Fragile X syndrome (FXS), caused by a trinucleotide expansion (>200 CGG repeats) in the fragile X mental retardation gene (FMR1), is currently not included in newborn screening (NBS) panels in the United States as it does not meet the standards for recommendation. Although in the past few years FXS has met many of the criteria for population screening and studies have shown that NBS for FXS is feasible, the idea is still controversial and the debate is open. The recent advances in genomic testing as well as groundbreaking advances in targeted treatment for FXS have been challenging the dogma and principle of the national NBS program: screen only if you can intervene. Arguments in favor of NBS include benefits of early intervention and follow-up for the identified baby, which would justify NBS even in the absence of medical benefit to the child. In addition, the extended family members may benefit from genetic and reproductive counseling, informed decision making before a subsequent pregnancy, and access to treatment and services. However, communicating the results and the potential consequences to families is a challenge and could lead to a heavy psychosocial burden. A controversial issue is the identification of premutation carriers (55-200 CGG repeats), because it not only can lead to information on the reproductive possibility of having a child with FXS but also leads to information about personal health risks associated with the premutation. Yet, knowledge of carrier status could stimulate and encourage lifestyle changes and preventive measures likely to reduce the risk of medical problems reported in premutation carriers. If NBS for FXS is developed, it must be carried out with clear awareness of the potential impact on the lives of the children, and it should be done after counseling and parents’ informed consent. Importantly, the infrastructure to support testing, counseling, treatment, and follow-up will have to be made available to the families.

Mandatory newborn screening (NBS) programs in the United States, using a simple blood test on blood spot cards, have been designed as a public health program to identify a number of rare conditions that are not apparent at birth. If not diagnosed at birth and not treated, these disorders will cause significant disability or death. Indeed, since the development of the screening test for phenylketonuria using blood spots dried onto a filter paper card, the NBS health program has been a national priority designed to save or improve the lives of affected babies. Although NBS has primarily implemented a short-term follow-up process, the need for long-term follow-up has been recently recognized as essential for optimal treatment and comprehensive care. Every state in the United States has mandated independent NBS programs, which include different conditions based on the severity of the condition, the availability of effective treatment, and the cost of the test. The NBS program has seen a rapid increase in the number of known genetic conditions screened, particularly owing to technological developments, which have led to increased accuracy and reduced costs of the tests. Conversely, expanded NBS has raised a number of concerns, including lack of evidence-based research for treatment, shortage of follow-up services, unclear financial responsibilities, and ethical, legal, and social implications of situations such as disclosing carrier status or susceptibility to future disease.

History of Newborn Screening in Fragile X Syndrome

Fragile X syndrome (FXS), the most well-known and most common single-gene cause of inherited intellectual disabilities and autism, is due to a trinucleotide CGG repeat expansion (>200 CGG repeats; full mutation) in the 5′ untranslated region of the fragile X mental retardation 1 gene (FMR1; OMIM 309550). Epigenetic modification of the CGG-rich region turns off the gene, which results in the absence or deficit of the encoded product, fragile X mental retardation protein. This leads to reduced synaptic plasticity, which is important for learning and memory. Although FXS is the most common cause of inherited intellectual disabilities, the mean age at diagnosis is approximately 36 months in males and is later in females. Many families have a second child with FXS before the first one has been diagnosed.

Newborn screening for FXS is controversial; FXS was not recommended for inclusion in the panel of conditions for NBS as described in the 2006 American College of Medical Genetics report partly because there was no medical advantage for early detection. However, in the past few years, reports from large screening studies demonstrating the technical feasibility of widespread test-
individuals, the benefits from genetic counseling, and advances in treatment may change this scenario.

Spectrum of Clinical Involvement

Individuals with a full mutation present with a constellation of involvement, including intellectual disabilities, autism spectrum disorder, social anxiety and withdrawal, language deficits, hyperactivity, aggression, and self-injurious behaviors, in addition to physical features such as hypertensible finger joints, prominent ears, and macro-orchidism in puberty.6

