UNIPARENTAL DISOMY STUDIES
BY PCR ANALYSIS

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Joshua Dain Paynich
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This thesis for honors recognition has been approved for the Department of Biology and Chemistry by

Dr. Marilyn Schendel

Dr. John Addis

Dr. Ron Stottlemeyer

March 24, 1996

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ABSTRACT

Uniparental disomy is a condition that results in many genetic anomalies. The condition involves an individual who has inherited both members of a homologous pair of chromosomes from one parent, while all other chromosome pairs come from biparental inheritance. That is, each parent contributes one chromosome to each homologous pair. The intent of the study was to find individuals with this condition. To test for uniparental disomy PCR technology was used to amplify regions of polymorphic microsatellite repeats. This PCR technique allows the experimenter to determine the origin of the child's chromosomes. Two individuals suspected of uniparental disomy were tested. The result was negative for uniparental disomy in both cases.

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INTRODUCTION AND LITERATURE REVIEW

Most often, the fusion of a human sperm and egg creates a diploid zygote. This zygote contains 22 homologous pairs of autosomal chromosomes and one pair of sex chromosomes, 46 chromosomes in all. Normally, biparental inheritance occurs during this fusion. Biparental inheritance means that a haploid sperm (containing 23 chromosomes) has fertilized a haploid egg (also containing 23 chromosomes). Thus, the sperm contributes one chromosome to each homologous pair and the egg contributes the other homologous chromosome.

An abberation of biparental inheritance, uniparental disomy, was discovered by Eric Engel in 1980 (James et al. 1994). In individuals with uniparental disomy, both chromosomes of a homologous pair are inherited from only one parent; the other parent simply provides no genetic contribution to that pair. For example, the sperm contributes 24 chromosomes while the egg contributes only 22 chromosomes. The resulting zygote contains the normal diploid number of chromosomes, but the chromosomes have not been obtained by biparental inheritance. Consequently, clinical problems such as severe mental retardation and

physical anomolies may arise.

Causes of Uniparental Disomy

How then can one inherit an abnormal number of chromosomes from one or both parents? Four mechanisms that may cause uniparental disomy have been proposed:

1) *Gamete complementation*: this mechanism involves the fusion of a nullisomic gamete with a gamete disomic for the same chromosome. That is a nullisomic gamete containing only 22 chromosomes (a certain autosomal chromosome is missing) fuses with a disomic gamete (containing 24 chromosomes, with the extra chromosome being the homologous equivalent of the autosomal chromosome missing in the nullisomic gamete).

2) *Monosomy duplication*: this mechanism results from a monosomic gamete (one containing a normal haploid number of chromosomes) fusing with a nullisomic gamete (one missing an autosomal

chromosome). After fusion, duplication of the chromosome in the monosomic gamete that has no homologous chromosome creates a diploid zygote; however, this newly completed homologous pair contains two chromosomes from one parent rather than one chromosome from each, as seen in biparental inheritance. The result is a zygote with uniparental iso-disomy (iso- infers that both chromosomes involved are identical). Not only are both chromosomes of the pair involved from the same parent, but they are identical rather than simply homologous.

3) *Trisomic rescue*: this mechanism occurs when a monosomic gamete fuses with a disomic gamete. The resulting zygote contains 47 chromosomes. Thus, it is aneuploid (containing a chromosomal triplet rather than the normal pair). In this case, the aneuploid zygote contains a homologous triplet rather than pair for the chromosome pair involved. Aneuploid may also refer to a cell missing a chromosome (45 chromosomes), but here anneuploid

refers to a total of 47 chromosomes. Although most aneuploid zygotes spontaneously abort before amniocentesis sampling is performed, the aneuploid zygote may be rescued by natural selection and restored to a diploid state (Uehara et al. 1992). The zygote restores the diploid state by correcting the aneuploidy. To do this the zygote rids itself of the extra chromosome. Elimination of the extra chromosome occurs randomly; thus, elimination may lead to uniparental disomy or biparental inheritance. Because there are three chromosomes (two from one parent and one from the other), uniparental disomy will result in 1/3 of the occurrences and biparental inheritance in 2/3 of them.

An extension of trisomic rescue arises when there is a postzygotic nondisjunction occuring during the development of the aneuploid embryo. Specifically, during mitotic division complications arise with the separation of the newly synthesized chromosomes. These complications lead to the uneven distribution of chromosomes. These zygotes develop a mosaic of cell

lines. Each line differs in its genetic content; some cell lines contain a normal complement of 46 chromosomes (where uniparental disomy is possible) while others maintain the original aneuploidy of 47 chromosomes (James et al. 1994).

