Using DNA-based Technologies in Newborn Screening
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Meeting Charge
Thomas Murray, PhD
President, The Hastings Center

The meeting was intended "to provide a clear a picture as possible of the scientific, technical, clinical, and the economic landscape" of these technologies. The meeting was not planned to "tease out" the medical, social, and ethical implications of the use of DNA-based technologies in newborn screening (NBS), explained Dr. Murray, but rather to address the science directly.

Introductions

The participants introduced themselves, identified their institutions, and explained their interest in the topic. A list of participants is provided at the end of this document.

Overview of the Convergence of NBS and New Technologies
R. Rodney Howell, MD
Special Assistant to the Director, National Institute of Child Health and Human Development

Dr. Howell opened by telling participants that this discussion was intended to explain a little about the history of NBS, its current status, a recent study conducted by the American College of Medical Genetics (ACMG) under a contract from the Health Resources and Services Administration (HRSA), and about some of the conditions that at present require new technology before they can be effectively introduced into NBS programs.

Dr. Howell began his review of the history of NBS by describing the discovery of phenylketonuria (PKU) in 1934. Working with patients who were profoundly retarded, Dr. Asbjorn Folling of Norway surveyed severely retarded patients with simple screening tests. Dr. Folling discovered that phenylpyruvic acid, which is excreted in the urine of children with untreated PKU, turns green in the presence of ferric chloride. Later, it was shown that these patients lack the enzyme that converts phenylalanine to tyrosine, and excrete large amounts of phenylpyruvic acid due to this blockage.

In 1953, Horst Bickel of Germany, working with colleagues in Birmingham, England, developed a low-phenylalanine diet as the first treatment of PKU. The phenylalanine-free

diet was developed by first removing all the essential aromatic amino acids, then putting a very strictly controlled amount back in order for growth and development to occur without damage. Bickel and his colleagues discovered that the earlier the patient started on the diet, the better his or her health outcome. Thus, the possibility was considered that NBS might be useful in establishing who needed to go on the diet as early as possible. Clearly, early detection would be a way to identify patients with PKU at a point when they could receive the most effective treatment; however, mass screening was considered to be impractical. Over the years, it became popular to look at patient populations using simple screening tests for PKU.

In 1959, Dr. Robert Guthrie developed a simple test using a dried blood spot that could be collected from a baby at birth and a bacterial inhibition assay. This made mass screening of all newborns possible. In this test, small circles are punched out of the blood spot so the phenylalanine levels can be measured. In many places in the world, the Guthrie test is still used, especially since it has been expanded over the years to include other metabolites. At the same time, the field of medicine began to discuss when NBS should be used. The fundamental criteria for screening became those established by J.M.G. Wilson and G. Jungner in *Principles and Practice of Screening for Disease* (Geneva: World Health Organization (WHO), 1968) and have remained the standard since the publication of this work.

In their preface, Wilson and Jungner wrote that the book was commissioned by the WHO because screening for disease is now a subject of growing importance in developed countries. Wilson and Jungner further stated that "screening is an admirable method of combating disease, since it should help detect it in its early stages and enable it to be treated adequately before it obtains a firm hold on the community."

The criteria outlined by Wilson and Jungner are as follows:

- The condition sought should be an important health problem.
- There should be an accepted treatment for patients with recognized disease.
- Facilities for diagnosis and treatment should be available.
- There should be a recognizable latent or early symptomatic stage.
- There should be a suitable test or examination.
- The test should be acceptable to the population.
- The natural history of the condition, including development from latent to declared disease, should be adequately understood. This criterion has rarely been satisfied, Dr. Howell noted.
- There should be an agreed policy on whom to treat as patients.
- The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
- Case-finding should be a continuing process and not a "once and for all" project.

As a result of these advances, and still largely using these criteria, routine NBS has been carried out in all 50 states since the 1970s. PKU was the test with which the hospitals in

the United States started, and tests for congenital hypothyroidism and a handful of other diseases have been added on a state-by-state basis.

NBS guidelines vary extraordinarily from state to state, and little systematic evaluation of either the rationale for screening and/or the outcomes of such screening has been conducted. In March of 2003, the General Accountability Office reported to Congress that more than 4 million infants were screened annually in this country, making NBS by far the most common type of genetic testing. Most states have similar programs for funding and administering their programs, supported largely through fees collected by health care providers. Dr. Howell noted that the primary reason that states do not screen for specific illnesses is that they cannot afford to do so. The state that has the largest mandated number of screening tests today is Mississippi. Dr. Howell reviewed a chart of the disorders screened in the United States.

In 2001, the Maternal and Child Health Bureau of HRSA contracted with ACMG to convene an expert group to evaluate the scientific and medical information related to screening for specific conditions and to make recommendations based on this evidence. A widely representative group of 125 individuals worked over a two-year period to complete this report, which is to be released in late 2004-early 2005. The report identifies two predominant goals, with additional goals that were interrelated and supportive of the primary goals. The first of the primary goals was the establishment of a uniform condition panel for NBS programs. The second goal was the development of a model decision matrix for program expansion of testing for other diseases.

The report identifies these overriding principles for the development of NBS guidelines:

- Universal NBS is an essential public health responsibility that is critical to improve the health outcome of affected children.
- NBS policy development should be driven by what is in the best interest of the affected newborn, with consideration of the interests of unaffected newborns, families, health professionals, and the public.
- NBS is more than testing; it is a coordinated and comprehensive system consisting of education, screening, follow-up, diagnosis, management, and program evaluation.
- The medical home and the public and private components of the screening programs should be in close communication to ensure confirmation and the appropriate follow-up and care of identified individuals.
- Evaluation and recommendation of conditions appropriate for NBS should be based on scientific evidence and expert opinion. (The report, Dr. Howell commented, includes all the scientific evidence relevant to diseases that are tested. Some, however, are so rare that there is little or no scientific evidence for them.)
- To be included in a NBS program, a condition should meet the following criteria:
    - It is identifiable at a phase in which it would not ordinarily be recognized clinically.
    - There is an available test with appropriate sensitivity and specificity.

