THESIS

MICHIGAN STATE UNIVERSITY LIBRARIES

3 1293 01595 1779

This is to certify that the

thesis entitled

AN INVESTIGATION OF
NEONATAL INTENSIVE CARE UNIT INFANTS
FOR UNIPARENTAL DISOMY - CHROMOSOME 16

presented by

Qaisra Zubair

has been accepted towards fulfillment
of the requirements for

Masters of Science degree in Clinical Laboratory Science

David P. Thorne

Major professor

Date May 1996

O-7639

*MSU is an Affirmative Action/Equal Opportunity Institution*

**LIBRARY**
**Michigan State University**

**PLACE IN RETURN BOX** to remove this checkout from your record.
**TO AVOID FINES** return on or before date due.

| DATE DUE | DATE DUE | DATE DUE |
|---|---|---|
| MAR 2 2 2000 | | |
| NOV 1 9 2002 | | |
| | | |
| | | |
| | | |
| | | |
| | | |

MSU is An Affirmative Action/Equal Opportunity Institution
c:\circ\datedue.pm3-p.1

THESIS
MICHIGAN STATE UNIVERSITY

# AN INVESTIGATION OF NEONATAL INTENSIVE CARE UNIT INFANTS FOR UNIPARENTAL DISOMY - CHROMOSOME 16

By

**Qaisra Zubair**

A Thesis

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

**MASTER OF SCIENCE**

in

**Clinical Laboratory Sciences**

of Medical Technology Program

and

Department of Pediatrics and Human Development

1996

**ABSTRACT**

**AN INVESTIGATION OF NEONATAL INTENSIVE CARE UNIT INFANTS FOR UNIPARENTAL DISOMY - CHROMOSOME 16**

By

**Qaisra Zubair**

The Concept of Uniparental Disomy (UPD) was introduced by Engel in 1980. UPD is defined as the abnormal inheritance of both members of a chromosome pair from the same parent with no homologue from the other parent. UPD may be sub-classified as isodisomy (two identical copies of same parental chromosome) or heterodisomy (two different chromosomes but both from the same parent). The mechanisms producing UPD are not clear but post-fertilization loss of a chromosome in a trisomic conceptus, fertilization of a disomic gamete with a nullisomic gamete and duplication of a monosomic gamete are proposed to be possible causes of UPD. UPD has been reported for a number of chromosomes, in some cases associated with human genetic disorders, fetal growth retardation, mental retardation or fetal death. The objective of this study was to establish a procedure for DNA anlysis using microsatellites in order to investigate whether UPD 16 could be a significant cause of prematurity and intra uterine growth restriction in infants. The study group was infants from Neonatal Intensive Care Unit. The investigation required DNA from infants and their parents. The DNA was amplified by PCR using primers for highly polymorphic DNA markers on chromosome 16. The amplified products were analyzed on denaturing polyacrylamide gel to separate alleles in a family. The segregation patterns of the alleles were examined to determine whether UPD 16 was present.

**ACKNOWLEDGEMENTS**

I wish to express my sincere feelings of thanks to my academic advisor, Dr. David P. Thorne, Medical Technology Program, and Dr. Rachel Fisher, Department of Pediatrics and Human Development, for their continued guidance and support during the period of my graduate studies. I truly appreciate their invaluable help that significantly contributed to both my personal as well as my academic accomplishment, including the completion of this study and presentation of this thesis.

I am extremely grateful to Dr. Karen A. Friderici, Department of Pathology, for allowing me to work in the Molecular Biology Laboratory and for the invaluable guidance in developing the molecular techniques employed in this study. I am also thankful to Dr. Gerald L. Davis, Department of Medical Technology, and Dr. Claudia B. Holzman, Department of Human Medicine, for being members on my guidance committee.

Finally, my special thanks to Dr. Magid Farid and Dr. Said Omar, Department of Neonatology, Edward Sparrow Hospital, Lansing, for their cooperation in obtaining the required blood and buccal wash samples for this study.

**TABLE OF CONTENTS**

## LIST OF FIGURES

## LIST OF TABLES

# CHAPTER 1

# INTRODUCTION AND RESEARCH BACKGROUND

## INTRODUCTION

Chromosomal abnormalities are associated with many types of human disorders. The commonly recognized chromosomal abnormalities are numerical or structural involving either autosomes or sex chromosomes. The numerical autosomal abnormalities involve either the gain or loss of all or a part of one or more chromosomes (aneuploidy) resulting in trisomies (an addition of an extra chromosome), and monosomies (loss of an autosome). These abnormalities may sometimes result in the gain of a whole chromosomal set (3n, 4n) which is referred to as polyploidy. Autosomal monosomies and polyploidy are usually lethal in humans and result in early abortions. On the other hand, trisomies have been observed in several genetic disorders in humans. The commonest trisomies observed are: trisomy 21 (Down syndrome); trisomy 13 (Patau syndrome); and trisomy 18 (Edward syndrome). These trisomies are associated with multiple congenital abnormalities and mental retardation. Trisomies usually occur due to non-disjunction during meiosis in gamete formation or in early mitotic divisions of the developing zygote, though the former is probably more

frequent. Common numerical abnormalities involving the sex chromosomes are Turner syndrome (45,X0) and Klinefelter syndrome (47,XXY) which are both associated with a mildly abnormal phenotype, infertility and mild or no mental retardation.

The structural chromosomal abnormalities observed in autosomes and sex chromosomes are translocations, deletions and inversions. The commonest translocation seen in Down syndrome is t(14,21). Translocations are also seen in cancer cells, for example in chronic myelogenous leukemia (CML) t(22q-;9q+). Translocations may occur as balanced or unbalanced rearrangements of chromosomes. An individual with a balanced translocation rearrangement would have a normal phenotype since no chromosomal material is lost. However, such an individual would have an increased risk of producing gametes with an unbalanced chromosomal complement. The partial monosomy or trisomy for a portion of a chromosome may result in early spontaneous abortion, congenital abnormalities and other abnormal phenotypes in liveborn. The microdeletions are observed in Beckwith-Wiedemann syndrome (11p-); Angleman syndrome (15q-); DiGeorge syndrome (22q-); Cri-du-chat (5p-); and in Prader-Willi syndrome (15q11-q13). The deletion, whether involving a large or minute segment of a chromosome often results in dysmorphic features, congenital anomalies, or mental retardation. Chromosomal inversions can be either paracentric (excluding centromere) or pericentric (including

centromere). Individuals carrying inversions with a balanced rearrangement do not have any physical or mental features as they have the correct total amount of chromosomal material but they can produce abnormal gametes which may lead to offspring with an abnormal phenotype. For example, carriers of inversion of chromosome-3, inv(3)(p25-q21), are normal but their offspring may have an abnormal phenotype [1,2].

The chromosomal abnormalities described above, which relate to the gain or loss of the normal genetic material, are detectable microscopically or by banding techniques which were developed twenty years ago. Recently high resolution banding techniques and fluorescent in situ hybridization (FISH) have allowed detection of even very small structural changes (e.g., microdeletion in Prader-Willi Syndrome 15q11-q13). However, with the development of molecular genetic techniques, a different class of genetic abnormalities have been discovered. These abnormalities do not relate to the gain or loss of the total amount of normal genetic material but instead to the parental origin of the genetic material.

Human somatic cells contain 46 chromosomes (23 pairs). Of these, 22 are like pairs and are referred to as autosomes whereas the remaining pair is comprised of two Xs in females and a X and a Y in males. The X and Y chromosomes are referred to as sex chromosome. The members of a pair of homologous chromosomes carry matching genetic material at specific loci which may be identical or slightly different creating

different alleles. One member of each pair of chromosomes is inherited from the mother and one from the father during normal fertilization to complete the total number of 46 chromosomes for normal development of fetus. However, if both homologues of a pair, of a specific chromosome, are inherited from the same parent, the disorder is referred to as Uniparental Disomy (UPD) [3-4].

Individuals with UPD have a normal karyotype with no structural or numerical abnormalities detected either by low and high resolution cytogenetic banding techniques or fluorescent in situ hybridization FISH techniques. These cases appear to have a normal and balanced amount of the total genetic material. However, inheritance of two copies of homologous chromosomes with imprinted genes or recessive genes from one parent can result in an imbalanced state. This imbalanced state further results in various adverse effects on humans (such as spontaneous abortions) and probably contributes significantly to the etiology of mental retardation, physical dysmorphology and failure to thrive [5,21,22]. Within uniparental chromosome pairs, each chromosome can be genetically identical (isodisomy) or dissimilar (heterodisomy) depending on when during meiosis the error in chromosome division occurs. In isodisomy, the uniparental pair is a duplicate of a same chromosome containing extensive segments of identical gene sequences which causes an increased risk of recessive disorders by

reduction to homozygosity. In heterodisomy, the uniparental pair of chromosomes remains heterozygous made up of two non-recombinant homologous segments. Isodisomy would result from lack of separation of the chromosome pair during the second meiotic division or after fertilization, while heterodisomy could be produced during the first meiotic division. Thus in cases of UPD, not only both alleles for a gene are inherited from one parent, but in cases of isodisomy, the genomic content is identical [5-6].

**LITERATURE REVIEW**

Until the late 1970's, all chromosomal anomalies were considered to result in an imbalance of total chromosomal material that could be depicted by a karyotype with a higher or lower than normal compliment of genetic material. In 1980, the concept of UPD was first introduced by Eric Engel as a probable consequence of high rate of germ cell aneuploidy in man [3]. At that time no cytogenetic evidence existed to support the concept of uniparental inheritance and chromosome pairs were believed to segregate independently, one from the mother and one from the father. Engel's UPD theory received attention after a decade when polymorphic DNA probes were able to discern the parental origin of chromosomes and cases of uniparental inheritance of a chromosome pair were reported in experimental mice. In 1985, Cattanach and Kirk, and in 1987 Beechey and Searle, using translocated inbred parents produced

offspring with UPD in experimental mice which received both copies of one specific chromosomal segment from one or the other parent [7-8]. These offspring mice had balanced sets of chromosomes, but both copies of the chromosomes or a part of the chromosome were derived from one parent. They observed effects on growth, behavior, and survival. In some cases, the phenotypic effect was observed only from maternal duplication of chromosomes with paternal deficiency. In other cases, the phenotypic effect was observed from paternal duplication with maternal deficiency of chromosomes while in some other cases, the phenotypic effects were different and opposite with paternal versus maternal uniparental inheritance. This led to an idea that in the balanced somatic genome, some diploid sequences can derive from only one parent and if identical alleles are inherited could lead to isodisomy which can increase a risk of recessive disorder by reduction to homozygosity.

In 1988, Spence et. al. [9] reported a proven case of UPD in which they detected a uniquely maternal origin of the chromosome 7 in a diploid patient with cystic fibrosis, very short stature, and deficiency of growth hormones. The patient was reported to have inherited two identical sequences for most or all of chromosome 7 from her mother. A similar case of cystic fibrosis and short stature was reported by Voss [10] in 1989 who found maternal isodisomy in a four year old girl. Grundy at. al. [11] also reported UPD as a cause of Beckwith-

Wiedemann syndrome due to inheritance of paternal homologous chromosomes 11 causing segmental isodisomy.

A later study in 1989 by Nicholls et. al. [12] reported several cases of Prader-Willi syndrome in which no deletion was found, but instead the two chromosomes (15) in the effected individuals had both been inherited from the mother, (i.e., UPD of chromosome 15). A study by Cassidy et. al. [13] in 1992 found that some cases of Angleman syndrome have two homologous chromosome 15 but inherited from the father.

In 1993, Kalousek et. al. [14] studied nine cases of pregnancies with trisomy 16 confined to the placenta that were prenatally diagnosed by Chorionic Villus Sampling (CVS) with subsequent confirmation of a diploid fetus by amniocentesis. Their study reported four cases of UPD for chromosome 16 showing intrauterine growth retardation (IUGR) and fetal death. In 1994, Vaughan et. al. [22] found maternal UPD 16 in two growth retarded fetus with trisomy 16 placental mosaicism. Early onset IUGR was present in both cases and was also the consistent feature in all previously reported cases by Kalousek et. al. [14]. These studies [14,15] suggest either mosaic placenta and/or UPD may cause impaired fetal growth.

So far UPD has been demonstrated in humans for chromosome 3, 4, 6, 7, 9, 10, 11, 13, 14, 15, 16, 21 and 22. Some of these chromosomal UPDs have been associated with abnormal phenotype and others with normal human development. An abnormal phenotype has been observed with UPD of chromosome 7,

11, 14, 15, and 16. The parental origin and the phenotypic effects of the above mentioned chromosomal UPD are displayed in Table 1-1.

