis in

the presence of ethidium bromide and visualised under ultra-violet light (Karp *et al.*, 1996).

The technique aside its advantage of not requiring DNA probes or sequence information for primer design is also quick and simple since no blotting or hybridisation is required (Williams *et al.*, 1990). Also very small quantities of genomic DNA are required. The technique, however, has a setback of not being able to give reproducible profiles. In order to obtain reproducible profiles, it is required that investigators maintain a strictly constant PCR reaction conditions. RAPDs are dominant markers (Karp *et al.*, 1996: Mondini *et al.*, 2009) because they cannot tell heterozygotes from homozygotes (fig. 5). However, Riedy *et al.*, (1991) demonstrated that this technique leads to unacceptable number of non-parental bands within a family tree pedigree, thus raising serious concern regarding its use in paternity testing.

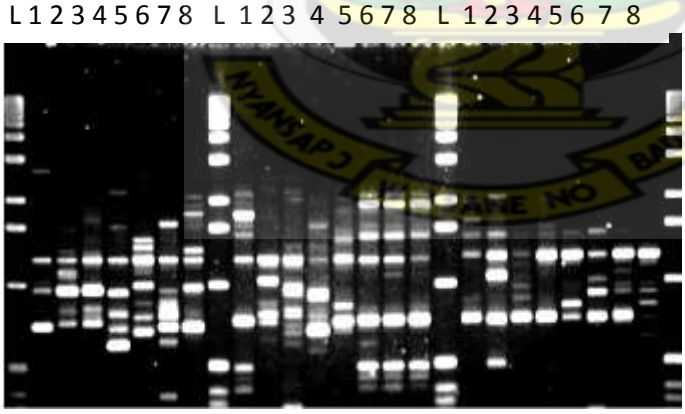


Figure 5. Multiple arbitrary amplicon profiling (MAAP) showing analysis of different Rhododendron spp. using RAPDs (Karp *et al.*, 1997 IACR-LARS)

2.7.2.2.3 Variable number of tandem repeats (VNTRs) marker

A special type of polymorphism in the human genome referred to as variable number of tandem repeats (VNTRs) is composed of repeated copies of DNA sequences that are adjacent to one another on the chromosome (Applied Biosystems, 2011). VNTRs are polymorphic DNA sequences composed of different numbers of a repeated "core" sequence arranged sequentially (Applied Biosystems, 2011). VNTRs are found in the non-coding regions of the DNA and they are not responsible for the observable phenotypical traits. The size of the core sequence can vary from 8 to 100bp in different VNTRs, and the number of repeats present at a VNTR locus also varies widely (Alford *et al.*, 1994).

Although many different VNTR loci have been identified in the human genome, their functions are not currently known. VNTR locus D1S80 has repeats of 16 bps and is currently used in most laboratories for determining genetic relationships. The D1S80 locus is on chromosome one, the largest human chromosome (Kasai *et. al.*, 1990). To date, twenty-nine different alleles of D1S80 have been identified, which range in size from 200-700, because they have different numbers of repeats (Applied Biosystems, 2011).

Every individual has two copies of the D1S80 locus, one inherited from the father and the other from the mother. Approximately 86% of the population is heterozygous at this particular locus because each VNTR allele is inherited from a parent. Most individuals have alleles containing between 14 and 40 repeats, and are inherited in a Mendelian fashion on the maternal and paternal copies of chromosome one (Bloom, 1994). Although

the use of PCR helps in amplifying VNTR loci to make several copies, excessive amplification cycles results in generation of additional aberrant fragments which can interfere electrophoresis of PCR products (Decorte and Cassiman, 1993).

The primers used in this experiment bracket the D1S80 locus and selectively amplify this region of chromosome one (Bloom, 1994). Other VNTR loci used for parentage testing are D2S44, D14S13, D4S139, D3S1358, D5S818, D7S820, D8S1179, D13S317,D16S539, D18S51, D21S11 and D16S85 and these provide power of exclusion of about 99.93% (Bloom, 1994).

Although PCR-based amplification of VNTR loci is limited with average heterozygosity rate less than 70%-80%, Pena (1995) states that PCR based typing can be used as a confirmative tool when exclusion is unraveled using multi-locus probes which might be due to technical pitfalls, such as partial digestion and band shifts. Despite the low heterozygosity ranges, PCR-based amplification of VNTR loci gives a high exclusion probability of about 99.99% which is statistically very significant in determining paternity of a child (Pena, 1995).

Compared to all the marker systems available for human identification, there is a high polymorphism of the VNTR locus D1S80 and, thereby making, its use in paternity very reliable (Jeffrey *et al.*, 1991; Aronson, 2007). Also, the use of multi-locus mini-satellite probes (MLP) of 5 or 6 single-locus probes provides about 99.9999% average power of paternity exclusion and, therefore, resolves disputes with certainty (Pena, 1995). According to Alford *et al.* (1994), VNTRs are able to form stable inheritance patterns and provides accurate results when used in paternity testing.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study site and sample size

The study involved randomly selected thirteen (13) families involving 29 children, residing in the Anwomaso Township in the Kumasi Metropolis. The members within a family were at least four, which is, father, mother and two children.

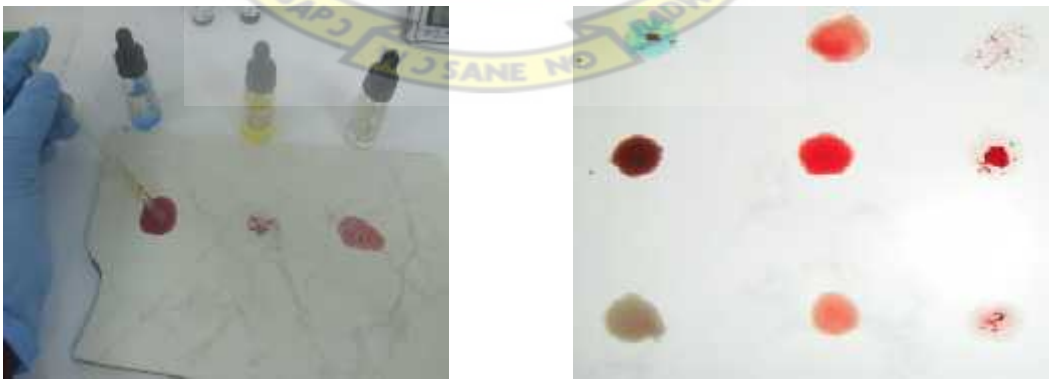
3.2 Blood sample collection

4 ml of whole blood samples were collected from each subject using hypodermal needle syringes and stored in ethylene diamine tetraacetate, EDTA vacutainer tubes (fig.6). The tubes were then transported to the Molecular Biology Laboratory of the Department of Biochemistry and Biotechnology, Kwame Nkrumah University of Science and Technology (KNUST), Kumasi. Part of the blood sample was used immediately for the ABO blood group analysis and the remaining stored at -20°C for two weeks. The blood samples from families were labelled with codes F1, F2, F3… and F13, where the letter (F) and the numbers (1, 2, 3, 4 …13) represented the individual families. Each family code was subdivided to identify the subjects, for example, family one (F1) is divided into F1-1, F1-2, F1-3 and F1-4 where F1-1 represents the father, F1-2 represents the mother, F1-3…F1-n represent the children.