4) A final mechanism creates a partial uniparental disomy. According to Antonarakis, this formation involves, "mitotic recombination in somatic cells that results in uniparental disomy for a portion of a chromosome." That is these individuals have uniparental disomy only for a portion of a chromosome due to translocation. This mechanism is implicated in certain instances of Beckwith-Wiedemann Syndrome (Antonarakis et al. 1993).

Anomalies Associated with Uniparental Disomy

Severe clinical anomalies are associated with individuals who have developed uniparental disomy. Usually these anomalies arise from two general sources:

complications caused by parental imprinting and the expression of recessive traits. The first source, imprinting, is differential expression of genes based on the parent of origin (James et al. 1994). Just one of the human chromosomes known to be affected by imprinting is chromosome 15. A region on chromosome 15 is chemically modified (imprinted) in both the sperm and egg during gamete formation. Maternal imprinting suppresses expression of the region of DNA on chromosome 15. Lack of maternal imprinting causes Angelman's syndrome. Paternal imprinting modifies the same region of chromosome 15 but with a different chemical. This paternal imprinting also suppresses expression, but lack of paternal imprinting leads to Prader-Willi syndrome. Therefore this region shows differential gene expression based on parent of origin. If the child inherits only maternally imprinted chromosome 15, Prader-Willi syndrome develops. Conversely, inheritance of only paternally imprinted chromosomes leads to Angelman's syndrome. Both syndromes are caused by the expression of one region of chromosome 15, but which syndrome depends on the parent of origin. Thus, uniparental disomy affects imprinting by causing the inheritance of only maternal (or

only paternal) chromosomes for the pair involved. Thus, it leads to congenital anomalies such as Angelman's syndrome or, more commonly, Prader-Willi syndrome.

For example, maternal uniparental disomy 15 produces a child that lacks a paternally imprinted chromosome 15. Consequently, Prader-Willi syndrome is not suppressed, and the child develops clinical symptoms of Prader-Willi syndrome.

Another way to develop Prader-Willi must be noted. Prader-Willi can be caused by a deletion of the imprinted region of the father. With this in mind one must consider the fact that maternal uniparental disomy only causes Prader-Willi syndrome in a limited number of all cases. According to Holm, 70% of all clinically typical Prader-Willi cases are caused by deletion of the proximal part of the paternal chromosome 15. Only 10-15% of all cases are caused by uniparental disomy. The rest result from some other cause, such as undetectable deletion (1993).

The second source by which uniparental disomy causes anomalies is the unmasking of recessive traits. Normally recessive traits are expressed only when two carriers of that trait produce a child; however, uniparental disomy

causes expression of a recessive trait when only one parent is recessive or only one parent is a carrier. In the first case, expression of a recessive trait results when a homologous pair, each containing a recessive allele, is passed to the child. Thus, if one parent displays a recessive trait, while the other parent has only dominate alleles, the child with uniparental disomy will express that recessive trait. This inheritance of a homologous pair is uniparental hetero-disomy, inheritance of a homologous pair from one parent. Examples of recessive traits unmasked this way are cystic fibrosis (gene on chromosome 7) (Antonarakis et al. 1993) and rod monochromacy (gene on chromosome 14) (Pentao et al. 1992).

The second case, where only one parent is a carrier for a recessive trait, adds another dimension to the uniparental disomy thesis. That dimension is uniparental iso-disomy. With uniparental iso-disomy both the chromosomes involved are identical. This inheritance results in the production of a child who is homozygous, recessive for a trait like cystic fibrosis on chromosome 7. The child has one parent who is heterozygous for the trait; the other parent is not even a carrier (Antonarakis et al. 1993). In this case

neither parent displays the recessive phenotype, but the child expressed the recessive phenotype.

Another example, illustrated by Pentao, of the unmasking of recessive traits is rod monochromacy. Rod monochromacy is characterized by a complete absence of retinal cones. Rod monochromacy may result from biparental inheritance of two recessive alleles, uniparental hetero-disomy, or uniparental iso-disomy. Through the two types of uniparental disomy only one parent need be associated with the condition. The genetic sequence for rod monochromacy lies on chromosome 4; so, any test for uniparental disomy would involve chromosome pair 4 (1992).

With rod monochromacy as well as cystic fibrosis, different mechanisms cause the same clinical result--expression of a recessive trait. The mechanism of biparental inheritance of two recessive alleles leads to phenotypic expression of that recessive trait. In addition, uniparental disomy may cause expression of a recessive trait when only one parent either carries or expresses the recessive trait.