**Table 1-1. Reported Cases of UPD in Humans [Source: 15,45].**

| Chromosome | Parental Origin | No. of Cases | Phenotypic/Imprinting Effects |
|---|---|---|---|
| 2 | Maternal | 1 | Possible |
| 4 | Maternal | 1 | Unlikely |
| 5 | Paternal | 1 | Unlikely |
| 6 | Paternal | 4 | Possible |
| 7 | Maternal | 8 | Yes |
| 7 | Paternal | 2 | Likely |
| 9 | Maternal | 2 | Unlikely |
| 10 | Maternal | 1 | Unlikely |
| 11 | Paternal | >15 | Yes |
| 13 | Maternal | 3 | Unlikely |
| 14 | Maternal | 9 | Yes |
| 14 | Paternal | 2 | Yes |
| 15 | Maternal | >30 | Yes |
| 15 | Paternal | 9 | Yes |
| 16 | Maternal | 9 | Yes |
| 20 | Paternal | 1 | Likely |
| 21 | Maternal | 3 | Unlikely |
| 21 | Paternal | 2 | Unlikely |
| 22 | Maternal | 3 | Unlikely |
| 22 | Paternal | 1 | Unlikely |
| XX | Maternal | 3 | Unlikely |
| XX | Paternal | 1 | Yes |
| XY | Paternal | 1 | Unlikely |

## MECHANISM FOR UPD

There are at least four possible mechanisms (Figure 1-1) which could lead to UPD. These include:

1) Gamete Complementation: Fertilization of nullisomic gamete by a disomic gamete resulting in a zygote with UPD referred to as gamete complementation [5,9,15-19]. With gamete complementation, the

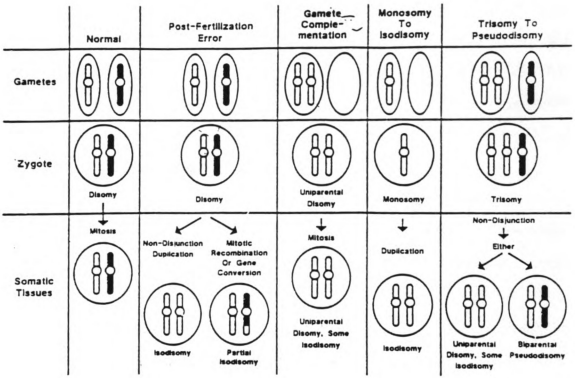



**FIGURE 1-1. The Proposed Mechanism for the Occurrence of UPD [Source: 9].**

chromosomes would be heterodisomic (different) if the disomic gamete result from a meiosis I error, whereas the chromosomes would be isodisomic (same) if the disomic gamete result from a meiosis II error. In gamete complementation, a varying extent of isodisomy is possible depending on where the meiotic error occurred and on the number of

crossovers occurring during meiosis. This mechanism was observed in experimental mice by Searle and Beechy [8]. In humans, so far the only case of gamete complementation of UPD is reported by Wang et al. [16], who found paternal heterodisomy for chromosome 14 in a 45XX,t(13q14q)der,pat, abnormal propositi, whose two parents were balanced heterozygotes for a translocation involving chromosome 14.

2) Compensatory UPD: Chromosome duplication in a monosomic conception could lead to complete isodisomy, referred to as compensatory UPD. This mechanism has been documented in monosomy 21 [17].

3) Post Fertilization Errors: Post fertilization error such as mitotic recombination, non-disjunction with reduplication, or gene conversion may lead to complete or partial isodisomy. This has been proposed as a possible mechanism for paternal disomy of distal 11p in Beckwith-Wiedemann syndrome and pathogenesis of retinoblastoma [18].

4) Trisomy Rescue: Post zygotic loss of a extra homologue by a trisomic conceptus is referred to as either trisomy correction or trisomy rescue. As a result of trisomy rescue when the loss of third chromosomes occurs randomly, the remaining pair in two third of cases would be biparental as normal and in one third of cases it would be uniparental.

This mechanism has already been documented to occur for maternal UPD 14, maternal UPD 15 and maternal UPD 16 [5,13,14,19].

**ASSOCIATION OF CONFINED PLACENTAL MOSAICISM WITH UPD.**

UPD has been most commonly observed in aneuploidic conceptions in which there is confined placental mosaicism (CPM) detected on chorionic villus sampling (CVS). CPM is defined as the presence of two or more cell lines in one fetoplacental unit derived from a single zygote. Chromosomal mosaicism originates during early embryonic cleavage which may take the form of generalized mosaicism or may be confined to the placenta only (Figure 1-2).

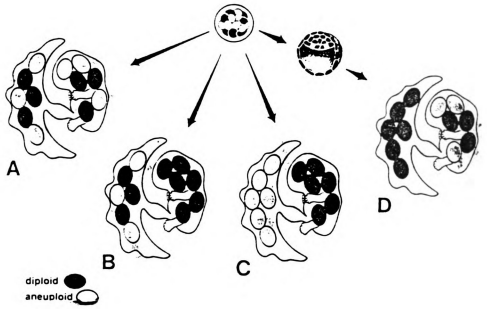



**FIGURE 1-2. Different Types of Mosaicism. A: Generalized Mosaicism; B&C: Confined Placental Mosaicism; D: Mosaicism Confined to Embryo [Source 20].**

The CPM may persist to any stage of pregnancy with different outcomes depending upon the area involved in the placenta. CPM is classified as type I when it is confined to the trophoblast, type II when it is confined to the chorionic villus, and type III when it is confined to trophoblast and stroma of placenta [20]. CPM is usually associated with early spontaneous abortions and fetal deaths. However, still-births and intrauterine growth restriction (IUGR) may occur if pregnancy persists to term. The most commonly involved chromosomes in CPM and trisomy are chromosomes 2, 7, 9, 15 and 16. These chromosomes are also associated with UPD either with phenotypic or no phenotypic effects. The diploid fetus most probably occurs as a result of post zygotic loss of one of the three chromosome in the embryonic/fetal progenitor cells (Figure 1-3).

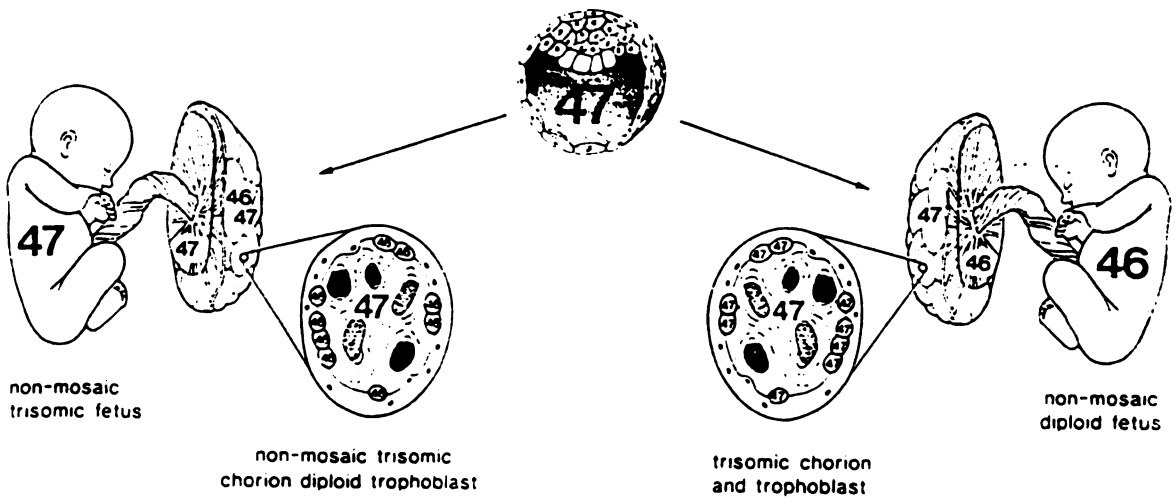



**FIGURE 1-3. Outcome of Trisomic Zygote Rescue Thorough the Postzygotic Loss of Trisomic Chromosome Resulting in A: Trisomic Fetus; B: Diploid Fetus [Source: 20, 23].**

In two third of the cases of mosaic or nonmosaic trisomy, loss of an extra chromosome will result in biparental disomy and in one third of the cases it will result in UPD with both chromosome originating from one parent as shown in Figure 1-4.

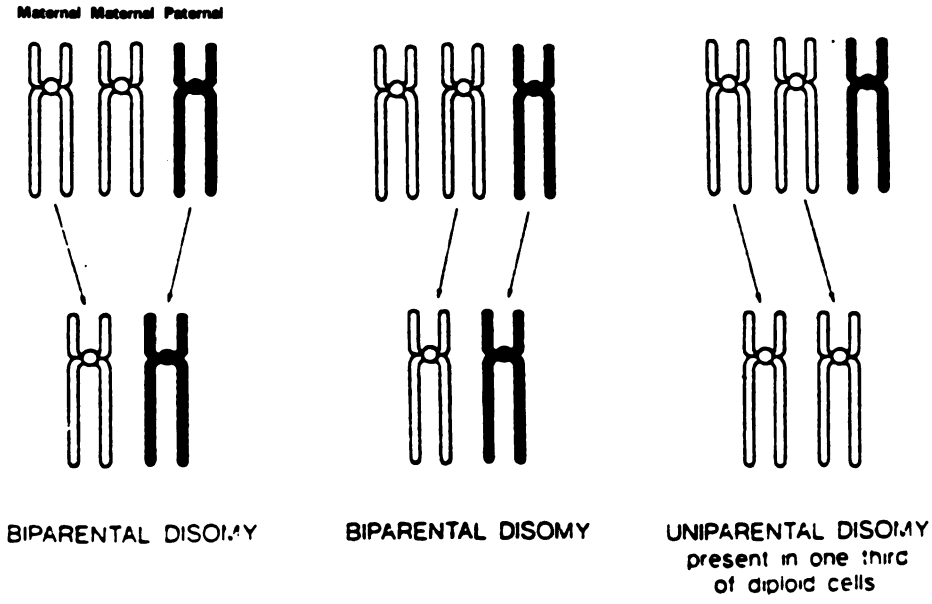



**Figure 1-4. Diagram showing the Origin of UPD in Trisomic Conceptus [Source: 20]**

The above described mechanism has been proposed to be the most common cause for occurrence of UPD. Trisomy 16 is the most frequently observed trisomy associated with spontaneous abortion accounting for 31% of all autosomal trisomies. Mosaic or nonmosaic trisomy 16 with subsequent loss of one copy of chromosome can result in either biparental or uniparental disomy [21,22].

**CONSEQUENCES OF UPD**

UPD may or may not affect the embryonic/fetal development. An effect may be observed if the involved pair carries an imprinted DNA segment or recessive disease genes. Genomic imprinting is described as a genetic phenomenon which induces genetic marking of genes before fertilization so that they are transcriptionally silenced at one of the parental alleles in the offspring [23-28]. The deleterious effects of UPD have been well documented in experimental mice and in some disorders in man as a result of imprinting. The effects of imprinting are observed in Beckwith-Wiedemann syndrome due to UPD 11. UPD 15 has been associated with some cases of Angleman syndrome and Prader-Willi syndrome, and UPD 16 has been associated with fetal growth retardation [14]. Different types of phenotypic effects are observed resulting in UPD from imprinted segments. These include: 1) No phenotypic effect, i.e., maternal and paternal genes are functionally equivalent; 2) A lethal effect, i.e., when the chromosomes originate from one or the other sex. For example, maternal UPD 6 is lethal in mouse; 3) Production of similar phenotypic abnormalities whether UPD is maternal or paternal; and 4) Production of different or even opposite phenotypic effects depending upon the sex of the contributing parent. The best studied example are Prader-Willi syndrome and Angleman syndrome. These two syndromes are associated with distinct and opposite features. Individuals with Prader-Willi syndrome are overweight and slow moving, while individuals with Angleman syndrome are

hyperactive with a characteristic happy puppet appearance and inappropriate laughter. In most cases, both syndromes occur from microdeletion of chromosome 15 segment 15q11-13 which is detected cytogenetically. In some cases, these syndromes occur due to UPD in which a pair of chromosome 15 is either inherited from father or mother. The inheritance of two copies of maternal chromosome 15 results in Prader-Willi syndrome, whereas inheritance of two copies of paternal chromosome 15 results in different clinical phenotype known as Angleman syndrome. This proves that humans need both maternal and paternal contribution to have normal offspring [30].

UPD can also have serious consequences if the individual carries a recessive gene defect. Examples include, a case of transmission of hemophilia (a recessive X-linked disease) by a father to a son as a result of uniparental inheritance of both X and Y chromosomes from the father [31]; UPD 7 in cases of cystic fibrosis where it was discovered that only mothers of these children were the carriers for cystic fibrosis but the fathers were not [10]; and expressions of X-linked disorders in women as a result of inheritance of both X chromosomes from a carrier mother.

**ROLE OF MICROSATELLITES FOR DETECTION OF UPD**

The human genome contains approximately $6x10^9$ nucleotides per diploid genome. These nucleotides are distributed unequally between the 23 pairs of chromosomes. Each chromosome consists of two long, linear strands of polynucleotide which

are hydrogen bonded and coiled around each other in a form of double helix. About 70% to 75% of DNA in human genome is organized as single copy or unique DNA sequence (i.e., DNA whose nucleotide sequence is represented only once per haploid genome). The rest of the genome consists of several classes of repetitive DNA which is characterized by its repetition of sequence in the human genome (Figure 1-5).

