From the Kilobase to the Kilogram—Molecular Diagnosis in the Newborn Nursery

Alan Shanske
The Center for Congenital Disorders
Children’s Hospital at Montefiore
Albert Einstein College of Medicine
Bronx, New York 10461

ABSTRACT

Screening for genetic disorders in newborns began in 1962 with the use of Guthrie’s bacterial inhibition test for phenylalanine quantification. It resulted in the development of many other tests used in the mass screening of newborns for metabolic defects, endocrinopathies, hemoglobinopathies, and cystic fibrosis. Despite the explosion in molecular biology and the recent decoding of the human genome, molecular diagnostic techniques are not widely used in the newborn nursery. However, molecular diagnostic techniques will become increasingly important part of the newborn assessment. This review will summarize the recent experience with molecular diagnosis in the newborn nurseries of the Children’s Hospital at Montefiore.

INTRODUCTION

The principle aim of modern human genetics is to identify the mutations that are responsible for genetic disease then use this information to improve the diagnosis and treatment of human disorders. The sequencing of the human genome marked a milestone for this process. Molecular genetic and recombinant deoxyribo nucleic acid (DNA) techniques have begun to be used in the newborn nursery to supplement or replace the biochemical and cytogenetic methods of the past. Genetic screening during the newborn period is the most common application of medical genetics in the newborn nursery. Newborn screening for genetic disorders began in 1962 with the application of Guthrie’s bacterial inhibition test for phenylalanine quantitation. It resulted in the mass screening of newborns for phenylketonuria (PKU). Newborn screening programs have become very successful. It is estimated that about 60% of newborns in the world have a screen for a genetic disorder. Despite the explosion in molecular biology and the recent sequencing of the human genome, molecular diagnostic techniques are not widely used in the newborn nursery. Currently, molecular biology is primarily used for confirmation of more traditional technologies. In fact, the screening for metabolic diseases still relies on bacterial inhibition or biochemical assays, the detection of hemoglobinopathies relies on electrophoretic techniques, and the diagnosis of congenital hypothyroidism relies on a radioimmunoassay. New paradigms for newborn screening using DNA technologies are being developed and undoubtably will be an increasingly important part of newborn assessment.

TRADITIONAL GENETIC SCREENING METHODS

The bacterial inhibition assay (BIA) for phenylalanine quantitation was first used in a mass screening program for the identification of PKU. The New York State Newborn Screening Program uses the Guthrie BIA to detect levels of three amino acids in newborn dried blood samples. Elevated levels of phenylalanine, leucine, and methionine, may be indicative of PKU, maple syrup urine disease (MSUD), and homocystinuria, respectively. The BIA uses a mutant strain of bacteria that requires a specific amino acid to grow on agar. Blood spot discs from newborns are place on agar plates with media containing chemical analogues that inhibit growth. If there is a significant elevation of the amino acid being tested (e.g., above that of the inhibitory analogue), bacterial growth will be observed.

Because of their success, screening tests have been developed for other disorders including other metabolic defects (e.g., galactosemia and biotinidase deficiency), endocrinopathies (e.g., congenital hypothyroidism and congenital adrenal hyperplasia), hemoglobinopathies (e.g., sickle cell anemia and Sβ-Thalassemia), infectious diseases (e.g., human immunodeficiency virus, HIV), and monogenic diseases (e.g., cystic fibrosis, CF). Testing for the transferase enzyme in red blood cells identifies classical galactosemia. New York State utilizes a semi-quantitative colorimetric screening test for biotinidase activity.

