Genetic Screening for the Next Decade:
Application of Present and New Technologies

EDWARD R.B. McCABE, M.D., Ph.D.

Institute for Molecular Genetics and Department of Pediatrics, Baylor College of Medicine, Houston, Texas

Received May 31, 1990

Molecular genetic technology is diffusing from the research laboratory to the clinical laboratory, where it has already begun to influence prenatal diagnosis and counseling. In the very near future, this technology will be applied more generally, using population-based screening strategies. Pilot programs are beginning to evaluate the technical feasibility and efficacy of recombinant DNA techniques for newborn screening follow-up. DNA-based population screening is being considered for heterozygous carriers of an autosomal recessive disorder such as cystic fibrosis in order to identify carrier couples at risk of having an affected child. We will review the current DNA methodologies in the context of three genetic disorders: sickle-cell disease, Duchenne muscular dystrophy, and cystic fibrosis. We will then consider the requirements for implementation of these new technologies. We will conclude that implementation will require two key factors: machines and people. Machines are required to automate molecular genetic procedures, which are currently personnel-intensive, so that the expense can be reduced and the procedures made more cost-effective. The people who are required are health professionals knowledgeable in the clinical aspects of the target disorders, as well as in the DNA laboratory testing. These professionals will be able to facilitate sample acquisition and information exchange among the laboratory, the primary health care provider, and the families requesting consultation.

One of the primary goals of basic research in human genetics is to improve our understanding of the underlying molecular mechanisms responsible for human disease. The recent explosion of information in this area has provided medical genetics with the opportunity to achieve improved diagnosis of patients affected with genetic diseases and improved ascertainment of individuals heterozygous for autosomal recessive disorders.

We will review three genetic disorders, sickle-cell disease (SCD), Duchenne muscular dystrophy (DMD), and cystic fibrosis (CF), and discuss the recent advances in molecular genetic technology as they relate to screening and diagnosis for these diseases. The application of the techniques of molecular biology to neonatal screening relies on our ability to manipulate DNA from dried blood specimens on filter paper blotters. These blotters, frequently referred to as “Guthrie cards” after their originator, Dr. Robert Guthrie, have proven their value in newborn screening due to ease of sample collection and transport and to stability of a variety of analytes [1–3]. Examples of analytes routinely analyzed from Guthrie cards include phenylalanine for detection of phenylketonuria (PKU), thyroxine (T₄) and thyroid stimulating

Abbreviations: ASO: allele-specific oligonucleotide  CF: cystic fibrosis  DMD: Duchenne muscular dystrophy  HRP: horseradish peroxidase  PCR: polymerase chain reaction  PKU: phenylketonuria  RFLP: restriction fragment length polymorphism  SCD: sickle-cell disease  T₄: thyroxine  TSH: thyroid stimulating hormone

Copyright © 1991 by The Yale Journal of Biology and Medicine, Inc.
All rights of reproduction in any form reserved.
hormone (TSH) for congenital hypothyroidism, and hemoglobin for sickle-cell disease and other hemoglobinopathies. The illustrations which we will provide will demonstrate that DNA is another analyte which can be analyzed, using these dried blood specimens. In addition, we will consider the requirements for implementation of this molecular genetic technology so that it may have an even broader effect in clinical medicine.

SICKLE-CELL DISEASE

Sickle-cell disease is a common genetic disorder occurring with an incidence of 1 in 400 births among individuals in the U.S. of African-American origin [3]. Universal newborn screening for sickle-cell disease was recommended by an NIH Consensus Development Conference [4], because of the evidence that prophylactic treatment with penicillin is effective in preventing the infant mortality associated with this disorder [5].

Knowledge of sickle-cell disease has consistently been at the forefront in our understanding of the molecular pathogenesis of human disease, and this phenomenon has been true also in the area of molecular genetic diagnosis. In 1978, Kan and Dozy demonstrated the use of a restriction fragment length polymorphism (RFLP) for the prenatal diagnosis of sickle-cell disease [6,7]. They showed that there was linkage disequilibrium between the sickle-cell mutation and a 13 kb HpaI restriction fragment: 87 percent of individuals with the hemoglobin S allele had this restriction fragment. This fragment was, however, only a linked marker for the sickle-cell mutation; it required knowledge of the RFLP pattern of the proband and was not informative in all families. In 1982, it was shown that the sickle-cell mutation resulted in an alteration in the restriction recognition sequence for MstII [8,9]. Detection of the presence or absence of this restriction site by MstII digestion provided a sensitive and specific DNA test which did not require analysis of other family members and would identify all individuals with the βS allele. In 1983, Conner et al. utilized allele-specific oligonucleotide (ASO) probes for detection of the βS allele by differential hybridization [10]. By controlling the conditions of hybridization and washing, the presence of the βA and/or BS alleles could be determined with the appropriate labeled ASOs hybridized to Southern blots [11]. Electrophoresis of the human DNA was required to separate the specific diagnostic band from other cross-hybridizing sequences [10].

