Newborn and Carrier Screening for Spinal Muscular Atrophy

Thomas W. Prior, Pamela J. Snyder, Britton D. Rink, Dennis K. Pearl, Robert E. Pyatt, David C. Mihal, Todd Conlan, Betsy Schmalz, Laura Montgomery, Katie Ziegler, Carolee Noonan, Sayaka Hashimoto, and Shannon Garner

1Department of Pathology, The Ohio State University, Columbus, Ohio
2Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, The Ohio State University, Columbus, Ohio
3Department of Statistics, The Ohio State University, Columbus, Ohio
4Department of Obstetrics and Gynecology, Riverside Methodist Hospital, Columbus, Ohio

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Spinal muscular atrophy (SMA) is a common autosomal recessive neuromuscular disorder caused by mutations in the survival motor neuron (SMN1) gene, affecting approximately 1 in 10,000 live births. The homozygous absence of SMN1 exon 7 has been observed in the majority of patients and is being utilized as a reliable and sensitive SMA diagnostic test. Treatment and prevention of SMA are complementary responses to the challenges presented by SMA. Even though a specific therapy for SMA is not currently available, a newborn screening test may allow the child to be enrolled in a clinical trial before irreversible neuronal loss occurs and enable patients to obtain more proactive treatments. Until an effective treatment is found to cure or arrest the progression of the disease, prevention of new cases through accurate diagnosis and carrier and prenatal diagnosis is of the utmost importance. The goal of population-based SMA carrier screening is to identify couples at risk for having a child with SMA, thus allowing carriers to make informed reproductive choices. During this study we performed two pilot projects addressing the clinical applicability of testing in the newborn period and carrier screening in the general population. We have demonstrated that an effective technology does exist for newborn screening of SMA. We also provide an estimate of the carrier frequency among individuals who accepted carrier screening, and report on patient’s knowledge and attitudes toward SMA testing. © 2010 Wiley-Liss, Inc.

Key words: spinal muscular atrophy; carrier testing; newborn screening; SMN1; SMN2

INTRODUCTION

The autosomal recessive disorder proximal spinal muscular atrophy (SMA) is a severe neuromuscular disease characterized by degeneration of alpha motor neurons in the spinal cord, which results in progressive proximal muscle weakness and paralysis. SMA is the most common fatal autosomal recessive disorder, with an estimated incidence of 1 in 6,000 to 1 in 10,000 live births [Pearn, 1978]. Childhood SMA is subdivided into three clinical groups on the basis of age of onset and clinical course: type I SMA (Werdnig–Hoffmann) is characterized by severe, generalized muscle weakness and hypotonia at birth or within the first 3 months [Munstat and Davies, 1992; Zerres and Rudnik-Schoneborn, 1995]. Death from respiratory failure usually occurs within the first 2 years. Type II children are able to sit, although they cannot stand or walk unaided, and often survive beyond 4 years. Type III SMA (Kugelberg–Welander) is a milder form, with onset during infancy or youth: patients learn to walk unaided. Adult-onset SMA, referred to as type IV, while less frequent, has also been reported. However, the large majority of SMA patients have onset of symptoms in infancy or early childhood.

SMA is caused by mutations in the survival motor neuron (SMN) gene [Lefebvre et al., 1995]. The SMN gene comprises nine exons, with a stop codon present near the end of exon 7, and has been...
shown to be the primary SMA determining gene. Two almost identical SMN genes are present at 5q13: the telomeric or SMN1 gene, which is the SMA-determining gene, and centromeric or SMN2 gene. The coding sequence of SMN2 differs from that of SMN1 by a single nucleotide (840C > T), which does not alter the amino acid but has been shown to be important in splicing. Both copies of the SMN1 exon 7 are absent in about 95% of affected patients. Although SMA patients lack SMN1, they always carry at least one copy of SMN2, which is partially functional but unable to compensate for the absence of SMN1. The remaining 5% of affected cases are compound heterozygotes for SMN1 exon 7 deletion and small intragenic mutations. The molecular diagnosis of the SMA consists of the detection of the absence of exons 7 of the SMN1 gene in the majority of cases. The absence of detectable SMN1 in SMA patients is being utilized as a reliable and powerful diagnostic test for the majority of SMA patients.