The hypothyroidism screening test utilizes a radioimmunoassay (RIA) for T4 and for thyroid stimulating hormone (TSH) for verification. An enzyme immunoassay for measurement of elevated 17-hydroxyprogesterone (17-OHP) is used to screen for congenital adrenal hyperplasia (CAH) due to 21-hydroxylase deficiency. HIV is the only infectious disease currently screened for by New York State and is only one of two tests that rely upon DNA technology. A positive serology screen for HIV using
an enzyme-linked-immunosorbent assay (ELISA) for antibodies to HIV or a Western blot for HIV antigens is followed by a polymerase chain reaction (PCR) analysis for HIV DNA. The screening procedure for hemoglobinopathies is cellulose acetate electrophoresis at pH 8.6. The screening procedure will identify homozygous sickle cell anemia (SS disease), other sickling disorders (SC disease, Sα-Thalassemia, SD disease, and Sαα Arab) disease as well as other hemoglobinopathies (CC, Cα-Thalassemia, AS, and AC). The immunoreactive trypsin (IRT) test is the screening test for CF. New York State will perform DNA analysis on specimens with high IRT values (the second routine use of DNA technology by the New York State screening program).

The recent development of tandem mass spectrometry (MS/MS) has added more than 30 disorders of fatty acid oxidation, organic acid metabolism, and amino acid metabolism that can potentially be screened for (Korson, 2000). Metabolites impregnated on blood filter paper specimens are ionized producing daughter ions that are subjected to mass spectrometry. The spectra are compared to stable isotope-labeled internal standards that permit the identification of amino acids and acylcarnitine derivatives. Recently, New York State has added MS/MS screening for MCADD (medium chain acyl-CoA dehydrogenase deficiency), which is the most common disorder of fatty acid oxidation. The measurement of octanoylcarnitine is the initial screening modality. At the present time, eleven analytes are screened for by the New York State Department of Health. Ten of the 11 analyses depend upon a BIA, enzyme reaction, electrophoresis, or immunologic assay. Only testing for MCADD depends on MS/MS.

SCREENING USING MOLECULAR TECHNOLOGY

It is important to first review the structure of genes and genomes as well as the methods of their analysis before examining their specific applications in the newborn nursery. Currently, applications are primarily for confirmation of a screening test or identification of a rare genetic disorder. After discussing the molecular basis and common applications of DNA analysis, the recent experience in the nurseries of the Children’s Hospital at Montefiore of the Albert Einstein College of Medicine will demonstrate some of the exciting possibilities for these methods. The recombinant DNA revolution has resulted in the identification of more than 2000 human genes and made available a rapidly growing list of new diagnostic tests. DNA technology is not yet used for routine newborn screening but rather for the confirmation of specific genetic disorders in the sick newborn. It is especially useful in those conditions where an analyte cannot be evaluated by biochemical analysis, where the gene product is unknown, or where the mutation is the result of a mutation beyond the resolution of the light microscope.

Structure of genes and genomes

Most organisms store their genetic information as the macromolecule DNA. This is a linear polymer with four different monomeric units called nucleotides. These are linked together in a chain by phosphodiester bonds. According to the Watson-Crick model, a DNA molecule consists of two intertwined polynucleotide chains. A chain may typically be made of thousands of millions of monomers. Each monomer in one chain is linked specifically by hydrogen bonds to a nucleotide in the other chain. There are two kinds of nucleotide pairings in DNA. Deoxyadenosine monophosphate pairs with deoxythymidine monophosphate (A-T pairs), and deoxyguanosine monophosphate pairs with deoxyctydine monophosphate (G-C pairs). The sequence of the four nucleotides along the polynucleotide chain varies from individual to individual and is the molecular basis for genetic diversity. The sequence of nucleotides of one chain determines the sequence of the other, hence the two chains are complementary to each other. It is the power of base complementarity that is the basis for currently used methods of DNA analysis. There are many excellent reviews of molecular genetics and technology (Gelehrter et al., 1998; Griffiths et al., 1999; Nussbaum et al., 2001).