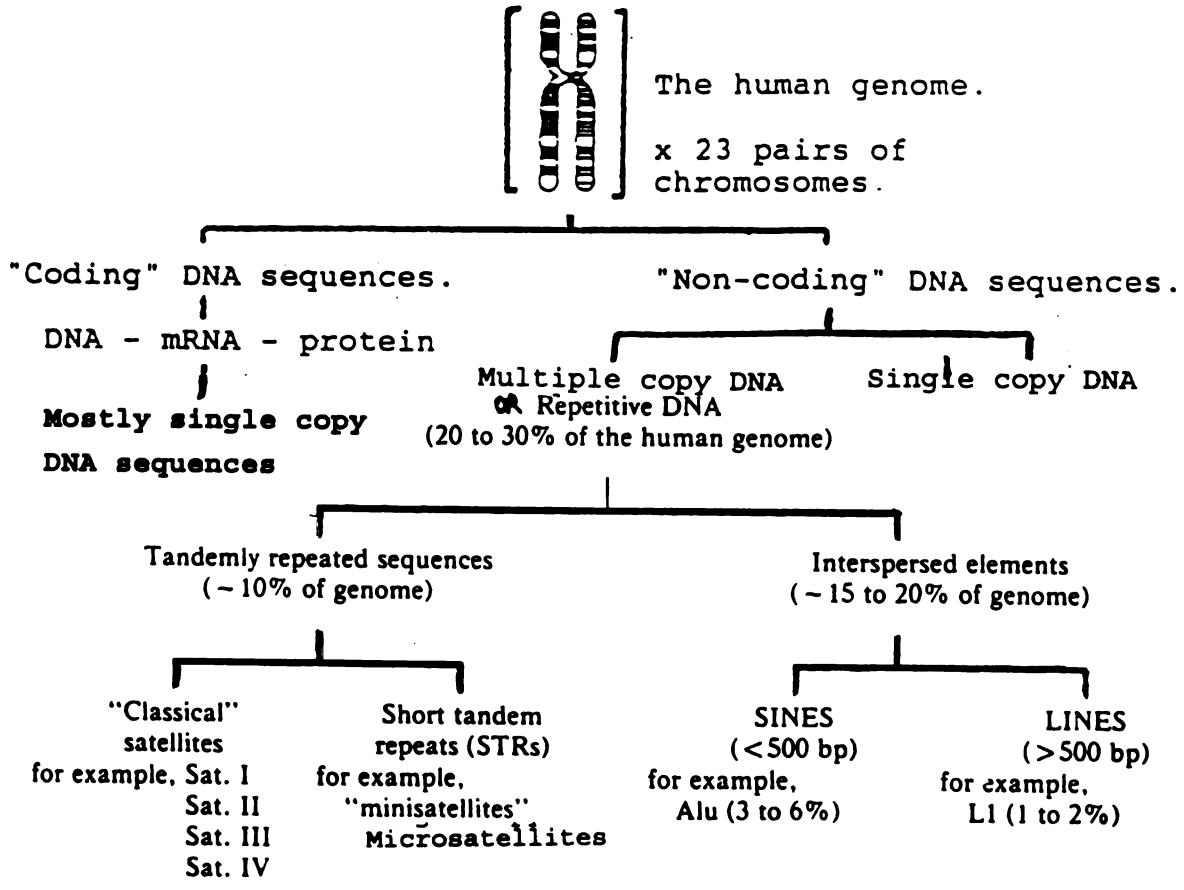



**Figure 1-5. Classification of Repetitive DNA in Human Genomes [Source: 33].**

The single copy sequence of DNA consists of coding regions with encoding sequences (genes) which constitute only a portion of all the single copies of DNA, and non coding regions. These act as a spacer between the coding regions. The rest of the human genome (25% to 30%) consists of repetitive DNA containing sequences which do not include any functional gene members and are composed of arrays of tandem repeat units, or of individual repeat units interspersed with other DNA sequences. Approximately 5% to 10% of the human genome is comprised of tandem repetitive sequences which are characterized by head to tail repetition of lengths of DNA of some common sequence. They are termed as "hypervariable DNA" as they exhibit polymorphism due to presence of variable numbers of tandem repeats of a short DNA sequence.

The variation in lengths of tandem repeats are considered to arise from: 1) unequal exchange between two homologous chromosomes during recombination in meiosis; 2) unequal exchange of sister chromatids during mitosis; and 3) slippage during replication. They are further classified according to the average size of arrays of tandem repeats into satellite DNA, minisatellite DNA, and microsatellites DNA. Some of the satellite DNA sequences have a different density and can be isolated from bulk genomic DNA using buoyant-gradient centrifugation and are referred to as classical satellites. These satellite DNA sequences are usually located at or near chromosome centromeres in the area of heterochromatin.

Satellite DNA is comprised of large arrays of tandemly repeated DNA consisting of repeat lengths of 5 to several thousand base pairs (bp) with $10^3$ to $10^7$ repetitions at each locus. The minisatellites are different from satellites as they have a moderate degree of repetition and the length of the repeat units range from 9 to 100 bp. They are found interspersed throughout genome but are often clustered in the telomeric regions of chromosomes. Microsatellites are relatively short runs of tandemly repeated DNA which are simple in sequence (generally 1-4 bp in length) and are interspersed through out the human genome. They are also known as short tandem repeats and are highly polymorphic due to variation in the number of tandem repeats. Approximately 50% of short tandem repeats studied are polymorphic due to variation in the repeat number.

These microsatellites are further classified into mononucleotide repeats, dinucleotide repeats, trinucleotide repeats, and tetranucleotide repeats depending upon the number of base pairs in each repeat. Of the mononucleotide repeats, runs of A and T are very common and account for about 0.3% of the nuclear genome. Dinucleotide repeat sequences are the most abundant interspersed repetitive DNA elements in the human genome. The most common dinucleotide repeat consist of arrays of CA repeats which are highly polymorphic (TG on the complementary strand) and account for 0.2% of the human genome. Other microsatellites such as trinucleotide and tetranucleotide repeats are less common compared to

dinucleotide repeats but they also exhibit length polymorphism. Although all types of tandem repeats exhibit polymorphism, it is difficult to use the large satellite repeat regions for polymerase chain reaction (PCR) as they are too large to be efficiently amplified. Instead microsatellites can be amplified with PCR, as they consist of a core repeat of 2-4 bp long and their overall length is often only a few hundred base pairs making them ideal for amplification. Microsatellites with repeat lengths of more than 3 bp are more useful because PCR artifacts (due to strand slippage) are reduced. These repeats can be amplified by PCR using primers flanking the region. The amplified products are used to determine alleles segregation in families using different molecular detection techniques. For example, one may use high resolution sequencing gel for separation of the amplified products and identify DNA fragments (bands) differing by as little as one repeat unit [32-37].

These microsatellites are also utilized as gene markers in other areas of genetics such as mapping of the human genome, chromosomal analysis, and diagnosis of inherited diseases. Moreover, these microsatellites can be used for identification of individuals and in disputed paternity cases in the area of forensic medicine. In this study, a PCR procedure has been developed to detect or rule out uniparental inheritance in premature infants utilizing microsatellites for chromosome 16.

**OBJECTIVES**

The main objectives of this study were: 1) to develop a procedure to determine the parental origin of chromosome 16 by DNA analysis; and 2) to apply this procedure to a group of infants born prematurely and/or with intra uterine growth restriction (IUGR) for the presence of UPD 16.

To accomplish these objectives, a base procedure was initially developed for evaluation of polymorphic markers (microsatellites) on chromosome 16 by DNA analysis. The microsatellites evaluated were either dinucleotide or tetranucleotide repeat sequences distributed along the length of chromosome 16. Polymerase chain reaction (PCR) was used to amplify DNA by specific primers. The amplified products were analyzed on denaturing polyacrylamide gel to separate alleles based on variable number of repeats. Inheritance patterns between family members were accomplished by comparison of alleles migration patterns developed by gel electrophoresis. The details of the above procedure and its application are described in the following chapter.

# CHAPTER 2

# MATERIALS AND METHODS

## COLLECTION OF SAMPLES

This study required DNA samples from premature and IUGR infants and their biological parents. Cord blood samples were obtained from the infants admitted to the Neonatal Intensive Care Unit (NICU) of Edward C. Sparrow Hospital, Lansing, Michigan. The blood samples were drawn from these premature and IUGR infants at the time of birth. If a cord blood sample was not available then DNA was extracted from cheek cells. These samples were obtained using small sterile brushes or swabs which were rubbed against the inner cheeks of infants. These brushes were placed in saline solution contained in 15 ml tube and rotated for 30 seconds. The brushes were discarded and DNA extracted by the method described below. Buccal wash samples were collected from the infant's parents [38]. They were provided with a 50 ml test tube containing 15 ml of sterile saline solution which they were asked to swish in their mouth for about 45-60 seconds and then spit back into the test tube. The DNA from infants' blood samples and parents' mouth wash samples was extracted according to the protocol as described below.

## DNA EXTRACTION FROM BLOOD AND MOUTH WASH SAMPLES

To extract DNA from blood, RBC were lysed by adding 500 ul of a TE buffer [Tris 10 Mm EDTA 1 Mm] to 100 ul of blood in

a 2 ml centrifuge tube. The mixture was vortexed for 10 seconds and then centrifuged at 10,000 rpm for 10-15 seconds. The red colored supernatant containing lysed RBC was discarded and the pellet was re-suspended in TE buffer solution. This procedure was repeated until the pellet cleared. For DNA extraction, the white blood cell pellet was suspended in 400 ul of 50 Mm NaoH and heated at 95°C for 10 minutes. It was then cooled on ice for 10 minutes and neutralized by addition of 40 ul of 1 M, Ph 8 Tris buffer. The sample was then stored at 4°C for further use. The buccal wash samples collected from the parents were centrifuged at 2,500 rpm for 10 minutes. The supernatant was discarded and pellet was re-suspended in 400 ul of 50 Mm NaoH. This mixture was transferred into 2 ml storage tube and heated at 95°C for 10 minutes and cooled on ice for 10 minutes. 40 ul of 1 M Tris Ph 8 was added and the sample stored at 4°C for further use in PCR analysis [38-40].

**PROCEDURE FOR DNA AMPLIFICATION BY PCR**

The PCR reaction was performed using specific pairs of oligonucleotide primers which flanked one of the dinucleotide or tetranucleotide repeat sequence (microsatellite) distributed along the length of chromosome 16. The primer sequences used for these polymorphic markers on chromosome-16 (Table 2-1) were obtained from the Research Genetics Map Pairs [Huntsville, Alaska, USA].

The following dinucleotide and tetranucleotide repeat markers were used in this study: D16S400, D16S407, D16S415, D16S423, D16S310, D16S539, D16S540, D16S541, D16S749, D16S750, D16S751, D16S752, and D16S753.

**Table 2-1. Chromosome-16 Microsattelite Amplification Primers**

| Marker | Locus | Location of Markers | No of Alleles / Type of STR | Heteroz-ygosity | PCR Product Size | Amplification Primers Sequence |
|---|---|---|---|---|---|---|
| AFM024xg1 | D16S400 | 16 | 3 DINUC/STR | 0.61 | 192-202 | GTCATCCGACTTCTCACAGG<br>AATATGAACCCTCCATGCTG |
| AFM249yc5 | D16S423 | 16p13.3 | 10 DINUC/STR | 0.73 | 121-139 | AACAGGCTTGAAAGTCTCTGTC<br>GCCTATTTGATAATGCTGTACG |
| AFM113xe3 | D16S407 | 16p13.2 | 10 DINUC/STR | 0.85 | 150-170 | CTCGCGCTGGGTACAGTTAT<br>AGATCAGAGGAGTGGGTTCC |
| AFM205ze5 | D16S415 | 16q13.3 | 8 DINUC/STR | 0.72 | 208-234 | CCAGTAATGTTATGTAAGTCAATGC<br>TAGCCACTGTACCCCAGC |
| GATA11CO6 | D16S539 | 16 | N/A TETNUC/STR | 0.60 | 157 | ACGTTTGTGTGTGCATCTGT<br>GATCCCAAGCTCTTCCTCTT |
| GATA7BO2 | D16S540 | 16q12.1 | N/A TETNUC/STR | 0.68 | 237 | AATCTCCCCAACTCAAGACC<br>CCTCCATAATCATGTGAGCC |
| GATA7E02 | D16S541 | 16q12.1 | N/A TETNUC/STR | 0.77 | 160 | CCACACCAGCGTTTTTCTAA<br>CACACTTTACACACACCTATACCC |
| GAAT1E9 | D16S749 | 16 | N/A TETNUC/STR | 0.69 | 144 | CTCCTACTCTGCACCAGGAA<br>TCCTAGTTACAGTGCCTGGC |
| GAAT2B10 | D16S750 | 16 | N/A TETNUC/STR | 0.47 | 113 | ATAGCAAGTACTGAATGACCTGG<br>GCAAAGCACTGGGAGATTTA |
| GATA49B09 | D16S751 | 16 | N/A TETNUC/STR | 1.00 | 224 | TAATGTACCTGGTACCAAAAACA<br>ATCCCTCCAGCTAAAGTGCT |
| GATA51G03 | D16S752 | 16 | N/A TETNUC/STR | 0.92 | 102 | AATTGACGGTATATCTATCTGTCTG<br>GATTGGAGGAGGGTGATTCT |
| GGAA3G05 | D16S753 | 16 | N/A TETNUC/STR | 0.88 | 265 | CAGGCTGAATGACAGAACAA<br>ATTGAAAACAACTCCGTCCA |
| MIT-MH20 | D16S310 | 16 | N/A TETNUC/STR | 0.67 | 162 | GGGCAACAAGGAGAGACTCT<br>AAAAAAGGACCTGCCTTTATCC |

PCR reactions were performed using 5 ul of DNA in a total volume of 50 ul containing: 2 ul (0.75 Um) of each primer; 1 ul (200 Um) of each dNTP; 5 ul (1.5 Mm) of $MgCl_2$; 5 ul of 10 x PCR buffer (10 Mm Tris, 50 Mm KCl Ph 8.3); and 2.5 units of AmpliTaq Polymerase. These samples were processed through 28 cycles each consisting of: denaturation at 95°C for 1 minute; annealing at 55°C for 45 seconds; extension at 72°C for 45 seconds; and final extension done at 72°C for 5 minutes. PCR products were analyzed for amplification on 3% agarose (Nusieve) gel electrophoresis.

**AGAROSE GEL ELECTROPHORESIS**

The equipment required was:

1) Horizontal gel electrophoresis apparatus
2) Power supply
3) Microwave oven
4) UV transparent plastic gel mold
5) UV transilluminator

The 3% agarose gel was prepared by adding 3 gm of agarose (Nusieve) to 100 ml of 0.5X TBE buffer (Appendix B). Agarose was solubilized by heating in the microwave oven and allowed to cool but not to set. After this, 5 ul of ethidium bromide (10 mg/ml) was added and swirled to mix. The agarose solution was poured into a taped gel former mold. The well forming comb was placed near one edge of the gel. The gel was left to set till it became milky and opaque. Comb and tapes were removed and the gel slab was placed in the electrophoresis tank completely submerged in 0.5X TBE buffer solution. The samples were prepared by adding 10 ul of PCR product and 2 ul of

loading buffer (Appendix D). Each well was loaded with 10 ul of sample. Marker-V (DNA molecular weight marker of known fragment sizes ranging from 587 bp to 50 bp) was loaded in one well in order to determine the size of PCR products. The gel was run at 90-100 volts for 1.5 to 2 hours and examined on UV transilluminator for ethidium bromide stained bands. The gel was photographed on UV transilluminator by polaroid camera [41,42].