Newborn Screening

The purpose of newborn screening is to identify affected infants prior to the presentation of clinical symptoms. Newborn screening has been an extremely successful program and has improved the quality of life of many children with a variety of disorders. There has now been an expansion of the number of conditions included in many newborn screening panels. Many newborn screening labs utilize tandem mass spectrometry, which allows for the detection of more than 30 rare disorders simultaneously [Tarini et al., 2007]. The benefits achieved through newborn screening have traditionally referred to the direct benefits to the affected child. However, there are currently a number of disorders screened which do not have a well-defined medical treatment. Additional benefits from implementing a screening program may also include improvement in the quality of life and a decrease in early mortality. The results from newborn screening are also important for the child’s family as a result of the identification of at-risk parents and the possibility for the prevention of additional cases through genetic counseling and carrier testing.

While a number of potential therapies are currently in clinical trials for SMA [Brahe et al., 2005; Weihl et al., 2006; Brichita et al., 2006; Tsai et al., 2007; Swoboda et al., 2009], their success may depend on identifying individuals as early as possible in order to begin treatment before potentially irreversible neuronal loss. In infants with type I SMA, rapid loss of motor units occurs in the first 3 months and severe denervation with loss of more than 95% of units within 6 months of age [Swoboda et al., 2005]. Therefore a very small window for beneficial therapeutic intervention exists in infants with type I SMA. Therapies would need to be administered within the newborn period for maximum benefit which could potentially be accomplished through a newborn screening program for SMA. Lack of an early diagnosis also presents a quandary for the ongoing clinical trials that are assessing the efficacy of agents in symptomatic cases rather than during the presymptomatic phases of the disease. Since these individuals have already experienced motor neuron degeneration, they may not represent the ideal population for the evaluation of an agent to improve motor neuron retention or delay the onset of symptoms. A newborn screening program would not only allow patients to be enrolled in the clinical trials at the earliest time period, but will allow patients to obtain proactive treatment earlier in the disease progression with regard to nutrition, physical therapy, and respiratory care.

SMA also presents a unique case in which prognostic information, regarding disease severity, is also available which can further benefit the family. Several studies have shown that the SMN2 copy number modifies the severity of the disease [Campbell et al., 1997; McAndrew et al., 1997; Wirth et al., 1999; Feldkotter et al., 2002; Mailman et al., 2002]. The copy number varies from zero to three copies in the normal population, with approximately 10–15% of normals having no SMN2. However, milder patients with type II or III SMA have been shown to have more copies of SMN2 than type I patients. The majority of patients with the severe type I form have one or two copies of SMN2; most patients with type II have three SMN2 copies; and most patients with type III have three or four SMN2 copies. This type of prognostic information will obviously be of interest to the parents, and can easily be obtained along with the SMN1 status. An accurate and early diagnosis, particularly for an often misdiagnosed disorder, can provide closure to the family and reduce the cost and anxiety by preventing unnecessary investigations.

The identification of SMA patients during the newborn period can be accomplished only by DNA testing because the disorder does not have a biochemical marker. Thus SMA presents a unique challenge since the testing requires DNA as the substrate, which differs from current newborn screening practices. Although direct DNA testing maybe the next innovation in newborn screening, it is currently being used primarily for reflex testing for 1st-tier-positive results. With its sizeable capacity for multiplexing, array technology has the potential to be utilized for the analysis of DNA in newborn screening [Saxena, 2003; Green and Pass, 2005]. Using liquid microbead array for the detection of the homozygous SMN1 exon 7 deletion, we previously demonstrated that newborn screening for SMA could be technically accomplished [Pyatt et al., 2007]. In a series of 367 blood spots, all 164 affected samples were correctly identified, while 157 unaffected samples and 46 carriers were excluded. The method was shown to perform well using DNA extracted from blood spots and demonstrated both a high sensitivity and specificity.

Carrier Screening

Although the targeted mutation analysis has a high sensitivity in identifying affected homozygotes for the SMN1 deletion, it cannot detect SMA carriers who have heterozygous deletions of SMN1. Rather, SMN1 gene dosage analysis is required to detect carriers and is highly accurate when performed in an experienced laboratory. Since SMA is one of the most common lethal genetic disorders, with a carrier frequency of 1/40–1/60, direct carrier dosage testing has been beneficial to many families with affected children. A number of quantitative assays have been used for the identification of SMA carriers [McAndrew et al., 1997; Feldkotter et al., 2002; Gerard et al., 2000; Anhuf et al., 2003; Su et al., 2005; Huang et al., 2007].