Applications of DNA Analysis in the Newborn Nursery

Since the beginning of mass screening programs, newborn screening has utilized dried blood specimens on a filter paper matrix. This method was designed for ease of sample transport to centralized laboratories, usually a centralized state laboratory. The stability of various analytes including intermediary metabolites, proteins, and hormones on the filter paper was soon established. McCabe et al. (1987) demonstrated that DNA was also stable on filter paper. Descartes et al. (1992) showed that these DNA samples could be amplified using PCR for diagnostic purposes. Jinks et al. (1989) demonstrated the feasibility of confirming a diagnosis of sickle cell disease and other hemoglobinopathies using the original dried blood specimen. The identification of the common mutations in the CF gene done on the original specimen has been used as a confirmatory test for CF neonatal screening using initial measurement of immunoreactive trypsinogen (Seltzer et al., 1991) and has recently been added to the newborn screening program in New York State. Newborn screening for CAH is accomplished by measuring the level of 17-OHP in the dried blood specimen. The level of 17-OHP does not always predict disease severity. Accordingly, genotyping the 21-hydroxylase gene responsible for this disorder in dried blood specimens serves both to confirm the diagnosis and predict severity (Witchel et al., 1997). Recent recommendations have been made for universal neonatal hearing screening. Confirmatory tests traditionally include more sophisticated tests of sensory perception. Approximately 50% of childhood deafness is
due to genetic causes. About 80% of these cases are non-syndromic and recessively inherited. Fifty percent of these cases are caused by mutations in the gene encoding the gap junction protein, connexin 26 (Cx26). It has been suggested that molecular testing be part of the confirmatory process of an abnormal neonatal hearing screen (McCabe and McCabe, 1999).

A DNA sequence can be analyzed using either the Maxam-Gilbert chemical cleavage method or the Sanger enzymatic chain termination method (Jandreski, 1995). The Sanger method is the most commonly employed today and uses four different enzymatic reactions to synthesize a strand of DNA using DNA polymerase and a synthetic primer. The primer is from a flanking sequence and is used to initiate DNA synthesis. DNA synthesis occurs in the presence of dideoxynucleotides triphosphates (ddNTPs) which when incorporated terminate synthesis. Four reactions are prepared containing a single-stranded DNA sequence, DNA polymerase and either ddATP, ddTTP, ddCTP, or ddGTP along with the four deoxynucleotide triphosphates (dNTPs). A different fluorescent dye is attached to each of the four oligonucleotide primers. All possible truncated fragments are generated by the four reactions. The fragments are visualized by electrophoresis of the four samples in four lanes on an acrylamide gel. Fluorescent detection is accomplished in an automatic DNA sequencing machine that can read as many as 1000 bases in one separation. The nucleotide sequence is fed into a computer that scans all the reading frames beginning with an initiation codon and ending with a stop codon. Throughput of sequencing is still limited and the costs are prohibitive for mass screenings. An alternative approach with the promise of faster and more economical results is DNA sequencing using “chip” technology.

Fluorescent in situ hybridization (FISH) is a technique based on the principle that when double-stranded DNA is heated it denatures into single-stranded DNA. On cooling, the single-stranded DNA reanneals with its complementary sequence into double-stranded DNA. An appropriately labeled DNA sequence (probe) is denatured and added to denatured nuclei or chromosomes and then reannealed by cooling. The labeled DNA hybridizes to its complementary sequence in the chromosomal DNA. Detection of the labeled DNA identifies the site of hybridization and the region of complementary chromosomal DNA. Probes conjugated with a hapten, usually biotin or digoxigenin, are detected by fluorochromes coupled to avidin or anti-digoxigenin, respectively. The signal is read using a fluorescent microscope and a digitalized imaging system to acquire and store the images. Digital fluorescence microscopy allows for multicolor FISH using fluorochromes for each individual chromosome at the same time.

Comparative genomic hybridization (CGH) is a reverse FISH technique that allows for the identification of chromosomal gains or losses by scanning the entire genome in a single step. It is accomplished by in situ hybridization of differentially labeled total genomic specimen DNA and normal reference DNA to normal human metaphase chromosome spreads. Hybridization of the specimen and reference DNA can be distinguished by their different fluorescent colors using a digital imaging analysis system.