Premutation FMR1 alleles, harboring between 55 and 200 CGG repeats, are unstable and can expand to a full mutation within 1 generation; consequently, female premutation carriers are at increased risk for having children with FXS. An expanding number of medical disorders, including the well-established fragile X-associated tremor/ataxia syndrome (FXTAS) and fragile X-associated primary ovarian insufficiency, occur in some carriers of premutation alleles; in the premutation population, these problems include seizures (13%), hypertension (60%), neuropathy (40%), migraines (30%), sleep apnea (30%), immune-mediated problems (44%), neurological and psychiatric problems (60%), autism spectrum disorder (10%), and attention-deficit/hyperactivity disorder (40%). Although attention-deficit/hyperactivity disorder and autism spectrum disorder occur in childhood, the other disorders have been found throughout the lifespan of carriers, and some commonly occur before the onset of FXTAS.3 Studies in the premutation mouse model have demonstrated developmental problems, with the degree of involvement correlating with CGG repeat number and FMR1 messenger RNA (mRNA) levels,6 as well as ovarian abnormalities involving both oocytes and granulosa cells,7 mitochondrial dysfunction,8 and altered calcium regulation.9

Individuals who carry an intermediate (or gray-zone) allele of 45 to 54 CGG repeats, which are unstable when transmitted across generations, may be at risk for FXTAS and fragile X-associated primary ovarian insufficiency.5 However, the role of gray-zone alleles is currently unclear, and future research is necessary to help us understand the potential impact on human health.

Population Screening and Prevalence

Estimated prevalence of individuals with the full mutation and the premutation varies depending on the geographical area and population assessed. In the general population, the range for full-mutation alleles is 1 in 2500 to 1 in 8000 females and approximately 1 in 5000 males. The prevalence of premutation alleles ranges from 1 in 130 to 1 in 256 females and 1 in 250 to 1 in 810 males in the general population. Although CGG allele size distribution appears to be similar between sexes and among ethnic and racial groups, differences in the prevalence of expanded alleles have been reported among different populations.10

While molecular characterization of FMR1 is complex because of the presence of the high CG-rich sequence, recent advances in genetic testing methods have led to the development of efficient methods that have enabled several polymerase chain reaction-based screening studies using DNA isolated either from whole blood or from blood spot cards (Figure). These large-scale, population-based screening studies span the entire spectrum of FMR1 mutations in the United States.10

Of these US screening studies, 8 reported on NBS, 6 of which were conducted on males only and 2 of which were conducted on both males and females (Table). The largest US pilot study of NBS for all FMR1 mutations demonstrated that using blood spots is possible and reliable, and it suggested that the prevalence of the premutation, particularly in males, is higher than had been previously reported,10 in agreement with rates from a large population-based study of approximately 20,000 men and women.11 Interestingly, most of the identified carriers had a very low CGG repeat number, with more than 70% having an allele of less than 70 CGG repeats,10 representing a relatively lower rate of reproductive and neurological risk problems than those with longer premutation expansion alleles with a higher CGG repeat number. Additionally, Tassone et al10 documented differences in prevalence rates among various ethnic groups; Tassone and colleagues found a lower premutation prevalence in African American males (1 in 780) compared with white males (1 in 358) and Hispanic males (1 in 595).

Understanding the Need for NBS

The pilot NBS study by Tassone et al10 involved a voluntary informed consent process whereby families were offered the opportunity to participate in NBS for the FMR1 mutations. Remarkably, the investigators found that most families consented to the study; the acceptance rate was approximately 60% to 76%, depending on the investigation site. Although a recent Australian study12 reported a much higher rate of participation, overall findings indicate a high level of maternal acceptance and voluntary support of NBS for FMR1 mutations. The NBS study identified a cohort of babies with the premutation, generating an unselected premutation population that could be followed up developmentally.10 Preliminary data presented at the First International Conference on the FMR1 Premutation12,13 suggested that newborns with the premutation have early developmental problems, particularly in social and emotional domains and behavior compared with age-matched controls, and show evidence of developmental problems in visual perception. Although very preliminary, the data emphasize the great need to study the processing capabilities and developmental trajectories of infants and toddlers carrying a premutation.

The pilot study by Tassone et al10 was designed to determine the feasibility of screening in anticipation of a possible future scenario of NBS for FMR1 mutations, and in this respect it succeeded. However, because it identified both premutation and gray-zone allele carriers, the study findings have raised questions about carrier screening.

Should We Screen for the Premutation?

