

Article Title: Genetic Implications of Newborn Screening

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Screening asymptomatic individuals for present or future diseases is a mainstay of current medical practice. A natural and important extension of that activity has eventuated in various newborn screening programs in each of the 50 United States as well the District of Columbia. The evolution of these programs has derived from a clear goal, namely the avoidance of the devastating consequences of having a specific disease that can be potentially prevented by early detection and specific interventions. The justifications for inclusion of a specific disease in the "Newborn Screening Panel" are just as varied as each of the state programs, but generally they are selected based upon fulfillment of criteria developed in the late 1960s. For example Wilson and Jungner suggested that if a disease was to be screened for, the disease should be 1) an important health concern; 2) be able to be recognized during its early stages (prior to symptomatic presentation); 3) testing for the condition should be non-intrusive and acceptable to society at large; 4) have an accepted treatment; 5) have facilities available for diagnosis and therapy; and 6) all while attempting to be cost effective.¹ These guidelines, though clear and generally succinct, also provide for a bit of varied interpretation that can result in one state justifying inclusion of screening for a specific disease while another state does not. Although the manner in which state run newborn screening programs decide upon which diseases to screen for can vary greatly, there are several points of commonality that have evolved in most programs, and it is these that I will touch upon in this brief review, and specifically as they relate to genetic diseases.

In most states, screening and then medically managing newborns identified as having a specific disease is a mandatory function of the health care professional responsible for a newborn infant. Furthermore, lack of instituting newborn screening or appropriate medical intervention can result in legal liability for the health care worker that fails to fulfill this obligation. Screening of newborn infants in most states tends to be a mandatory function, but parents can opt out in most states by either refusing to consent to having their children tested, or by providing to the appropriate state health agency(s) a justified request refusing mandatory testing. Typically these options obligate the parents to sign a waiver indicating that by refusing

newborn screening, they acknowledge that they will jeopardize the health and well-being of their child, (i.e.: severe mental and/or physical impairment or death) and that they also release the health care providers, hospital, institution and/or state newborn screening program from liability should such an outcome occur.²

In the early to mid 1960s, the first newborn screening programs instituted presymptomatic testing of newborns for the devastating neurological, and genetic disorder, phenylketonuria (PKU). PKU is an inborn error of amino acid (phenylalanine) metabolism that has an incidence of ~1 in 20,000, and is generally due to inheritance of significant mutations in the phenylalanine hydroxylase gene. Lack of phenylalanine hydroxylase activity causes high phenylalanine levels to accumulate in the blood, resulting in severe neurologic impairments should the condition not be detected prior to symptom onset. Rapid and sustained institution of a phenylalanine free diet prior to both phenylalanine ingestion and symptom onset can avoid most, if not all of the devastating clinical consequences of PKU. Dr. Guthrie developed the use of a filter card based assay for PKU testing, using blood specimens spotted onto the cards (Guthrie card) after the heel stick of a newborn infant. Within 2-3 days of obtaining the dried blood spot sample, an assay performed on the blood sample could indicate with fairly high certainty whether the newborn's blood showed evidence of accumulation of phenylalanine. All state newborn screening programs currently screen for PKU.²

As humans, we are diploid organisms, therefore we receive one copy of all of our genetic information from our biological father, and the other copy from our biological mother. Note that in a newborn affected by PKU, the cause of PKU is due to inheritance of genetic mutations in both copies of the infant's phenylalanine hydroxylase genes. This makes PKU an autosomal recessively inherited disorder, therefore each parent must pass on a defective, or mutated copy of the phenylalanine hydroxylase gene to their offspring in order for the child to be affected. In such an instance, the unaffected parents of a child affected by PKU must be heterozygous carriers, that is one copy of their phenylalanine hydroxylase gene is not affected, while the second copy carries a mutation. Therefore, identifying a newborn with PKU immediately has genetic implications for the PKU affected newborn's family. As both biological parents of the PKU

affected infant are heterozygous carriers of phenylalanine hydroxylase gene mutations, they will have a 25% chance of having another child with PKU per pregnancy that they conceive as a couple. Furthermore, unaffected siblings of the affected newborn each have at least a 50% chance of also being a heterozygous carrier of a mutated phenylalanine hydroxylase gene as well.