    - There are demonstrated benefits of early detection and timely intervention.
- The primary targets of NBS should be conditions that meet the criteria listed in the previous bullet item. The NBS program also should report any other result of clinical significance.
- There should be centralized data collection for longitudinal assessment of disease-specific screening programs.
- Total quality management should be applied to all NBS programs.
- NBS specimens are valuable health resources and are preserved in many states. Every program should have a policy to ensure confidential storage and appropriate use.
- Public awareness coupled with professional and family education and training are significant program responsibilities that must be part of the complete NBS system.

These criteria were developed to "score" the value of the disease screening. The ACMG group agreed that screening for medium-chain acyl-CoA dehydrogenase deficiency (MCAD) was necessary and identified 29 conditions that also met the criteria. Tandem mass spectrometry provides a great deal of useful information and identifies a number of results that relate to disease conditions about which little is known. The group agreed that any information that could be valuable should be reported whether a treatment is available or not.

The report listed a number of conditions that were excluded from recommended testing because no test is available. Dr. Howell noted in particular severe combined immunodeficiency disease (SCID) and Pompe disease. In the latter disease, the average age of diagnosis is five months and the average age for death is seven months, although lifesaving treatment is becoming available. Clearly, this is an area where the technology needs to be developed to provide appropriate NBS tests.

Some of the significant barriers to the creation of a model NBS system in the United States include:

- Financing across state and county lines that is constrained by Medicaid rules;
- Fragmentation on a disease basis;
- Insufficient support to bridge geographic barriers;
- The lack of experienced providers for complex care;
- The requirements of the Health Insurance Portability and Accountability Act of 1996;
- Underutilization and lack of uniformity of information technology;
- Constraints on collaborative management and care imposed by systems of reimbursement;
- Variability in state mandates;
- State sovereignty; and
- Variability in the financing of screening programs.

The need to identify other diseases during the newborn period is rapidly growing, Dr. Howell emphasized, because therapies are being developed for previously fatal, untreatable diseases, including:

Pompe Disease
Hurler Syndrome
Morquio Syndrome
Fabry Disease
SCID, and
Duchenne Muscular Dystrophy, for which there are treatments in development.

These diseases will require new technologies for the identification of products such as lysosomal enzymes and structural proteins. To this end, Department of Health and Human Services Secretary Tommy G. Thompson has appointed the Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children, which was mandated in the Children's Health Act of 2000. According to this legislation, "This Committee shall advise and guide the Secretary regarding the most appropriate application of universal NBS tests, technologies, policies, guidelines, and programs for effectively reducing the morbidity and mortality in newborns and children having, or at risk for, heritable disorders." A number of members of the HRSA/ACMG contract committees have been appointed to this national advisory committee and this work will aid the development of a more standardized approach to NBS.

In view of the rapidly changing landscape in NBS, the National Institutes of Health's (NIH's) National Institute of Child Health and Human Development is launching a new program to examine its scientific bases. This program will consider what would be required to put into place the testing and other required systems necessary to perform NBS for all genetic conditions that would have clinical value to children and their families.

Technical Tools, Opportunities, and Challenges

DNA Technologies
Robert Vogt, MD
Newborn Screening Branch of the Division of Laboratory Sciences, National Center for Environmental Health

Dr. Vogt opened by noting that the he represents the laboratory branch at the Centers for Disease Control and Prevention (CDC) that operates the Newborn Screening Quality Assurance program. All the states and territories are enrolled in this program, which also interacts with more than 300 laboratories in 60 nations around the world. This is a very active program in a small branch.

Dr. Vogt indicated that one of the most important considerations for laboratories working in the field of NBS is cost. To re-emphasize what Dr. Howell had noted, Dr. Vogt

reminded the group that NBS is more than testing. Getting the samples to the laboratory and conducting follow-up on positive results may cost more than doing the actual bench work involved. The cost of conducting the test, in other words, may not be the major contributor to the cost of NBS. Even so, the impact of adding new tests to an NBS panel must be considered carefully, since NBS laboratories are under the constant pressure of a large sample load and the demand for rapid turnaround.

No public health newborn screening laboratory in the United States at present uses DNA testing as a first-tier screening. Many, however, conduct DNA screening on a confirmational or research level. Molecular biology is a relatively new and specialized area of laboratory science, and graduates of training programs are in high demand. The need to staff public health newborn screening laboratories with trained molecular biologists may raise the costs of human resources at the laboratory level.

The production of quality control and proficiency testing materials in quantities that can be used for DNA-based tests by hundreds of different laboratories also presents special challenges. CDC is in the process of developing alternative approaches for producing these materials, such as spiking blood with cultured cells having known genetic mutations.

A major concern of any DNA-based testing facility is contamination of laboratories. This is a special problem for NBS laboratories, since widespread contamination may require a complete shutdown of the affected facilities. CDC and some of the more recently-constructed state public health laboratories have specially-designed facilities to avoid contamination, but other public health laboratories that do not have such facilities may be reluctant to engage widely in DNA-based NBS testing.

In terms of detection strategies, the signals that are measured in the laboratory are often the same regardless of the analyte used. Radioactive DNA probes, the original approach to molecular biology, are now used only in research-based settings, since radioisotopes presents biosafety and environmental concerns. Most DNA-based tests use fluorescence-labeled probes, which provide excellent sensitivity and are safe to handle and dispose. One of the most common microtiter plate technologies currently used in NBS laboratories is fluorescence based, and the reagents and systems for conducting this kind of test are already commercially available for selected DNA analytes. Further improvements can be anticipated from newly-developed fluorochromes and fluorometer technology incorporating light emitting diodes (LEDs) and charge coupled devices (CCDs). These systems can be coupled with robotic sample handling and large-capacity microtiter plates. The maximum utility of fluorescent-based technology will probably be realized over the next decade.