The PCR amplification was confirmed by measuring the size of the various DNA bands (PCR products) produced by gel electrophoresis. The size of the DNA band was estimated by measuring the distance that the fragment had migrated during gel electrophoresis. The actual measurement was made on the developed film on which position of the DNA was evident as bands and the position at which the DNA was loaded in the gel (the origin). The migration was measured either by ruler or with a set of calipers. There is a inverse relationship between the migration distance and the DNA fragment size. This relationship was established for each gel by measuring the migration distance of a number of fragments of known sizes. The standard fragment sizes were obtained by running DNA molecular weight markers on the gel with the DNA samples. The migration distances of a series of DNA fragments of known size (molecular weight markers) were plotted on a graph against its known size and a curve was drawn through the points. This curve could be drawn by hand or by computer program using a standard curve formula. The migration distance

of DNA fragments of various samples were measured from the same gel and the sizes of fragments were determined from the curve by reading the fragment size which correspond to its migration distance. Once the amplification was confirmed the alleles were separated for different families on 6% polyacrylamide 8 M or 7 M urea denaturing gel.

**PREPARATION OF DENATURING POLYACRYLAMIDE GEL**

Denaturing polyacrylamide gels allow resolution of single stranded DNA ranging in length from 20bp to 1kb. This gel was used in this study to separate alleles in premature/IUGR infants and their parents to investigate the possibility of uniparental inheritance in the infant.

**Equipment Required**

1) Sequencing gel apparatus (GIBCOBRL)
2) Glass plates
3) Spacers and combs for 0.4 mm thickness gel
4) Syringes, cylinders, beakers etc.

**Reagents Required**

1) Acrylamide
2) Bisacrylamide
   [Both acrylamide chemicals were required to prepare (19:1) 40% polyacrylamide solution (Appendix E) or purchased as ready-to-use 40% Polyacrylamide (19:1) solution from Bio-Rad Laboratories].
3) Urea
4) Dichlordimethylsilane
5) Bind silane (Appendix G)
6) Temed
7) 10% Ammonium Persulphate
8) Loading solution (Appendix F)
9) 1X TBE solution (Appendix C)
10) 1% agarose in 0.5% TBE solution

A 6% polyacrylamide 8 M or 7 M urea solution was prepared for 0.4 mm thick gel according to the formula given below [41-43].

6% POLYACRYLAMIDE 8 M UREA GEL SOLUTION

| | |
|---|---|
| 40% Polyacrylamide Solution | 75 ml |
| 5X TBE solution | 50 ml |
| Urea (Ultrapure) | 230 gm |
| Dd $H_2O$ | 300 ml |

[volume adjusted to 500 ml with deionized distilled water]

6% POLYACRYLAMIDE 7 M UREA GEL SOLUTION

| | |
|---|---|
| 40% Polyacrylamide Solution | 22.5 ml |
| 10X TBE solution | 15 ml |
| Urea (Ultrapure) | 63 gm |
| Dd $H_2O$ | 100 ml |

[volume adjusted to 150 ml with deionized distilled water]

The chemicals were dissolved completely and dd $H_2O$ was added to adjust the volume. The solution was filtered through a nitrocellulose filter (Nalge, 0.45 micron) and stored in dark bottles at 4°C for up to several weeks.

**Preparation of Plates for Polyacrylamide Gel**

Two glass plates (one large and one short) were required to prepare a polyacrylamide gel 0.4 mm thick and 32 cm x 16 cm in size. The plates were treated with KOH/methanol (5 gm of KOH pellets in 100 ml of methanol) to remove old silicon, cleaned with nonabrasive detergent, rinsed thoroughly with deionized water, and wiped dry. The inner surface of the smaller plate was treated with bind silane (methacryloxypropyl

trimethoxysilane from Sigma) to fix the gel (when polymerized) to the inner surface of the small plate. The inner surface of the larger plate was treated with siliconizing reagent (dimethyldicholrosilane from Sigma) to avoid adhesion between the two plates. Both the plates were rinsed with water and wiped dry.

The glass plates treated with bind saline and siliconizing reagent were rinsed with ethanol and wiped dry with lint free paper towel before pouring the gel. The glass plate sandwich was assembled by putting 0.4 mm vinyl spacers on both sides and sealing the sides and bottom with gel sealing tape. The tape was applied in a smooth and firm manner to avoid forming air channels or bubbles along the edges. A few spring clips were also applied on the sides and bottom to prevent the glass plates from sliding [44].

**Pouring of Gel**

Approximately 50 ml of 6% polyacrylamide 8M urea solution was required to prepare a 32 cm x 16 cm and 0.4 mm thick gel. For polymerization, 150-180 ul of 10% ammonium persulphate and 50-100 ul of TEMED were added to the above solution. The gel mixture was swirled and poured immediately (using either syringe or beaker) between the glass plates held at 45° angle. Constant flow was maintained to reduce the chance of forming bubbles between the plates. Wells were created by inserting either a square tooth comb or a sharkstooth comb in the top of the gel. The glass plates were left in a horizontal position for 1 to 2 hours until the acrylamide had polymerized.

## POLYACRYLAMIDE GEL ELECTROPHORESIS

### Pre-Electrophoresis

Following the polymerization, clamps and tape were removed and the top of the gel was rinsed with electrophoresis buffer to remove any unpolymerized acrylamide. The comb was slid out carefully from the plates. If the sharkstooth comb had been used, it was reinserted between the glass plates with the teeth toward the gel, until it made contact with the surface of the gel. The gel sandwich was placed in the sequencing apparatus with the short plate facing inside and secured with the clamps. First the upper and then the lower buffer chambers of the electrophoresis apparatus were filled with approximately with 300 ml of 1% TBE solution making sure no buffer was leaking from the upper chamber. The power was connected and the gel was pre-electrophoresed for 15 to 30 minutes at 40 W or 1425-1450 V as recommended for 0.4 mm thick and 32 cm x 16 cm dimension gel. The temperature of the gel surface was kept between 45-50°C for best result and monitored by a thermostrip applied to the surface of the glass plate.

### Loading of Samples

One ul of PCR product was mixed with 9 ul of loading buffer in a centrifuge tube and samples were prepared for loading in the wells by heating at 90°C to 100°C for 5-10 minutes and then chilling on ice for 5 minutes. Before loading the samples, wells were rinsed with a syringe or Pasteur pipette using electrophoresis buffer to wash away the accumulated urea. The samples (8-10 ul) were loaded in a square tooth well, and (2-3 ul) in a sharkstooth comb well, as

recommended by the instruction manual. Marker-V or a 10 bp ladder marker were prepared and loaded on both sides of the samples. The gel was electrophoresed for 1 to 1.5 hour as recommended [44].

After the electrophoresis was done, the buffer was drained from the chambers. The gel sandwich was released from the sequencing apparatus and allowed to cool for 10 minutes. The gel sandwich was disassembled by prying the siliconized glass plate from the bottom corner with a thin spatula. The gel which was fixed to small the plate, was stained with the silver stain according to the following protocol [42,44].

**PROTOCOL FOR SILVER STAINING**

The acrylamide gel was stained with silver stain immediately after the electrophoretic run according to the protocol given by the Bio-Rad. The following steps were done to fix, stain and develop the gel.

| | Reagent | Volume | Duration |
|---|---|---|---|
| 1) | Fixative 40% methanol | 500 ml | 30 min |
| 2) | Fixative 10% ethanol | 500 ml | 15 min |
| 3) | Fixative 10% ethanol | 500 ml | 15 min |
| 4) | Oxidizer | 500 ml | 5 min |
| 5) | Deionized water | 500 ml | 5 min |
| 6) | Deionized water | 500 ml | 5 min |
| 7) | Deionized water | 500 ml | 5 min |
| | [Repeat wash 5,6,7 until all the yellow color is removed from the gel] | | |
| 8) | Silver reagent | 500 ml | 20 min |
| 9) | Deionized water | 500 ml | 1 min |
| 10) | Developer | 500 ml | ~30 sec |
| | [Develop until solution turns yellow or brown. Then pour off the developer and add]: | | |
| 11) | Fresh Developer | 500 ml | ~5 min |
| 12) | Stop 5% acetic acid | 500 ml | 5 min |

The above volumes of the different staining reagents were prepared to stain a 32 cm x 16 cm gel attached to a glass plate with bind saline in a large pyrex baking dish (it is important that the size of the dish should be appropriate to allow free movement of the gel during shaking while maintaining a volume of reagent sufficient to cover the gel completely). After completing the staining procedure as described above, the gel was photographed on Kodak's Electrophoresis Duplicating Film (EDF) according to the following protocol.

**PROTOCOL FOR DEVELOPING (KODAK) EDF**

The following equipment was required for developing EDF.

1. Cellulose acetate sheet.
2. Desk lamp or any other source of light with two 15-watt fluorescent cool white bulb.
3. Yellow, red, or reduced room light.
4. Kodak Dektol Developer.
5. Kodak Rapid Fixer or Kodak Fixer.

The photographic film was placed on a dry and clean surface with the emulsion side up and the cellulose sheet directly on the top of it. Then the wet gel was placed directly on the top of cellulose sheet. The source of light which was positioned 8-10 inches above the gel was turned on and EDF was exposed for 10-30 seconds. The film was developed in Kodak Dektol Developer (1:1) for 1 minute. Then it was rinsed with running water for 10 seconds and fixed in Kodak fixer for 2-4 minutes with rapid agitation. The film was again

washed in running water for 5-10 minutes and dried in dust free place. The developed film was used to analyze the segregation of alleles in different families.

**DEVELOPMENT OF BASE PROCEDURE**

The development of a base procedure was required so that it could be subsequently applied in this study to evaluate polymorphic DNA markers in order to determine parental origin of chromosome 16 in premature and IUGR infants. To begin the study, DNA samples were required from such infants and their biological parents. Since the required samples were not readily available from the designated hospital, buccal wash samples were obtained from a volunteer family (comprised of mother, father and two daughters) for developing the base procedure. DNA was extracted by the method described in the previous sections of this chapter.

Different CA dinucleotide repeat polymorphisms with high heterozygosity were selected which were distributed along the length of chromosome 16. The CA repeats used were: D16S400 D16S402, D16S405, D16S407, D16S411, D16S412, D16S415, and D16S423. PCR reactions were performed by as described using amplification primers which flanked each side of CA repeat sequences. The DNA samples obtained were amplified in a single and multiplex PCR reaction in thermal cycler (Perkin Elmer). The presence of PCR products was evaluated on ethidium bromide agarose gels. To achieve optimal amplification results, magnesium ion concentration, primers concentration, annealing

temperatures of primers and time for primer extension were adjusted. Single locus PCR reactions yielded good results whereas multiplex PCR reactions did not yield DNA amplification. The PCR products were separated by electrophoresis on 0.8 mm thick 8% polyacrylamide 8 M urea gel. The gel was first tried to be stained with ethidium bromide but was finally stained with silver stain for better resolution. Using the above method, only two dinucleotide primers, for the loci D16S400 and D16S423, yielded satisfactory results.

When dinucleotide repeats did not yield satisfactory results, other microsatellite loci (tetranucleotide repeats) on chromosome 16 were investigated. The microsatellites used were: D16S310, D16S539, D16S540, D16S541, D16S749, D16S750, D16S751 and D16S752. These markers were analyzed by electrophoresis on a thinner polyacrylamide gel of 0.4 mm thickness. This extremely thin polyacrylamide gel resulted in ripping of gel into fragments during staining and developing procedures. Gel-bond (a membrane which binds the gel to it surface) was used to alleviate the ripping problem. Though the application of gel-bond solved the problem of gel ripping, it resulted in inadequate resolution during electrophoresis. A gel bind silane mixture (Appendix G) was then applied to one glass plate to keep the gel attached to its surface in order to prevent ripping during staining and developing procedures. Moreover, the application of gel bind silane mixture did not interfere with the electrophoresis and yielded satisfactory

resolution. The gel was finally stained with silver stain which produced distinct separation of alleles. Electrophoresis duplicating films (EDF) were developed from this gel to determine segregation of alleles in the family investigated.

## Results Obtained From Base Procedure

The results of DNA analysis in the volunteer family using the developed base procedure are given in Table 2-2.

**Table 2-2 Results of DNA Analysis for the Volunteer Family**

| MARKERS | (M) | (C1) | (C2) | (F) | ORIGIN OF CHROMOSOME 16 IN THE CHILD | CHILD'S UNIPARENTAL DISOMY |
|---|---|---|---|---|---|---|
| D16S310 | 1,2 | 1 | 1 | 1 | undetermined | markers noninformative |
| D16S540 | 2 | 2 | 2 | 1,2 | undetermined | markers noninformative |
| D16S541 | 1,3 | 2,3 | 1,2 | 1,2 | maternal/paternal | UPD ruled out |
| D16S749 | 1,3 | 2,3 | 2,3 | 2 | maternal/paternal | UPD ruled out |
| D16S750 | 1 | 1 | 1 | 1,2 | undetermined | markers noninformative |

**(M)** = Mother
**(C1)** = Child 1
**(C2)** = Child 2
**(F)** = Father

Out of the different markers investigated for chromosome 16 for the volunteer family (Table 2-2), only two markers, D16S541 and D16S749, were informative. Both children, C1 and C2, inherited two alleles, one from the mother and one from the father, thus ruling out UPD 16.