Therefore, even as early as 1965, identification of newborns by newborn screening programs immediately entailed providing genetic information to affected individuals as well their immediate family members, information that they may not have anticipated by participating in the newborn screening program. I raise these simple points to shed light on the notion that newer newborn screening tests are raising new concerns in regard to providing genetic information to families. In fact, many times these concerns are duplicative of issues that have been dealt with in routine newborn clinical care since the early 1960s.

As newborn screening programs took hold in various states, they expanded to test for other disorders that fulfilled the Wilson and Jungner criteria I have alluded to earlier in this review.¹ Rapidly, screening for hypothyroidism, and hereditary galactosemia gained wide acceptance for inclusion in newborn screening programs, as a result, these are now typically offered in all states and the District of Columbia.² Again, many forms of these diseases can be inherited, and require appropriate genetic counseling to at risk family members (see below).

Additional newborn screening tests were developed for Maple Syrup Urine disease (MSUD), Homocystinuria, and Biotinidase deficiency. MSUD is due to autosomal recessive inheritance of mutations in the genes encoding proteins composing the catalytic subunits of the branched chain alpha-keto acid dehydrogenase complex. MSUD is detected by measuring increased blood levels of the branched chain amino acids leucine, isoleucine and valine in blood samples of affected individuals. Homocystinuria is also due to autosomal recessive inheritance of mutations in the genes encoding proteins composing the cystathionine beta-synthase complex. Homocystinuria is detected by measuring increased blood levels of

methionine. Biotinidase deficiency is due to autosomal recessive inheritance of mutations in the genes encoding proteins composing the biotinidase protein. Biotinidase deficiency is confirmed when blood levels of biotinidase activity are 0-10% of normal levels. Each of these inborn errors of metabolism are again also genetic conditions, and have some unique characteristics that should be noted.

Homocystinuria is a disease that manifests with increased risk of stroke and cognitive impairment (as well as visual problems and skeletal manifestations,) however clinical symptoms may not be present until the first or second decades of life. Biotinidase deficiency manifests skin abnormalities as well neurological problems and can be treated with biotin supplementation. There are however, early and late onset forms of the disorder, and newborn screening can also identify both forms of the condition. Clearly, these examples confirm that newborn screening has already been utilized to detect genetic disorders that may not manifest symptoms for decades, despite identification in the newborn period. Despite this, clinicians attempting to deal with these disorders can successfully manage these clinical dilemma'. For example, by treating all partial or complete biotinidase deficiency patients with biotin supplementation. For newborns identified with Homocystinuria, physicians routinely implement vitamin B6 supplementation, protein intake restrictions, and methionine restricted diets, while clinically monitoring the patients over time for other progressive problems (i.e.: ectopia lentis) that can develop due to having Homocystinuria.

The addition of newborn screening tests for metabolic diseases such as MSUD and Homocystinuria fueled interest in developing newer methods, and/ or technologies that could detect any one of these, or other inborn errors of metabolism simultaneously, rapidly, and cheaply. This need primarily focused upon the ability to detect and quantify abnormal levels of either specific amino acids, or various species of acylcarnitines, that are present at abnormal levels in blood samples derived from affected patients. Acylcarnitines are organic acids or fatty acids that are bound to carnitine, which is a transporter of fatty acids into and out of the mitochondria. Rapid, and accurate quantification of these specific compounds in small

amounts of blood would allow for early detection of metabolic defects in amino acid, urea cycle, and/or fatty acid oxidation pathways.