Two types of arrays to be considered in DNA-based testing are the surface array and the microbead suspension array. The “chip” can be considered a subset of the surface type of array; it involves the examination of individual units on a surface, using probes point by point to measure the degree of hybridization. Most scientists think of micro-arrays as having tens of thousands of targets. But a slide can be made into what one might consider

a "super" microtiter plate. That is, instead of using 30,000 targets, 30,000 patient samples can be put on a slide and overlaid with a solution containing a few selected probes. This "reverse microarray" promises to be a highly economical approach.

The microbead suspension array is a highly-flexible technology because different populations of microbeads can be easily labeled with a DNA probes and mixed together to form customized application-specific arrays . The system that is most well known is the Luminex system, which can measure many as 100 different hybridization targets in a single suspension of microbeads that have been "fluorescent bar-coded." A number of the human leukocyte antigen test (HLA) laboratories doing cross-matching in preparation for organ transplants are using a Luminex system to do medium- to high-resolution HLA genotyping. These systems are considered practical and cost effective in clinical use.

Over the previous few weeks, Dr. Vogt had the opportunity to work with assays based on real-time polymerase chain reaction (also called "quantitative PCR"). For this process, fluorescence is measured after each amplification cycle. Real-time PCR has two advantages: the fact that researchers can observe the amplification as it occurs, which generates more confidence in the result, and the fact that there is no need to worry about laboratory contamination, because the PCR amplification occurs only in sealed tubes that are disposed of after reading. One of the participants later noted that PCRs are notorious for nonamplification, which can be resolved only through the use of gels. The question becomes one of how to incorporate a quality control measure to ensure that what is intended for amplification is in fact what is amplified. Dr. Vogt said that the only way to provide this assurance is to use a mature and locked-down system. Those working in real-time PCRs do not run gels because they have an inadequate level of confidence in the results. Laboratory at both NIH and CDC, he noted, have developed real-time PCR assays to evaluate dried blood spots for severe combined immunodeficiency disorder (SCID). These assays include controls to insure PCR amplification.

Regarding information and interpretation, Dr. Vogt commented that measures of population sensitivity, specificity, and predictive value must be based upon well-defined endpoints. In terms of DNA analysis, the question being addressed is that of the health endpoint. The NBS laboratory has the difficult task of assuring normalcy. How can a DNA test assure a normal health status based on *not* finding a genetic abnormality? There is no way to be certain that every possible combination of genetic markers, as well as the effect of the environment can be taken into account. Since phenotypic measures are more closely related to the actual expression of the disease process, the most appropriate use of DNA testing may be for confirmatory follow-up (as currently practiced for hemoglobinopathies and cystic fibrosis) or to determine inborn risk factors that influence the probability of future disease. Dr. Vogt concluded that DNA testing will play an increasing role in NBS but is unlikely to supplant phenotypic tests entirely.

Microarrays
John F. Palma, PhD
Director, Molecular Diagnostics R&D at Affymetrix, Inc.

Microarrays are one of the new technologies that will be useful to the NBS community and will help revolutionize the characterization of heritable, treatable newborn illnesses. The needs of this community are affected by a number of factors, including:

- The development of specific types of assays that can in a parallel manner address the research/clinical question with improved accuracy, reliability, and sensitivity;
- Anticipation of the types of content that can be generated in the future and the development of a platform flexible enough to incorporate that content in the future;
- The economic feasibility of and access to the technology developed; and
- Clarity regarding how the information will be used in a clinical setting.

Dr. Palma told participants that issues that private companies must address regarding these technologies include the development of:

- A NBS test that is sensitive to regulatory, social and advocacy groups;
- Custom assays that allow researchers to obtain quality genetic information from the most reliable platforms
- Reporting systems that are required to deliver that information.

Dr. Palma proceeded to describe the products that his company offers. Invented over a decade ago using photolithography, it involves a glass wafer that can be diced up into tens, hundreds, and even thousands of individual square chips, also called arrays. Each square is packaged into a cartridge, and the sample is then hybridized to the array. Each array contains millions of DNA probes. The arrays that are designed to perform gene expression experiments reflect all of the well annotated genes of the human genome. The arrays focused on genotyping applications, or those that examine DNA rather than RNA, examine the genome more directly–identifying thousands of single nucleotide polymorphisms (SNPs) in a single experiment. Essentially, the power of the technology is its ability to simultaneously answer many questions about what any given sample looks like at a genetic level.

In the future, incorporation of a wide range of genetic tests on a single array will require the use of what Dr. Palma calls "real estate," or space on the array. Currently, the company will be able to make features that are 8 microns in size, resulting in up to 2½ million features on each array. This is an evolving technology, he explained, that can be used as necessary to address other scientific and clinical issues. Future technology development will drive the features to 5 microns or less to allow the inclusion of newly characterized screening tests.

In terms of cost, manufacturing each wafer represents a fixed cost. As features shrink and more and more data can fit onto a single wafer, it will be possible to dice that wafer up into hundreds or thousands of individual chips. Over time the cost will continue to fall as the amount of content included on each array continues to increase. This is a flexible format that ultimately will allow the production of high-resolution, cost-effective genome

scanning chips. Dr. Palma's company has also developed a high-throughput technology to ask the same questions using a 96-well format more suited to industrial or high-volume laboratories. This technology will not only reduce costs by reducing the human effort involved in processing samples, but it also will affect configurations by using smaller volumes.