Fragmentation of DNA facilitates its identification. The most extensively used method of fractionating DNA is by electrophoresis. A mixture of linear DNA molecules is placed on an agarose gel and exposed to an electric field and separated by size. Restriction enzyme digestion of genomic DNA results in so many fragments that a stained electrophoretic gel shows a smear of DNA. E.M. Southern developed a technique to identify a single fragment in this mixture using a probe complementary to the DNA fragment of interest (Southern, 1975). An absorbent membrane is laid over the gel and the DNA bands are transferred (“blotted”) onto the membrane by capillary action. The membrane is bathed with a radioactive probe, and an autoradiogram is used to reveal the presence of any bands homologous to the probe. By analogy, the analysis of RNA molecules using a similar technique is called Northern blotting and of proteins using specific antibodies is called Western blotting.

The remarkable ability of PCR to amplify nucleic acid sequences has made this an invaluable tool to obtain diagnostic quantities of DNA. It was developed by Kary Mullis in 1983 and essentially involves three steps. First, the nucleic acid target is denatured by heating in the presence of a pair of sequence specific primers (one complementary to the 3’ strand and the other to the 5’ strand). Heating causes the two strands of the molecule to separate. The solution is then cooled enough to allow the primer pair to hybridize to the target sequence. Finally, DNA polymerase is added with deoxynucleotide triphosphates. The cycle is repeated and results in an exponential reproduction of the nucleic acid sequence bounded by the primers. Detection of the amplified sequences can be done with agarose gel electrophoresis and ethidium bromide staining. Simultaneous amplification of multiple loci can be done using so called “multiplex PCR.” The PCR reaction is not limited to DNA templates and can also use RNA.

The use of microarray technology for neonatal screening offers the possibility to greatly expand the number of treatable diseases screened for and of consolidating existing assays (i.e., biochemical, spectrometric, or immunologic) into a single more efficient format. Newborn blood filters are the source of high quality DNA sample which can be amplified using multiplexed PCR and which can then be applied to a DNA chip. The concept behind sequencing using a DNA chip involves the hybridization of a fragment of labeled DNA with all possible DNA oligomers (i.e., a short chain of nucleotides) of a fixed
length of usually ten to fifteen bases (Persidis, 1999). The oligomers are attached to a solid substrate and are selected to test for overlapping sequences. A small group of the oligomers, say of ten bases, will react specifically with the ten base sequence of the unknown DNA. The result is a labeled pattern detected by laser confocal fluorescence microscopy using a computer program to detect the DNA sequence of the unknown fragment. The DNA chips currently used contain high-density, miniaturized arrays of DNA probes on a silicon solid support. Semiconductor type photolithography similar to the process used in manufacturing microchips for computers and solid phase chemical synthesis techniques are used to manufacture the chips. High-density arrays contain thousands of probes that can cover the length of an entire gene. Chip technology will become widespread when new manufacturing techniques have brought down the cost of the microarrays. In fact, the feasibility and practicality of using multiplex amplification and microarray analysis has already been demonstrated. Dobrowolski et al. (1999) used DNA samples from dried blood cards to screen for sickle cell disease, alpha-1-antitrypsin deficiency, and Factor V Leiden, simultaneously. Unlimited potential exists for multiplex amplification and DNA chip analysis applied on a population-wide basis for treatable genetic diseases such as sickle cell disease, alpha-1-antitrypsin deficiency, Factor V Leiden, myoclonic epilepsy ragged red fibers (MERRF) syndrome, mitochondrial encephalopathy, lactic acidosis, stroke-like episodes (MELAS), and hemochromatosis.

**RECENT CASES FROM OUR NEWBORN NURSERIES WHERE MOLECULAR ANALYSIS WAS UTILIZED**

**Prader-Willi Syndrome**

JG was the 2180 gm product of a 37-week gestation complicated by diabetes treated with insulin. She was delivered by C-section because of intrauterine growth retardation (IUGR) and breech. When examined at 12 days of age, she was feeding poorly and had generalized hypotonia. The physical examination was otherwise unremarkable and a routine karyotype revealed a normal female complement. The clinical picture of a floppy newborn with feeding difficulty suggests the possibility of the Prader-Willi syndrome (PWS), a contiguous gene or microdeletion syndrome involving chromosome 15 that often is missed by standard karyotypic means. The PWS is a dysmorphic syndrome characterized by short stature, obesity, small hands and feet, hypogonadism, and mental retardation (Shaffer et al., 2000). FISH using a probe for the SNRPN gene on the proximal long arm of chromosome 15 showed a microdeletion at CH 22 (del q11).

