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New Technologies in Newborn Screening

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In our Supplemental Newborn Screening Program in western Pennsylvania, we have introduced not only a unique approach to newborn screening, but also some innovative concepts in follow-up confirmation and prognostic diagnosis. The Pennsylvania Department of Health routinely screens only for phenylketonuria and congenital hypothyroidism. In an attempt to extend this basic state-mandated screening program, we developed, in 1986, the concept of an independent supplemental fee-for-service screening program. At present we are screening 37 hospitals plus a number of nurse midwifery programs in western Pennsylvania and eastern Ohio. We routinely screen for 11 inherited metabolic disorders: sickle-cell disease, congenital adrenal hyperplasia, galactosemia, biotinidase deficiency, maple syrup urine disease, homocystinuria, cystic fibrosis, Duchenne muscular dystrophy, glucose-6-phosphate dehydrogenase deficiency, pyroglutamic aciduria, and arginase deficiency. Having the opportunity to design our program from the start has permitted us to include screening for conditions with extensive worldwide experience and acceptance, as well as for lesser-known and less widely accepted conditions. It has also allowed us to develop and introduce some exciting new technologies as part of our routine confirmation procedures.

Newborn screening for cystic fibrosis (CF), using immunoreactive trypsinogen (IRT), was first introduced in New Zealand over ten years ago [1]. Today, well over 3.2 million newborns have been screened worldwide, using this procedure [2]. In the United States, screening is only being done in Colorado, Wisconsin, at 13 hospitals in Conneticut, and by our program. A major justification for newborn screening for CF has been that at least half of the patients with CF go undiagnosed during the first year of life, while 25 percent remain undiagnosed by the end of their second year. In addition, a number of clinical benefits have been shown to be associated with early diagnosis and early treatment [3–5].

One limitation of newborn CF screening using IRT is the age-dependence of the elevation. By three to six months of age, the pancreas has begun to burn out or has, in fact, already burned out, with the result that reliable screening and retesting is limited to the early weeks or months of life; that circumstance requires a declining cut-off with increasing age. This complexity potentially increases the risk of false-negatives. A second problem with newborn IRT screening is a higher than comfortable false-positive rate. Depending on the program, between two and ten patients are referred for sweat testing in order to confirm one case of CF. While this number is not an extremely high false-positive rate as screening tests go, it is higher than we would like because of the severe degree of anxiety created for the family. Therefore, if there is anything that we can do to decrease this false-positive rate, while at the same time increasing our detection rate, we have attempted to do it.

In the case of newborn CF screening, our approach has been to use molecular genetic techniques to back up our initial IRT results. Using polymerase chain

Abbreviations: CF: cystic fibrosis DMD: Duchenne muscular dystrophy IRT: immunoreactive trypsinogen PCF: polymerase chain reaction

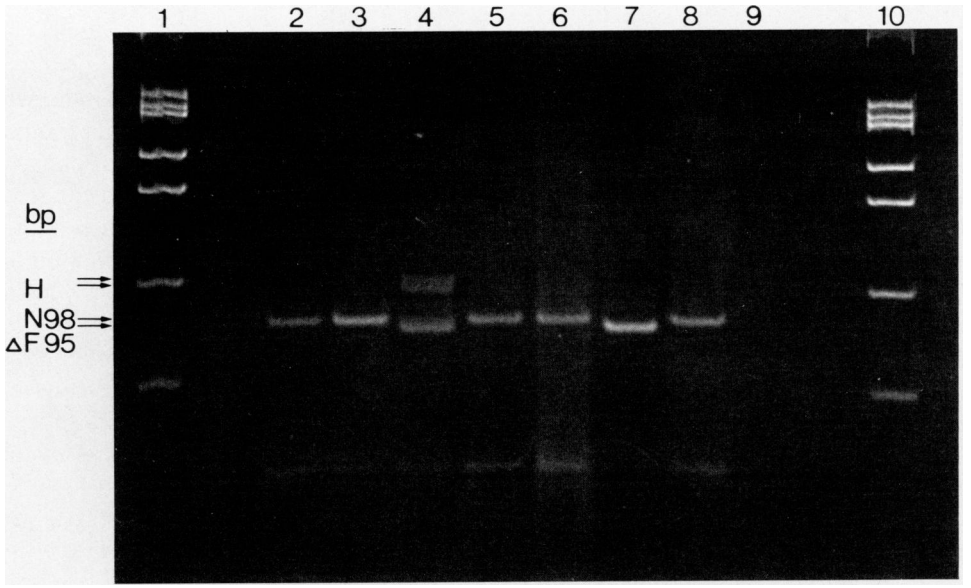

FIG. 1. Diagnosis of cystic fibrosis: amplification product of DNA, using the ΔF508 mutation as a primer from neonatal specimens (lanes 2–8). The reaction product of the negative control (no DNA) is seen in lane 9. The amplified product of an individual heterozygous for the deletion is in lane 4; one homozygous for the deletion is in lane 7. Lanes 1 and 10 contain the DNA size markers ϕX174 Hae III restriction fragments.

reaction (PCR) amplification of the DNA eluted from a single filter paper blood spot, we are able to screen for the presence of the common three base pair deletion (delta F508) associated with CF [6]. Among known patients with CF, 50 percent are homozygous for this deletion, while another 41–44 percent are mixed heterozygotes for the delta F508 deletion plus a second mutant allele. The remaining 6–9 percent have two copies of mutations other than the delta F508 deletion. This information is illustrated in Fig. 1.