The company has developed an assay by which, in a single tube, it is possible to query 100,000 SNPs at one time and hybridize an array that will provide the profiles for all 100,000 SNPs. Over the next year this technology will be scaled to represent 500,000 SNPs. It is possible to build redundancy into the test by examining a SNP or genomic region of interest using multiple different probes. This increases the confidence in each data point. The company also has developed a number of resequencing methods that will be critical to the NBS community. Dr. Palma concluded by noting that the ability to use DNA as a primary test in NBS will depend on the quality of arrays, accuracy, automation, and content.

Analytic Validity *CFTR* Mutation Testing in a Prenatal Screening Program for Cystic Fibrosis
Glenn Palomaki, BS, BA
Director of Biometry/Epidemiology at the Foundation for Blood Research

Mutations in the CFTR (Cystic fibrosis transmembrane conductance regulator) gene are associated with the presence of cystic fibrosis. Dr. Palomaki opened by differentiating between analytic validity, analytic specificity and analytic sensitivity. The latter is the proportion of positive test results correctly reported by the laboratory among samples with mutations that the laboratory's test is designed to detect. Analytic specificity is the proportion of negative test results correctly reported among samples with no detectable mutation present. For quality control, the procedures for ensuring that results fall within specified limits are assessed. Assay robustness is determined by how resistant the assay is to changes in pre-analytic and analytic variables (e.g., sample degradation).

The current sources of data for computing analytic validity raise more issues:

- Method comparisons are of limited use; usually, only two methods are compared.
- Pre-analytic errors may not be reported.
- Small numbers of samples are tested.
- A "true" genotype often is unknown.
- These current sources may not represent actual clinical practice.
- External proficiency testing schemes are the only major reliable sources currently available for computing analytic sensitivity and specificity.

The advantages include:

- Most clinical laboratories participate.
- A wide range of methodologies are represented.
- Samples have confirmed genotypes.

The disadvantages include:

- Over-representation of 'difficult' samples due to 'educational' nature of the program.
- Mixing of 'screening' and 'diagnostic' challenges Generally, ? 508 women will not be pregnant.
- Limited number of DNA tests covered.
- 'Research,' 'manufacturers', and 'non-U.S.' laboratories participate.
- Artificial nature of sample preparation, shipping, and handling. This is not currently a problem.

The laboratories were challenged with mutations for which they already test, and it was noted that it is best to focus on false negatives. The analytic sensitivity for CFTR mutations in a prenatal population of 100,000 rose from 97.9 percent to 98.2 percent. Of this group, 400 have the mutation, and 350 of that group have a panel that can be identified. The others have mutations that cannot be identified.

Inevitably, some of this group includes carrier couples and affected fetuses who will be identified when they are diagnosed, but the parents have been screened and not identified. One example already has been reported in the literature (Cunningham S *et al*., Arch Dis Child 1998;78:34508). Confirmatory testing is not helpful, as negatives are not subject to such efforts. A detectable mutation is uncommon in the population (1 in 60 chromosomes) but common in the survey (1 in 2 chromosomes). The rate of wrong mutations found in the survey should be discounted by a factor of 30.

Dr. Palomaki emphasized the need to identify false-positive carrier couples. The analytic specificity is 99.7. The incidence of false negatives is more common than that for false positive results, and this must be taken into account when estimating specificity.

Participants briefly discussed the complications of and knowledge base governing the research. Although the technology is stable, more work needs to be done to ensure validity. One of the participants noted that although some of the technology has been around for a long time, NBS was not considered marketable in the past. Another participant responded that NBS was now considered more marketable because the ACMG had called for carrier testing in cystic fibrosis (CF). After this, private companies began to explore other opportunities, and NBS became a major market in Europe. The British, French, and German governments have recognized it as a standard of care, and while markets in Europe do not have to go through the Food and Drug Administration to get tests approved, there are a number of hurdles that need to be overcome in other countries.

In addition, supporters of NBS have become an organized community, which has helped facilitate market entry. Finally, the technology has evolved to the point that it is less risky to enter more sensitive market areas and can be used in a variety of other markets.

There are differences between selecting a CFTR mutation panel for prenatal screening and selecting one for newborn screening. In the former, the panel must be limited to known highly penetrant alleles, because one available option when an affected fetus is identified is selective termination of the pregnancy. In the latter, the identification of a "mild" mutation(s) (or even a polymorphism) would have less impact, most likely including incorrect labeling and unnecessary sweat testing.

Clinical, Social, and Economic Implications

Cystic Fibrosis and Other Disorders
Wayne Grody, MD
Professor in the Departments of Pathology and Laboratory Medicine, Pediatrics, and Human Genetics, UCLA School of Medicine

Dr. Grody began by noting that he has not been as involved in NBS as he has been in carrier screening, but that he believed there are many lessons that have been learned regarding carrier screening that would be useful to participants.

He began by noting that CF screening was the first mass population carrier screening program to identify recessive traits. The carrier rates are high in all populations, and the only way to identify them is at the DNA level.

The gene for CF is extremely complex, with more than 1,300 mutations spread across it, and considerable debate occurred regarding how the testing would be marketed. In a pilot study, a number of factors were considered, and participants in that study were screened for the six most common mutations of the gene. The research indicates that specific allele probe results vary according to ethnic group. Participants in the study were educated about the meaning of results and received in-person counseling.

In 1997, an NIH consensus committee issued a report that addressed prenatal care. The committee recommended that CF carrier screening be offered to all pregnant couples and those planning pregnancy, but did not recommend how many mutations should be tested. Once the recommendation was published, a number of companies became interested in marketing platforms for testing; however, none of the tests that have been developed has entered the FDA's approval process. In addition, only approximately 50 percent of obstetricians are offering the 25-mutation panel, although it is supposed to be the standard of care.