Figure 6: Rack of blood samples from individuals stored in EDTA vacutainer tubes

3.3 ABO blood group determination

This test was conducted at the Clinical Analysis Laboratory (CAn Lab) of the Department of Biochemistry and Biotechnology, KNUST in Kumasi. Three drops of blood of an individual from a minipipette were spotted on different locations on a clean tile for the test. A drop of Anti-A (Mediclone A), Anti-B (Mediclone B) and Anti-D (Mediclone D) were added to the drop of the blood, respectively (fig.7). The presence or absence of agglutination was recorded and used to determine the blood groups.



A **B**

Figure 7: The panels show a scientist dropping blood samples on the tiles (A) and result obtained after reaction with the antisera. (B)

3.4 Genomic DNA Isolation

The blood genomic DNA isolation was done in the Molecular Biology Laboratory of the Department of Biochemistry and Biotechnology in KNUST.

For each blood sample, 300 µl was taken into different Eppendorf tubes and 700 µl of Cetyl trimethyl ammonium bromide, CTAB buffer warmed in water bath at 65^{o}C for 10 minute, was added. 5µl Proteinase K was added and the mixture was homogenized. The samples were kept at 60^{o}C in a sandbath for 45 minutes and vortexed intermittently after every 10 minutes. The samples were removed from the sandbath and allowed to cool at room temperature. 700 µl of chloroform: isoamyl alcohol (24:1) mixture was added to the cooled samples and inverted several times to mix.

The samples were then centrifuged using microfuge (Eppendorf 5415D, USA) at 14000 rpm for 15 minutes after which the supernatants were transferred into new Eppendorf tubes. 5 µl of RNase was added to the supernatant which was gently mixed after which equal volume of isoamyl alcohol was added. The mixture was centrifuged at 14000 rpm for 10 minutes. The supernatants were pipetted into new Eppendorf tubes and 500 µl ice cold isopropanol added to each tube. The tubes were kept in a freezer at -20^{o}C for one and half hours after which they were removed from the freezer and centrifuged at 14000 rpm for 5 minutes. The isopropanol portion was decanted and the DNA pellets washed using 400 µl of washing buffer and by placing the tubes on a rotary shaker. The washing buffer was decanted and the DNA pellets were washed with 200µl of 80% ethanol.

This was done by centrifuging at 1000 rpm for 4 minutes after which the ethanol was decanted. The DNA pellets (fig. 8) were dried in a vacuum for 30 minutes at 37ºC. The DNA pellets were then suspended in 60 µl of TE buffer and stored in a freezer at -20ºC.

Figure 8: Pellets of isolated DNA from blood.

3.5 Gel electrophoresis for determination of quality of genomic DNA

The isolated DNA was resolved on a 0.7% agarose gel in 1X TBE buffer (0.5 M Tris, 0.05 M boric acid and 1mM EDTA, pH 8.0). Staining was done with 2.5 µl ethidium bromide. 3 µl Bromophenol blue loading buffer was used for each of the samples. 5 µl each of the genomic DNA was loaded into the wells of the gel. The gel was run at 50V, 25A, 50W using a Consort power system.

3.6 VNTR Analysis

Primer sequences used were as described by (Kasai *et al., 1990*)

Primer 1: 5'- GAA ACT GGC CTC CAA ACA CTG CCC GCC G-3'

Primer 2: 5'- GTC TTG TTG GAG ATG CAC GTG CCG GC-3'

Each 0.2 ml PCR tubes containing 12.5 µl reaction volume included 7.0 µl sterile molecular water, 2.5 µl of Accupower mastermix (consisting of 1µl 10X buffer, 1 µl $MgCl_2$ (25mM), 0.4 µl dNTP mix (10mM), 0.1 µl Taq polymerase (5 µg/l)), 1 µl primer mix (0.5 µl each of forward and reverse primers) and 2 µl of DNA template.

The DNA amplification reactions were carried out in a Bio RAD Mycycler™ thermal cycler (fig.9) using the following programme: initial denaturing of 3 mins at 95°C, 30s cyclic denaturation at 95°C, 30s primer annealing at 68°C and 60s extension stage at 72°C. After 25 cycles, a final extension stage at 72°C for 5min was performed. The products were held at 4°C for 15min before being taken out of the PCR block.

Figure 9: Bio RAD Mycycler™ thermal cycler used for DNA amplification

The amplified PCR products were resolved on a 2.5% agarose gel in 1X TBE (0.5 M Tris, 0.05 M boric acid and 1mM EDTA, pH 8.0).

Staining was done with 4 µl ethidium bromide. 3 µl orange G loading buffer was used for each of the samples. 5 µl each of the PCR product was loaded in the wells of the gel.

A PCR ranger 100 bp DNA ladder from NORGEN (50 bp – 1000bp) was used to estimate band sizes. The gel was run at 120V, 100mA, 50W using a Consort power system.

Figure 10: Set-up used to resolve amplified PCR products on 2.5% agarose gel.

3.7 Analysis of Genetic Relationship

Genetic relationships among the individual members of the 13 families sampled were evaluated using the VNTR primers. Each fragment size was treated as a unique characteristic and scored as 1 for presence or 0 for absence. The data was used to calculate genetic similarities using NTSYS, which was used to compute pairwise similarity matrices and dendrograms.

CHAPTER FOUR

4.0 RESULTS

4.1 ABO blood grouping results for the selected families

Table 3.0 shows the result of blood groups and possible genotypes of members of the selected families studied. The genotypes consist of alternative forms of the same gene transferred from parents to offspring randomly during fertilization. Individuals shared the same blood group but with different alleles.

Table 3.0 Blood group and possible genotypes of the parents and the children of the various families.

FAMILY		Father	Mother	Child1	Child2	Child3
1	CODE	F1-1	F1-2	F1-3	F1-4	-----
	BLOOD GROUP	AB	O^+	A^+	A^+	-----
	Possible genotypes	$I^A I^B$	$I^O I^O$	$I^A I^A$,$I^A I^O$	$I^A I^A$,$I^A I^O$	-----
2	CODE	F2-1	F2-2	F2-3	F2-4	-----
	BLOOD GROUP	O^+	A^-	O^+	O^+	-----

	Possible genotypes	I^OI^O	I^AI^A ,I^AI^O	I^OI^O	I^OI^O	-----
3	CODE	F3-1	F3-2	F3-3	F3-4	-----
	BLOOD GROUP	B^+	O^+	O^+	B^+	-----
	Possible genotype	I^BI^B. $I^B I^O$	I^OI^O	I^OI^O	$I^B I^B$,$I^B I^O$	-----
4	CODE	F4-1	F4-2	F4-3	F4-4	-----
	BLOOD GROUP	AB	B^+	B^+	B^+	-----
	Possible genotype	$I^A I^B$	$I^B I^B$,$I^B I^O$	$I^B I^B$,$I^B I^O$	$I^B I^B$,$I^B I^O$	-----
5	CODE	F5-1	F5-2	F5-3	F5-4	-----
	BLOOD GROUP	O^+	O^+	O^+	O^+	-----
	Possible genotype	I^OI^O	I^OI^O	I^OI^O	I^OI^O	-----
6	CODE	F6-1	F6-2	F6-3	F6-4	F6-5
	BLOOD GROUP	O^+	A^+	A^+	O^+	O^+
	Possible genotype	I^OI^O	I^AI^A ,I^AI^O	I^AI^A ,I^AI^O	I^OI^O	I^OI^O
7	CODE	F7-1	F7-2	F7-3	F7-4	F7-5
	BLOOD GROUP	O^+	O^+	O^+	O^+	O^+
	Possible genotype	I^OI^O	$I^O I^O$	$I^O I^O$	$I^O I^O$	$I^O I^O$
8	CODE	F8-1	F8-2	F8-3	F8-4	-----