In fact, use of Tandem mass spectrometry (MS/MS) based technologies allows one to detect these and various other compounds in a small amount of blood sample. MS/MS not only simultaneously identifies various compounds in an experimental sample based upon their molecular weights, but also quantifies the amounts of the respective compounds present in the sample, as compared to internal standards or control samples. By implementing MS/MS technology into newborn screening to detect diseases such as phenylketonuria or MSUD, the number of additional diseases that could potentially be screened for using the same blood sample was also greatly expanded. Typically, in those states in which MS/MS technology has been introduced, well over 20 additional inborn errors of metabolism can now be detected pre-symptomatically.³ All of these conditions are typically genetic in nature, and though most are inherited in an autosomal recessive fashion, some are due to alternative inheritance patterns. Clearly, for each of these conditions, the health care professional must be adept at understanding and transmitting the nuances of these hereditary conditions to their patients and their families, or be prepared to send their patients to other health care professionals trained in these issues. Otherwise, great liabilities might be incurred should incorrect genetic counseling be provided.

For example, the Urea Cycle Disorder Ornithine Transcarbamylase Deficiency (OTC) can lead to episodes of hyperammonemia after protein ingestion, episodes that can lead to profound mental deficiency, and death if left untreated. OTC is inherited in an X-linked recessive pattern, typically from a non-manifesting carrier female. Empirically, recurrence risk of OTC would be at 50% in each and every future male offspring born to a confirmed OTC carrier female, while 50% of all future female offspring born to the same female could also be carriers of the mutated OTC gene. What may not be clear to a practitioner is that female carriers of OTC mutated genes can also manifest hyperammonemia symptoms, sometimes as severe as males, due to non-random patterns of X-inactivation. Similarly, newborn screening based identification of an

offspring with OTC can sometimes identify previously undiagnosed, but manifesting female OTC carrier mothers, allowing for their treatment as well. Detection of affected newborns with a specific disease should also prompt further testing of at risk biological siblings of the identified newborn, that may either have been not tested or detected previously. Gonadal mosaicism for OTC gene mutations has also been documented in families as a cause for a presumably non-carrier mothers to have more than one child affected by OTC deficiency.⁴ This fact that will further complicate the counseling of OTC families identified by newborn screening, and needs to be understood by health practitioners attempting to counsel families for this and other genetic diseases subject to gonadal mosaicism, such as Duchenne Muscular Dystrophy and Achondrodysplasia.

There are no set guidelines as to disease incidence rates dictating inclusion of a disease for newborn screening. Some of the additional metabolic conditions detected by implementation of MS/MS technology are exceedingly rare, while some are more common than the original diseases screened for prior to implementation of MS/MS technology for newborn screening.³ An example of this is the fatty acid oxidation defect disease known as Medium-Chain Acyl-Coenzyme A Dehydrogenase Deficiency (MCAD) (Table 1). This recessively inherited disease can have symptoms present at birth, or be asymptomatic for years. MCAD patients that are under metabolic stress, (i.e.: due to extended fasting, increased durations of time between feedings, or having an illness causing catabolism) for significant periods of time can precipitate a sustained, non-ketotic hypoglycemia, that can result in liver damage, neurological sequela, and even death if an affected individual is not identified and treated with appropriate glucose and other dietary supplementations.

| Genetic Disease | Approximate Incidence |
|---------------------------|------------------------------|
| Phenylketonuria | 1 in 10,000-15,000 |
| Homocystinuria | 1 in 200,000-335,000 |
| Maple Syrup Urine Disease | 1 in 185,000 |

| | |
|------------------------|-------------------|
| Biotinidase Deficiency | 1 in 60,000 |
| MCAD | 1 in 5,000-17,500 |
| CH | 1 in 3,000 |
| CF | 1 in 4,000 |

Many newborn screening programs also utilize blood spot testing for other diseases as well, for example congenital adrenal hyperplasia (CAH) , congenital hypothyroidism (CH), sickle cell disease as well as other forms of hemoglobinopathy. Diagnosing any of these conditions should not only institute specific medical therapies and supportive interventions, but should also begin genetic evaluations as well. For example, most causes of CAH are recessively inherited (due to 21-hydroxylase deficiency) but may require testing of at risk siblings as well genetic counseling of the biological parents as to the 25% recurrence risk in future offspring.