During the course of the research, a mutation on the panel that was not yet known to exist was discovered and it was found to be rarer than was initially thought. Another mutation was found to be 100 times more common than expected. In fact, it turned out not to be a mutation at all, but rather a benign polymorphism.

The best data available were derived from research being conducted at Kaiser:

- 30,000 patients were screened.
- 1,776 carriers were detected (1/28).
- There were 50 at-risk couples and 63 pregnancies.
- The incidence of CF births in the population was reduced by half.

Dr. Grody reminded the group that this research was based on carrier screening, but he also emphasized that some of the science may be applicable to NBS. He explained the strategies that some states have used by presenting the following chart:

Newborn Screening Strategies

First Tier	Second Tier	Third Tier
IRT*	Sweat test	
IRT	IRT	Sweat test
IRT	F508	Mutation panel, sweat test
IRT	Mutation panel	Sweat test

*IRT refers to the immunoreactive trypsin test, a blood test for elevated levels of trypsinogen.

CF screening remains controversial. However, its pros and cons are important to consider. The potential benefits of including CF in NBS are improved nutrition and growth, prevention or delay of lung disease, prevention of salt depletion, and the opportunity to make timely reproductive decisions. Potential risks, however, include:

- The use of overly aggressive/inappropriate prophylaxis;
- Earlier lung infections (*Pseudomonas*);
- Miscommunication of results;
- Inadequate genetic counseling resources;
- Identification of child, carriers, and parental obligate carriers; and
- Revelation of false paternity.

A highly promising area of research lies in the work being done at the University of California, Los Angeles, on newborn hearing screening. At present, infants must fail three consecutive tests before they are referred to audiology services, which means time is lost during which a critical intervention could have occurred. The logic behind conducting this study is that recessive mutations are responsible for 50 percent of congenital nonsyndromic deafness. The study is using Connexin-26 variants, and more than 90 variants have been described (http://www.crg.es/deafness/). These tests are designed to:

- Determine the etiology of hearing loss in as many of 50 percent of babies with nonsyndromic hearing loss;
- Eliminate the need for expensive additional medical workup in babies known to have GJB2-related hearing loss;
- Encourage more timely referrals for audiologic intervention; and
- Provide information about recurrence risk for parents.

Carrier frequency in the general population is 3 percent. The pickup rates in Hispanics are low. The study identified the following carrier frequencies of common Connexin-26 mutations:

35delG	Caucasian	~1/33
167delT	Ashkenazi Jewish	~1/25
235delC	Asian	~1/100

In general, Dr. Grody concluded, guidelines for including a disorder in NBS should include the following elements:

- The disorder must be relatively common.
- The disorder must be serious.
- The natural history of the disorder must be consistent and well defined.
- There must be an effective treatment that should be initiated early in life.
- The screening test must be acceptable to the population, technically reliable, and relatively inexpensive.
- Resources must be in place for rapid communication of positive results, tracking, follow-up of affected infants, and expedient delivery of medical care and genetic counseling.
- There must be high predictive value between the abnormal analyte being detected and the phenotypic outcome.

Discussion

One of the participants inquired whether, considering the diversity of the populations to be tested, there was some governance that could be provided regarding adding tests to the panels. The committee that made the recommendation of 25-mutation test for CF has struggled with other disorders that meet the criteria. The question is whether there are usable panels specific to different ethnicities or whether the disorders occur at great enough frequency to warrant inclusion.

A question also was raised about how to increase obstetricians' use of the testing for CF. Dr. Grody responded that as a rule this community has been very intimidated by the nature of genetic testing and considers CF a pediatric issue rather than an obstetrical one. Another concern is that obstetricians do not fully understand the nature and interpretation of the testing, largely because of communication breakdowns between obstetricians and

parents. Pediatricians, on the other hand, are beginning to use tests and genetic counseling effectively.

What sort of resources would be required to do some level of genetic counseling *prior* to the test? For the hearing test study, for which pretest counseling was required, the child already has undergone some testing; therefore, some counseling is necessary. However, if DNA testing becomes a first-tier test, the issue becomes more complicated. In addition, some populations do not consider deafness a disease and would respond negatively to what they might consider an intrusion.

Another concern involves determining an appropriate clinical response to positive test results. The communication between parents and clinicians is critical, but also delicate. Over-reaction can be detrimental to the child, resulting in stigmatization.

Costs
Scott Grosse, PhD
Health Economist, Centers for Disease Control and Prevention, Department of Health and Human Services National Center for Birth Defects and Developmental Disabilities

Dr. Grosse began by identifying the economic questions relevant to DNA testing:

- How much does it cost?
- How much will we save by implementing the intervention?
- Is it cost effective or cost beneficial? (not the same)

Questions that also should be asked include:

- Does it provide value for our money?
- Is this the best use of resources?
- Who gains and who loses?

Dr. Grosse emphasized that economics is not the same thing as accounting. Economists think in terms of resources and how they are consumed, and resources are valued in terms of opportunity cost, or their value in terms of foregone alternatives. That is, once the resource is used, it is no longer available. Incremental cost is the difference in total cost between conducting the intervention and not conducting it. For example, in considering whether to add a test to a NBS panel, the cost of obtaining a blood spot does not need to be factored in—it already has been obtained.

Dr. Grosse reviewed a model based on hypothetical data on IRT/DNA NBS algorithms. If one assumes a prevalence of CF of 1 in 4,000, if 30 percent are detected early without NBS, then 1 in 5,700 are detected early by NBS. Assuming a protocol with a single mutation and a sensitivity of 94 percent, at a cost of $3 per infant screened, screening costs $18,000 per additional case detected (165 new cases detected out of 1 million infants screened). Using a protocol with a multiple mutation panel and a sensitivity of 98 percent at an average cost of $4.50 per infant screened, the average cost is $26,000 per

case detected (172 cases detected). The incremental cost of going from a single mutation to a multiple mutation panel would be $1.5 million for 7 additional cases, or $214,000 per extra case detected.