There are known limitations of the carrier test. First, approximately 2% of SMA cases arise as the result of de novo rearrangement events [Wirth et al., 1997]. The high rate of de novo mutations in SMN1 may account for the high carrier frequency in the general
population despite the genetic lethality of the type I disease. The large number of repeated sequences around the SMN1 and SMN2 loci likely predispose this region to unequal crossovers and recombination events and results in the high de novo mutation rate. Second, the copy number of SMN1 can vary on a chromosome; we have previously observed that about 4% of the normal population possess three copies of SMN1 [McAndrew et al., 1997]. It is therefore possible for a carrier to possess one chromosome with two copies and a second chromosome with zero copies [Wirth et al., 1999; Mailman et al., 2001; Ogino et al., 2002; Prior, 2009]. This is referred to as the “2 + 0” genotype. The finding of two SMN1 genes on a single chromosome has serious genetic counseling implications, because a carrier with two SMN1 genes on one chromosome and a SMN1 deletion on the other chromosome will have the same dosage result as a noncarrier with one SMN1 gene on each chromosome. Lastly, the dosage testing does not identify carriers of other types of intragenic mutations in the SMN1 gene, which are present in about 5% of affected patients [Parsons et al., 1998; Wirth, 2000; Alias et al., 2009]. Thus, the finding of normal two SMN1 copy dosage significantly reduces the risk of being a carrier; however there is still a small recurrence risk of future affected offspring for individuals with two SMN1 gene copies. Risk assessment calculations using Bayesian analysis are essential for the proper genetic counseling of SMA families [Ogino and Wilson, 2002].

In our experience, individuals with a family history of SMA are most often referred for carrier testing. However, more broad-based population carrier screening is currently recommended for a number of other genetic disorders with a similar diseases profile. The prototype for heterozygote screening was testing for Tay–Sachs disease in the Ashkenazi Jewish population, where carrier testing has been offered since 1969. Carrier screening, followed by prenatal diagnosis when indicated, has resulted in a dramatic decrease in the incidence of Tay–Sachs disease in the Jewish population [Kaback, 2001]. It is generally accepted that the following criteria should be met in order for a screening program to be successful: (1) disorder is clinically severe, (2) high frequency of carriers in the screened population, (3) availability of a reliable test with a high specificity and sensitivity, (4) availability of prenatal diagnosis and (5) access to genetic counseling. SMA fits the criteria and should be considered for inclusion in population-based genetic screening as recommended by the American College of Medical Genetics (http://www.acmg.net). Since the carrier frequency is high (1/40–1/60) and the carrier test has a relatively good sensitivity (~90%), why has the test not been performed as a more massive screen or on a population basis? One major factor is that the general population is virtually unfamiliar with SMA. Since the affected type I children die within the first 2 years of life, most individuals have no exposure or appreciation of the severity of this very common genetic disorder.

As results from pilot studies often help project outcomes and help to complete a realistic national picture of what can be expected from large-scale programs, and given the devastating nature of SMA, this two tiered study is designed to address: the clinical applicability of testing in the newborn period and carrier screening in the general population. Over 40,000 blood spots, obtained from the Ohio Department of Health, were used to demonstrate the utility and effective nature of SMA newborn screening. The second portion of the pilot study focused on carrier detection among couples seeking prenatal care and queried patient’s knowledge and attitudes toward SMA testing as well as an assessment of the education material utilized during genetic counseling.

METHODS

Dried Blood Samples

Dried blood spots were collected from the Ohio Department of Health. Each sample was collected sequentially after the state had completed all newborn screening. The samples were all deidentified and blood spot punches were collected in 96-well plates. DNA was extracted using the Gentra Capture Card Kit (Gentra Systems, Minneapolis, MN) into a volume of 50 μl according to the manufacturer’s protocol using a Biomek NX Laboratory Automation Workstation (Beckman Coulter, Brea, CA). The Ohio Department of Health approved this study.