Indications of uniparental disomy also exist at a microscopic level. Often a history of miscarriages will

compel a couple to have a karyotype produced. These karyotypes may uncover balanced translocations which are important to uniparental disomy research because they increase the probability of its occurrence. Especially important are balanced Robertsonian translocations. Robertsonian translocations involve the fusion of two chromosomes within the short arm region, that is end to end. Although these individuals contain a normal amount of chromosomal material, the fusion of two chromosomes leaves them with only 45 chromosomes. Because of this odd number of chromosomes, separation becomes complicated during meiosis. Complicated separation increases the probability of trisomic rescue. One in 500 newborns has a balanced Robertsonian translocation; these individuals have a high risk for uniparental disomy (Wells et al. 1991).

Reguardless of possible problems with meiotic distribution of chromosomes associated with Robertsonian translocations, these individuals may develop phenotypically normal. One phenotypically normal female who has a Robertsonian t(13q13q) shows no anomalies. This indicates that the individual has two chromosome 13s fused short arm to short arm into one chromosome. Another female with a

Robertsonian t(22q22q)--again two chromosome 22s fused short arm to short arm--shows no anomalies (Stallard et al. 1995). These patients support the assumption that there are no maternally imprinted regions on chromosome 13 because no phenotypic anomalies are associated with the uniparental disomy.

Uniparental disomy has been associated with many mental and physical anomalies as well as normal development. Because of its association with various anomalies, uniparental disomy has the potential to surface as a cause of many diseases. Consequently, prenatal diagnosis for uniparental disomy holds potential importance for fully informing parents of risks concerning their child.

Because of this potential, a summer research project intended to search for cases of uniparental disomy came about. This project took place at Shodair Children's Hospital under the direction of Linda Beischel. The intention of the project was to employ PCR technology using commercially marketed primers to compare parental genetic material to that of their offspring. The analysis of the information gained from this particular research will provide insight into the origin of the chromosomes from two

children. This insight is useful in determining which chromosomes are inherited from which parent; subsequently, the results will allow diagnosis of either biparental inheritance or uniparental disomy.

MATERIALS AND METHODS

Using PCR to test for uniparental disomy requires expensive reagents and labor. To be efficient we decided to increase our chances for success by looking for patients that display clinical symptoms associated with uniparental disomy. Thus, testing could be limited to those individuals with a high probability of the condition. This high probability is seen with Robertsonian translocations and Prader-Willi syndrome. First, an individual possessing a balanced Robertsonian t(13q;14q) translocation who also displays the delays in physical and mental development associated with uniparental disomy was chosen. The other subjects were chosen because they had Prader-Willi syndrome. These subjects did not show the characteristic deletion associated with 70% of Prader-Willi cases, but they did fit clinical description of Prader-Willi syndrome. The clinical description used to select subjects is based on symptoms adopted from "Prader-Willi Syndrome: Consensus Diagnostic Criteria" (Holm et al. 1993). Each symptom was classified as major or minor with respect to Prader-Willi and given a point value (one point per major and one-half point per minor). These points were then added up; a total of eight (or more with at least five from major symptoms) confirms existence of Prader-Willi syndrome.

By combining the diagnosis of Prader-Willi (based on

the above criteria) and the lack of characteristic Prader-Willi deletion, individuals could be classified into the 30% of Prader-Willi cases in which one third to one half of that 30% have maternal uniparental disomy.

Once likely candidates were identified, the families were contacted through a genetics counselor at Shodair Children's Hospital. Those who responded to the request had blood samples taken by local doctors. These samples were then sent to Shodair where Linda Beischel purified the DNA.

DNA Purification

The DNA used throughout the experiment was isolated from the blood samples mentioned above. The procedure for isolation uses the Purgene DNA Isolation Kit D-5000 and proceeds as follows:

--Obtain a specimen and assign a laboratory number and log sheet.

--Open a vacutainer tube in the laminar flow hood in pathology, observing all of the applicable precautions.

--Pour the sample into a 15 or 50 ml centrifuge tube and estimate the volume to nearest ml. This is "one volume."

--At the bench in the DNA lab area to the left of the sink, add a total of three volumes of "RBC lysis solution" to the sample. Use some of this volume to rinse out the vacutainer tube. Invert to mix the solution and incubate 10 minutes at room temperature.

--Centrifuge the solution for 10 minutes at 32000 RPM (program 3, IEC CENTRA, 2000G) pour off the

supernate into sink. Leave behind the visible white cell pellet.

--Vortex the tube vigorously to evenly resuspend the cell pellet.

--Add one volume "cell lysis solution" to resuspend the cells and pipette up and down to lyse.

--Add 0.005 ml RNase solution per 1 ml of the original volume to the cell lysate. Invert solution 25 times to mix and incubate at 37 degrees C in a water bath for 15 min.

--Cool the sample to room temperature. Add one-third volume "protein precipitation solution" to lysate. Vortex vigorously for 20 seconds. Centrifuge program 3.