Dr. Grosse noted that many confuse cost savings with cost effectiveness. If screening were cost saving it would mean that total costs are lower than without screening. This is often not the case, but although many interventions are not cost saving, they may be cost effective or cost beneficial. Cost effectiveness involves determining whether good value is obtained for money that is expended on any given intervention.

Once data on the effectiveness of screening are obtained, one can determine the value of the outcomes. The conventional approach is to consider only the health outcomes, which involves attaching values, such as quality-adjusted life years. For cost-benefit analysis, there are two different ways of assigning dollar values to health outcomes: the human capital approach (how much the productivity would have been worth if the patient had not died or had been disabled) and the willingness-to-pay approach (how much society is willing to pay to prevent a death or a disability). A third approach would be to look at *all* outcomes that people care about. If people care about more intangible outcomes, such as reassurance and the ability to plan families, there are ways of aggregating estimates of willingness to pay so that total benefits can be identified.

Economics concerns the use of limited resources matched against unlimited wants, Dr. Grosse explained. Prioritization of resources and considering alternative uses for resources are necessary. Screening involves the question of how to determine the importance of one test over another, with the determining factor being the level of advocacy. Every method for establishing a value on an outcome assumes the existence of certain social values—be they utilitarian or psychosocial. Society must determine these values before it can begin to invest resources.

Discussion

Dr. Grosse was asked if there had been any examples of the willingness-to-pay paradigm being used in health care or in the field of NBS in particular. The comment was made that although the concept makes sense, the decision to place a higher value on one life than on another (for example, the value of an American life versus the value of the life of a native of sub-Sahara Africa) is controversial and offensive to many. The most common examples of use of these methods, said Dr. Grosse, are in analyses of regulations. All regulations have to be supported by cost-benefit analyses, which generally include willingness-to-pay estimates of prevented deaths. In addition, the Office of Management and Budget (OMB) and the Institute of Medicine (IOM) are collaborating on a project addressing valuation of health outcomes in cost-effectiveness analysis of regulations.

Another question involved whether data from supplemental screening programs could be substituted for willingness to pay as a measure. This would be an example of “revealed preferences” in which economists look at data on how much people pay for an actual

service. One problem, said Dr. Grosse, is that if insurance pays for tests we may not observe individual preferences.

One challenge is that cost effectiveness estimates may be used to further advocacy agendas rather than to provide objective information to decision makers. At present, there are few sources of funding in this country to support objective research into health care cost effectiveness.

The "stated preferences" approach to soliciting information on willingness-to-pay involves posing hypothetical scenarios to people. Participants are asked a series of questions including escalating figures (e.g., would you be willing to pay $50? $100?). This tool is called "contingent valuation." Another approach to determining willingness to pay is conjoint analysis, in which respondents are presented with scenarios involving combinations of different attributes and asked to indicate their willingness to pay for different bundles of attributes. The information and perceptions that respondents already have will govern the responses.

The ACMG included recommendations for a "report only" category, which could require follow-up by public health personnel and that would increase costs. A participant noted that, in addition, if mutations are added endlessly to panels, virtually every newborn would require follow-up screening.

There was some debate about how the "value points" were assigned by the ACMG committee to benefits to the child (200) and benefit to the family (100). It was noted that the individuals in the group had a bias that affected their assignment of value. These discussions, Dr. Howell said, were extensive and controversial, and the group was diverse in its representation of views. These variances in views, of course, do not necessarily reflect the range of views of society. He explained that a critical issue is that any discussion of information that could lead to a discussion of pregnancy termination raises the specter of eugenics.

Practical and Public Health Implications (Working Lunch)

Anne Marie Comeau, PhD
Deputy Director, University of Massachusetts Medical School New England Newborn Screening

Dr. Comeau opened by discussing the applications of the various types of molecular testing. Testing for DNA or RNA testing can be a qualitative or quantitative assay. Results may indicate a genotype/phenotype relationship or may indicate the amount of exogenous nucleic acids present in a person with an infectious disorder.

If there is HIV in a Guthrie spot, it can be detected at a level of 1 to 10 copies, and once detected it is possible to detect sequences and trace lineages. Tests for single copy genes such as CF are far less complicated. Dr. Comeau used CF NBS as a model to demonstrate

the differences between using DNA assays in a second-tier versus a first-tier test. She extrapolated from data acquired in Massachusetts.

The current Massachusetts algorithm uses DNA assays in a second tier; only infants with elevated IRT have DNA assays. Approximately 5,000 out of every 100,000 babies prompt DNA testing in the second tier. Detection of one or two mutations prompts referral for diagnostic testing at a CF Center. Of the 5000, approximately 400 will be referred for sweat testing, most of these being referred with detection of only one mutation and elevated IRT. Though most of these infants will have a negative diagnosis (and families will be offered genetic counseling for the one detected mutation), some infants do have a second mutation that is not picked up by the screen. This screening algorithm provides the opportunity to diagnose these affected infants without placing too much burden on the CF center for sweat testing and genetic counseling.

In contrast, if we were to use the DNA assays in the first tier, of 100,000 infants, 3,653 carriers would be identified. By current Clinical Laboratory Improvement Amendment (CLIA) standards, all would need to be reported. By current ethical standards, all would need some level of genetic counseling. By current information, we still do not know enough about genotype phenotype—even in as well-studied a disease as CF—that such an algorithm would enhance predictive values.

There are situations where first-tier DNA screening might be appropriate, such as when no other analyte is available. Even then, however, results would need to be reported. Still, our capacity for reporting and recording into the medical record is limited by our knowledge. One solution to this issue that should be pursued is the development of informatics that will allow sequence information to be stored into the medical record for future queries. Without that, it seems hard to justify first-tier DNA assays for population screening.