Liquid Bead Array

An assay to amplify both for SMN1 exon 7 and GAPDH was developed in conjunction with EraGen Biosciences and reactions were based on standard protocols. Multiplex reactions for SMN1 exon 7 and the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) promoter included 2 μl of template, 1X MC PCR 555 solution (Eragen Biosciences, Madison, WI), 50 nM PCR primers (SMN1 exon 7 forward: (isoC) AGC TAT TTT TTA CTG CCT), SMN1 exon 7 reverse: (isoC) TCA TAA TGC TGG CAG ACT TA/GAPDH forward: (isoC) TTT CAT CCA AGC GTC TAA, GAPDH reverse: TGA GAT TGG CCC GAT, and 1× Titanium Taq Polymerase (Clontech, Mountain View, CA) in a final volume of 10 μl. Samples were cycled for 95°C for 2 min followed by 30 cycles of 95°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec. Allele specific amplification was carried out by the addition of 5 total μl to the previous reaction volume containing 1× MC TSE 555 solution (Eragen Biosciences) and 10 nM each primer for SMN1 exon 7 reverse (TAG3-c3-CAC CCT CCT TCT TCT TTT TAA TGG TAT TCT), and GAPDH (TAG05-(C3spacer)-GAC ACT AGG GAG GAG TCA AGG A). Samples were cycled for 95°C for 30 sec followed by 5 cycles of 95°C for 10 sec and 65°C for 2 min and a final extension of 65°C for 5 min.

For the bead hybridization, 1 μl of the Fast-Shot 30 bead array (Eragen Biosciences) containing a mixture of microbeads 1–30 diluted in 34 μl of MC Hybridization solution (Eragen Biosciences) were added to each sample and incubated for 10 min in the dark at room temperature. Finally, 2 μg of streptavidin R-phycocerythrin conjugate (Prozyme, San Leandro, CA) in 34 μl of standard Luminex sheath fluid (Luminex) was added to bead-DNA extension product mixture and incubated in the dark at room temperature for 15 min. The reactions were read and fluorescent signals were recorded using a Luminex 200 cytometer (The Luminex Corporation, Austin, TX) with IS software version 2.3, collecting a minimum of 30 events for each bead per and the median fluorescent intensity (MFI) was recorded.
Subjects

Women or couples, referred to two perinatal centers in Columbus, Ohio (Ohio State University or Riverside Methodist Perinatal Centers) for genetic counseling and consultation with a maternal fetal medicine specialist, were eligible to be offered SMA carrier screening free of charge. Testing was paid for through a donation from the Claire Heine Foundation, Inc. The Ohio State University institutional review board approved this study. The carrier testing was performed as previously described [McAndrew et al., 1997]. Individuals with a family history of SMA were excluded. Pretest and posttest counseling was required and performed by a genetic counselor. Written information about SMA and screening was also provided (Claire Altman Heine Foundation, Inc., Prevention of Spinal Muscular Atrophy at: http://www.preventsma.org). An instrument to assess basic demographics, knowledge, and experience with SMA as well as attitudes toward SMA screening was created for two scenarios: female and/or male partners who elected to proceed with carrier screening and those who declined testing. Those undergoing carrier screening were asked whether carrier testing contributed to anxiety about pregnancy, factors that contributed to their decision to test and whether cost would influence their decision to have the test. Patients who declined testing were queried regarding reasons they chose not to pursue testing. In both surveys, participants had the option of making comments after each question. Exploratory data analysis included examination of the data for missing, extreme and influential values. Chi-square tests were used for comparisons of categorical variables in the survey data and analyzed using Stata 10.0 (StataCorp, College Station, TX). Confidence intervals used the exact binomial method for the screening study and the maximum likelihood method for the carrier study under the assumptions of a Hardy–Weinberg equilibrium in the population studied, that no chromosome had more than three copies of SMN1, and that individuals with homozygous deletion do not survive to be tested at adulthood.

RESULTS

Newborn Screening

Previously we had shown that the liquid microbead array was a successful platform for the identification of the homozygous SMN1 deletion [Pyatt et al., 2007], found in about 95% of the SMA affected patients. Furthermore we showed that the analysis could be performed on DNA extracted from blood spots which we artificially created from 367 affected, 46 carriers, and 157 unaffected individuals. In addition to the accuracy of the assay, its robustness is a most important consideration. In order to show that the assay can perform in a newborn screening setting as a high-throughput methodology, it must be able to handle the 400–500 Guthrie cards received daily from the Ohio State Department of Health Laboratory. We have now automated the assay, which is amenable to a 96-well plate format and automated processing. The bloodspot is punched from the Guthrie card (Ohio State Department of Health Laboratory) and DNA is extracted using a Biomak NX Laboratory Automation Workstation (Beckman Coulter), which requires 1-hr per 96-well plate. The array platform is composed of four steps: multiplex of the target SMN1 exon 7 and control GAPDH promoter, genotyping by multiplex allele specific elongation, array sorting by bead hybridization, and detection on the Luminex 200 cytometer. The total processing time from the end of the DNA isolation to the placement on the Luminex instrument was approximately 3 hr. The Luminex instrument is capable of analyzing 30 samples per hour. Thus the entire procedure can easily be completed in a single 8-hr shift, and this includes all labor and instrument time. Utilizing two Luminex instruments and two technologists, we were able to perform the assay on the typical number of 400–500 Guthrie cards received daily in the state of Ohio.