--Prepare the newly labeled tube for the specimen. Pour into each new tube one volume of isopropanol (Fisher). Pour the supernate of the protein precipitation step into isopropanol, leaving the precipitated protein pellet behind to be discarded.

--Mix the supernate and the isopropanol by gently inverting 50 times. The white threads of DNA should form a visible clump. Use a sterile plastic inoculating loop to transfer the precipitated DNA into a 1.5 ml microfuge tube containing 1 ml of 70% ethanol in water.

--Microfuge 1 minute at 14000 RPM to pellet DNA. Pour off the supernate and drain, remove the drops of supernate with a sterile swab. Allow to air dry 10 minutes.

--Add one-tenth volume of "DNA hybridization solution." Mix to dissolve. Heat the solution to about 50 degrees C for maximum of one hour to speed dissolution of DNA.

--After DNA is dissolved, estimate its concentration (see Hoefer Fluorometer Procedure) and store the DNA at 4 degrees C.

Once extracted, specific loci (regions of DNA) that were amplified using the PCR process.

PCR Amplified Alleles

Polymerase chain reaction (PCR) utilizes thermocycling to replicate the natural process of DNA synthesis; however, this synthesis is limited to specific loci (alleles). First, PCR denatures the double helix by "melting" the helix at high temperature. Once denatured, primers anneal with the DNA as the temperature is lowered. As the temperature drops further, DNA polymerase synthesizes the loci "primed." Then the thermocycling repeats indefinitely and results in an amplified (replicated many times) DNA locus. Only the locus to which the primer has annealed is amplified.

The sizes of the loci amplified are important. Each locus is a polymorphic microsatellite repeat sequence. Roughly translated this phrase means each locus is the equivalent of an allele, many different conformations of this allele exist in the population, and the region contains many repeats of a short nucleotide sequence. Consider our alphabet as an analogy to the polymorphic microsatellite repeat sequence. DNA is a string of letters (nucleotides). The letters form words (loci). Last, the words form long sentences or chromosomes that define life. The loci are polymorphic. By analogy this means, one person may contain the locus HAT-HAT-HAT-HAT-HAT-HAT, while another person carries the locus HAT-HAT. Both loci are composed of the same repeating segment, but each has a different number of

repeats. The same primer starts the synthesis of both forms of the locus (because the sequences are the same), but synthesis stops sooner in the later locus because the number of repeats is shorter. Thus, the same locus will produce different length products in different individuals because of variation in the number of repeating units.

The above mentioned primers of polymorphic microsatellite repeat sequences can be obtained commercially. Primers for multiple loci on each of the 23 chromosome pairs can be purchased. These primers enable the experimenter to identify the parents' loci on each of the 23 pairs of chromosomes and test for their contribution to the child's genetic makeup. This comparison requires electrophoresis.

Gel Electrophoresis

First, simple gels were prepared from agarose and run in TAE buffer (0.04 M Tris, 0.020 M acetic acid, 0.001 M EDTA, pH 8.5). These gels ranged from 5-7 mm in thickness. The running times and voltages varied because the purpose was to test whether or not gene product was created during the PCR and to give an indication whether the product produced was within the expected size range. Once electrophoresed the gels were viewed by labeling the DNA with ethidium bromide combined with ultraviolet light

illumination. Ethidium bromide inserts into the DNA double helix and fluoresces in ultraviolet light. The gels were soaked in ethidium bromide of a concentration of 1 microgram/ml for about 20 min, then destained in distilled water.

Once it was determined that product of the expected size had been produced, a higher resolution gel was needed to compare the loci of parents and child. To gain the high degree of resolution needed to determine chromosomal inheritance, another type of gel had to be prepared. These gels were 2 mm thick and ran vertically. The composition of the gels allowed resolution of as little as a two-base pair difference in size. The gels were composed of 3 or 3.5% metaphor in TBE buffer (0.089 M Tris, 0.089 M boric acid, 0.002 M EDTA, pH 8). These vertical gels were run at 350 volts, 60 milliamps for 1.5 to 3 hours depending on when the dye ran off the gel.

In preparation for analysis by vertical "gel loading solution" (Perkin-Elmer) was added to the PCR product. This solution added density and dye to the product. Upon completion of electrophoresis, these vertical gels were also analyzed using ethidium bromide and ultraviolet light illumination. Once studied with ultraviolet light, Polaroid photographs were taken to preserve the results.

RESULTS

The original focus of this project was a systematic search for individuals with uniparental disomy; however, the direction of the project shifted toward optimizing the polymerase chain reaction (PCR) used to test for the disorder since PCR is used here to amplify polymorphic microsatellite repeat sequences. To read these amplified sequences, each primer must be optimized to an ideal band resolution. These sequences are then read as bands by gel electrophoresis.