In 1985, Saiki et al. described the polymerase chain reaction (PCR) and demonstrated the application of this new enzymatic amplification technique for the diagnosis of sickle-cell disease [12]. This methodology permitted amplification of a specific DNA region of interest, such as that spanning the sickle-cell mutation in the β-globin gene. There would be approximately 220,000-fold enrichment in the region of interest at the end of 20 PCR cycles with 85 percent efficiency of amplification per cycle. This process resulted in marked diminution of the cross-hybridization that had previously been observed using ASOs, since now the amplified region of interest represented the majority of the DNA present. This process permitted one to hybridize the ASOs directly to dot blots of the PCR reaction products or, alternatively, to separate the PCR reaction products on a gel and stain directly with ethidium bromide. The diagnostic application of PCR was facilitated by the introduction of a thermostable (Taq) DNA polymerase which permitted automation of this process [13].
Our group had been interested in the analysis of DNA on the dried blood specimens used for newborn screening [14]. PCR amplification of microextracted DNA from the specimens facilitated genotypic analysis of SCD [15,16]. One could successfully distinguish AA homozygotes, SA heterozygotes, and SS homozygotes using ASO probes labeled with $^3$P [15] or horseradish peroxidase (HRP) [17], as well as by direct ethidium bromide staining of gels after digestion with restriction enzyme [16,18]. Chehab and Kan recently presented an elegant fluorescence assay for detection of the sickle-cell mutation which readily lends itself to automation [19].

We have proposed that DNA follow-up to newborn screening would permit rapid genotypic confirmation of positive newborn screening specimens and clarification of those which were questionable after the initial protein-based evaluation [15]. We are currently involved in a project, in collaboration with the newborn screening laboratory in the Texas Department of Health, to evaluate this approach. Preliminary results indicate that PCR-based strategies can be effective in providing genotypic confirmation and clarification with a very low recall rate for second specimens [18]. Such an approach would reduce one of the major expenditures for newborn screening programs, the personnel time required to contact the families to obtain the confirmatory liquid blood specimens [15]. In addition, we feel that contacting the family in a more timely fashion with the knowledge of the genotype would convey to them the appropriate sense of urgency and immediacy regarding the diagnosis of SCD and might reduce the frequency of noncompliance with antibiotic prophylaxis [15].

**DUCHEINNE MUSCULAR DYSTROPHY**

Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder with incidence estimates of 1:3,000 to 1:5,000 male live births or 1:11,500 overall births [3]. This disorder is characterized by progressive deterioration of the muscles, resulting in death in the second or third decade. Recent advances in the cloning and characterization of mutations in the DMD locus at Xp21 have improved remarkably the diagnostic capability for this disorder [20,21]. Utilizing the DMD cDNA probes, approximately 56 percent of patients reveal detectable deletions [21]. Because of the frequency of deletions, this disorder is particularly amenable to rapid diagnosis by PCR, since deletions which remove one or both of the priming sites would prevent amplification. Multiplex PCR permits the amplification of many sequences simultaneously and results in detection of 80 percent to 90 percent of all DMD gene deletions [22,23]. Thus, utilizing nine sets of PCR primers, the vast majority of DMD deletions can be detected, and nearly 50 percent of all DMD patients will have a recognizable deletion. This procedure greatly facilitates the diagnosis of these patients and in many cases can replace biopsy confirmation of the DMD diagnosis. In the DNA laboratory, multiplex PCR replaces the personnel-intensive efforts required to perform Southern blotting with multiple probes. Multiplex PCR should accelerate implementation of DNA technology for diagnosis of DMD in the general medical community and would facilitate rapid genotypic confirmation in at least 50 percent of patients ascertained by infant screening programs for DMD.

**CYSTIC FIBROSIS**

Cystic fibrosis is an autosomal recessive disorder with an average incidence among Caucasians of 1:2,000 births and a heterozygote frequency of approximately 1:22
individuals [3]. Death usually results in the second to fourth decades from obstructive pulmonary disease and infection.