Carrier screening is usually seen in the context of reproductive health care whereby the outcome may inform parents about reproductive options and family planning. In the case of FXS, the identification of carriers not only can advise on the reproductive risk of having a child with FX but may also inform about personal health risks;
The latter raises concerns for genetic counseling regarding the potential to develop, for example, late-onset neurological problems (FXTAS), anxiety, depression, or other medical conditions associated with the \textit{FMR1} premutation.\textsuperscript{5}

The identification of the proband through NBS also raises the need for cascade testing of other family members and subsequent identification of individuals who may be affected by the premutation or full mutation, which can present ethical and legal issues because family members did not directly consent to the screening. Identifying a baby with expanded alleles could lead to increased anxiety, stress, and depression in parents and related family members. However, a recent study\textsuperscript{12} of parents of babies with expanded alleles showed that the quality of the parents’ lives, as defined by scores from measures of anxiety, depression, and stress, was not more affected compared with those who did not have an affected infant and agreed to participate in NBS. If accepted, screening for FXS should be voluntary and therefore involve a consent process, which could overwhelm parents and burden hospitals. However, recently reported data do not indicate reduced participation in screening programs because of these concerns; indeed, a high participation rate was observed.\textsuperscript{10,12,14}

The identification of gray-zone alleles is problematic because the research regarding clinical involvement is very limited and the prevalence of that expansion is very high.\textsuperscript{10,11} Although abnormal molecular phenotypes (higher \textit{FMR1} mRNA and lower fragile X mental retardation protein expression levels) have been reported and some studies have indicated a potential risk for both FXTAS and fragile X–associated primary ovarian insufficiency in individuals carrying a gray-zone allele, more studies of an unselected sample of gray-zone allele carriers are necessary to understand the penetrance of a phenotype in this CGG repeat size range.

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**Figure. Newborn Screening Process**

From the left, blood collected on a blood spot card from a heel prick is used to screen newborns for fragile X syndrome. Isolation of DNA, polymerase chain reaction (PCR), and capillary electrophoresis analysis allow measurement of CGG repeat allele size. The identification of individuals with an \textit{FMR1} expanded allele can lead to cascade testing of extended family members. The benefits of cascade testing include early intervention, genetic and reproductive counseling, access to behavioral and pharmacological treatment, and long-term follow-up services.
Arguments in favor of NBS for FXS include benefits of early detection and possibilities for families to access early intervention programs. A family could avoid a prolonged search to explain the baby’s developmental problems and could plan for better treatments for all in the family who are affected. While babies who have the full mutation and therefore have FXS can benefit from early intervention programs that begin in the first year, such as the Early Start Denver Model, babies carrying the premutation who present with developmental delay also benefit from the Early Start Denver Model and other programs, but treatment data are lacking. Important reasons to diagnose babies as carrying the premutation at the time of birth are similar, including treatment and follow-up for the baby and benefits for family members. Although babies carrying the premutation are far less likely to show developmental problems than those with the full mutation, some do develop autism spectrum disorder, intellectual disabilities, and/or seizures; thus, early intervention is important for this group. Indeed, preliminary results seem to indicate that several forms of clinical involvement can occur very early in life.

Some family members may struggle with premutation or full-mutation problems and can benefit from treatment. Reproductive counseling for the mother and other female carriers in the family tree who may present with clinical symptoms related to the premutation should be considered. However, the needs of the extended family members may be significant and extensive, so time and funding for counseling professionals could limit how many individuals in a family tree can be identified through cascade testing. Therefore, screening could overwhelm and saturate genetic counseling resources and comprehensive care infrastructures, important factors to consider in support of the families with an identified newborn.

Finally, in the past few years we entered into a new age of targeted treatments with a number of clinical trials for pharmacological interventions, such as minocycline hydrochloride, sertraline hydrochloride, metabotropic glutamate receptor 5 antagonists, and γ-aminobutyric acid A and B agonists, showing benefits and efficacy for those with FXS, including young children. New targeted treatments will continue to be developed in the future, and multiple family members who are identified through newborn diagnosis and familial cascade testing and present with clinical involvement will also benefit from early intervention and long-term follow-up.