The results from the DNA extracted from 40,103 anonymized blood spots, from the Ohio State Department of Health, are shown in Figure 1. Each 96-well plate consisted of 91 samples, three controls positive for SMN1 exon 7 homozygous deletions, and two randomly placed no template controls (blanks). Cluster analysis shows three distinct collections including: the no template controls which collect at the origin (back circles), nonaffected bloodspots (blue squares) which cluster together with SMN1 MFI values ranging from 1,500 to 11,000 and GAPDH MFI values from 2,000 to 19,000 and positive controls (purple triangles) with low SMN1 MFI values (<1,000 MFI) and normal GAPDH MFI values. The GAPDH primer serves as an amplification control and ensures that all samples have amplifiable DNA. We have previously shown that carriers are not distinguished from normals [Pyatt et al., 2007]. The microbead assay is designed to exclusively identify only homozygous deletions and preclude the unwanted identification of SMA carriers. Four positive blood spot samples were identified (yellow squares) and show clear differentiation from the unaffected group and cluster along the Y-axis with the positive controls (purple triangles) with low SMN1 MFI values (<1,000 MFI) and normal GAPDH MFI values. Allele specific reactions for SMN1 exon 7 were also conducted in the forward direction in each multiplex for further confirmation of all SMN1 genotyping calls (data not shown). Samples were targeted for repeat analysis based on either ambiguous clustering or by a lack of concordance in the genotyping calls between the SMN1 forward and reverse reactions. Analysis of the entire 40,103 blood spot series produced a total of seven specimens which required repeat re-extraction from the original blood spot. On repeat testing, all seven demonstrated robust signal intensity and correct genotype clustering from SMN1 forward, SMN1 reverse, and GAPDH primers. There were no cases in which we were not able to make a determination from the original blood spot. Thus a new heal stick from the child would have never have been necessary during this pilot study.

From our data, we estimate the incidence of SMA in the general population to be 1 in 10,026 (95% CI: 1 in 4,517 to 1 in 38,541). The results of the newborn screen are thus in agreement with the 1/10,000−1/10,000 incidence most commonly reported in the literature. The four positive homozygous deletions were confirmed using a competitive PCR [McAndrew et al., 1997] and the SMN2 copy numbers were also determined. Two of the positives samples had two SMN2 copies, which is most consistent with the more severe type I phenotype, while the other two positives were shown to have three SMN2 copies which would be more consistent with the milder type II or III phenotype. Thus, there were two patients
possessing SMN2 levels who may have benefited from earlier enrollment into a clinical trial.

**Carrier Screening**

The Ohio State University Maternal Fetal Medicine and Riverside Methodist Hospital are both referral centers in Columbus Ohio for women seeking prenatal genetic counseling services. SMA carrier screening was offered to patients seeking prenatal counseling, beginning in October of 2007 and ending in June of 2009. Cost of testing was paid through a grant from the Claire Altman Heine Foundation, Inc., and therefore gratuitously to patients (Claire Altman Heine Foundation, Inc., Prevention of Spinal Muscular Atrophy at: http://www.preventsma.org). During the initial visit all patients were offered screening and given brochures about SMA and carrier testing. Patients with a family history were excluded. Overall approximately 60% of patients accepted SMA carrier testing at the time of counseling, of which approximately 90% were Caucasian. Carrier testing was performed on 500 preconceptional or pregnant women and the DNA testing detected 16 carriers for a carrier frequency of approximately 1 in 31 (95% CI: 1 in 19 to 1 in 54). Among the 16 carriers, 14 had their partners tested and all partners tested negative for carrier status. There were two carriers whose partners were not tested: one carrier was not concerned over the increased risk of having an affected offspring, while the other carrier elected to have a prenatal test for the homozygous deletion which was negative.