In September of 1989, we received the exciting news that the gene for cystic fibrosis (CF) had been cloned and the mutation determined [24-26]. The common Caucasian CF mutation is referred to as ΔF508, because it results in the loss of three nucleotides in frame and, consequently, the deletion of a phenylalanine residue, amino acid 508, in the CF protein. Knowledge of this mutation permitted PCR amplification and hybridization with ASOs for analysis of the presence or absence of the ΔF508 mutation among homozygotes and heterozygotes for this disorder [27]. The ΔF508 mutation was present on 75.8 percent of the CF chromosomes. The results of this analysis indicated that population-based screening would identify approximately 57 percent of the non-Ashkenazic white couples at risk for CF [27]. A recent NIH-sponsored workshop recommended that, at this time, population-based carrier screening should not be undertaken and that mutation analysis should be used in families only where there was an affected individual with this disorder [28].

Pilot studies on the efficacy of newborn screening for cystic fibrosis are in progress [3]. Although reports of PCR amplification for detection of the ΔF508 mutation in dried blood specimens are not yet available, such samples have been used for haplotype analysis in this disorder. In one remarkable example from the United Kingdom, a specimen that had been stored for 17 years was obtained for amplification and restriction enzyme digestion [29]; the specimen, obtained in 1971 and analyzed in 1988, permitted informed genetic counseling for the sibling of that deceased patient with CF. This experience showed that such newborn screening specimens can be quite valuable as a molecular genetic resource. It also showed that, in addition to providing a simple form of sample acquisition and transport, dried blood specimens on filter paper blotters also provide a convenient medium for storage of DNA in a stable form.

IMPLEMENTATION OF MOLECULAR GENETIC TECHNOLOGY FOR THE FUTURE

The key to implementation and broad-ranging diffusion of this technology will be twofold: machines and people. Automated equipment is essential in order to reduce the current labor-intensive molecular genetic techniques. In our current trials to interface recombinant DNA methodologies with newborn screening, the major costs are the salaries of the technical personnel who perform the DNA microextractions and analyses. The ability to automate this technology would make it available at an acceptable cost. In addition, equipment automation will reduce personnel involvement in the repetitive manipulations associated with these procedures, in turn decreasing the opportunity for human error [30]. Just as there is the need for quality assurance and quality control in other aspects of neonatal screening [31,32], there also will be the need for analogous standards to be adopted for the molecular genetic components of such programs.

But, having said that we should automate in order to reduce personnel costs and human error, why have I said that the second important factor in implementation of this technology is people? The people who are needed are individuals knowledgeable in both the clinical sector and the DNA laboratory, who are able to operate at the interface of these two arenas. These health professionals will facilitate the movement
of samples to the DNA laboratory and will ensure that the information from the laboratory is transferred to the physician and the patient in a clear, effective manner.

In summary, the successful implementation of this technology will require research and innovation. Research will be required in order to provide automated and reliable methods. Innovation will be required in order to introduce this novel technology effectively and to train the health professionals who will facilitate implementation of molecular genetic technologies for improved patient care.