Primer Optimization

Ideally, the PCR reaction should produce high resolution bands (one or two crisp bands at each locus). To improve band resolution, PCR parameters had to be adjusted. One of these parameters involves a variable number of cycles. Each cycle has three steps: denaturing, primer annealing, and elongation. First, the highest possible denaturing temperature (94 degrees C) is used. This temperature should be kept high to insure complete DNA

denaturing. The denaturing temperature is determined by the stability of the DNA polymerase used during the reaction. Thus, this step provides little or no help to improve resolution. The next step, primer annealing temperature, must be optimized to a high temperature (near 60 degrees C). This high temperature will insure that only the target sequence is primed. Any nonspecific primer binding is minimized due to unstable annealing at high temperature. The difference between nonspecific and specific primer binding becomes quite obvious as seen in Figure 1.

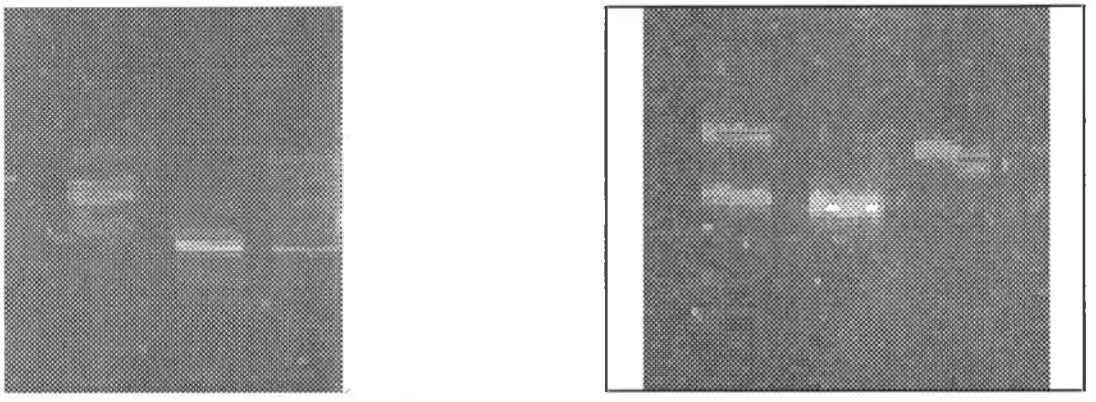


Figure 1. Comparison of primer annealed at 55 C (left) and 65 C (right).

The other important variable responsible for increasing band resolution is the number of cycles used during PCR amplification. A balance must be found between too few cycles (<20) and too many cycles (>35). Too few cycles will

not create enough product to be easily read by gel electrophoresis. Too many cycles, on the other hand will increase the probability of mistakes: since mistakes create extra bands, lower band resolution results. The increase in resolution achieved by simply lowering the number of cycles can be quite significant as seen in Figure 2.

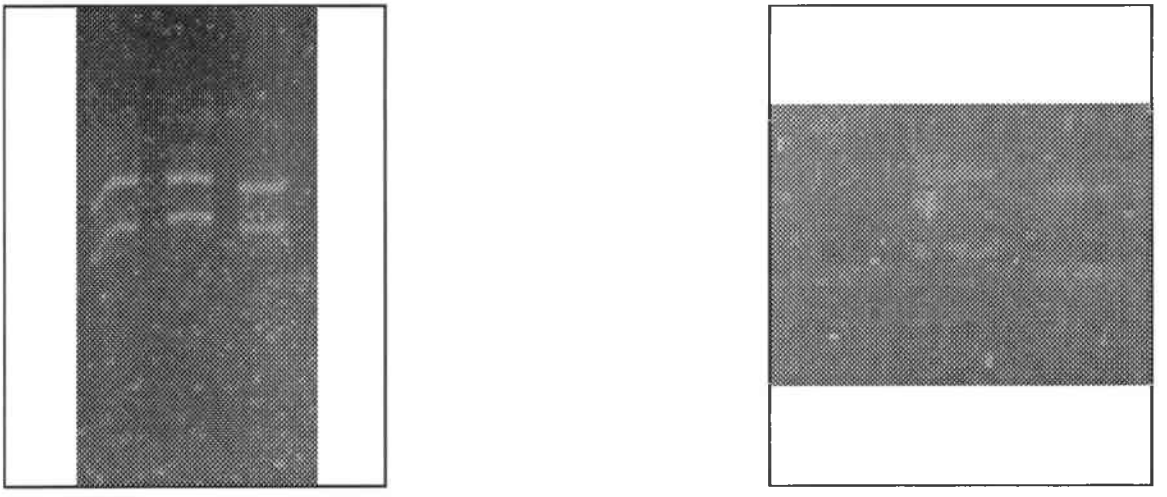


Figure 2. Difference in product by varying the number of cycles: 35 (left) and 25 (right).