	BLOOD GROUP	B^+	B^+	B^+	B^+	-----
	Possible genotype	I^BI^B, $I^B I^O$	$I^B I^B$,$I^B I^O$	I^BI^B, $I^B I^O$	I^BI^B,$I^B I^O$	-----
9	CODE	F9- 1	F9- 2	F9-3	F9- 4	-----
	BLOOD GROUP	O^+	AB	A^+	A^+	-----
	Possible genotype	I^OI^O	I^AI^B	I^AI^A ,I^AI^O	I^AI^A ,I^AI^O	-----
10	CODE	F10-1	F10-2	F10-3	F10-4	F10-5
	BLOOD GROUP	B^+	O^+	O^+	O^+	B^+
	Possible genotype	$I^B I^B$,$I^B I^O$	$I^O I^O$	$I^O I^O$	$I^O I^O$	I^BI^B,I^BI^O
11	CODE	F11-1	F11-2	F11- 3	F11- 4	-----
	BLOOD GROUP	A^+	O^+	A^+	A^+	-----
	Possible genotype	I^AI^A ,I^AI^O	$I^O I^O$	I^AI^A ,I^AI^O	I^AI^A ,I^AI^O	-----
12	CODE	F12-1	F12-2	F12-3	F12-4	-----
	BLOOD GROUP	A^+	A^+	A^+	A^+	-----
	Possible genotype	I^AI^A ,I^AI^O	I^AI^A ,I^AI^O	I^AI^A ,I^AI^O	I^AI^A ,I^AI^O	-----

4.2 Genomic DNA quality determination

Figure 11 shows the electro gram of the isolated genomic DNA from the blood samples resolved on agarose gel. The isolation of DNA was successful in all the samples collected.

Figure 11: Agarose electrophoretic gel of genomic DNA isolated from the individual blood samples

4.3 PCR – Amplified VNTR regions separated on 2.5% agarose gel

Figures 12, 13 and **14** are clear and scorable bands on electrophoretic gel representing amplified regions of the VNTR DIS80 locus in DNA templates of individuals from the families F1, F2, F3, F4, F5, F6, F7, F8, F9, F10, F11, F12, F13 alongside DNA ladder (lane L) ranging from 50bp to 1000bp. The primers amplified the target sequences in all the individuals in the respective families. Unfortunately, two families F3 and F4, failed to produce scorable PCR products when separated on the agarose gel.

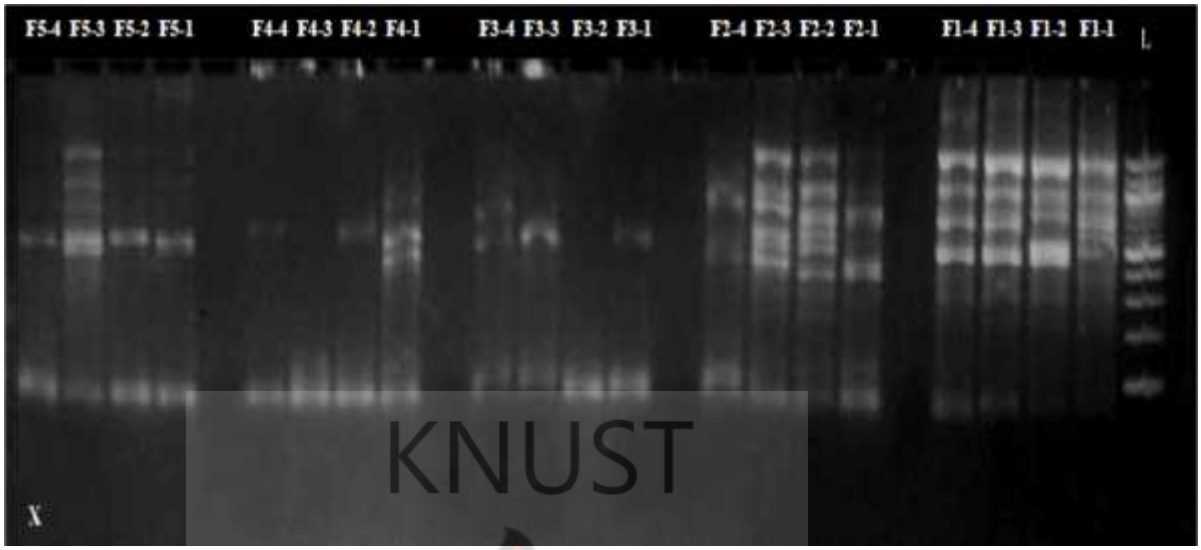


Figure 12: Agarose electrophoretic gel of PCR amplified allelic fragments of five families F1, F2, F3, F4, and F5. The bands are scored as present (1) or absent (0) for all the amplified alleles at their respective lengths.

Figure 13: Agarose electrophoretic gel of PCR amplified allelic fragments of four families F6, F7, F8 and F9. The bands are scored as present (1) or absent (0) for all the amplified alleles at their respective lengths.

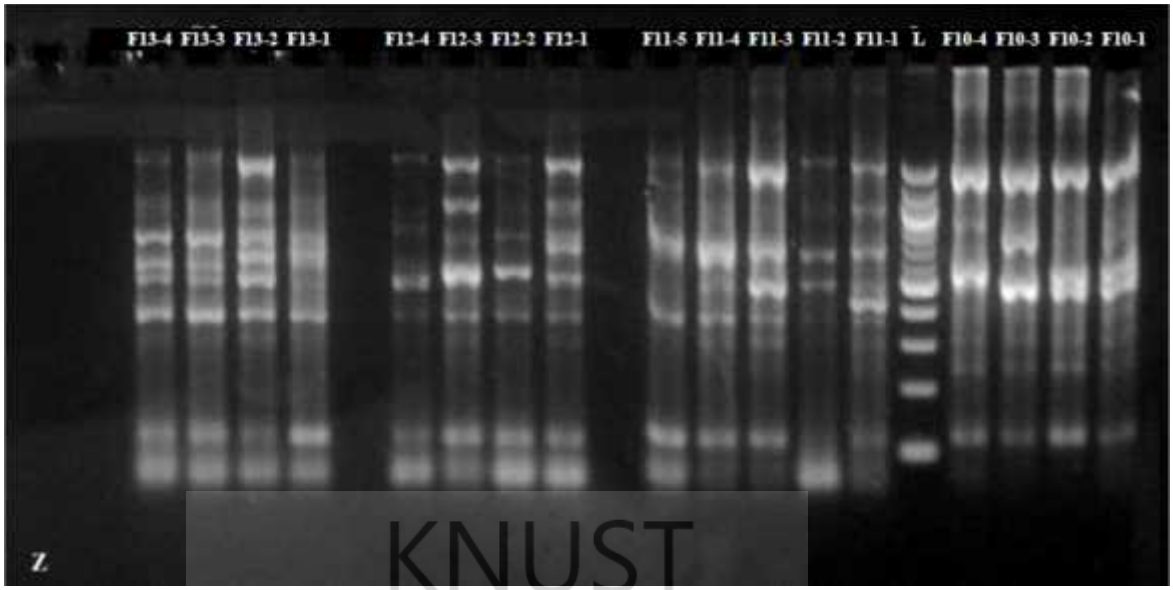


Figure 14 Agarose electrophoretic gel of PCR amplified allelic fragments of four families F10, F11, F12 and F13. The bands are scored as present (1) or absent (0) for all the amplified alleles at their respective lengths.