During this investigation the ratios of SMN1/SMN2 present in the population were determined (Table I). Among the 16 carriers there were: 1 with a 1:0, 3 with a 1:1, 7 with a 1:2, 4 with a 1:3, and 1 with a 1:4 SMN1/SMN2 ratios. There were also 46 individuals identified with three or more copies of SMN1. Using our observed genotype results from the newborn screen and the carrier analysis, and assuming Hardy–Weinburg 2pq equilibrium, it is possible to approximate the frequency of carriers with the “2 + 0” genotype. This is the genotype which results in false negative results when performing SMA carrier testing. Combining the carrier and screening data, the allele frequency of the 0 SMN1 chromosome is estimated to be approximately 0.0124 (95% CI: 0.008–0.016).

**TABLE I. SMN1/SMN2 Genotype Frequencies**

<table>
<thead>
<tr>
<th>SMN1/SMN2</th>
<th>Total</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>1,0</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>1,1</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>1,2</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>1,3</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>1,4</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>2,0</td>
<td>29</td>
<td>5.8</td>
</tr>
<tr>
<td>2,1</td>
<td>180</td>
<td>36</td>
</tr>
<tr>
<td>2,2</td>
<td>215</td>
<td>43</td>
</tr>
<tr>
<td>2,3</td>
<td>14</td>
<td>2.8</td>
</tr>
<tr>
<td>3,0</td>
<td>6</td>
<td>1.2</td>
</tr>
<tr>
<td>3,1</td>
<td>28</td>
<td>5.6</td>
</tr>
<tr>
<td>3,2</td>
<td>8</td>
<td>1.6</td>
</tr>
<tr>
<td>4,0</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>4,2</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>5,0</td>
<td>1</td>
<td>0.2</td>
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Using the maximum likelihood method, the estimated frequency of the \( >2 \) copy chromosome is 0.0485 and therefore the frequency of the \( \geq 0 \) SMA carrier is 2 (0.012) (0.0485) = 0.0012 (0.12%). Thus if an individual has two SMN1 copies, the probability of being an SMA deletion carrier is approximately 1 in 736. From our data approximately 4.9% of carriers will therefore have the \( \geq 0 \) genotype and will not be detected by carrier testing.

Survey responses were available from 392 patients (92% female, 87% Caucasian, 8% African American, and 5% other). Of those women surveyed, 163 (41.6%) declined testing (several individuals had multiple responses): 58.7% cited low anxiety about SMA, 38.0% responded that a positive result would not change pregnancy management or would not elect prenatal diagnosis if the result was positive, 27.3% did not wish to know their genetic status and 13% stated that the testing would be associated with increased anxiety. After adjusting for ethnicity and age, the odds of agreeing to SMA testing was 79% lower among African Americans as compared to Caucasians (\( P < 0.01 \)). Of the 229 (38.4%) patients who underwent testing (Table II) 76.4% had no knowledge about SMA prior to the genetic counseling session. The patient education material (Claire Altman Heine Foundation, Inc., Prevention of Spinal Muscular Atrophy at: http://www.preventsma.org) was read by 169 of the 229 patients and was reported to be very helpful by 74% of the respondents and somewhat helpful by the remaining 26% of respondents. Among those who accepted testing: 74.5% pursued testing because they were interested in their carrier status, 57.3% were worried about the risk for pregnancy, 45% had the test because there was no additional cost, and 47% expressed an interest to contribute to the SMA knowledge base. Following disclosure of results, 98.7% of patients reported that they were glad they pursued screening. Furthermore 96.9% of the participants who underwent screening reported that they would pursue screening if the testing was covered by insurance. If their insurance would not cover the cost of testing, 29% of the participants would still have the testing at a cost of $500. Only one participant who underwent testing reported a negative experience due to the added anxiety of the testing.