CH is typically non-hereditary and due to an isolated lack of thyroid gland development, however upwards of 15% of cases can have a normal appearing thyroid gland but yet be absent of functional thyroid hormone activity. Most of these latter cases are typically due to autosomal recessive inheritance of mutations in specific genes, such as those encoding the thyroid stimulating hormone receptor, or in the PAX8 transcription factor gene, a factor that induces formation of the thyroxine produce thyroid cells.⁵ X-linked and autosomal dominant forms of inherited CH have also been described, and may be deduced by careful review of the clinical phenotype of the affected newborn, specific laboratory testing, as well as careful review of the newborn's family history. Clearly, each genetic form of CH would not only entail specific medical consequences and interventions for the affected newborn, but these interventions may also need to be instituted in primary or distant biological relatives. Identification of a genetic form of CH in a newborn would also have specific genetic consequences (in regard to recurrence risk) for at-risk biological relatives as well.

Many newborn screening programs have expanded their capabilities to also include hearing screens for congenital deafness in the newborn. The long term economic and societal costs of lack of proper speech development in so-identified individuals has justified institution of this screening test. I would suggest to the health care worker that identification of the "symptom" of Deafness in a newborn should rapidly trigger not only interventions to improve hearing and speech development, but also clinical assessment as to the underlying cause of the deafness. In many cases, deafness may be due to an underlying genetic cause.

Traditionally, it is thought that up to 50% of the causes of childhood deafness can be due to an underlying genetic causation. Of these, ~1/3 are thought to be due to a "syndromal" form of genetic deafness, (associated with other congenital anomalies or problems); with the other ~2/3 being "isolated" or "non-syndromal" forms of genetic deafness that are not associated with other congenital defects or problems. Dependent upon the specific underlying genetic cause, genetic deafness can be inherited in an autosomal recessive, autosomal dominant or X-linked fashion. While 50% of all of the causes of childhood deafness may be acquired (i.e.: due to infectious causes, exposure to toxins) I again would suggest that many of these cases may be due to an unrecognized form of genetic deafness as well, and it is up to the health care professional treating such individuals to discern this possibility. Together, the high number of underlying potential genetic causes of deafness should immediately prompt health care workers in a number of directions when a newborn they are caring for is determined to have a congenital form of hearing loss.

Further exemplifying this complexity, in the Mendelian Inheritance in Man series (an evolving and rapidly updated compendium listing all known forms of genetic disease), there are well over 700 identified genes that in one way or the other have been implicated in the isolated or syndromal causations of deafness.⁶ Specific identification of any one of these, or other genetic forms of deafness requires a thoughtful and detailed evaluation of the affected newborn, as well detailed interrogation of the newborn's family history.⁷ The implications are numerous, not only from a genetic counseling point of view relative to recurrence risks to other family members, but also medically to the affected newborn as well at-risk family members.

Syndromal forms of deafness can entail involvement of other organ systems, such as facial development and the renal system in the autosomal dominant form of the Branchio-Oto-Renal Syndrome, the integumentary and ocular systems in the autosomal dominant forms of Waardenburg Syndrome, and the cardiac system in the autosomally recessively inherited Jervell syndrome. Lack of recognition of these disorders can have dire consequences, for example having the Jervell syndrome can lead to sudden cardiac death should underlying long QT intervals or other cardiac arrhythmias go untreated in the so-affected individual. These medical complexities make it imperative that health care workers are confident that they have ruled out a syndromal form of genetic deafness before assigning a deaf individual to the "non-syndromal" or "isolated" form of congenital deafness category.

Recently, specific DNA testing for detection of diseases has also been implemented in a number of newborn screening programs. Primary of this is testing newborns potentially affected by the recessively inherited, multi-organ disease known as Cystic Fibrosis (CF). Justification of newborn screening for CF arises from the need to intervene medically before substantial lung, pancreatic, growth or other organ systems are significantly affected by unrecognized CF. Furthermore, identification of CF newborns can provide families with genetic knowledge in regard to the 25% recurrence risks (per pregnancy) for this autosomal recessive disorder. Currently, most newborn screening programs testing for CF utilize a two tiered approach. Blood spots are first assessed for elevated blood levels of immunoreactive trypsinogen (IRT). Those samples exhibiting high IRT levels are then subjected to DNA mutation detection analysis. Typically, 8-25 of the more common mutations found in the CF transmembrane conductance regulator (CFTR) gene are queried to assess whether the infant should be further medically evaluated as well assessed by sweat chloride testing.²