This base procedure was finally adopted to test and validate the applicability of this procedure and to determine the parental origin of chromosome 16 in premature and IUGR infants.

# CHAPTER 3

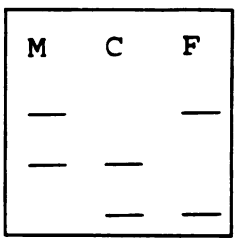
## ANALYSIS AND RESULTS

The results reported in this chapter are PCR-based genetic analysis to investigate uniparental inheritance of chromosome 16 in premature and IUGR infants from NICU. Cord blood samples were drawn from the infants at the time of birth whereas buccal wash samples were collected from the parents. A set of 11 samples was obtained to be used in this study. The DNA was extracted, and microsatellite markers (tetranucleotide repeats) for chromosome 16 were used to detect uniparental disomy 16. These polymorphic loci were analyzed by PCR, and were run on polyacrylamide gel to detect the parental origin of chromosome 16 in each case.

### CRITERIA USED TO EXCLUDE UPD FOR CHROMOSOME 16

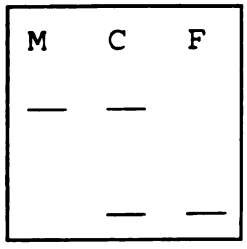
In order to exclude, or to confirm, uniparental inheritance in a proband (IUGR or premature infants), the following criteria were used in this study.

1) If the mother and the father were found to be heterozygous for the microsatellite locus investigated on chromosome 16 with different alleles for that locus and the child's DNA contained one allele from the mother and one allele

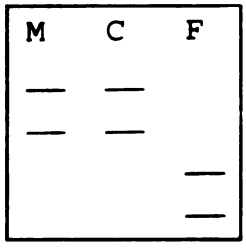


from the father, it would completely exclude uniparental inheritance from either of the parents and the condition would be referred to as biparental disomy.

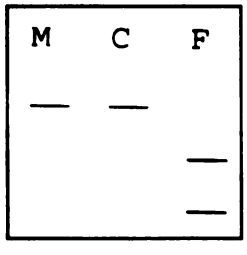
2) If the mother and the father were found to be homozygous for the same microsatellite locus investigated but having different alleles and the child's DNA contained two alleles, one from the mother and one from the father, it would rule out uniparental inheritance from either of the parents.




3) If the mother and the father were found to be heterozygous for the locus on chromosome 16 and both of them had two different alleles for that locus, and the child's DNA contained two alleles same as his mother without any contribution from the father, it would confirm maternal uniparental inheritance and the condition would be referred to as heterodisomy.

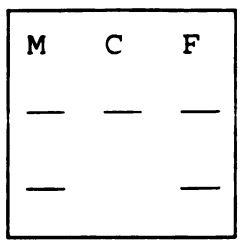



4) If the mother was found to be homozygous for different loci on chromosome 16 and for each of these loci the father had different alleles than the mother, and the child's DNA contained exactly the same homozygous alleles as his mother, without any contribution from his



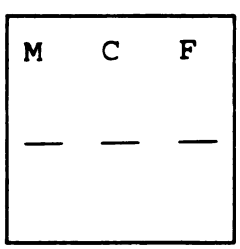
father, it would confirm maternal uniparental inheritance and the condition would be referred to as isodisomy.

5) If the parents were found to be heterozygous and the child was homozygous for the same locus on chromosome 16, the uniparental inheritance would not be ruled out.




In such cases, a number of other markers on the same chromosome would have to be investigated to rule out UPD.

6) UPD 16 would not be ruled out, if the parents were found to be homozygous for the locus on chromosome 16, and the child's DNA contained the same allele as his mother and father. In such cases, number of other markers on the same chromosome would have to be investigated to exclude UPD.




**INFORMATIVE AND NONINFORMATIVE MARKERS**

The markers investigated would be referred to as fully informative if they could rule out both maternal and paternal UPD, whereas they would be referred to as noninformative if parental inheritance could not be determined. The markers would be partially informative if they could rule out either paternal or maternal UPD.

**CASE REPORTS**

In the following pages, eleven case reports are presented using an abbreviated terminology of G, P, and A (each word followed by a number) which describes the mother's obstetrical history. The word G refers to gravidity; P refers to parity; and A refers to abortion.

**Case: 1 A**

In this case, the mother was a 22 years old G2 P1 A0 who went into pre-term labor and delivered premature twin baby boys at 32 weeks of pregnancy by caesarian section. The patient had splenectomy earlier for idiopathic thrombocytopenic purpura and a history of previous c-section at 36 weeks. The mother was a smoker without any history of drugs and alcohol intake. There was no history of diabetes or hypertension during this pregnancy. The twins were appropriate for gestational age according to the percentile weight. At the time of birth, the weights of twins A and B were at the 19th and the 31st percentile respectively. No dysmorphic features or congenital anomalies were detected at the time of birth. These twins were shifted to NICU where they were treated for prematurity, pulmonary immaturity syndrome, hyperbilirubinemia and neonatal hypoglycemia. They stayed in NICU for 22 days and were discharged to be followed up by their pediatrician. Only twin A blood sample was available for this study.

**Family A**

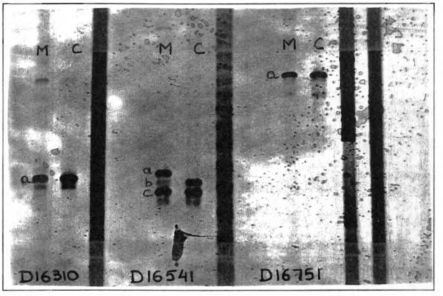



**Figure 3.1 (Family A).**

EDF showing PCR microsatellite alleles segregation in the proband (C) and mother (M) for the loci D16S310, D16S541 and D16S751 located on chromosome 16. For D16S310, alleles were M(a); C(a), D16S541, M(a,c); C(b,c), D16S751, M(a); C(a).

**Results:**

In family A, polymorphic microsatellite markers D16S310, D16S541 and D16S751 were investigated for UPD 16. The father was not available in this study. Markers D16S310 and D16S751 were noninformative as the mother was homozygous for above loci and the child had inherited the same allele from the mother. In this case marker D16S541 was informative as the mother and the child were both heterozygous for this locus. The child's DNA contained two alleles, one from the mother and other probably from the father, thus ruling out maternal UPD but not paternal UPD for chromosome 16 in this case.

**Case: 2 B**

In this case, the mother was 39 years old G1 A0 P0 who was diagnosed with twin pregnancy by ultrasound before delivery. She went into premature labor at 31 weeks of gestation and delivered twin A vaginally and twin B by caesarean section for transverse lie. The mother was not a smoker and did not take drugs or alcohol during the pregnancy. She had a history of multiple fibroids. There was no history of diabetes or hypertension during this pregnancy. The infants were appropriate for their gestational age. No dysmorphic features or congenital anomalies were detected at the time of birth. Both babies were shifted to NICU and treated for neonatal hyperbilirubinemia, transient hypoglycemia and conjunctivitis. They were discharged after 21 days to be followed up by their pediatrician and eye specialist. Only twin A blood sample was available for this study.

**Family B**

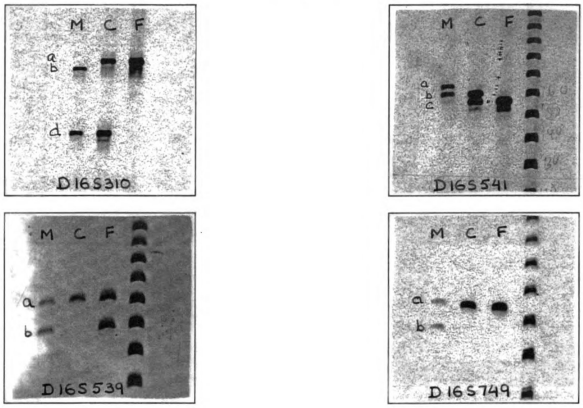



**Figure 3.2 (Family B).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for loci D16S310, D16S541, D16S539 and D16S749 located on chromosome 16. For D16S310, alleles were M(b,d); C(a,d); F(a); D16S541, M(a,b); C(b,c); F(c); D16S539, M(a,b); C(a); F(a,b); D16S749, M(a,b); C(a); F(a).

**Results:**

In this case markers D16S539 was noninformative and D16S749 was partially informative ruling out maternal UPD. The other two markers D16S310 and D16S541 were fully informative for ruling out UPD 16. The mother was heterozygous and the father was homozygous for D16S310 and D16S541 loci on chromosome 16 but had different alleles. The child inherited one allele from the mother and one allele from the father thus ruling out UPD 16 from either parents.

**Case: 3 C**

In this case, the mother was a 33 year old G2 P1 A0 at 32 weeks of pregnancy who delivered a premature baby girl by emergency caesarean section for non-assuring heart rate and decreased fetal movement.

The mother was not a smoker and did not take drugs or alcohol during the pregnancy. There was no history of diabetes or hypertension during this pregnancy. No congenital abnormalities or dysmorphic features were detected at the time of birth. The infant was premature and small for gestational age. The infant was admitted in NICU for prematurity, hyperbilirubinemia, respiratory distress. The infant stayed in NICU for 24 days and was discharged to be followed by her pediatrician and developmental assessment clinic.

**Family C**

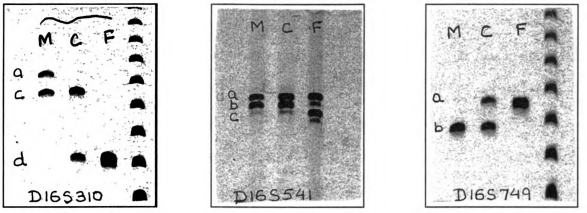



**Figure 3.3 (Family C).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for loci D16S310, D16S541 and D16S749 located on chromosome 16. For D16S310 the alleles were M(a,c); C(c,d); F(d); D16S541, M(a,b); C(a,b); F(a,c); D16S749, M(b); C(a,b); F(a)

**Results:**

To rule out UPD 16 in family C, D16S310, D16S541 and D16S749 loci on chromosome 16 were investigated. D16S310 and D16S749 were informative whereas D16S541 was noninformative. The mother was heterozygous and the father was homozygous for D16S310 locus. The child inherited two alleles, one from the mother and one from the father, thus ruling out UPD 16. The mother and the father were homozygous for D16S749 locus but the child was heterozygous inheriting one allele from the mother and one allele from the father, thus ruling out UPD 16 from either parents.

**Case: 4 D**

In this case, the mother was 25 years old G2 P1 A0 who delivered a premature baby girl at 35 weeks of pregnancy by spontaneous vaginal delivery. The mother was non-smoker, did not take any drugs or alcohol in pregnancy. The pregnancy was uncomplicated with no history of diabetes, hypertension or vaginal bleeding. No dysmorphic features or congenital anomalies were detected at the time of birth. The baby was premature but appropriate for gestational age with a 19th percentile weight at birth. She was shifted to NICU and treated for respiratory distress and transient tachypnea. She was discharged after 8 days to be followed up by her primary physician.

**Family D**

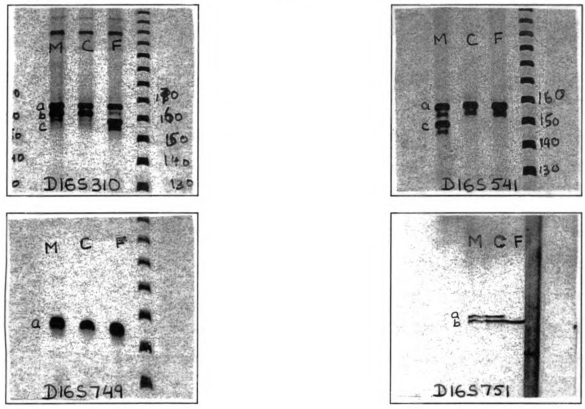



**Figure 3.4 (Family D).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for loci D16S310, D16S541, D16S749 and D16S751 located on chromosome 16. For D16S310 alleles were M (a,b); C(a,b); F(a,c); D16S541, M(a,c); C(a); F(a); D16S749, M(a); C(a); F(a); D16S751, M(a,b); C(a,b); F(b).

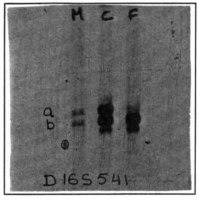
**Results:**

On their own, all the markers investigated (D16S310, D16S541, D16S749 and D16S751) were noninformative. For marker D16S310 and D16S751, the child has inherited two alleles but we can not be sure if both these alleles were inherited from the mother, or one from the mother and other from the father. Since the child has not inherited both maternal alleles for the D16S541 locus or both paternal alleles for D16S310 and D16S751 loci, UPD 16 can be ruled out from either parents.

**Case: 5 E**

In this case, the mother was 17 years old G1 P0 A0 who delivered a premature baby girl spontaneously by vaginal delivery at 26 weeks of pregnancy. The mother was a smoker. However, there was no history of alcohol or drug intake during the pregnancy. The pregnancy was uncomplicated with no history of vaginal bleeding, diabetes or hypertension. The infant was premature and small for gestational age. His weight was below the 10th percentile at the time of birth. No dysmorphic features or congenital anomalies were detected at the time of birth. The infant was shifted to NICU and treated for hyper-bilirubinemia, retinopathy, intraventricular hemorrhage, respiratory distress and hypoglycemia. He was discharged after 79 days to be followed up by his pediatrician and developmental assessment clinic.