Ken Pass, PhD
Chief of Laboratory, Wadsworth Center Division of Genetic Disorders at the New York State Department of Health

A long-term outcome of NBS, Dr. Pass said, would reduce newborn testing to a single assay, with the reporting of only one marker analyte, the mutation found (e.g., diabetes). This could greatly simplify the laboratory testing portion of NBS, in which four or more technologies are currently used. For many years, NBS programs have been doing DNA screening for sickle cell screening. The electrophoresis used for hemoglobin screening is almost diagnostic and does, in fact, identify carriers.

With the appropriate DNA technology it should be possible to identify carriers and perhaps even screen for wild type. When Dr. Pass met with the directors of the CF centers they said that they did not want the carriers reported to them, a task that is impossible with today's screening technology. However, he believes it is an advantage to identify carriers, which provides options for addressing diagnosis and treatment. With the ability to use DNA first, a whole range of opportunities present themselves. The carriers

can be managed with a modicum of risk and it is possible to identify those infants who are at minimal risk and those who are at increased risk. The advantage of identifying carriers is that fewer resources could be expended in the follow-up phase of NBS, since it is known that these infants are not at risk for phenotypic expression of the condition. However, a problem with DNA-based testing is identifying people who remained healthy even though they tested positive by exhibiting the presence of a mutation. Much more work on genotype/phenotype correlations must be done to make DNA screening practical.

Bill Hagopian, MD, PhD
Clinical Associate Professor of Medicine/Metabolism, Endocrinology and Nutrition, University of Washington

Type 1 diabetes (T1D) accounts for only 5 to 10 percent of individuals with diabetes. It occurs at all ages but the highest incidence is in childhood. Most new cases do not occur in families with a close relative with T1D; they are sporadic.

The frequency of type 1 diabetes mellitus (T1DM)-associated HLA DQ alleles in the U.S. Pacific Northwest- is as high as in Scandinavia. The high regional rate of islet autoimmunity observed among DPT-1 relatives supports this notion. Fortunately, Washington State archives dried blood spots from NBS programs. The Diabetes Evaluation in Washington (DEW-IT) study aims to show that population-based prospective prediction of T1DM by HLA genotype screening followed by autoantibody surveillance can be performed within the public health infrastructure.

A previous study, the Washington State Diabetes Prediction Study, simultaneously analyzed the three most informative islet cell-related autoantibodies in a large cohort of children for prediction of type 1 diabetes. The results had a 95% confidence interval for sensitivity (58–100%) and positive predictive value (25–75%), which supported the use of this strategy to predict the disease in the general population. Because T1D is a sporadic disease, the ability to predict it in the general population using multiple autoantibody measurement is quite promising. The use of HLA-DQ typing may be helpful, but when used alone it does not have the same predictive value of multiple autoantibodies to islet cells. HLA-DQ is most effective when used as a first screening step to identify children for whom subsequent auto-antibody surveillance is warranted.

Results of this study have opened the way for large screening programs among the general population, thus enabling implementation of preventive measures as they become available. However, in order for such NBS to be performed, the laboratory used inexpensive assay tests in scalable formats similar to what is currently available for thyroid antibodies. Thus, we are not yet ready for large-scale implementation. Although we still do not possess any tools to stop the autoimmune disease process once it has begun, there is now strong evidence that auto-antibody testing leads to earlier diagnosis of new T1D, with significantly less morbidity at onset. Therefore, the prediction of T1D, currently an exercise limited to the research setting, may become reality within the next decade.

Consumer Concerns: DNA-based Technologies and Newborn Screening
Sharon F. Terry, MA
President, Genetic Alliance, Inc.

Ms. Terry noted that the Genetic Alliance, which represents 600 members, operates on the overarching premise that we are constrained by economic models developed in our crippled health care system. She said that the Alliance feels that the conversation would be different in a world in which resources are aligned more equitably.

Consumers want healthy children at any cost, she emphasized, and the benefit analyses are not conducted from a medical model. In addition, parents are not aware of the nuances involved and often are not aware that their infants are being screened. In addition, they do not understand that there is variability in screening across states, as well as gray zones in sensitivity and specificity, in analytical and clinical concerns, and in the utility and validity of tests. She said that in lived experience the odds of being affected are either none or 100 percent, because for parents the issue is black and white, and the disease becomes the prism through which all of life is assessed.

That is, consumers do not experience the testing, the diagnosis, or the day-to-day struggles on a population level—for the consumer, it is a completely personal experience. How we define health in society must be examined with the understanding that professionals and consumers define health and disease in different ways. When consumers look at harms and benefits, they see a series of continuums. Ms. Terry quoted an e-mail she had received from the mother of a child dying of a genetic disease:

A year or two after Rick's diagnosis, when he was still apparently well, I asked him if he ever wished that he did not know that he had Neiman-Pick disease, that he had never been tested. He said, "Oh no, Mum, now I know I am not stupid. I know there is a reason for some of the things I can't do." When we had thought that he had no obvious symptoms, Rick had been struggling to understand why he was not able to keep up with his peers, why there were some things that he could not do as well as he felt he should.

She noted that the choices that are relevant to consumers are addressing lifestyle issues for both children and families, choosing caregivers and specialists, conducting financial planning, choosing a job, making educational choices, finding a support group, securing insurance, building registries, and participating in research.

Furthermore, DNA-based technologies exacerbate the following problems faced by consumers:

- In general, there is an inadequate understanding about NBS and rare diseases. *Solution:* Parents and advocacy groups must raise the awareness of health professionals.
- Many communities lack information and resources. *Solution:* Outreach by parents and advocacy groups should be promoted.