4.4 Analysis of genetic relationship using Jaccard's Index

The results in Tables 4 to 16 give Pair-wise similarity matrices generated from binary matrix of allelic scores from VNTR primers using NTSYSpc version 2.1 with Jaccard's index for the analysis of genetic relationship among the families. Evidence of paternity between a father and a child showed a similarity range of 0.5(50%) to 1.0(100%) and this indicates that both subjects (father and child) have at least half of the bands (alleles) from the PCR product. On the contrary, a similarity coefficient less than 0.5(50%) showed that individuals do not share half of the alleles in common.

Table 4.0 Similarity matrices among 1st Family (F1).

F1	F1-1	F1-2
F1-3	0.66	0.66
F1-4	0.50	0.50

Table 5.0 Similarity matrices among 2nd Family (F2)

F2	F2-1	F2-2
F2-3	0.55	0.91
F2-4	0.33	0.45

Table 6.0 Similarity matrices among 3rd Family (F3)

F3	F3-1	F3-2
F3-3	0.67	0.33
F3-4	0.57	0.29

Table 7.0 Similarity matrices among 4th Family (F4)

F4	F4-1	F4-2
F4-3	0.13	0.67
F4-4	0.29	0.67

Table 8.0 Similarity matrices among 5th Family (F5)

F5	F5-1	F5-2
F5-3	0.89	0.89
F5-4	0.50	0.50

Table 9.0 Similarity matrices among 6th Family (F6)

F6	F6-1	F6-2
F6-3	0.56	1.0
F6-4	0.25	0.43
F6-5	0.88	0.67

Table 10.0 Similarity matrices among 7th family (F7)

F7	F7-1	F7-2
F7-3	0.50	0.50
F7-4	0.90	0.90
F7-5	1.00	1.0

Table 11.0 Similarity matrices among 8th family (F8)

F8	F8-1
F8-3	0.89
F8-4	0.70

Table 12.0 Similarity matrices among the 9th Family (F9)

F9	F9-1	F9-2
F9-3	0.38	0.89
F9-4	0.50	0.50

Table 13.0 Similarity matrices among the 10th Family (F10)

F10	F10-1	F10-2
F10-3	0.63	0.63
F10-4	0.86	0.86

Table 14.0 Similarity matrices among the 11th Family (11)

F11	F11-1	F11-2
F11-3	0.89	0.44
F11-4	0.78	0.33
F11-5	0.50	0.22

Table 15.0 Similarity matrices among 12th Family (12)

F12	F12-1	F12-2
F12-3	0.80	0.56
F12-4	0.60	0.71

Table 16.0 Similarity matrices among the 13th Family (13)

F13	F13-1	F13-2
F13-3	0.67	1.00
F13-4	0.67	1.00

4.5 Dendograms showing relationships between individuals in the families

The Figures 15-27 show dendograms generated from binary matrices of allelic scores from VNTR alleles to show relationship between individuals in families at a particular coefficient. Individuals having more bands in common are linked up at a higher coefficient to show close relatedness, whereas individuals having fewer bands in common are linked up at a low coefficient.

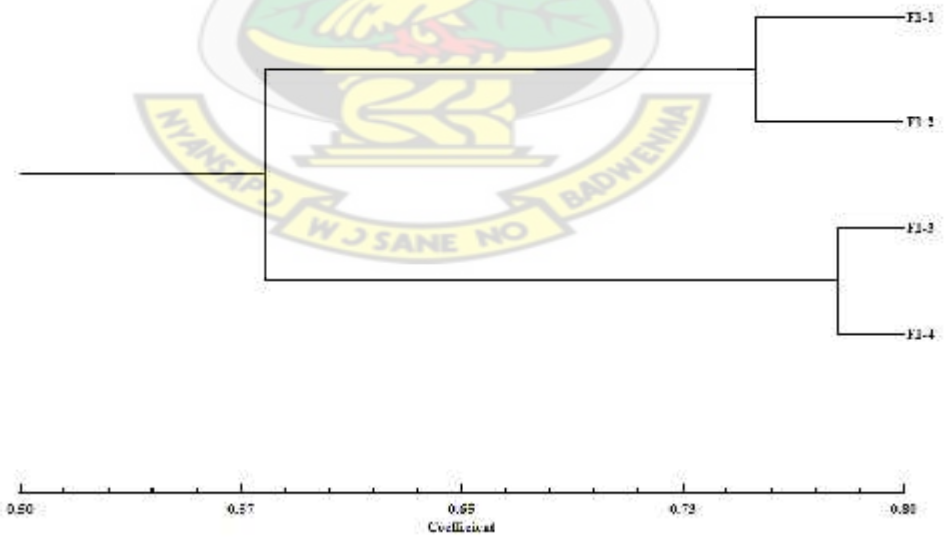


Figure 15: Dendogram showing similarity coefficient range of 0.50 to 0.80 for members of family one (F1) using NTSYSpc version 2.1.

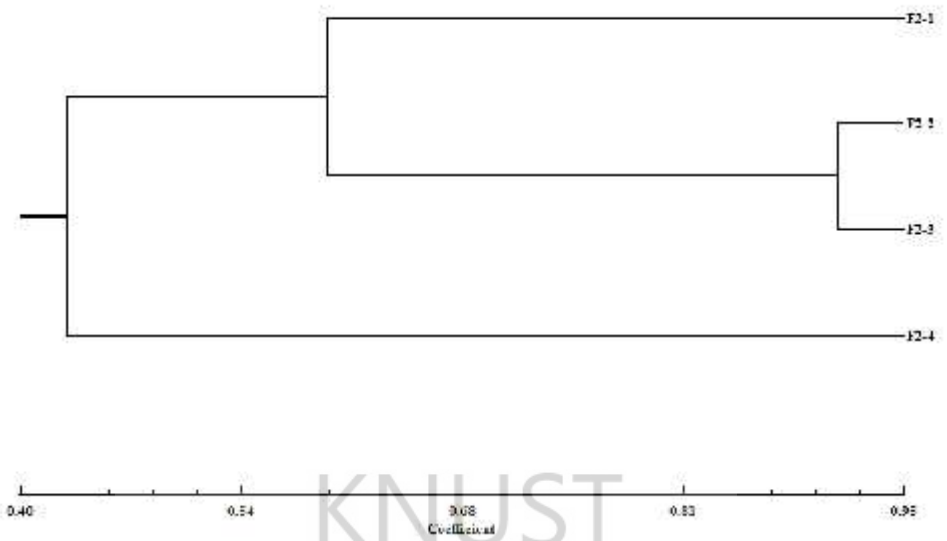


Figure 16: Dendogram showing similarity coefficient range of 0.43 to 0.95 for members of family two (F2) using NTSYSpc version 2.1