**Family E**



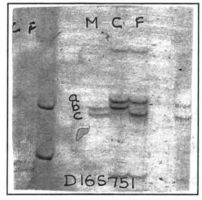




**Figure 3.5 (Family E).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for loci D16S541 and D16S751 located on chromosome 16. For D16S541 alleles were M (a,b); C(a,b); F(b); D16S751 M(b,c); C(a,b); F(a,c).

**Results:**

For family E, marker D16S541 and marker D16S751 were investigated to rule out uniparental inheritance for chromosome 16. Marker D16S541 was noninformative but marker D16S751 was fully informative. The mother and the father were heterozygous for D16S751 locus. The child's DNA contained two alleles inheriting one allele from the mother and one from the father, thus ruling out UPD 16 in this case.

**Case: 6 F**

In this case, the mother was 43 years old multigravida G5 P2 A2 who developed hypertensin during pregnancy. The mother used alcohol and tobacco during pregnancy. IUGR was diagnosed by ultrasound before delivery. The patient went into premature labor at 36 weeks of pregnancy and delivered a premature baby girl by caesarean section. No dysmorphic features or congenital anomalies were detected at birth. The child was small for gestational age and his weight was below the 10th percentile at the time of birth. Cytogenetic analysis revealed a normal karyotype. The baby was kept in NICU for 11 days and treated for respiratory distress, hyperbilirubinemia, fetal alcohol syndrome and cardiac murmur. She was discharged to be followed up by her pediatrician, physical therapist, geneticist, and developmental assessment clinic. The mother was a single parent and the father was not available for this study.

**Family F**

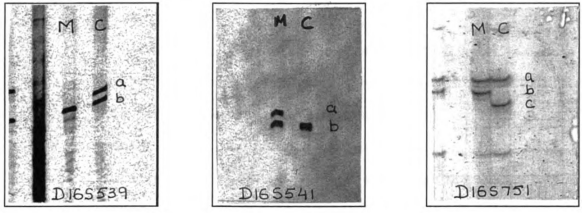



**Figure 3.6 (Family F).**

EDF showing PCR microsatellite alleles segregation in the proband (C) and mother (M) for loci D16S539, D16S541 and D16S751 located on chromosome 16. For D16S539 alleles were M(b); C(a,b); D16S541, M(a,b); C(b); D16S751, M(a,b) C(a,c).

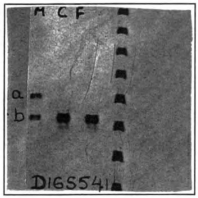
**Results:**

In this case the father was not available to investigate UPD 16. The markers D16S539 and D16S751 were informative but D16S541 was noninformative. The mother was homozygous for D16S539 locus and heterozygous for D16S751 locus located on chromosome 16. The child's DNA contained two alleles, one same as the mother and other presumably from the father, thus ruling out maternal UPD 16.

**Case: 7 G**

In this case, the mother was 19 years old G2 P1 A0 who delivered spontaneously a premature baby boy at 32 weeks of pregnancy. The mother was a smoker with no history of alcohol or drug intake during this pregnancy. She had a prior history of pre-term delivery at 33 weeks in which the infant died at 6 weeks from necrotizing enterocolitis. No dysmorphic features or congenital anomalies were detected at the time of birth. The infant was premature but appropriate for his gestational age. His weight was at the 67th percentile at the time of birth. The infant was kept in NICU for 29 days and treated for respiratory distress syndrome, hyperbilirubinemia, bradycardia and apnea. He was discharged to be followed up by his pediatrician.

**Family G**



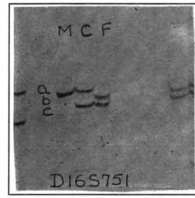




**Figure 3.7 (Family G).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for D16S541 and D16S751 loci located on chromosome 16. For D16S541 alleles were M (a); C(a,c); F(b,c); D16S751, M(a,b); C(b); F(b)

**Results:**

In this case, marker D16S541 was partially informative and could rule out maternal UPD 16. The mother was homozygous and the father was heterozygous for D16S751 locus. The child's DNA contained two alleles, one from the mother and other from the father, thus ruling out UPD 16 from either parents.

**Case: 8 H**

In this case, the mother was 26 years old G2 P1 A0 who was delivered by emergency caesarean section for antepartum hemorrhage and breech presentation. She delivered a premature baby boy at 25 weeks of pregnancy who was appropriate for gestational age with no dysmorphic features. However, the baby had immature male external genitalia and undescended testis. The mother was a nonsmoker. She had a previous history of still birth at 33 weeks but had no history of diabetes or hypertension during the pregnancy. The mother had a congenital abnormality, a bicornuate uterus and a single kidney. The infant was shifted to NICU and treated for respiratory distress syndrome, anemia, hyperbilirubinemia, retinopathy and renal insufficiency. The infant was born with ductus arteriosus diagnosed on echocardiogram; and intraventricular hemorrhage grade 1 diagnosed on cranial ultrasound. He was discharged after 90 days to be followed up by chest clinic, physical therapist, eye specialist and developmental assessment clinic.

**Family H**

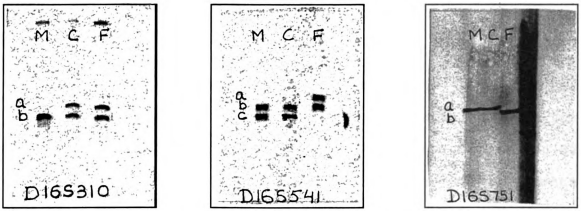



**Figure 3.8 (Family H).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for D16S310, D16S541 and D16S751 loci located on chromosome 16. For D16S310 alleles were M (b); C(a,b); F(a,b); D16S541, M(b,c); C(b,c); F(a,b); D16S751, M(a); C(a); F(b).

**Results:**

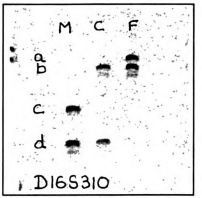
In this case, none of the three markers were fully informative on their own. The D16S310 pattern could be due to paternal UPD, whereas D16S541 and D16S751 patterns could be due to maternal UPD. Thus D16S541 and D16S751 rule out paternal UPD and D16S310 rules out maternal UPD for chromosome 16 in this case.

**Case: 9 I**

In this case, the mother was 28 years old G12 P6 A6 who delivered a premature, small for gestational age baby boy by caesarean section at 32 weeks of pregnancy. Poor fetal growth, placenta previa and oligohydramnios were diagnosed before delivery. The mother was a smoker and used marijuana and alcohol during pregnancy. She had a history of spontaneous abortion during first trimester, still birth in second trimester, and a twin pregnancy. There was no history of diabetes and hypertension. The infant was born with reducible umbilical hernia and undescended testis without any other dysmorphic features and congenital abnormalities. The infant developed pneumonia, hyperbilirubinemia, anemia and cardiac murmur for which he was admitted and treated in NICU. He was discharged after 36 days to be followed by his pediatrician and developmental assessment clinic later on.

For DNA analysis, the microsatellite markers D16S310 and D16S541 were analyzed to determine UPD 16 in the infant.

**Family I**



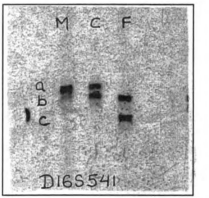




**Figure 3.9 (Family I).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for D16S310 and D16S541 loci located on chromosome 16. For D16S310 alleles were M (c,d); C(b,d); F(a,b); D16S541, M(a); C(a,b); F(b,c).

**Results:**

In this case, markers D16S310 and D16S541 were investigated and were found to be fully informative to rule out UPD 16. The parents were heterozygous for D16S310. The child inherited two alleles, one from the mother and one from the father, thus ruling out UPD 16. The mother was homozygous for D16S541 and the father was heterozygous for D16S541. The child inherited two alleles, one from the mother and one from the father, thus ruling out UPD 16.

**Case: 10 J**

In this case, the mother was 29 years old G1 P0 A0 who delivered premature baby girl by vaginal delivery at 33 weeks of pregnancy. The mother was not a smoker and did not take any drug or alcohol during this pregnancy. The pregnancy was uncomplicated with no history of diabetes, hypertension or vaginal bleeding. The infant was appropriate for gestational age and his weight was at the 90th percentile at the time of birth. No dysmorphic features or congenital anomalies were detected at the time of birth. The infant developed respiratory distress and hyperbilirubinemia and was admitted to NICU. He was treated and discharged after 13 days to be followed by his primary care physician.

To determine UPD 16 in the infant, microsatellite markers D16S310 and D16S541 were analyzed.

**Family J**

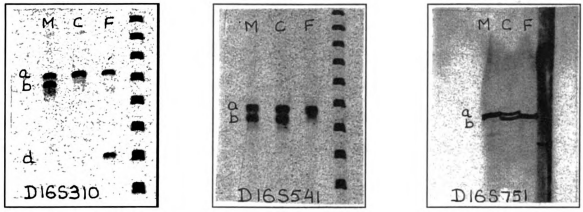



**Figure 3.10 (Family J).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for loci D16S310, D16S541 and D16S751 located on chromosome 16. For D16S310 alleles were M (a,b); C(a); F(a,d); D16S541, M(a,b); C(a,b); F(a); D16S751, M(a); C(a,b); F(b).

**Results:**

In this case, markers D16S310 and D16S541 were investigated to rule out UPD 16. The markers D16S310 was partially informative and could rule out maternal UPD as the child had not inherited two maternal alleles. Both parents were homozygous for the locus D16S751. The child's DNA contained two alleles, one from the mother and one from the father, thus ruling out UPD 16 from either parents.

**Case: 11 K**

In this case, the mother was 29 years old G1 P0 A0 who went into pre-term labor and delivered a premature baby boy by vaginal delivery at 29 weeks of pregnancy. The mother was a nonsmoker with no history of diabetes or hypertension. Earlier in this pregnancy, she had a premature onset of labor due to incompetent cervical os for which cervical cerclage was performed. The baby was kept in NICU and treated for pulmonary insufficiency, hyperbilirubinemia, anemia, candida dermatitis and cardiac murmur. No dysmorphic features or congenital anomalies were detected at the time of birth except right lacrimal duct stenosis. The baby stayed in NICU for 28 days and was discharged to be followed up by his primary care physician, ophthalmologist and developmental assessment clinic.

**Family K**

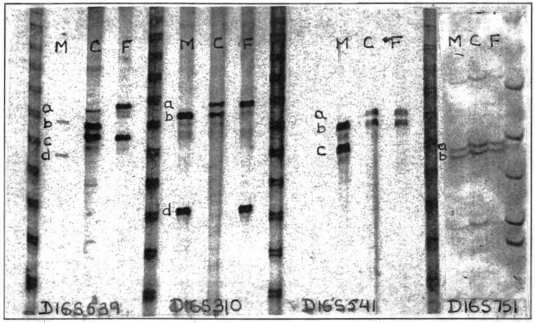



**Figure 3.11 (Family K).**

EDF showing PCR microsatellite alleles segregation in the proband (C), mother (M) and father (F) for D16S310, D16S539, D16S541 and D16S751 loci located on chromosome 16. For D16S539 alleles were M(b,d); C(b,c); F(a,c); D16S310 M(b,d); C(a,b); F(a,d); D16S541, M(b,c); C(a,b); F(a,b); D16S751, M(a,b); C(a,b); F(a,b).

**Results:**

In this case, the markers D16S541 and D16S751 are noninformative whereas D16S310 and D16S539 are informative. Both parents were heterozygous for these loci with different alleles. The child inherited two alleles, one from the mother and one from the father, thus ruling out UPD 16 from either parents.

**SUMMARIZED DNA ANALYSIS RESULTS FOR ALL FAMILIES**

The results of DNA analysis and the parental origin of chromosome 16 for each family is summarized in Table 3-1.

**Table 3-1. DNA Analysis Results: Parental Origin of Chromosome 16 in Premature and IUGR Infants**

| No. | CASE | MARKERS | MOTHER | INFANT /CHILD | FATHER | ORIGIN OF CHROMOSOME 16 IN THE CHILD | INTERPRETATION OF UNIPARENTAL DISOMY |
|---|---|---|---|---|---|---|---|
| 1 | A | D16S310 | a | a | father N/A | undetermined | markers noninformative |
| | | D16S541 | a,c | b,c | father N/A | maternal/paternal | UPD ruled out |
| | | D16S751 | a | a | father N/A | undetermined | markers noninformative |
| 2 | B | D16S310 | b,d | a,d | a | maternal/paternal | UPD ruled out |
| | | D16S541 | a,b | b,c | c | maternal/paternal | UPD ruled out |
| | | D16S749 | a,b | a | a | undetermined | markers noninformative |
| | | D16S539 | a,b | a | a,b | undetermined | markers noninformative |
| 3 | C | D16S310 | | | | maternal/paternal | UPD ruled out |
| | | D16S541 | a,b | a,b | a,c | undetermined | markers noninformative |
| | | D16S749 | b | a,b | a | maternal/paternal | UPD ruled out |
| 4 | D | D16S310 | a,b | a,b | a,c | undetermined | markers noninformative |
| | | D16S541 | a,c | a | a | undetermined | markers noninformative |
| | | D16S749 | a | a | a | undetermined | markers noninformative |
| | | D16S751 | a,b | a,b | b | undetermined | markers noninformative |
| 5 | E | D16S541 | a,b | a,b | b | undetermined | markers noninformative |
| | | D16S751 | b,c | a,b | a,c | maternal/paternal | UPD ruled out |
| 6 | F | D16S539 | b | a,b | father N/A | maternal/paternal | UPD ruled out |
| | | D16S541 | a,b | b | father N/A | undetermined | markers noninformative |
| | | D16S751 | a,b | a,c | father N/A | maternal/paternal | UPD ruled out |
| 7 | G | D16S541 | a,b | b | b | undetermined | markers noninformative |
| | | D16S751 | a | a,c | b,c | maternal/paternal | UPD ruled out |
| 8 | H | D16S310 | b | a,b | a,b | undetermined | markers noninformative |
| | | D16S541 | b,c | b,c | a,b | undetermined | markers noninformative |
| | | D16S751 | a | a | b | undetermined | markers noninformative |
| 9 | I | D16S310 | c,d | b,d | a,b | maternal/paternal | UPD ruled out |
| | | D16S541 | a | a,b | b,c | maternal/paternal | UPD ruled out |
| 10 | J | D16S310 | a,b | a | a,d | undetermined | markers noninformative |
| | | D16S541 | a,b | a,b | a | undetermined | markers noninformative |
| | | D16S751 | a | a,b | b | maternal/paternal | UPD ruled out |
| 11 | K | D16S539 | b,d | b,c | a,c | maternal/paternal | UPD ruled out |
| | | D16S310 | b,d | a,b | a,d | maternal/paternal | UPD ruled out |
| | | D16S541 | b,c | a,b | a,b | undetermined | markers noninformative |
| | | D16S751 | a,b | a,b | a,b | undetermined | markers noninformative |

# CHAPTER 4

# DISCUSSION AND SUGGESTED RESEARCH

## DISCUSSION

The work reported in this thesis is a part of the pilot project undertaken by the Department of Pediatrics and Human Development at Michigan State University. The main objectives of this pilot project were to develop a standard procedure for DNA analysis using microsatellites as genetic markers; and to investigate UPD 16 in premature and/or IUGR infants from the Neonatal Intensive Care Unit (NICU).