Since January 1990, we have been performing PCR amplification for the delta F508 deletion on every filter paper blood specimen in which we find an elevated IRT. We expect that this practice will improve both the sensitivity and specificity of our CF screening program in two ways. The ability to combine IRT elevations with delta F508 status should reduce the risk of a potential false-negative resulting from an inaccurate sweat test. We also hope to be able to redefine our IRT screening cut-off level and follow-up criteria, using a combination of IRT and delta F508 status, in the hope of reducing the number of false-positives that are referred for sweat testing.

A second area where we have introduced new molecular genetic technologies as part of our routine confirmation procedures is in our newborn screening program for Duchenne muscular dystrophy (DMD). The worldwide screening experience, which uses elevated creatine kinase as a marker, is not nearly as extensive as that for CF and many other disorders. Nevertheless, over 800,000 newborns have been screened, primarily in West Germany, France, Manitoba, Canada, and New Zealand [7–10]. Our program has screened approximately 73,000 newborns, including 20,000 specimens from Sao Paulo, Brazil, through March 31, 1990. We have had 111 initial

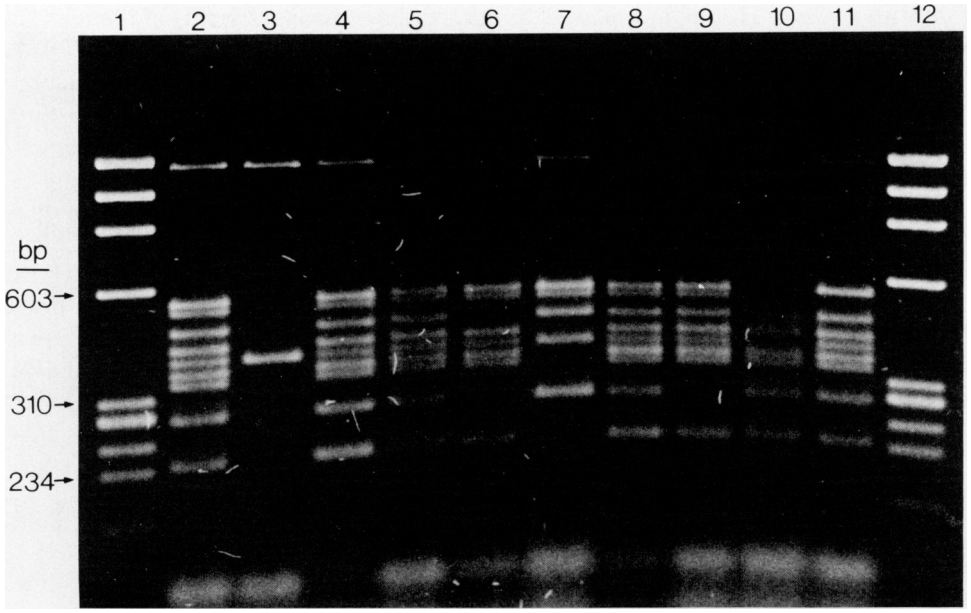

FIG. 2. Diagnosis of Duchenne muscular dystrophy (DMD): Multiplex amplification products of DNA from eight neonatal specimens (lanes 4–11), using the DMD gene as primer. Lane 2 demonstrates the nine normal bands generated by DNA from a normal individual. Lane 3 contains DNA from an individual with a deletion of eight of the nine exons in the DMD gene. Lanes 1 and 12 contain the DNA size markers ϕX174 Hae III restriction fragments.

elevations, 12 persistent elevations on repeat testing, and ten confirmed cases of DMD, giving us an incidence of 1/7,300 newborns of both sexes.

Unlike the situation with CF, we are not primarily concerned about eliminating false-positives or false-negatives with DMD screening. Rather, we are trying to provide rapid confirmation of the diagnosis as early as possible, preferably before the patient is even referred to a DMD clinic for clinical evaluation and muscle biopsy. To do this, we are using multiplex PCR amplification of DNA from the initial filter paper blood specimens of newborns with two creatine kinase elevations [11,12]. Due in part to very large size, the gene mutations responsible for DMD can be directly identified in 65 percent of patients as deletions of specific exons within the dystrophin gene [13]. Using multiplex PCR, we are able to identify 80 percent of these deletions and, therefore, confirm the diagnosis in approximately half of our patients before we even request a follow-up quantitative serum creatine kinase. We feel that this confirmation helps significantly in the early management of the patient and family. Figure 2 illustrates an example of a multiplex PCR amplification of a newborn DMD patient with a significant deletion.

As part of an expanded confirmation protocol, we routinely request quantitative serum creatine kinase analysis, clinical evaluation, family history, and routine histology of a muscle biopsy for confirmation of the diagnosis. We also carry out additional Southern blot analysis on the patient, using various probes to define further and characterize the mutation or deletion at the DNA level. This practice, together with the multiplex PCR studies, can provide the basis for carrier testing of at-risk family members and potentially has some prognostic value. Also, in collabora-

tion with Dr. Eric Hoffman, we are determining the dystrophin status of the muscle, using both immunoblot and immunofluorescent assays on biopsies [14–15]. This technique permits us to distinguish the more severe DMD from the milder Becker muscular dystrophy in an asymptomatic newborn with no positive family history. Without this expanded follow-up protocol, one might have to wait for years to determine the patient's prognosis.

In summary, what we have done is to adapt several molecular genetic techniques for use with DNA eluted from filter paper blood specimens and to incorporate these into the routine follow-up and confirmation protocols for our supplemental newborn screening program for DMD and CF. While we are not using multiplex PCR or delta F508 analysis for primary screening or carrier testing, we are using these techniques to improve the sensitivity and specificity of our existing creatine kinase and IRT screening for DMD and CF, respectively. As automated procedures are developed and as the cost decreases, however, we are optimistic that the use of these or similar molecular tests on filter paper blood specimens will become practical for primary screening in the not too distant future.

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