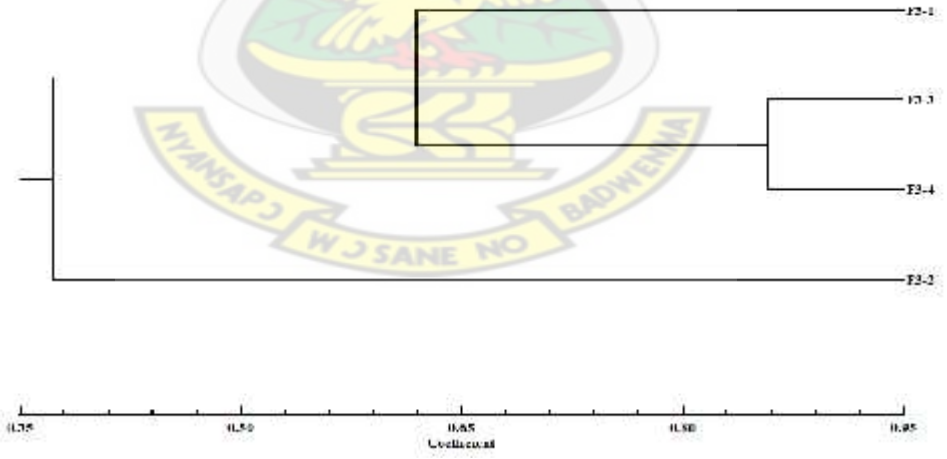


Figure 17: Dendogram showing similarity coefficient for members of family three (F3) using NTSYSpc version 2.1.

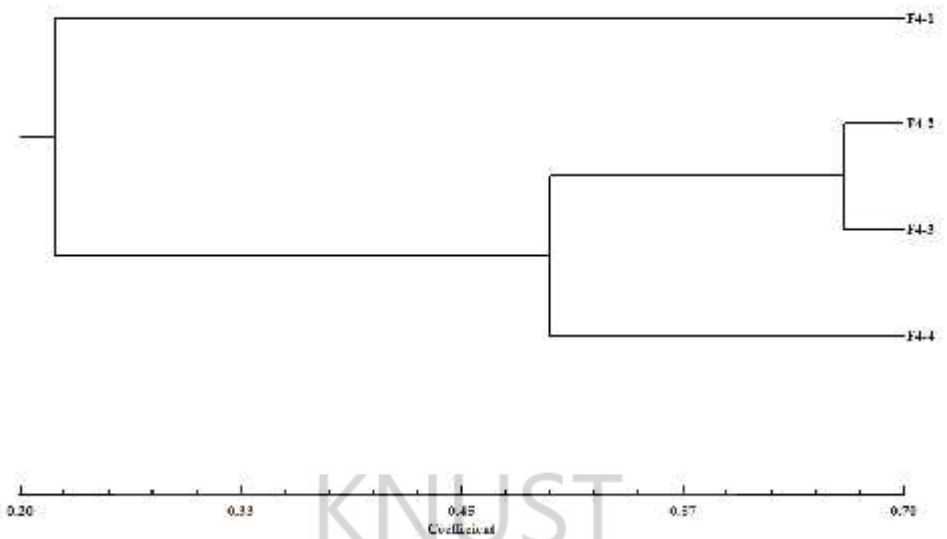


Figure 18: Dendogram showing similarity coefficient for members of family four (F4) using NTSYSpc version 2.1.

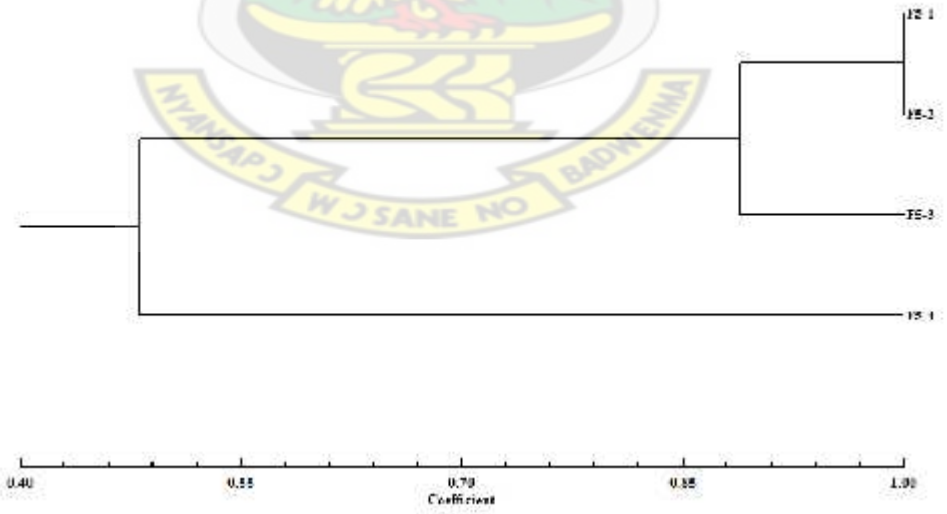


Figure 19: Dendogram showing similarity coefficient for members of family five (F5) using NTSYSpc version 2.1.

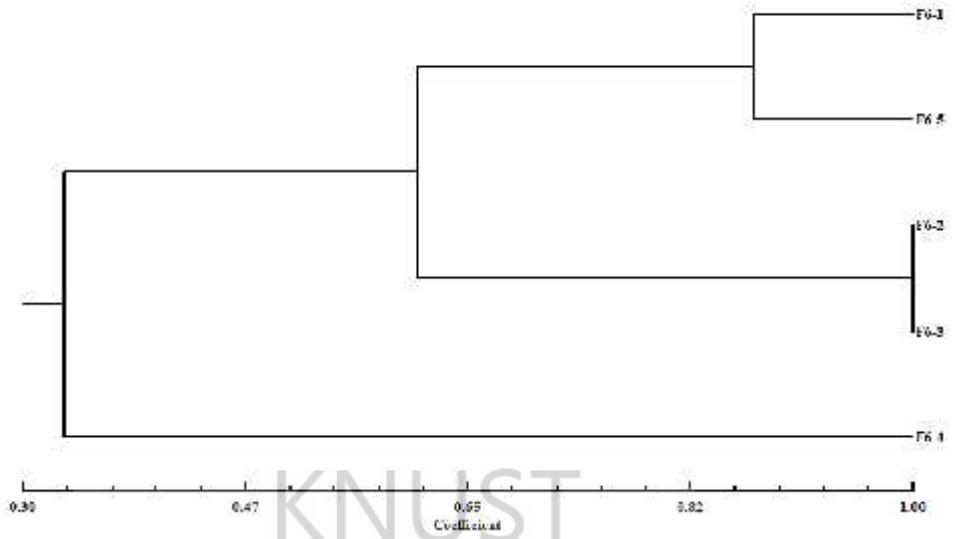


Figure 20: Dendogram showing similarity coefficient for members of family six (F6) using NTSYSpc version 2.1.