**FIGURE 1** Cartoon of FISH staining of chromosome 22 (courtesy of Dr. Bernice Morrow).
FIGURE 2 | Karyotype of Twin B with two ring chromosomes, ring 1 and ring 16 (denoted by arrows).
FIGURE 3 1  CGH of patient with Waardenburg-Shah syndrome indicating the loss of material in the interstitial region of the long arm of chromosome 13.
15q11.2. About 70% of patients with the PWS are deleted for this area and the remainder have uniparental disomy for chromosome 15 and are missing the paternal homologue of this imprinted chromosome region.

**Velocardiofacial syndrome**

SC was the 3500 gm product of term pregnancy delivered by emergency C-section because of fetal distress. The newborn exam revealed a broad nasal tip and root, malar hypoplasia, abnormal helices, pre-axial polydactyly and anal atresia. He developed cyanotic episodes. An echocardiogram was consistent with Tetralogy of Fallot. These findings strongly suggested a relatively common microdeletion syndrome, the Shprintzen syndrome also known as the velocardiofacial syndrome (VCFS) which is a multisystem disorder characterized by a cleft palate, unusual facial appearance, and a conotruncal congenital heart defect (Shprintzen et al., 1978). A routine karyotype showed a normal male complement. However, FISH using a probe for a DNA marker, D22S75, on the proximal long arm of chromosome 22 showed a microdeletion of region 22q11.2, visible as the loss of a fluorescent signal on the proximal portion of the long arm (Figure 1). The velocardiofacial syndrome is associated with a deletion in this region in about 90% of cases. It is felt that haploinsufficiency for genes in this critical region are responsible for the phenotype (McDermid and Morrow, 2002).

**Supernumerary or Marker (Unknown) Chromosomes**

Twin A was the 672 gm product of a twin 25/26 week gestation. Examination of the fetal membranes showed a single chorion and two amniotic sacs consistent with monozygosity. The newborn examinations were discordant. The exam of twin A revealed no dysmorphic features. Twin B weighed 632 gms and had a bifid right thumb. Routine chromosome analysis revealed the presence of two supernumerary or extra ring chromosomes in both twins (Figure 2). Ring chromosomes are quite rare and are formed by breakage and reunion of the short and long arms of a chromosome. The origin of the marker chromosomes in this case was established by hybridization with appropriate satellite probes that showed the large ring to be derived from chromosome 1 and the small ring to be derived from chromosome 16. This was only the second occurrence of two rings reported in the same individual. The presence of even a single de novo marker or ring chromosome presents great difficulty in diagnosis. Delineation of the two rings could only have been done using FISH techniques as in this case (Shanske et al., 1999).

**Interstitial Deletion of Chromosome 2**

LR was the 288 gm product of a term pregnancy and spontaneous vaginal delivery (SVD). The newborn examination revealed craniofacial disproportion, relative macrocephaly, a large fontanelle, hypertelorism, abnormal pinna and preauricular fistulae. He required several admissions because of chronic intestinal obstruction resulting in a derotation of a malrotation and lysis of Ladd bands at 11 months. His growth and development were severely delayed. Conventional cytogenetic investigation done in the newborn nursery revealed a de novo apparently balanced translocation, 46,XY,t(1;2) (p22;q14.1). Reanalysis using CGH revealed a loss of chromosomal material in the mid-portion of the long arm of chromosome 2 (Shanske et al., in press). Balanced translocations are for the most part not associated with any clinical phenotype. The imbalance observed by CGH indicated that the translocation was not balanced and demonstrated the utility of CGH as an adjunct to identify subtle unbalanced rearrangements (Kirchhoff et al., 2001).