Optimized Primers

The following table lists the primers optimized during the course of the study. These results were obtained by modification of manufacturers' suggestions and experimental work with the previously mentioned aspects of PCR (the primer annealing temperature and the number of cycles).

Chromosome Pair	Primer	Annealing Temperature	Number of Cycles
13	D13S325	55	35
13	D13S796	55	35
14	D14S306	55	35
15	D15S659	55	35
15	D15S642	65	25
15	D15S175	60	25

Ideal Banding Results

Once the primers have been optimized, the product specific for the target locus can be produced. The target locus is selected by randomly choosing any one of the optimized commercial primers available for the chromosome

pair to be tested. The amplified product is then analyzed using vertical, agarose gels. The amplified product specific for each primed locus on each chromosome produces two types of banding patterns. The first type contains only one band for the locus. This means that the individual is homozygous for that locus. Another pattern shows two bands for one locus. That is, each chromosome (of the pair studied) has a different base pair number at that locus. More simply, that patient is heterozygous. A comparison of both parents' loci to the child's loci can give an indication of the origin of the child's loci. Determining the origin of the loci indicates the parent from which each of the child's chromosomes comes. Examples are illustrated in Figures 3, 4, and 5.

These figures show the banding patterns of a family that is not affected with uniparental disomy. Figure 3 illustrates the pattern of two homozygous parents. The mother shows only one band (a); the father also shows only one band (b), but the paternal band is different from that seen in the mother. The child shows both a and b bands and is heterozygous.

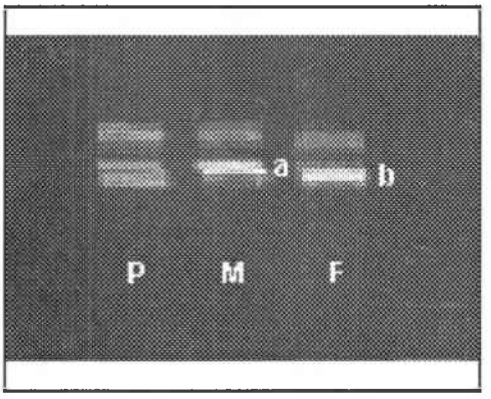


Figure 3. Homozygous parents with heterozygous child (chromosome 13).

The intensity of the parental bands should also be noted here. Parental bands are more intense than the child's bands. This intensity indicates a higher concentration of DNA in parental bands because both chromosomes contain the same allele, which causes a higher concentration of DNA product to be amplified.

Figure 4 shows an uninformative locus. The mother displays bands a and b while the father displays bands a and c. Because both parents have band a the origin of band a cannot be determined in the child, but band b is shown to be passed from mother to child.

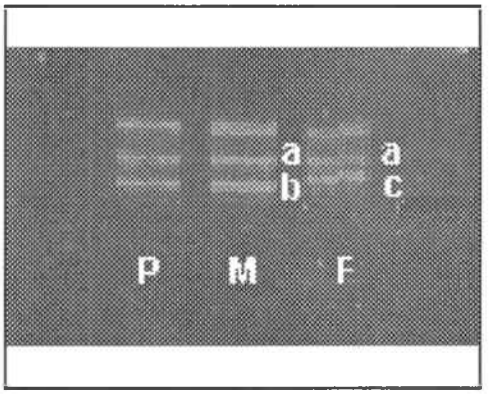


Figure 4. Uninformative locus. Heterozygous parents and heterozygous child (chromosome 13).

Figure 5 demonstrates another ideal band configuration. Both the mother and father are heterozygous and the child shows inheritance of one band from each parent: specifically, mother (c,d), father (a,b), and child (b,d). Here biparental inheritance becomes clear. The child has inherited the chromosome containing band b from its mother and the chromosome containing band d from its father. The expected result in a case of uniparental disomy is

inheritance of band(s) from only one parent.