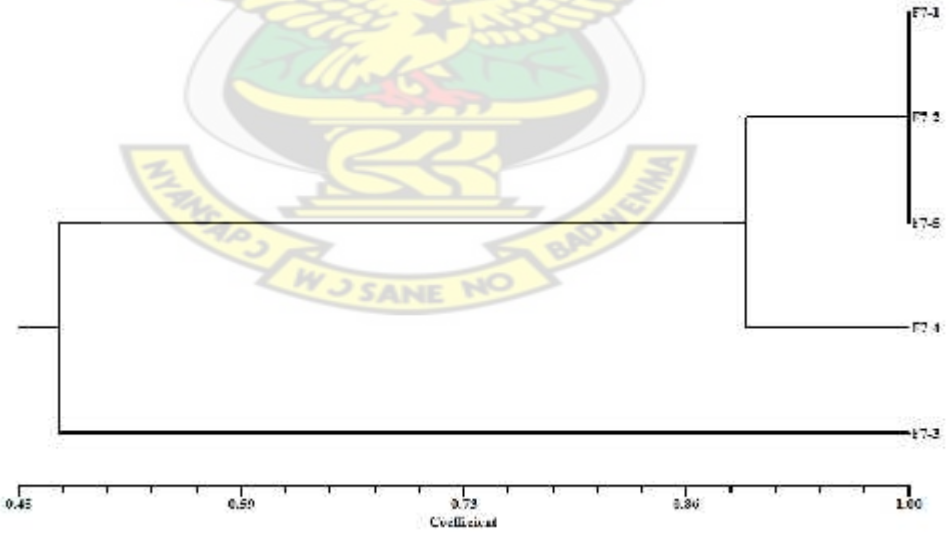


Figure 21: Dendogram showing similarity coefficient for members of family seven (F7) using NTSYSpc version 2.1.

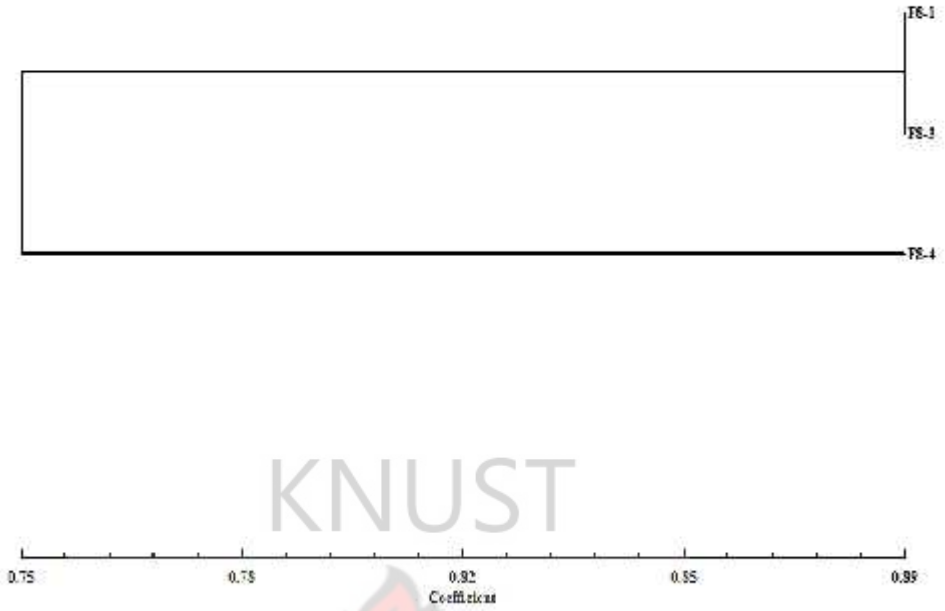


Figure 22: Dendogram showing similarity coefficient for members of family eight (F8) using NTSYSpc version 2.1.

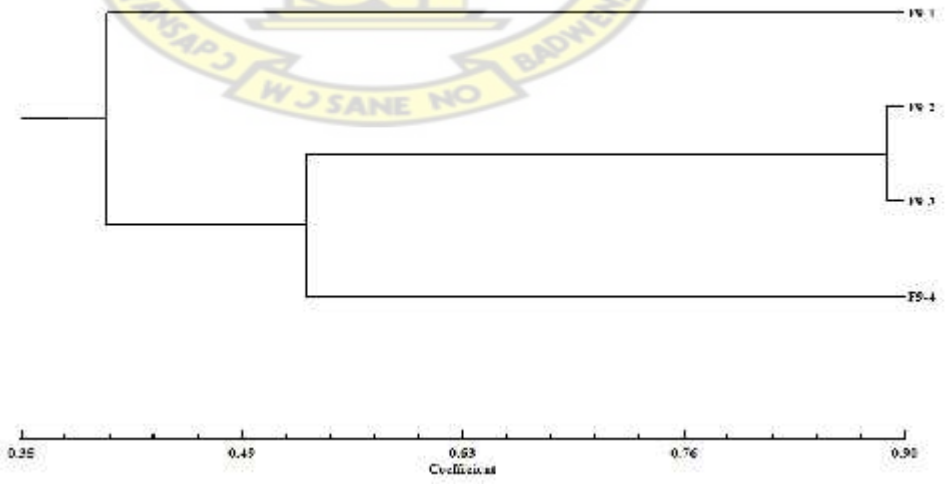


Figure 23: Dendogram showing similarity coefficient for members of family nine (F9) using NTSYSpc version 2.1.

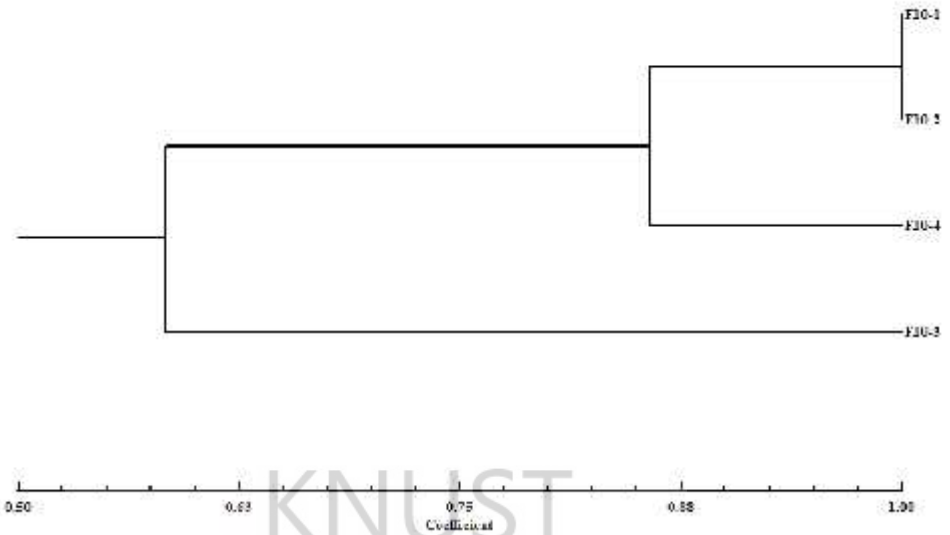


Figure 24: Dendogram showing similarity coefficient for members of family ten (F10) using NTSYSpc version 2.1.