**Waardenburg-Shah Syndrome**

NO was the 2722 gm product of a term uneventful pregnancy who fed poorly and did not pass meconium for several days. The newborn examination revealed bright blue irides in an African baby. A biopsy of the rectum and colon showed absence of ganglion cells. Hirschsprung disease is caused by the absence of ganglion cells in the myenteric plexis of the intestine. In the next few weeks, she underwent an endorectal pull through procedure. The highly unusual color of her irides suggested additional involvement of neural crest derived cells including the sensory cells of the cochlea. Accordingly, a hearing test was performed and unfortunately a profound bilateral neurosensory hearing loss was identified when she was about two months of age. Her karyotype revealed an interstitial deletion of the long arm of chromosome 13 [del(13)(q14.3q22.2)]. The deletion and its extent were delineated using CGH (Figure 3). Sequencing of the EDNRB gene, associated with Hirschsprung disease, revealed a mutation in the EDNRB gene on the homologous chromosome 13. The Waardenburg-Shah syndrome results from failure of neural crest differentiation and combines features of Waardenburg syndrome (sensorineural deafness and pigmentary skin changes) and Hirschsprung disease. It is an autosomal recessive disease that occurs as a result of homozygosity for mutations in EDNRB genes on chromosome 13. In our case, the infant had a mutant allele on one chromosome 13 and a deletion of the allele on the homologous chromosome 13 (Shanske et al., 2001a).

**Myotonic Dystrophy**

FC was the 3100 gm product of a 37-week gestation delivered by C-section because of pre-eclampsia, polyhydramnios, and arrest of labor to a 36-year-old mother with myotonic dystrophy. The newborn exam revealed profound generalized hypotonia with a weak suck and cry and no arthrogryposis (congenital contractures of the limbs). A likely diagnosis of congenital myotonic dystrophy confirmed by FISH with a probe for the EDNRB genes on chromosome 13.
**Normal:**  GTTAAGATGGCAGAGCCCGGTAATCGC

**MELAS:**  GTTAAGATGGCAGGGCCCGGTAATCGC

**HaeIII (GG/CC)**

**FIGURE 4** Analysis of the A to G tRNA-Leu(3243) mutation in MELAS by restriction digest.
dystrophy was made. The diagnosis can be established only with the aid of molecular analysis of the myotonic dystrophy gene (Hamshere et al., 1998). Molecular genetic testing is available using Southern blotting to analyze the size of the CTG repeats in the myotonic dystrophy gene (DMPK). The expansion of the gene is the molecular basis for this trinucleotide or dynamic repeat disorder. PCR and Southern analysis of DNA revealed an expansion of the CTG repeat in the DMPK gene on chromosome locus 19q13. A repeat size of 1537 CTG repeats was found in our patient. A normal individual has up to 37 CTG repeats in the polymorphic repeat region. One thousand to greater than 2000 CTG repeats correlates with the most severe phenotype, congenital myotonic dystrophy.

Holoprosencephaly

KG was the 3000 gm product of a term uneventful pregnancy born with severe microcephaly, hypotelorism, midfacial hypoplasia, and a midline cleft. A CAT scan of the head revealed semilobar holoprosencephaly (HPE). HPE is the most common structural defect of the developing forebrain. Its prevalence at birth is 1:10,000 to 1:20,000. HPE is etiologically heterogeneous with both genetic and environmental causes. The infant was the third born to this family with HPE. Sonic hedgehog (SHH) is one of at least four different loci implicated in familial HPE and maps to chromosome region 7q36 (Wallis and Muenke, 1999). The other genes identified so far in familial cases are ZIC2, SIX3, and TGIF. SHH is a segment polarity gene and plays a critical role in establishing the proper ventral orientation of the developing CNS. Sequencing of the infant’s DNA revealed a point mutation in SHH. Though identification of a specific mutation in this family has no prognostic significance, it has great value for the family in terms of genetic counseling and prenatal diagnosis.