**DISCUSSION**

There has been a major expansion in newborn screening throughout the United States with an increasing consensus that newborn screening should not be limited to only disorders for which there is a specific medical treatment. While a number of potential therapies for SMA are currently in clinical trials, their success may depend on identifying individuals as early as possible in order to begin treatment before potentially irreversible neuronal loss. Therefore, in the future, therapies will need to be administered earlier within the newborn period for maximum benefit. A newborn screening program for SMA would not only allow patients to be enrolled in the clinical trials at the earliest time period but would enable patients to obtain proactive treatment earlier in the disease progression with regard to nutrition, physical therapy, and respiratory care. Many infants with SMA have nutritional compromise as a function of their neurologic degeneration, resulting in an accelerated disease course. If identified early, this situation could be managed proactively via nutritional interventions. Furthermore, given the availability of noninvasive respiratory treatments, such as mechanically assisted cough devices to facilitate clearing of secretions, early implementation of such interventions may help to reduce respiratory morbidity and extend lifespan. The same types of arguments are used to support newborn screening for cystic fibrosis (CF): earlier intervention and more proactive treatments. Furthermore, identifying SMA-affected individuals at birth eliminates the pain and cost of unnecessary testing that often takes place in attempting to diagnose an affected patient.

A newborn screening program will also identify milder, late-onset cases of SMA. There is also the small possibility of identifying the exceptionally rare false positive asymptomatic individual with a SMN1 homozygous deletion [Prior et al., 2004]. This early diagnosis of a late onset or extremely rare asymptomatic condition may be met with controversy from the medical community and families who may not want this information. However, the well-documented genotype/phenotype association between the SMN2 copy number and clinical severity may help to specifically select those patients who will benefit most from early therapeutic intervention. The results from newborn screening would also be important for the child’s family because of the possibility for the prevention of additional cases through genetic counseling and carrier testing of at-risk family members. Thus, a newborn screen would provide an early and definitive diagnosis, facilitate prompt treatment and allow at-risk family members to make informed reproductive choices.

The identification of SMA patients during the newborn period can only be accomplished by DNA testing because the disorder does not have a biochemical marker. Thus SMA presents a unique challenge since the testing requires DNA as the substrate, which differs from present tests used in newborn screening laboratories. Previously, using liquid microbead array, we demonstrated that an accurate diagnosis of SMA can be made from DNA extracted from newborn blood spots [Pyatt et al., 2007]. To test the robustness and technical feasibility using the array approach, we have now screened 40,103 newborn blood spots and four SMN1 homozygous deletions were detected. The four positives were confirmed and the SMN2 copy numbers on each positive were also determined in order to obtain prognostic information. Two of the positives had a genotype most consistent with a type I phenotype (two SMN2 copies) and the other two, with three copies of SMN2, would be predicted to have a less severe form of SMA with the severity most often assessed as falling within the SMA II or III designations. We have shown that

**TABLE II. Responses Among Participants**

<table>
<thead>
<tr>
<th>Response</th>
<th>Percentage</th>
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<tbody>
<tr>
<td>76.4% knew nothing about SMA prior to the counseling session</td>
<td></td>
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<tr>
<td>74.5% pursued testing because they were interested in their carrier status</td>
<td></td>
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<tr>
<td>57.3% worried about risk for pregnancy</td>
<td></td>
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<tr>
<td>45% did it because it was free</td>
<td></td>
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<tr>
<td>47% wanted to contribute to the knowledge base about SMA</td>
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<tr>
<td>70.2% were not anxious about SMA after the session</td>
<td></td>
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<tr>
<td>98.7% were glad they were tested</td>
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the assay has a high-throughput and can handle a large number of samples processed in a newborn screening program. Given the trials underway for SMA treatments, this method could be used in newborn screening as a means of ensuring the earliest intervention. The clinical sensitivity of a SMA newborn screen would be approximately 95–98%, since it would not identify affected individuals who are compound heterozygotes possessing one deleted SMN1 allele and a second allele with a point mutation. Furthermore, like tandem mass spectrometry, the array approach allows one to multiplex and analyze many mutations simultaneously. Simultaneous mutation testing reduces both turnaround time and costs. The array platform has greater than 100 different tagged microbeads available, thus making it possible to test for multiple mutations. With a marginal increase in time and cost, new mutations for other genetic diseases can be easily implemented on the Luminex by the addition of different tagged microbeads in the multiplex reaction. Lastly, the Luminex instruments are relatively inexpensive and easy to operate and maintain.

SMA is a clinically severe autosomal recessive disease with a carrier frequency similar to CF, which population carrier screening is currently being offered in the United States. Since 2001, the American College of Obstetricians and Gynecologists and the American College of Medical Genetics has recommended offering CF carrier screening to all pregnant patients [American College of Obstetricians and Gynecologists and the American College of Medical Genetics, 2001]. These recommendations were based on the data from several funded CF carrier screening pilot projects. We have now completed a small pilot project addressing SMA population carrier screening.