Chromosome 16 was selected to investigate uniparental inheritance because previous studies [14,46] reported that chromosome 16 accounts for about 30% of the cases of trisomic conceptions which results in spontaneous abortions in first trimester of pregnancy. These figures were based on previous cytogenetic studies [46] on spontaneous abortions which had shown that 50% of first trimester abortions resulted from chromosomal aberrations (anomalies) in the conceptus. More than half of these abortions resulted from autosomal trisomies in which trisomy 16 accounted for about 30% of all trisomies. Most trisomies are lethal and so far no live births with trisomy 16 have been reported. It is only when a disomic cell arises through loss of the extra chromosome that a viable cell line will be produced.

Many previous studies [e.g., 14,22,23] on UPD 16 were associated with trisomic placenta or confined placental mosaicism (CPM) which resulted in fetal losses or live births with IUGR. The mechanism suggested for UPD 16 in these reported cases was loss of one copy of chromosome 16 due to mitotic nondisjunction after fertilization (trisomic rescue). The trisomy 16 zygote with the loss of one copy of chromosome 16 could result in either of the following three outcomes: disomic line with biparental disomy; fetal UPD as a result of early nondisjunction and random loss of trisomic line; or trisomy 16 mosaicism confined to placenta only. CPM occurs in about 1-2% of chorionic villus sampling (CVS) with trisomy 16 being most common. Based on these above mentioned probable outcomes, it was postulated that 33% of trisomy 16 would result in uniparental inheritance [4,29]. Since there is a 33% probability that UPD 16 could occur in trisomy 16 conceptions, premature and IUGR infants were selected from NICU to investigate UPD 16 in which no other chromosomal abnormalities were detected.

At present, the incidence of UPD in the general population, or in any specific group or population, has not been determined because no sequential studies have been made. However, a study [4] based on frequency of aneuploidy observed in sperms and oocytes suggested that the incidence of UPD could be as high as 16.5 per ten thousand conceptions. So far, a number of cases of UPD for different chromosomes have been reported in humans (Table 1-1, page 8). Some of these cases of

UPD were associated with phenotypes whereas others were not associated with any phenotypic effects.

There were two primary reasons for selecting the NICU as the main source of samples for this study. First, most of the premature and IUGR infants would be admitted to NICU for some type of postnatal problems and their parents could be contacted and requested to participate in this study. Second, the NICU would be the only place that could provide a number of patients to conduct this study. If UPD of chromosome 16 were to be found a significant factor associated with the cases of prematurity or/and IUGR, the procedure for detection of UPD could have diagnostic value in prenatal cases of CPM.

To accomplish this goal, PCR-based genetic analysis was performed to investigate parental origin of chromosome 16 in premature and IUGR infants admitted to NICU at the Edward Sparrow Hospital, Lansing, Michigan. A sample size of 30 cases (infants and their biological parents) was considered adequate to determine if UPD 16 was a significant cause of prematurity /IUGR. The proposed figure of 30 samples was not based on any statistical approach but was simply a plausible assumption of a number that could be attained within a reasonable time frame and available resources. However, only 11 sets of samples could be obtained for this study. Though the actual sample size was too limited to give statistically significant results, it provided a reasonable number of cases to initiate testing, develop and validate the procedures.

To begin this study, a base procedure was developed so that it could be subsequently applied in this study to evaluate polymorphic DNA markers (microsatellite) in order to determine parental origin of chromosome 16 in premature and IUGR infants. The details of the development and application of the base procedure are explained in Chapter 2 (page 32-34).

The previous studies [e.g., 9,14,22] used mostly DNA polymorphism such as CA repeats (dinucleotide) or variable number of tandem repeats (VNTR) to investigate UPD. These were analyzed either by Southern blotting or by PCR. The probes and amplification primers used were labelled with radioactive isotopes (such as $P^{32}$) to allow visualization of results by autoradiography following polyacrylamide gel electrophoresis.

In this study, two modifications were made to the procedures used in the above described studies: 1) Instead of using CA repeats (dinucleotide) or VNTR polymorphisms, different microsatellite markers (tetranucleotide repeats) for chromosome 16 were investigated. 2) Labelling of primers with radioactive isotopes was avoided by staining of PCR products with silver stain after electrophoresis.

Another important aspect of this study was that, as compared to use of CA repeats, use of tetranucleotide repeats which were highly polymorphic yielded discrete PCR product with better resolution (Appendix H). This resulted in use of relatively fewer markers to exclude UPD 16 in the families investigated.

**CONCLUSIONS**

- The microsatellites (tetranucleotide repeats) yielded discrete PCR products with better resolution as compared to dinucleotide repeats.
- Primers used were not labelled with radioactive isotopes for visualization of results by autoradiography. Staining with silver stain and development of eletrophoresis developing film was used as a method for studying allele segregation in the family.
- Uniparental disomy for chromosome 16 was excluded in 11 cases in this study by PCR based DNA anlysis using microsatellites as a genetic marker. This suggests that UPD 16 may not be the major cause of prematurity and intra growth retardation in infants.

**SUGGESTED RESEARCH**

The results of this study indicate that tetranucleotide repeats yield better results than dinucleotide repeats in PCR based genetic analysis. Based on this finding, it is suggested that tetranucleotide polymorphism should be used for investigating uniparental inheritance in premature and IUGR infants. In addition to chromosome 16, other chromosomes (i.e., chromosomes with known imprinted genes, or chromosomes involved in cases of fetal losses due to trisomy or CPM) should also be investigated to determine UPD in premature and IUGR infants in which other chromosomal abnormalities were not detected.

## REFERENCES


1. Emery, A.E.H., and Mueller, R.F. **Elements of Medical Genetics**. 8th Edition, Churchill Livingstone, 1992.

2. Thompson, M.W., McInnes, R.R and Willard, H.F. **"Genetics in Medicine"**. W.B. Saunders Company, 5the Edition, 1991.

3. Engel, E. **"A New Genetic Concept: Uniparental Disomy and its Potential Effects, Isodisomy"**. American Journal of Medical Genetics, 6: 137-143, 1980.

4. Engel, E. and DeLozier-Blanchet, C. Dawn. **"Uniparental Disomy, Isodisomy, and Imprinting: Probable Effects in Man and Strategies for their Detection"**. American Journal of Human Genetics, 40: 432-439, 1991.

5. Engel, E. **"Uniparental Disomy Revisited: The First Twelve Years"**. American Journal of Medical Genetics, Wiley-Liss, Inc., 46: 670-674, 1993.

6. Wilkins-Haug, Louise. **"The Emerging Genetic Theories of Unstable DNA, Uniparental Disomy, and Imprinting"**. Current Science, pp, 179-185. ISSN 1040-872X, 1993.

7. Cattanach, B.M., and Kirk, M. **"Differential Activity of Maternally and Paternally Derived Chromosome Regions in Mice"**. Nature, London, 1985.

8. Searly, A.G., Beechy, C.V. **"Mouse Chromosome"**. Genetic Research, 56: 237-244, 1985.

9. Spence, J.E., Perciaccante, R.G., Greig, G.M., Willard, H.F., Ledbetter, D.H., Hejtmancik, J.F., Pollack, M.S., O'Brien, W.E., and Beaudet A.L. **"Uniparental Disomy as a Mechanism for Human Genetic Disease"**. American Journal of Human Genetics, 42: 217-226, 1988.

10. Voss, R., Ben-Simon E., Avital, A., Godfrey, S., Zlotogora, J., Dagan, J., and Tikochinski, Y. **"Isodisomy of Chromosome 7 in a Patient With Cystic Fibrosis: Could Uniparental Disomy be Common in Humans?"**. American Journal of Human Genetics, 45: 373-380, 1989.

11. Grundy, P., Telzerow, P., Paterson, M.C., Haber, D., Berman, B., Li, F., and Garber, J. **"Chromosome 11 Uniparental Isodisomy Predisposing to Embropnal Neoplasms"**. Lancet, 338: 1079-1080, 1991.

12. Nicholls R.D., Knoll J.M.H., Butler M.G., Karam S., and Lalande M. **"Genetic Imprinting Suggested by Maternal Heterodisomy in Non-Deletion Prader-Willi Syndrome"**. Nature 324:281-285, 1989.

13. Cassidy S.B., Lai, L-W., Erickson, R.P., Magnuson, L., Thomas, E., Gendron R., and Herman, J. **"Trisomy 15 with a Loss of Paternal 15 as a Cause of Prader-Willi Syndrome Due to Maternal Disomy".** American Journal of Human Genetics, 51: 701-708, 1992.

14. Kalousek, D.K., Langlois, S., Yam, I., Wilson, D.R., Howard- Peebles, P.N., Johnson, M.P., and Giorgiutti. **"Uniparental Disomy for Chromosome 16 in Humans".** American Journal of Human Genetics, 52: 8-16, 1993.

15. Friedmann, T. **"Molecular Genetic Medicine".** Chapter: Genomic Imprinting in Humans, pp 37-77. Academic Press, 1994.

16 Wang, J-CC, et al. **"Uniparental Heterodisomy for Chromosome 14 in a Phenotypically Abnormal Familial Balanced 13/14 Robertsonian Translocation Carrier".** American Journal of Human Genetics, 48: 1069-1074, 1991.

17. Peterson, M.D. **"Uniparental Isodisomy due to Duplication of Chromosome 21 Occurring in Somatic Cells Monosomic for Chromosome 21".** Genomics 13, 269-274, Academic Press, Inc., 1992.

18. Henry, I., et al. **"Somatic Mosaicism for Partial Paternal Isodisomy in Beckwith-Wiedemann Syndrome: A Postfertilization Event".** European Journal of Human Genetics, 1: 19-29, 1993.

19. Antonarakis, S.E., Blouin, J.L., Maher, J., Avramopoulos, D., Thomas, G., and Talbot, C.C. Jr. **"Maternal Uniparental Disomy for Human Chromosome 14, Due to Loss of a Chromosome 14 From Somatic Cells with t(13;14 Trisomy 14".** American Journal of Human Genetics, 52: 1145-1152, 1993.

20. Kalousek, D.K. **"Current Topic: Confined Placental Mosaicism and Intrauterine Fetal Development".** Placenta, 15: 219-230, 1994.

21. Garber, A., Carlson, D., Schreck, R. et al. **"Parental Diagnosis and Dysmorphic Findings in Mosaic Trisomy 16".** Parental Diagnosis, Vol 14:257-266, John Wiley & Sons, Ltd. 1994.

22. Vaughan, J., Ali, Z., et al. **"Human Maternal Uniparental Disomy for Chromosome 16 and Fetal Development".** Parental Diagnosis, Vol 14: 751-756. John Wiley & Sons, Ltd. 1994.

23. Kalousek, D.K., and Barrett, I. **"Genomic Imprinting Related to Prenatal Diagnosis".** Parental Diagnosis, Vol 14: 1191-1201. John Wiley & Sons, Ltd. 1994.

24. Handerson, D.J., Sherman, L.S., Loughna, S.C., Bennett, P.R., and Moore, G.E. **"Early Embryonic Failure Associated with Uniparental Disomy for Human Chromosome 21"**. Human Molecular Genetics, Vol 3, No. 8, 1373-1376, 1994.

25. Reik, W. **"Genomic Imprinting: A Possible Mechanism for the Parental Origin Effect in Huntington's Chorea"**. Journal of Medical Genetics, 25:805-808, 1988.

26. Kotzot, D., Schmitt, S., Bernasconi, F. et al. **"Uniparental Disomy 7 in Silver - Russell Syndrome and Primordial Growth Retardation"**. Human Molecular Genetics, Vol 4, No. 4, 583-587, 1995.

27. Schinzel, A. **"Genomic Imprinting: Consequences of Uniparental Disomy for Human Disease"**. American Journal of Medical Genetics, 64:683-684, Wiley-Liss, Inc. 1993.

28. Wilson, G., Hall, J.G., and Cruz, F. **"Genomic Imprinting: Summary of an NICHD Conference"**. American Journal of Medical Genetics, Wiley-Liss, Inc., 46: 675-680, 1993.

29. Hall, J.G. **"Genomic Imprinting: Review and Relevance to Human Diseases"**. American Journal of Human Genetics, 46: 857-873, 1990.