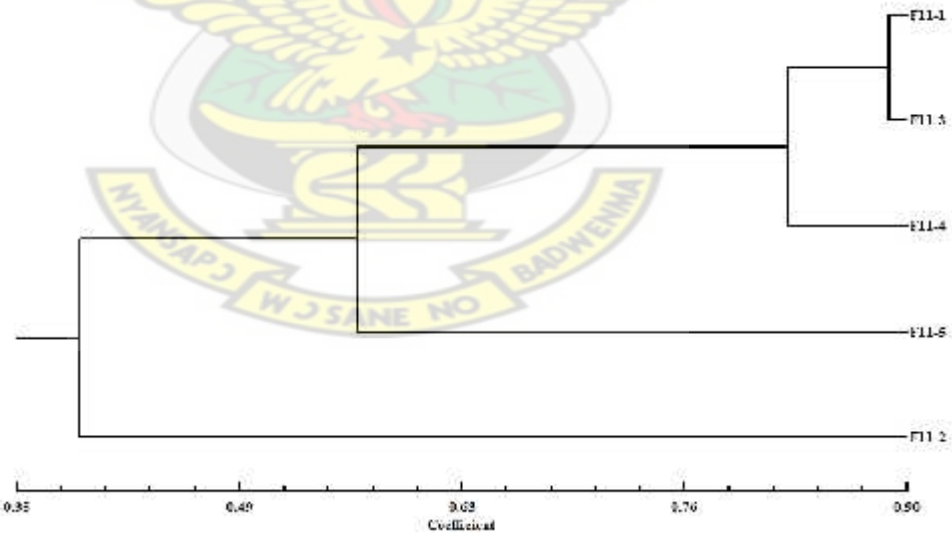


Figure 25: Dendogram showing similarity coefficient for members of family eleven (F11) using NTSYSpc version 2.1.

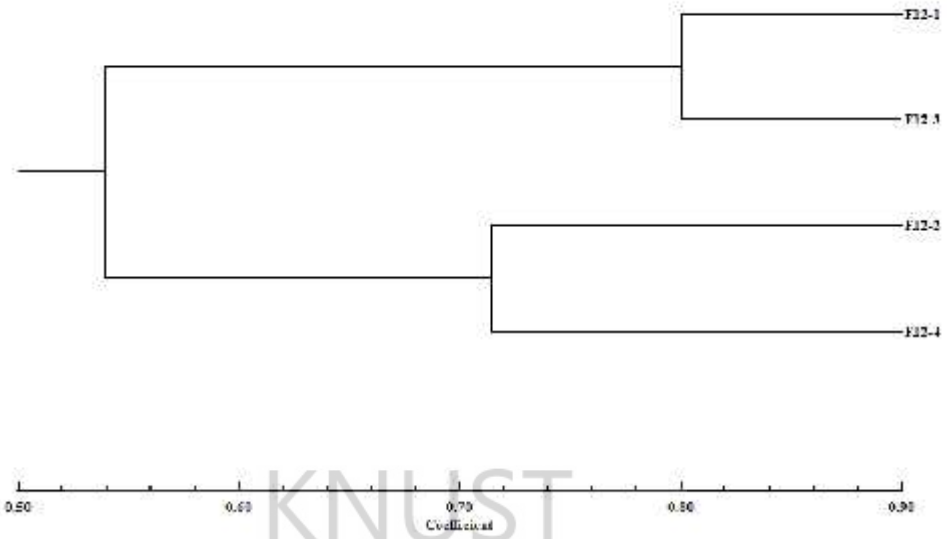


Figure 26: Dendogram showing similarity coefficient for members of family twelve (F12) using NTSYSpc version 2.1.

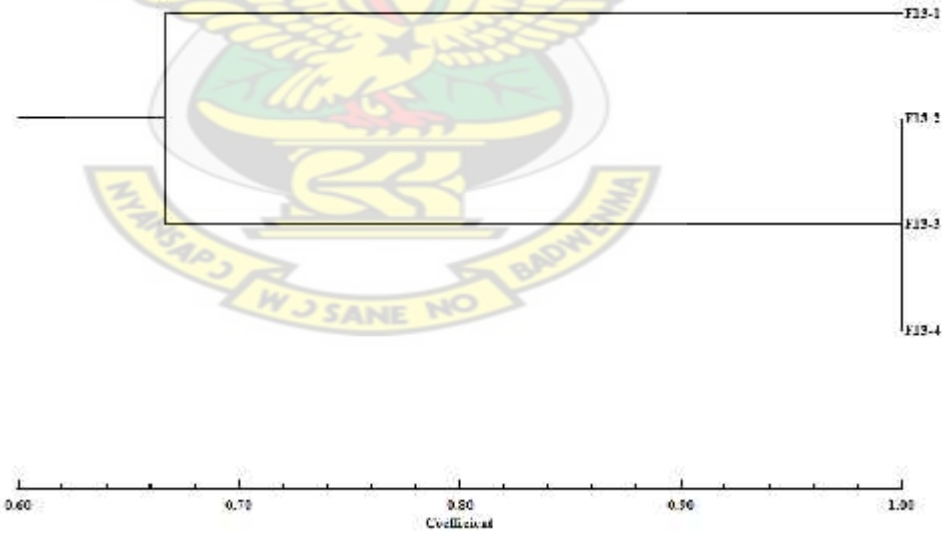


Figure 27: Dendogram showing similarity coefficient for members of family thirteen (F13) using NTSYSpc version 2.1.

CHAPTER FIVE

5.0 DISCUSSION

Many authors, including Silver (1999), pointed out that the weakness in the ABO blood grouping for determination of paternity of children led to the development of more reliable techniques including DNA fingerprinting. The limited power of exclusion of the ABO blood grouping is the major factor that limits the technique in paternity determination.

The ABO Blood typing depends on antigen – antibody reactions in the blood resulting in agglutination of non-compatible blood samples (Fig 7). In this study, the ABO blood group method conducted for the various families did not produce any interesting results. (Tab. 3). The combination of the possible paternal and maternal genotypes of the various families supported the ABO phenotypes of the children. All the children showed some level of relatedness between themselves and their corresponding fathers. Thus, interfamily relationships could not be properly accounted for because some fathers from different families shared the same genotypes with some children from other families. Definitive paternity could, therefore, not be established with the ABO test results. However, the data from the ABO testing is helpful for crude and on-the-spot determination of paternity within families and in blood transfusion. In legal setting, this is very significant as it will render the test results less useful in establishing reliable paternity.

Consequent to this pitfall, genomic DNA isolation followed by PCR amplification of the D1S80 locus was performed. The DNA fingerprinting method has over the years proven

to be the most reliable technique (Kirby, 1992) for example in the USA, UK and Asia. However such a reliable paternity tool is foreign to Ghanaian institutions. Isolation of high quality genomic DNA is essential for any successful molecular marker analysis, whether for phylogenetic studies or DNA fingerprinting. It is by far a major holdup in molecular diversity or DNA fingerprinting studies (Jayarama, 2009). This notwithstanding, good isolation protocol should give good quality DNA.

The genomic DNA isolation with CTAB as shown in Figure 11, gave higher yield of DNA which resolved well on the gel after a test run on agarose gel electrophoresis. This was indicative of good quality DNA. The presence of smears or DNA fragments is an indicative of poor quality DNA, due to degradation of the genomic DNA by mechanical or enzymatic actions.

The VNTR analysis involving a single pair of primers specific for VNTR locus D1S80 was carried out through amplification of all the DNA samples analyzed. A clearer difference in banding pattern of PCR products was obtained and the scored bands subjected to Jaccard's similarity coefficient analysis to establish genetic relatedness with the father (paternity).

Results from the Jaccard's similarity coefficient were used to draw pair-wise similarity matrices for the families in Tables 4 to 16. Amplification of the VNTR locus D1S80 on chromosome 1 of the individuals in the 13 different families showed polymorphism with respect to various bands generated per each sample.

From the Mendellian pattern of inheritance, it is known that children inherit half of their DNA from their biological father and the other half from their mother. On this basis, the

similarity coefficient between a parent and a child equals to or higher than 0.5, (50%) indicates that they are related genetically.