- Policies, legislation, and treatments are advancing at a faster pace than technology. *Solution*: Consumers can promote effective public dialogue and decisionmaking.
- Challenges exist regarding consistent, uniform, and continuous care. *Solution*: Develop strong partnerships between parents, professionals, and the pubic to address these challenges.

Parental involvement is a moral imperative, Ms. Terry said, emphasizing that there is a tactical urgency to making parents aware of the issues surrounding testing, including ensuring that they know that their newborns are being screened. Input from underserved and underrepresented communities also is required, and these groups deserve an even greater involvement. Proactive outreach will maximize the public health potential.

Consumer involvement also provides recommendations that reflect the lived experience, such as the following:

- Uniform NBS panels from state to state should be expanded.
- Professional education programs should be developed.
- Educational materials and programs must be developed.
- National standards and increased resources are needed.
- Information about expanded screening should be given to parents in states with fewer mandated tests.

From a consumer point of view, the bottom line is that the development of DNA-based technologies moves these critical issues to a boiling point, and the public must be made aware of the need to weigh the risks associated with implementing new technologies without a robust system in place against the risk of delaying the development and use of applications that will move basic science to produce health outcomes sooner. Consumer demand requires envisioning a different system—sooner rather than later, Ms. Terry said—one that includes the voices of consumers, changes in public policy, and funding for the service side.

One of the participants wondered what happens when parents receive information that they can do nothing to change. Ms. Terry responded that this information still helps them make decisions about the future, including those involving education and other needs. She also noted that in a broader framework, such information aids in the development of registries and surveillance that will be of use as the science moves forward. The argument could be made that identified patients could become involved in research whether they want to be or not, and that the only solution to this problem is to implement informed decision making.

Another participant asked how the public would prioritize the importance of the tests. It was suggested that a public dialogue must be initiated that would enable the identification of these priorities. Ms. Terry noted, however, that in the current health care environment, this is not possible. In addition, many people do not trust the health care system or the

information it provides. Participants noted that they have had a variety of different experiences with parents who want or do not want to know about any genetic anomalies.

Education is a critical element of a model for a "rapid action" approach. Another issue is the lack of a scientific definition for what is considered "normal" (as was noted earlier in the meeting), and there has been a failure to recognize that some cultures (such as the American Indian) do not have language to describe genetics.

Summing Up

Michele Lloyd-Puryear, MD, PhD
Chief, Genetic Services Branch, Division of Services for Children with Special Needs, Maternal and Child Health Bureau, Department of Health and Human Services

HRSA is the federal agency that deals with health care access and health services as opposed to research. It focuses on how to provide services such as NBS, how to ensure equity in the process, and how to translate research into practice. The only way to do so is to ensure that screening programs are universal and provided under the public health umbrella and therefore, not subject to one's ability to pay. She noted that as any new technology enters the marketplace, each of these issues has to be confronted. HRSA has included in its policies a requirement that parents should be counseled and should be directed to educational information about what new technologies are available and what they may offer. In addition, as the technology expands, it must be integrated into the health care service delivery system. Further, because the health care system may not have the needed technical expertise in these areas, it is important to consider models that partner public health agencies with universities or other research communities to develop new practice and research paradigms. For example, we need demonstration projects that reflect responses to the information and training needs of the public health community and the educational needs of the public. Projects also will need to come to consensus on translational research issues such as the use and storage of biological samples. Dr. Lloyd-Puryear said that all of these discussions will be part of the preparation for the next Advisory Committee on Heritable Disorders and Genetic Diseases in Newborns and Children meeting, and she urged participants to be active in Committee activities.

A draft report by ACMG will be released to this Committee in Fall 2004. The report will make recommendations to strengthen NBS programs. In anticipation of the ACMG recommendations, HRSA, through cooperative agreements, has funded seven regions as the focus for the development of collaborative genetics and NBS units to address the maldistribution of genetic and NBS programmatic expertise and other services across the country. The cooperative agreements will be implemented over the next three years. HRSA also expects to work with the CDC's birth defects programs and the NIH-Centers for Excellence that focus on rare diseases to enhance the collaboration between all three federal agencies. Most of the rare disease centers have fairly good relationships with state NBS programs. HRSA anticipates translational research relationships between the rare disease centers and the regional collaboratives.

The group discussed the lack of communication among the various elements of the community, with one of the most controversial issues noted as the need for informed consent. Although informed consent may not be as critical for screening to detect a metabolite as it is for detecting a specific disease; once researchers begin to run predictive DNA-based testing/screening, there is a responsibility to obtain informed consent to communicate both the risks and benefits of the screening. That is, once DNA-based tests are moved into use in the healthy population, multiple levels of information—about the individual, about the family, diagnostic, predictive—will be revealed.

Next Steps

This incorporation of new technology is an evolving process that must be informed by all the various communities that have a stake. Current activities involve surveying and recruiting members of a trans-NIH working group. This group will require identification of all the various partners and approaches to the use of DNA in NBS, as well as other technologies, such as those in the field of nanoscience, that emerge. The regional genetics and NBS collaboratives offer an opportunity to develop and undertake clinical trials that address these new technologies, help develop new partnerships, and provide access. New research initiatives will be developed, and NBS will continue to be the topic of various forums. In addition, scientists will have to consider follow-up testing if DNA-based technology becomes incorporated into NBS, which raise innumerable questions about mechanisms and ethics that must be discussed by all invested groups.

Next steps include identifying timely new targets and possible technologies to screen for various conditions. Moving forward in the area of providing medical and public education is essential, and the reports that emerge from various communities involved will provide critical resources in this regard. The ultimate goal is to obtain buy-in from any group that has credibility in this area, including advocacy groups and public health professional groups. This will help to generate the support needed to begin the important work of advancing the technology of DNA testing for NBS and to translate its importance to the general public.

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