Women or couples referred for a variety of unrelated indications to two large perinatal centers in Columbus, Ohio were offered free-of-charge SMA carrier testing at the time of genetic counseling. The goals of the SMA population carrier screening pilot program were to identify couples at risk for having a child with SMA, to gain an estimate of the carrier frequency, and to determine the frequency of the two copy SMN1 chromosomes. Furthermore this is the first study to provide data on patients’ knowledge of SMA, attitudes toward SMA screening as well as an assessment of the education material provided by the Claire Altman Heine Foundation, Inc. During the pilot study, there were 500 carrier tests performed. DNA testing detected 16 carriers for a carrier frequency of 1 in 31. The carrier frequency reported in the literature varies from 1/25 to 1/66, depending upon the population being tested [Feldkotter et al., 2002; Cusin et al., 2003; Smith et al., 2007; Hendrickson et al., 2009]. Furthermore the 4.9% of carriers with “& 0” genotype identified in this study, which will not be detected by carrier testing, is also consistent with the 2.4–5.0% reported in other Caucasian populations [Feldkotter et al., 2002; Chan et al., 2004; Smith et al., 2007; Hendrickson et al., 2009]. However, a recent report has shown that there is a significant difference in carrier frequencies and the 2-copy chromosome genotypes among the African American population [Hendrickson et al., 2009]. The results from this study provide adjusted detection rates based on ethnicity, and thus allow for more accurate Bayesian risk estimates.

During this study we have found a discrepancy between the carrier and newborn pilot studies. A carrier frequency of 1/31 would have predicted a disease prevalence of approximately 1/3,844. However there were only four bloodspots positive for the homozygous SMN1 deletion from a total of 40,103 blood spots tested. The lack of agreement between the higher carrier frequency and lower disease incidence may be the consequence of a ‘0 + 0’ SMN1/SMN2 genotype, which has not been reported and most likely results in fetal demise. However, the samples sizes are too small to allow a definitive conclusion and additional studies with larger cohorts are needed to determine if there is a significant lack of correlation between the carrier frequency and disease incidence. Lastly, differences in the racial makeup between the central Ohio carrier screening population and the state of Ohio newborn screening population may also contribute to the observed discrepancy.

Slightly more than half of individuals accepted SMA carrier screening when it was offered. The survey results indicated that approximately 75% of these individuals had no prior knowledge of the disorder. Race and cost were significant factors in patients’ acceptance of genetic testing for SMA. However, there may be ascertainment bias among the participants who have been referred for genetic counseling and whose desire to pursue testing may not be indicative of the general population. Limited demographic information was obtained, and only included age and ethnicity. The survey did not query additional information regarding level of education or socioeconomic status which may influence a patient’s desire to pursue testing. The patients were all cared for by two perinatal centers in Columbus, Ohio, a Midwestern city of approximately 1 million inhabitants. It was shown that a higher percentage of patients will accept SMA carrier testing if it was covered by insurance. The type of referral indication, especially for those patients being referred for advanced maternal age, may also influence a women’s acceptance of invasive prenatal diagnostic testing and therefore may lead to a higher uptake for carrier screening.

It is the current standard of care to offer routine carrier screening for other debilitating conditions with frequencies similar to SMA (e.g., CF, Tay–Sachs and Canavan disease among Ashkenazi Jewish population). Prospective pilot studies are still needed in order to determine if SMA carrier screening can be cost effective. However, the majority of patients (98.7%) who pursued carrier testing responded favorably to the experience and only one patient responded unfavorably due to added anxiety. Our study suggests that SMA carrier screening is an acceptable option for some individuals seeking prenatal genetic counseling. Since we found that about 75% of individuals are not familiar with SMA and the molecular test does not identify all carriers, it is important that formal genetic counseling services be made available to everyone having the carrier test. It is imperative that individuals understand the limitations of the molecular testing: two SMN1 genes in cis on the one chromosome 5, presence or rare de-novo mutations and the nondeletion mutations. The issue of these false negative results must be explained to all individuals undergoing carrier testing. As is true for carrier screening programs, the testing must be voluntary and informed consent and assurance of confidentiality are absolutely necessary.

We have found that the information necessary for individuals to make an informed decision regarding carrier testing can be presented effectively and efficiently through a counseling session enhanced by printed educational material.
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REFERENCES