**Conclusions**

Knowledge regarding prevalence is important to guide health policy decisions regarding the effects of *FMR1*-associated disorders on public health. Emerging, promising results from new targeted treatments for FXS and the feasibility of molecular detection could result in adding *FMR1* mutations to existing NBS programs in the near future. Ideally, an NBS program should include parental education, follow-up, diagnosis, and treatment to guarantee the benefits of

### Table. Prevalence Data Obtained From Newborn Screening Studies

<table>
<thead>
<tr>
<th>Location (Ethnicity [%])</th>
<th>Tested, No.</th>
<th>Sex</th>
<th>Mutation Genotype</th>
<th>CGG Repeats, Range, No.</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Georgia, US (white [45], African American [30], Hispanic [15], Asian [2], multicultural [2], American Indian [1], unknown [5])</td>
<td>36 124</td>
<td>Male</td>
<td>Full</td>
<td>&gt;200</td>
<td>...</td>
</tr>
<tr>
<td>Taiwan (Asian)</td>
<td>4843</td>
<td>Male</td>
<td>Gray</td>
<td>40-54</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
<td>55-200</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Full</td>
<td>&gt;200</td>
<td>...</td>
</tr>
<tr>
<td>Canada (Canadian)</td>
<td>1000</td>
<td>Male</td>
<td>Gray</td>
<td>40-60</td>
<td>...</td>
</tr>
<tr>
<td>Spain (Hispanic)</td>
<td>5267</td>
<td>Male</td>
<td>Gray</td>
<td>45-54</td>
<td>1/26</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
<td>55-200</td>
<td>1/251</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Full</td>
<td>&gt;200</td>
<td>1/2633</td>
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<tr>
<td>Catalan, Spain (Hispanic)</td>
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<td>Male</td>
<td>Gray</td>
<td>53-55</td>
<td>1/449</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
<td>56-200</td>
<td>1/1233</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Full</td>
<td>&gt;200</td>
<td>1/2466</td>
</tr>
<tr>
<td>South Carolina, US (NA)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1459</td>
<td>Male</td>
<td>Pre</td>
<td>55-200</td>
<td>1/730</td>
</tr>
<tr>
<td>Taiwain (Asian)</td>
<td>10 046</td>
<td>Male</td>
<td>Pre</td>
<td>45-54</td>
<td>1/143</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Full</td>
<td>&gt;200</td>
<td>1/730</td>
</tr>
<tr>
<td>US (white [29], Hispanic [25], African American [22], Asian [6], other [9])</td>
<td>7312</td>
<td>Male</td>
<td>Gray</td>
<td>45-54</td>
<td>1/112</td>
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<tr>
<td></td>
<td></td>
<td></td>
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<td>55-200</td>
<td>1/1430</td>
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<td></td>
<td></td>
<td>Pre</td>
<td>55-200</td>
<td>1/209</td>
</tr>
</tbody>
</table>

Abbreviations: NA, not applicable; US, United States; ellipses, not reported.

* Adapted from the article by Tassone et al.<sup>10</sup>

<sup>b</sup> Racial data were not collected for this study.

**Benefits of NBS for FXS**

Arguments in favor of NBS for FXS include benefits of early detection and possibilities for families to access early intervention programs. A family could avoid a prolonged search to explain the baby’s developmental problems and could plan for better treatments for all in the family who are affected. While babies who have the full mutation and therefore have FXS can benefit from early intervention programs that begin in the first year, such as the Early Start Denver Model, babies carrying the premutation who present with developmental delay also benefit from the Early Start Denver Model and other programs, but treatment data are lacking. Important reasons to diagnose babies as carrying the premutation at the time of birth are similar, including treatment and follow-up for the baby and benefits for family members. Although babies carrying the premutation are far less likely to show developmental problems than those with the full mutation, some do develop autism spectrum disorder, intellectual disabilities, and/or seizures; thus, early intervention is important for this group. Indeed, preliminary results seem to indicate that several forms of clinical involvement can occur very early in life.

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**Conclusions**

Knowledge regarding prevalence is important to guide health policy decisions regarding the effects of *FMR1*-associated disorders on public health. Emerging, promising results from new targeted treatments for FXS and the feasibility of molecular detection could result in adding *FMR1* mutations to existing NBS programs in the near future. Ideally, an NBS program should include parental education, follow-up, diagnosis, and treatment to guarantee the benefits of
screening. Thus, it is imperative that services, resources, and infrastructure for early-childhood developmental interventions be expanded and that counseling and education be made available so that treatment of emotional, behavioral, and developmental problems can be recommended for identified newborns and their families when needed.

ARTICLE INFORMATION

Accepted for Publication: August 16, 2013.
Conflict of Interest Disclosures: Dr Tassone has been a consultant for Novartis and Genentech. No other disclosures were reported.
Funding/Support: This work was supported by grant HD02274 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development.

Role of the Sponsor: The funding organization had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Additional Information: This work is dedicated to the memory of Matteo.

Additional Contributions: Randi J. Hagerman, MD, provided helpful comments.

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