REFERENCES

1. Guthrie R, Susi A: A simple phenylalanine method for detecting phenylketonuria in large populations of newborn infants. Pediatrics 32:338–343, 1963
2. Guthrie R: Organization of a regional newborn screening laboratory. In Neonatal Screening for Inborn Errors of Metabolism. Edited by H Bickel, R Guthrie, G Hammersen. Berlin, Springer, 1980, pp 259–270
3. American Academy of Pediatrics, Committee on Genetics: Newborn Screening Fact Sheets. Pediatrics 83:449–464, 1989
4. Wethers DL, Panel: Newborn screening for sickle cell disease and other hemoglobinopathies. National Institutes of Health Consensus Development Conference Statement 6(9):1–22, 1987
5. Gaston MH, Verter JI, Woods G, Pegelow C, Kelleher J, Presbury G, Zarkowsky H, Vichinsky E, Iyer R, Lobel JS, Diamond S, Holbrook CT, Gill FM, Ritchey K, Falletta JM, Prophylactic Penicillin Study Group: Prophylaxis with oral penicillin in children with sickle cell anemia—a randomized trial. N Engl J Med 314:1593–1599, 1986
6. Kan YW, Dozy AM: Polymorphism of DNA sequence adjacent to human β-globin structural gene: Relationship to sickle mutation. Proc Natl Acad Sci USA 75:5631–5635, 1978
7. Kan YW, Dozy AM: Antenatal diagnosis of sickle cell anemia by DNA analysis of amniotic fluid cells. Lancet ii:910–912, 1978
8. Chang JC, Kan YW: A sensitive new prenatal test for sickle cell anemia. N Engl J Med 307:30–32, 1982
9. Orkin SH, Little DFR, Kazazian HH, Boehm CD: Improved identification of the sickle mutation by DNA analysis—application to prenatal diagnosis. N Engl J Med 307:32–36, 1982
10. Conner BJ, Reyes AA, Morin C, Itakura K, Teplitz RL, Wallace RB: Detection of sickle cell βS-globin allele by hybridization with synthetic oligonucleotides. Proc Natl Acad Sci USA 80:278–282, 1983
11. Southern E: Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517, 1975
12. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Erlich HA, Arnheim N: Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354, 1985
13. Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA: Primer directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491, 1988
14. McCabe ERB, Huang S-Z, Seltzer WK, Law ML: DNA microextraction from dried blood spots on filter paper blotters: Potential applications in newborn screening. Hum Genet 75:213–216, 1987
15. Jinks DC, Minter M, Tarver DA, Vanderford M, Hejtmanek JF, McCabe ERB: Molecular genetic diagnosis of sickle cell disease using dried blood specimens on blotters used for newborn screening. Hum Genet 81:363–366, 1989
16. Rubin EM, Andrews KA, Kan YW: Newborn screening by DNA analysis of dried blood spots. Hum Genet 82:134–136, 1989
17. McCabe ERB, Zhang Y-H, Descartes M, Therrell BL, Erlich HA: Rapid detection of βS DNA from Guthrie cards by chromogenic probes. Lancet ii:741, 1989
18. Descartes M, Zhang Y-H, Huang Y, McCabe L, Therrell BL, McCabe ERB: Direct genotypic analysis for sickle cell disease using DNA from newborn screening specimens: Correlation with results from the state laboratory. Pediat Res 27:130A, 1990
19. Chehab FF, Kan YW: Detection of sickle cell anaemia mutation by colour DNA amplification. Lancet i:15–17, 1990
20. Koenig M, Hoffman EP, Bertelson CJ, Monaco AP, Feener C, Kunkel LM: Complete cloning of the Duchenne muscular dystrophy (DMD) cDNA and preliminary genomic organization of DMD gene in normal and affected individuals. Cell 50:509–517, 1987

21. Baumbach LL, Chamberlain JS, Ward PA, Farwell NJ, Caskey CT: Molecular and clinical correlations of deletions leading to Duchenne and Becker muscular dystrophies. Neurology 39:465–474, 1989

22. Chamberlain JS, Gibbs RA, Ranier JE, Nguyen PN, Caskey CT: Deletion screening of the Duchenne muscular dystrophy locus via multiplex amplification. Nucl Acids Res 16:11141–11156, 1988

23. Chamberlain JS, Gibbs RA, Ranier JE, Caskey CT: Multiplex PCR for the diagnosis of Duchenne muscular dystrophy. In PCR Protocols—A Guide to Methods and Applications. Edited by MA Innis, DH Gelfand, JJ Sninsky, TJ White. New York, Academic Press, 1990, pp 272–281

24. Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, Cole JL, Kennedy D, Hidaka N, Zsiga M, Buchwald M, Riordan JR, Tsui L-C, Collins FS: Identification of the cystic fibrosis gene: Chromosome walking and jumping. Science 245:1059–1065, 1989

25. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou J-L, Drumm ML, Iannuzzi MC, Collins FS, Tsui L-C: Identification of the cystic fibrosis gene: Cloning and characterization of complementary DNA. Science 245:1066–1073, 1989

26. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui L-C: Identification of the cystic fibrosis gene: Genetic analysis. Science 245:1073–1080, 1989

27. Lemna WK, Feldman GL, Kerem B, Fernbach SD, Zevkovich EP, O’Brien WE, Riordan JR, Collins FS, Tsui L-C, Beaudet AL: Mutation analysis for heterozygote detection and the prenatal diagnosis of cystic fibrosis. N Engl J Med 322:291–296, 1990

28. Beaudet AL, Kazazian HH, Panel: Statement from the National Institutes of Health Workshop on Population Screening for the Cystic Fibrosis Gene. N Engl J Med 323:70–71, 1990

29. Williams C, Weber L, Williamson R, Hjelm M: Guthrie spots for DNA-based carrier testing for cystic fibrosis. Lancet ii:693, 1988

30. Holtzman C, Slazyk WE, Cordero JF, Hannon WW: Descriptive epidemiology of missed cases of phenylketonuria and congenital hypothyroidism. Pediatrics 78:553–558, 1986

31. American Academy of Pediatrics, Committee on Genetics: New issues in newborn screening for phenylketonuria and congenital hypothyroidism. Pediatrics 69:104–106, 1982

32. National Committee for Clinical Laboratory Standards: Blood Collection on Filter Paper for Neonatal Screening Programs: Tentative Standards (NCCLS publication LA4-T). Villanova, PA, NCCLS, 1985