Leigh Syndrome (MELAS)

CM was the 2990 gm product of a term pregnancy that presented at birth with hypotonia, feeding difficulties and respiratory distress. His neurologic symptoms progressed with the onset of seizures, pyramidal signs, and cranial nerve palsies. Elevated levels of lactate and pyruvate were detected in the blood and cerebrospinal fluid. An MRI of the head revealed multiple symmetric foci of spongy degeneration of the brain stem and basal ganglia. The clinical presentation and biochemical profile were of a mitochondrial encephalomyopathy caused by a mutation in mitochondrial DNA. PCR amplification and diagnostic cutting with restriction enzyme endonucleases identified an A to G transition at nucleotide 3243 in the mitochondrial gene encoding leucine transfer RNA (Figure 4) (Shanske et al., 2001b). Leigh syndrome has been associated with a number of other mitochondrial DNA (mtDNA) mutations as well as deficiencies of pyruvate dehydrogenase (PDHC) and cytochrome oxidase (COX), complex four of the mitochondrial respiratory chain.

Neonatal Marfan Syndrome

AR was the 2615 gm product of a 38-week gestation complicated by IUGR. The newborn examination revealed a birth length of 52 cm, arachnodactyly, flexion contractures, and skin and joint hypermobility. A chest X-ray showed bulging of the liver into the right hemithorax consistent with a diaphragmatic hernia. She developed congestive heart failure at five weeks of age. An echocardiogram revealed dilation of four chambers, dysplastic and prolapsed mitral and tricuspid valves, and aortic root effacement. The excessive birth length, arachnodactyly (tapering of the digits) and valvular involvement suggested the possibility of neonatal Marfan syndrome (nMFS). A diagnosis of neonatal Marfan syndrome can only be established by sequencing fibrillin 1 (FB101), the gene responsible for Marfan syndrome. Sequencing of DNA fragments of the fibrillin gene on chromosome 15 corresponding to exons 22 through 33 which have been previously reported to contain mutations found in neonatal Marfan syndrome revealed a T to C transition at nucleotide 3277 (Figure 5) (Jacobs et. al., 2002). Neonatal Marfan syndrome is a rare member of the type 1 fibrillinoopathies associated with mutations of the connective tissue protein, fibrillin. This group of connective tissue diseases includes Marfan syndrome, the MASS phenotype, the Shprintzen-Goldberg syndrome, and familial arachnodactyly.

DISCUSSION

Molecular diagnosis in the nursery utilizes most of the commonly available diagnostic DNA techniques including Southern blotting, sequencing, PCR, and FISH. When employed, molecular methods are occasionally used as confirmatory assays for disorders such as the hemoglobinopathies, CF, congenital adrenal hyperplasia, and medium-chain acyl-CoA dehydrogenase deficiency. However, they are more commonly utilized to diagnose rare genetic disorders in the sick newborn as illustrated in the above examples including PWS, myotonic dystrophy, VCF, holoprosencephaly, and nMFS. The complexity, cost and technical limitations have thus far limited the routine application of molecular methods in newborn screening programs. In addition, many ethical, legal, and social issues will have to be resolved before this sort of analysis can be applied to universal screening. Until then, molecular techniques will be reserved primarily for those diagnostic situations where traditional biochemical, cytogenetic, electrophoretic, or immunologic analyses are inadequate.

Treatment outcomes in the diseases identified by newborn screening have been very gratifying. For example, the intelligence quota of children with PKU...
and treated with the appropriate diet is within a few points of their unaffected sibs and the mortality from complications of sickle cell disease among infants diagnosed in the newborn period and treated with prophylactic penicillin is now low. Using the lessons learned from our successes in newborn screening, in particular the availability of an effective treatment or intervention, we can be sure that in the future the new tools of molecular medicine will greatly enhance and accelerate our diagnostic abilities in the newborn nursery.

REFERENCES