30. Nicholls, R.D. **"Genomic Imprinting and Uniparental Disomy in Angelman and Prader-Willi Syndromes: A Review"**. American Journal of Medical Genetics, 46: 16-25, 1993.

31. Vivaud, D., et al. **"Father to Son Transmission of Hemophilia A due to UPD"**. American Journal of Human Genetics, Vol. 45 (suppl): A-226, 1989.

32. Strachan, T. **"The Human Genome"**. Medical Perspective Series, Bios Scientific Publishers.

33. Fowler, J.C.S., Burgoyne, L.A., Scott, A.C., Harding, H.W.J. **"Repetitive Deoxyribonucleic Acid (DNA) and Human Genome Variation - A Concise review Relevant to Forensic Biology"**. Journal of Forensic Science JFSCA, Vol 33, No. 5, pp 1111-1126, Sept 1988.

34. Tautz, D. **"Notes on the Definition and Nomenclature of Tandemly Repetitive DNA Sequences"**. DNA Fingerprinting State of the Science, Birk Hauser Verlag Basel, Switzerland, 1993.

35. Epplen, C., Melmer, G., et al. **"On the Essence of 'Meaningless' Simple Repetitive DNA in Eukaryote Genomes"**. DNA Fingerprinting State of the Science, Birk Hauser Verlag Basel, Switzerland, 1993.

36. Beckmann, J.S. and Weber, J.L. **"Survey of Human and Rat Microsatellites"**. Genomics 12, 627-631, Academic Press, Inc., 1992.

37. Easteal, S., McLeod, N., and Reed, K. **"DNA Profiling - Principles, Pitfalls and Potential"**. Harwood Academic Publishers, 1993.

38. Richards, B., Skoletsky, J., et al. **"Multiplex PCR Amplification from the CFTR Gene Using DNA Prepared from Buccal Brushes/Swabs"**. Human Molecular Genetics, Vol 2, No. 2, 159-163, Oxford University Press, 1993.

39. Cutting, G.R. **"Rapid Preparation of Genomic DNA from Dried Blood and Saliva Spots for Polymerase Chain Reaction"**. Human Mutation, 1:260-261, Wiley-Liss, Inc. 1992.

40. Lench, N., Stainer, P., and Williamson, R. **"Simple Non-Invasive Method to Obtain DNA for Gene Analysis"**. Lancet, 1:1356-1358, 1988.

41. Davis, L.G., Dibner, M.D., and Batty, J.F. **"Basic Methods in Molecular Biology"**. Elsevier, New York.

42. Maniatis, T., Fritsch, E.F., and Sambrook, J. **"Molecular Cloning: A Laboratory Manual"**. Cold Spring Harbor Laboratory, Cold Spring, New York, 1982.

43. Bio-Rad Laboratories, Inc. **"Sequi-Gen"**. Nucleic Acid Sequencing Cell Instruction Manual. Hercules, CA 94547.

44. Life Technologies, Inc. **"Model SA Adjustable Sequencing Gel Electrophoresis System"**. Instruction Manual, CAT Series 31096.

45 Ledbetter, D.H., and Engel, E. **"Uniparental Disomy in Humans: Development of an Imprinting Map and Its Implications for Prenatal Diagnosis"**. Human Molecular Genetics, Vol 4, Review 1757-1764, 1995.

# APPENDICES

## Appendix A

5X TBE solution:

| | |
|---|---|
| Tris base | 54 gm |
| Boric acid | 27.5 gm |
| EDTA 0.5 mM | 20 ml |

[Adjust volume to 1000 ml with dd water]

## Appendix B

0.5X TBE solution:

| | |
|---|---|
| 5XTBE | 100 ml |
| DD water | 900 ml |

## Appendix C

1X TBE solution:

| | |
|---|---|
| 5XTBE | 200 ml |
| DD water | 800 ml |

## Appendix D

The loading buffer for agarose gel electrophoresis:

0.25% of bromophenol blue
0.25% of xylene cyanol FF
0.40% of w/v sucrose in water

## Appendix E

40% polyacrylamide solution:

| | |
|---|---|
| Polyacrylamide (DNA sequencing grade) | 190 gm |
| N,N-methylbisacrylamide | 10 gm |
| Dd $H_2O$ | 300 ml |

[The mixture was heated to 37°C to dissolve all chemicals and dd $H_2O$ was added to adjust volume to 500 ml. The solution was filtered through nitrocellulose filter (e.g Nalge 0.45 micron pore size) and stored in dark bottles at 4°C].

## Appendix F

The loading buffer for polyacrylamide gel electrophoresis:

| | |
|---|---|
| Formamide | 10 ml |
| FF Xylene cyanol | 10 mg |
| Bromphenol blue | 10 mg |
| 0.5 M EDTA pH 8.0 | 200 ml |

## Appendix G

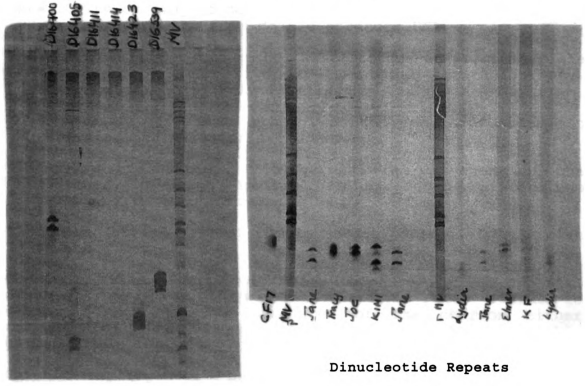
Bind silane mixture:

| | |
|---|---|
| Ethanol 95% | 1 ml |
| Acetic acid | 5 ul |
| Bind silane | 3 ul |

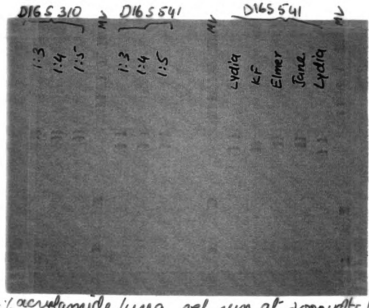
**Appendix H**

Separation of PCR Based Microsatellites Alleles on 6% Polyacrylamide Gel by Electrophoresis

**(COMPARISON OF RESOLUTION BETWEEN DINUCLEOTIDE AND TETRANUCLEOTIDE REPEATS)**




**Dinucleotide Repeats**




**Tetranucleotide Repeats**

## GLOSSARY

**Allele.** Alternative forms of a gene found at the same locus on homologous chromosomes.

**Anneal.** The pairing of complementary DNA or RNA sequences via hydrogen bonding, to form double-stranded DNA.

**Aneuploid.** A chromosome number which is not an exact multiple of the haploid number, i.e. 2N-1 or 2N+1 Autoradiography. Detection of radioactively labelled molecules on X-ray film.

**Autosome.** Any chromosome other than the sex chromosomes. In man there are 22 pairs of autosomes.

**Balanced Translocation.** A structural rearrangement of the chromosomes in which material is exchanged between two different pairs of chromosomes.

**Base Pair (bp).** A pair of complementary bases in DNA (A with T, G with C).

**Chorionic Villus Sampling (CVS).** A procedure used for prenatal diagnosis at 8 to 10 weeks gestation. Fetal tissue is withdrawn from the villous area of the chorion either transcervically or transabdominally under ultrsonographic guidance.

**Chronic Myelogenous Leukemia (CML).** See philadelphia chromosome.

**Confined Placental Mosaicism (CPM).** Presence of two or more cell lines confined to the placenta only.

**Congenital.** Any abnormality, whether genetic or not, which is present at birth.

**Cytogenetics.** A branch of genetics concerned with the study of chromosomes and their abnormalities.

**Deletion.** A type of aberration in which there is a loss of a part of a chromosome.

**Denaturation.** Conversion of DNA from the double-stranded to the single-stranded state usually accomplished by heating to destroy chemical bonds involved in base pairing.

**DNA (Deoxyribonucleic Acid).** The nucleic acid of the chromosome in which genetic information is coded.

**Dysmorphism.** Morphological developmental abnormalities, as seen in many syndromes of genetic or environmental origin.

**EDF.** Electrophoresis duplicating films.

**Electrophoresis.** The technique of separating charged molecules in a matrix to which is applied an electrical field.

> **Agarose Gel Electrophoresis.** Electrophoresis through a matrix composed of agar used to separate larger molecules of DNA or RNA molecules ranging from 100 to 20,000 nucleotides.
>
> **Polyacrylamide Gel Electrophoresis.** Electrophoresis through a matrix composed of synthetic polymer used to separate proteins small DNA, or RNA molecules up to 1000 nucleotides.

**FISH** (Fluorescence In Situ Hybridization). Molecular hybridization of a fluorescent labelled DNA probe to the metaphase chromosomes spread on a microscope slide.

**Flanking DNA.** Nucleotide sequence adjacent to the region being considered.

**Gamete.** A germ cell containing a haploid number of chromosomes.

**Genetic Marker.** A locus that has readily classifiable alleles and can be used genetic studies. It may be a gene or restriction enzyme site, or any characteristic of DNA that allows different of a locus to be distinguished from each other and followed through families.

**Genomic Imprinting.** Differing manifestations of genetic disorders dependent on the sex of the transmitting parent.

**Heterozygote (Heterozygous).** An individual who possesses two different alleles at one particular locus on a pair of homologous chromosomes.

**Homologous Chromosomes.** Chromosomes which pair during meiosis and contain identical loci.

**Homozygote (Homozygous).** An individual who possesses two identical alleles at one particular locus on a pair of homologous chromosome.

**Imprinting.** The phenomenon of a gene or region of a chromosome being more likely to be expressed when inherited from one parent than the other.

**Inversion.** A type of chromosomal aberration in which part of chromosome is inverted.

**IUGR** (Intra Uterine Growth Restriction). Newborns small for their gestational age.

**Karyotype.** The number, size and shape of the chromosomes of a somatic cell. A photomicrograph of an individual's chromosome arranged in a standard manner.

**Kilobase (kb).** 1000 base pairs.

**Locus.** The site of a gene on a chromosome.

**Marker.** A term for DNA polymorphism if shown to be linked to disease locus of interest, can be used in presymtomatic detection, determination of carrier status and prenatal diagnosis of inherited disease.

**Meiosis.** The type of cell division which occurs during gametogenesis and results in halving of the somatic number of chromosomes so that each gamete is haploid.

**Microdeletion.** A chromosomal deletion too small to be seen under the microscope.

**Microsatellite.** Polymorphic variation in DNA sequences due to variable number of tandem repeats of the dinucleotide or tetranucleotide repeats sequences.

**Minisatellite.** Polymorphic variation in DNA sequences due to variable number of tandem repeats of a short DNA sequences.

**Mitosis.** The type of cell division which occurs in somatic cells.

**Monosomy.** Loss of one member of a chromosome pair so that there is one less than the diploid number of chromosomes (2N-1).

**Mosaic.** An individual with abnormal genotypic or phenotypic variation from cell to cell within the same tissue or genotypic variation between tissue.

**Multiple Allele.** The existence of more than two alleles at a particular locus in a population.

**NICU.** Neonatal Intensive Care Unit.

**Non-Disjunction.** The failure of two members of a chromosome pair to separate during cell division so that both pass to the same daughter cell.

**Paracentric Inversion.** A chromosomal inversion that does not include centromere.

**Pericentric Inversion.** A chromosomal inversion that includes centromere.

**Phenotype.** The appearance (physical, biochemical and physiological) of an individual which results from the interaction of environment and his genotype.

**Philadelphia Chromosome ($Ph^1$).** The structurally abnormal chromosome 22 occurring in patients with chronic myelogenous leukemia (CML). It is a reciprocal translocation between the distal long arm of chromosome 22 and the distal long arm of chromosome 22.

**Polymerase Chain Reaction (PCR).** The repeated serial reaction involving the use of oligonucleotide primers and DNA polymerase which can be used to amplify a particular DNA sequence of interest.

**Polymorphic Information Content (PIC).** The amount of variation at a particular in the DNA.

**Polymorphism.** The occurrence in a population of two or more genetically determined forms in such frequencies that the rarest of them could not be maintained by mutation alone.

**Prenatal Diagnosis.** The use of tests during pregnancy to determine whether the unborn child is affected with particular disorder.

**Proband (= Index Case).** An affected individual through whom the family came to the attention of the investigator.

**Probe.** A labelled, single stranded DNA fragment which hybridizes with complementary sequences among DNA fragments.

**RBC.** Red blood cells.

**Recessive.** A trait which is expressed in individuals who are homozygous for a particular gene but not in those who are heterozygous for this gene.

**Reciprocal Translocation.** A structural rearrangement of the chromosomes in which material is exchanged between two different pairs of chromosomes.

**Recombination (= Cross Over).** The exchange of genetic material between chromosomes.

**Repetitive DNA.** DNA sequences of variable length which are repeated up to 100000 to over 100000 copies pergenome.

**RNA.** Ribonucleic Acid.

**Robertsonian Translocation.** A translocation between two acrocentric chromosomes by fusion at the centromere and loss of the short arms.

**Segregation.** The separation of alleles during meiosis so that each gamete contains only one member of each pair of alleles.

**Tandem Repeat.** Multiple copies of the same DNA sequence arranged in direct succession along a chromosome.

**Translocation.** The transfer of a segment of one chromosome to another non-homologous chromosome.

**Unbalanced Translocation.** A structural rearrangement of one homologue of two different chromosome pairs in which there will usually be partial monosomy of one the portions involved and partial trisomy of the other portion involved.

**Uniparental Disomy (UPD).** When an individual inherits both chromosomes from one parent.

**VNTR.** Variable Number of Tandem Repeats.


MICHIGAN STATE UNIV. LIBRARIES
31293015951779