The DNA finger print gave results which could not be highlighted by the ABO group testing. Paternity was confirmed for a total of 24 children representing about 82.7% of the children in all the 13 families with similarity matrix ranging from 0.5 (50%) to 1.0 (100%).

From Table 4 and Figure 15, similarity coefficients were 0.66 and 0.50 for comparison between father (F1-1) and children, (F1-3) and (F1-4) respectively; indicating that the two children are genetically related to their father. Also in family three (Table 6; Figure 17) the DNA fingerprint showed that all the children in the family are related to their father at a similarity coefficient of 0.67(67%) and 0.57(57%) for the first and second children, respectively. In addition to these, paternity was confirmed for all children and their fathers in families F5, F7, F8, F10, F11, F12 and F13 (Tables 8,10,11,13,14.15,16 and Figures19,21,22,24,25,26 and 27.

On the contrary, paternity was not confirmed for families F2, F4, F6 and F9 (Tables 5, 7, 9 and 12). In family two, for instance, although the ABO system indicated that the father and both children had the same blood group O+, the second child, F2-4 did not show any relatedness with the supposed father, (F2-1) as the similarity coefficients were way below 50% (0.33 or 33%). Since the Jaccard coefficient indices between the child F2-4 and the mother F2-2 was 0.45(45%) it could be concluded that, perhaps, that child was adopted. The results in Figure 18 involving family four was quite interesting in that, none of the children had coefficient more that 0.5(50%) for comparison between the father and

children. Both children had coefficients of 0.33 (33%) and 0.29 (29%). In addition to that, the second child in family six F6-4 compared with both the supposed parents F6-1 and F6-2 showed similarity at 0.25(25%) and 0.45(45%), respectively (Table 9 and Figure 20). Also from Table 12 and Figure 23, the first child F9-3 in family nine showed no biological relationship to father (F9-1), since the Jaccard index was 0.38(38%). This means that the DNA fingerprints established no biological relationship between the supposed fathers and children, though the blood groups of the children agreed with that of the fathers. Therefore, paternity could not be established for five children (17.2%) involved in the study.

This outcome, though quite interesting, cannot be conclusive since a minimum of about nine sets of VNTR primers each with 99.99% confidence are allowed in international practice and for legal purposes.

However, the current finding could lead to a number of interesting studies, since it has shown consistency with literature that VNTR DNA fingerprinting is more robust in refining paternity determination among individuals because the technique was able to highlight certain details that the ABO blood grouping failed to capture.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

In this study, the ABO blood grouping system produced results that agreed with the family relationships claimed by the recruited families. However, other fathers in different family units could claim paternity of children in different family units. Thus, the technique has very low genetic exclusion power.

The study also showed that high molecular weight genomic DNA from venous human whole blood can be obtained by using the CTAB protocol. The PCR amplification of the D1S80 locus using the appropriate VNTR primers was successful and the product separated on 2.5% agarose gel was successful. Individuals who were related gave high Jaccards similarity coefficient established by the band pattern from these gels.

Genomic DNA obtained was quite good for PCR work and for that matter forensic study. Again, this work has confirmed that paternity determination using DNA fingerprinting is superior to the ABO blood grouping system. Comparatively, DNA fingerprinting using just a pair of VNTR DS180 primers could exclude many genetic relatedness, which the ABO system failed to resolve.

6.2 RECOMMENDATIONS

1. The study should be expanded to cover more primers sets for the VNTR DIS80 locus to increase the level of confidence acceptable in legal settings.
2. A more comfortable method for taking biological samples such as buccal swabs should be used for the isolation of DNA instead of whole blood samples.

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APPENDIX A

SCORING OF BANDS AS PRESENCE (1) OR ABSENCE (0)

Ladder (bp)	1000	900	800	700	600	500	400	300	200	100	50
F1-1	1	0	1	1	1	1	1	0	1	0	0
F1-2	1	0	1	1	0	1	1	1	1	0	0
F1-3	1	0	1	1	1	0	1	1	1	0	1
F1-4	1	1	1	0	1	0	1	1	1	0	1
F2-1	1	0	1	1	1	0	1	0	1	0	1
F2-2	1	1	1	1	1	1	1	1	1	1	1
F2-3	1	1	1	1	0	1	1	1	1	1	1
F2-4	0	1	1	0	0	1	1	0	0	0	1
F3-1	0	0	0	1	1	1	0	0	0	0	1
F3-2	0	0	0	1	0	0	0	0	0	0	1
F3-3	0	0	1	1	1	1	1	0	0	0	1
F3-4	0	1	1	1	1	1	1	0	0	0	1
F4-1	1	1	1	1	0	1	1	0	0	0	1
F4-2	0	0	0	0	0	1	0	0	0	1	1
F4-3	0	0	0	0	0	0	0	0	0	1	1
F4-4	0	0	0	0	0	1	0	0	0	0	1
F5-1	1	1	1	1	1	0	1	1	0	0	1
F5-2	1	1	1	1	1	0	1	1	0	0	1
F5-3	1	1	1	1	1	1	1	1	0	0	1
F5-4	0	0	0	0	1	0	1	1	0	0	1
F6-1	1	1	1	0	1	1	0	1	0	0	1
F6-2	1	1	0	1	0	1	1	1	0	0	1
F6-3	1	1	0	1	0	1	1	1	0	0	1
F6-4	0	0	0	0	0	1	1	0	0	0	1
F6-5	1	1	1	1	1	1	0	1	0	0	1
F7-1	1	1	1	1	1	1	1	1	1	0	1
F7-2	1	1	1	1	1	1	1	1	1	0	1
F7-3	0	0	0	1	1	1	1	0	0	0	1

F7-4	1	1	1	1	1	1	1	1	1	0	0
F7-5	1	1	1	1	1	1	1	1	1	0	1
F8-1	1	1	1	1	1	0	1	0	1	0	1
F8-3	1	1	1	1	1	1	1	0	1	0	1
F8-4	1	1	1	1	1	1	1	1	0	0	1
F9-1	0	0	1	0	0	0	1	0	0	0	1
F9-2	1	1	1	1	0	1	1	1	0	1	1
F9-3	1	1	1	1	0	0	1	1	0	1	1
F9-4	1	0	1	0	0	0	1	1	1	0	1
F10-1	1	0	1	0	0	1	1	1	1	0	1
F10-2	1	0	1	0	0	1	1	1	1	0	1
F10-3	1	0	0	1	0	0	1	1	1	0	1
F10-4	1	0	1	0	0	1	0	1	1	0	1
F11-1	1	0	1	1	1	1	1	1	1	0	1
F11-2	1	0	1	0	1	1	1	0	0	0	0
F11-3	1	0	0	1	1	1	1	1	1	0	1
F11-4	1	0	0	1	1	0	1	1	1	0	1
F11-5	1	1	0	1	1	0	0	1	0	0	1
F12-1	1	1	1	1	1	0	1	1	1	0	1
F12-2	1	0	0	1	0	1	0	1	0	0	1
F12-3	1	1	1	1	1	1	0	1	1	0	1
F12-4	1	0	1	1	0	1	1	1	0	0	1
F13-1	1	1	1	0	1	1	0	1	0	0	1
F13-2	1	0	1	0	1	1	1	1	1	0	1
F13-3	1	0	1	0	1	1	1	1	1	0	1
F13-4	1	0	1	0	1	1	1	1	1	0	1