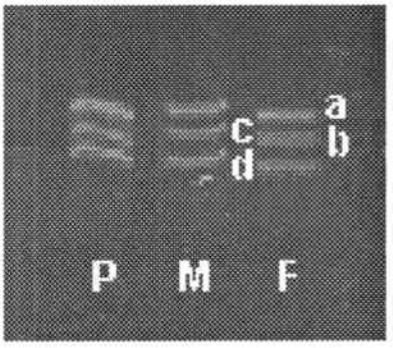
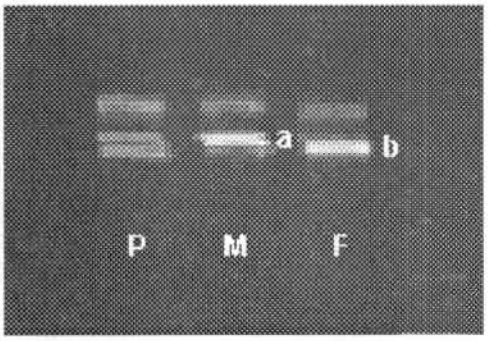


Figure 5. Informative locus, heterozygous parents and heterozygous child (chromosome 14).

Clinical Test Results

The next group is a family in which there is a paternal Robertsonian translocation t(13,14). One of the male children in this family has been shown to possees the Robertsonian translocation. This translocation combined with short height and speech delay makes the child a likely

candidate for uniparental disomy. Because the Robertsonian t(13,14) is inherited paternally, uniparental disomy in the child will be paternal and involve chromosomal pair(s) 13 and/or 14. Thus, we are specifically interested in demonstrating a maternal genetic contribution to chromosome pairs 13 and 14 of the child. Existence of this maternal contribution would refute the presence of uniparental disomy in this case.



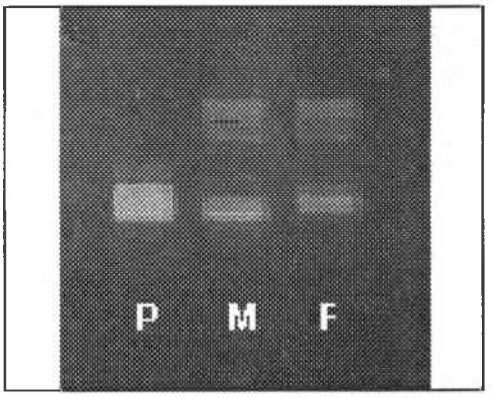


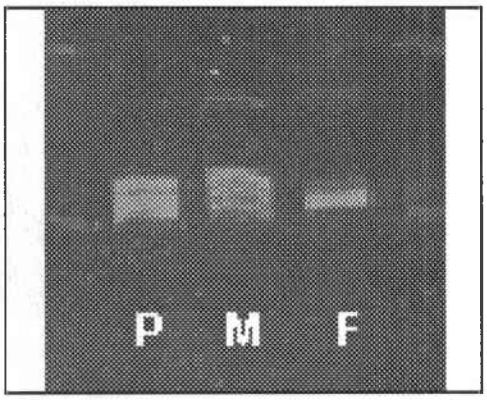
Figure 6. Biparental inheritance of chromosome 13 is proven with these two loci.

Figure 6, under primer locus D13S796 shows both parents to be uniquely heterozygous at that locus. Paternal alleles (a,b), and maternal alleles (c,d) combine in the child to create child allelee (a,c). Also on Figure 6, but under primer locus D13S232, one can see that there is a maternal contribution to the child even though the banding in the

reaction is not well resolved. Both primer loci indicate that there is a maternal contribution to chromosome 13. Thus, chromosome pair 13 shows biparental inheritance, not uniparental disomy.

The following group of results solidly proves normal inheritance of chromosome 14 in the group with the paternal Robertsonian t(13,14).

Figure 7 shows the primer locus D14S63. Although the resolution is poor here, a definite maternal contribution can be seen in the banding pattern. Additional proof for normal inheritance of chromosome 14 comes from Figure 7. Primer locus D14S306 depicted in this figure shows definite paternal bands (a,b) and maternal bands (c,d). The resulting child shows bands (b,d); thus, the child has both maternal and paternal genetic material for chromosome 14. Existence of maternal and paternal genetic material leads to the conclusion of biparental inheritance for chromosome 14.



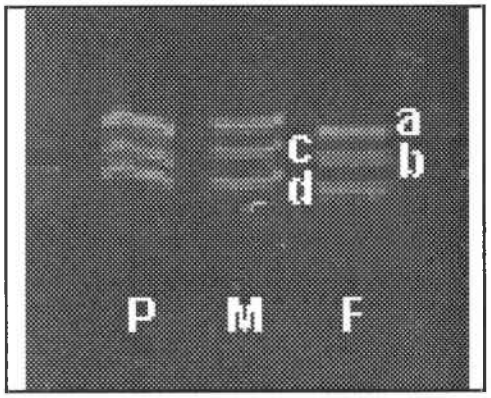


Figure 7. These gels prove biparental inheritance for chromosome pair 14.