There are several caveats that should be understood relative to newborn screening for CF. First, the IRT/DNA tiered approach, though sensitive, cannot detect all CF patients. It should be noted that well over 1,000 mutations have been found in the CFTR gene, so lack of detection of a CFTR mutation using a DNA test that is only capable of identifying the top 25 of those 1,000 potential mutations does not rule out a CF

diagnosis in a suspect case. Therefore, clinicians should still consider CF in the differential diagnosis of a child showing CF symptoms, despite the child having a normal newborn screen for CF.

Although not unique to CF, an additional genetic implication to the IRT/DNA newborn screening protocol needs to be recognized. In many instances, newborns with elevated IRT levels do not have CF, but yet may still be identified as a heterozygous carrier for a single CFTR gene mutation, as identified in the DNA mutation analysis portion of the IRT/DNA newborn screening protocol. This implies that at least one of the biological parents of the so-identified newborn is also at least a heterozygous carrier. Such genetic information may not have been anticipated by the parents of newborns subjected to CF specific newborn screening, and poses several important medical implications, as well as ethical dilemmas. For example, siblings of heterozygous CFTR mutation carrying newborns may need to be medically assessed further for possible CF, as both biological parents may be heterozygous CFTR mutation carriers until proven otherwise. In this light, the biological parents of the heterozygous CF carrier newborn may also be at risk for having a future child with CF if both parents are indeed CFTR mutation carriers. This latter scenario may be difficult to manage by some families, as many may not have anticipated that they would be identified as potential CF carriers by newborn screening of their unaffected but CF carrier newborn child. Careful consideration of these issues by health care professionals is warranted when faced with these scenarios. Furthermore, as the carrier frequency of CFTR mutations may be as high as 1 in 25, the genetic counseling services offered by any newborn screening program will likely be overwhelmed by the identification of CF carriers in this manner, therefore the burden of counseling and potential medical management of these individuals and their families will increasingly fall on the primary care providers caring for newborns.

In summary, each state has its own unique newborn screening program, each screening for a unique set and number of disorders. For a current listing of each individual state's current newborn screening portfolio, please visit the National Newborn Screening and Genetics Resource Center website; <http://genes-r-us.uthscsa.edu/nbsdisorders.pdf>. Newborn screening based recognition of each and every one of these

disorders has immediate medical consequences. However, identification of newborns with many of these diseases may also have immediate, and/or long term genetic consequences as well, not only for the identified newborn, but also for at-risk biological relatives of the newborn. The primary care physician responsible for the care of newborns must be prepared to understand and deal with the genetic implications that will necessary result from current newborn screening protocols being carried out in his or her state(s). As newer, high throughput technologies are developed (i.e.: machines that can rapidly sequence the entire human genome, multiplexed, chip-based technologies that can carry out hundreds of chemical reactions simultaneously) and as new therapies for once untreatable diseases are approved, a continued rapid expansion of newborn screening programs to detect greater number of diseases will necessarily occur. Furthermore, several genetic diseases that do not have current specific therapy, may yet be detectable using a simple test that can be utilized in newborn screening.

For example, many forms of the muscular dystrophies could be identified by newborn screening, by assessing elevated creatine phosphokinase (CPK) activity levels in the blood. By doing so, one can identify males affected by Duchenne Muscular Dystrophy.^{8,9} Most of these dystrophies are genetic in causation, but do not have a specific therapy, as yet. Early detection may however allow for improved induction of supportive measures, as well provide biological relatives of so-identified newborns important genetic information that may change future family decisions regarding recurrence risk in future pregnancies. The primary care provider will be increasingly called upon in these and other situations to calmly and knowledgeably educate these families in these and other regards, so that informed decisions can be made. It is the hope of this author that this brief review highlights some of the genetic caveats that are inherent in contemporary newborn screening programs, and how these caveats must be considered and thoroughly understood by health care workers medically managing newborn infants.

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