The second-case study analyzed by this procedure involves another family that has a child suspected of Prader-Willi syndrome. Thus tests were performed on chromosome pair 15. With Prader-Willi syndrome, the existence of a paternal contribution to chromosome pair 15 is enough to refute uniparental disomy because the paternal contribution suppresses Prader-Willi. The gel results show strong, but not conclusive, evidence to indicate biparental inheritance. That is, only one tested loci shows biparental inheritance.

In Figure 8, primer group D15S659 shows evidence supporting normal, biparental inheritance for this Prader-Willi case. First, the paternal bands (a,b) and maternal

bands (c,d) can be depicted. The child shows bands (a,d). Because band a is paternal, evidence of biparental inheritance is strong. Unfortunately, all other primer loci attempted on this group produced either unreadable results or uninformative markers. So, uniparental disomy, although unlikely, cannot be ruled out without at least one more informative locus indicating a paternal contribution (that is, evidence of biparental inheritance).

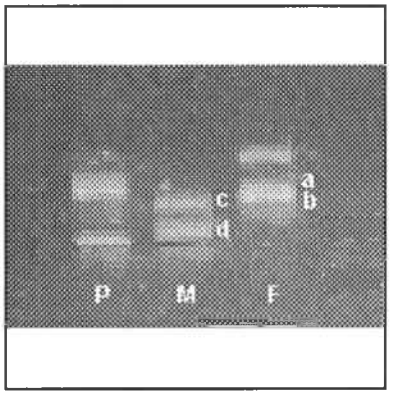


Figure 3. This gel shows a paternal contribution to the child (band a).

In sum, a large portion of the results created during this study involved the optimization of primers. During this optimization examples of ideal band configurations were created as well as an idea of what the banding pattern of an

individual with uniparental disomy should look like. Finally, two families suspected of having an individual with uniparental disomy were tested for the condition. One individual, with a Robertsonian translocation, was proven to have biparental inheritance for chromosome pairs 13 and 14. This rules out the possibility of uniparental disomy developing because of the translocation. A second individual, with Prader-Willi syndrome, was shown to be unlikely to have uniparental disomy.

DISCUSSION

A summer's worth of work produced few results but provided useful technical data. The reason for the lack of results was impractical testing methods. Experiments to optimize primers for each chromosome were quite involved. Also, random searching for uniparental disomy would be difficult because one must analyze the entire genome. To analyze the entire genome each of the 23 pairs of chromosomes must be tested. In addition, finding informative markers in each pair of chromosomes may take several attempts. Because random searching consumes so many resources, a search for uniparental disomy must employ certain "flags" or conditions that signal the existence of uniparental disomy.

Most of the energy spent in this project was used to optimize the primers used to test for uniparental disomy. Consequently, the original intent (a systematic search for uniparental disomy) was only briefly addressed. To this end, two children were actually tested for uniparental disomy. One child was proven to have biparental inheritance, and evidence strongly suggested the other child

also had biparental inheritance. Thus, in the families tested here uniparental disomy most likely is not present. These results correlate with results of other experiments that have found uniparental disomy very rare even in individuals with symptoms regularly associated with uniparental disomy.

The clinical importance of uniparental disomy is its ability to provide a mechanism for physical anomalies. Consequently, the condition must be understood. Understanding starts with the identification of a large group of individuals with the condition. Data from this group can then be used to generalize the symptoms of uniparental disomy as well as explore the possible effects of uniparental disomy. In addition, more susceptible chromosome pairs may be identified. Correlation between age of parents and occurrence of uniparental disomy may be useful to better inform couples of pregnancy risks, similar to informing them of the risk of Down's syndrome.

Regardless of possible implications of uniparental disomy, better testing methods must be developed before any real progress will be made in the area. Although the thermocycling takes relatively little time, primer

optimization takes time. Specifically, commercial primers do not provide necessary specificity; however they can be optimized individually. Adjusting temperatures during the stages of the PCR cycle as well as regulating the number of cycles has proven useful for optimizing primers. Unfortunately, this experiment does not provide results diverse enough to state whether or not the adjustments work universally (on any DNA sample), but the results show promise.

Another area that could have been improved was the fact only one loci could be tested per trial. Thus, work to combine primers so that more than one primer could be used in each trial may save time and increase the number of tested loci and thus, reduce cost.

In conclusion, research indicates that uniparental disomy occurs at a low frequency. On the other hand, it may exist without causing clinical effects, the possibility remains that the condition occurs at a much higher rate. No matter how often uniparental disomy occurs, its existence demands attention. Clinical problems resulting from the condition are often severe mental and physical anomalies. Thus, at the very least, researchers must uncover its

intricacies to help physicians identify the cause of those anomalies. Only with this knowledge may they begin to warn prospective parents of the severe impact on their lives and the lives of their children.

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