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ORIGINAL ARTICLE

The clinical utility of a risk-modifying SNP to detect carriers for spinal muscular atrophy with increased sensitivity

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Abstract

Background: Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease caused by biallelic inactivation of the survival motor neuron 1 (*SMN1*) gene. With a prevalence of ~1 in 11,000 live births (carrier frequency of ~1:50), SMA is one of the most common severe childhood-onset diseases; therefore, current guidelines recommend pan-ethnic carrier screening for SMA before or during pregnancy.

Routine *SMN1* copy number assessment detects ~96% of all SMA carriers, but not the remaining 4% who harbor two copies of *SMN1* arrayed in -cis [2 + 0]. The c.*3+80T>G risk-modifying SNP positively correlates with this chromosomal configuration and may be used to modify the residual risk of being a carrier for SMA.

Methods: One year after incorporating the detection of the c.*3+80>G risk-modifying SNP into our routine SMA carrier screen, we perform a retrospective chart review to evaluate its frequency and utilization in the prenatal clinic.

Results: In comparison with classic carriers for SMA, study data show that individuals with two copies of *SMN1* and the risk modifier were counseled less frequently about their increased risk of being a carrier for SMA.

Conclusion: Incorporating the c.*3+80T>G risk-modifying SNP is important for detecting carriers for SMA with a higher clinical sensitivity.

KEYWORDS

carrier testing, clinical diagnostics, genetic counseling, population screening, risk-modifying SNP, spinal muscular atrophy

1 | INTRODUCTION

Spinal muscular atrophy (SMA) is an autosomal recessive motor neuron disease characterized by symmetric muscle atrophy and weakness secondary to anterior horn cell degeneration (Lunn & Wang, 2008). The incidence of SMA is approximately 1 in 11,000 live births and has been considered the most common genetic cause of infant

Gardenier Ware and Cecelia Miller should be considered joint first authors.

mortality (Sugarman et al., 2012). Clinically, this disease presents with significant heterogeneity ranging from profound proximal muscle weakness in infancy, hypotonia, and early death (SMA type I) through later-onset neuromuscular phenotypes of decreasing severity (SMA types II, III, IV) (Kolb & Kissel, 2015).

Approximately 95% of SMA patients are homozygous for deletion or gene conversion alleles in the survival motor neuron (*SMN1*) gene (OMIM: 600354) (Prior et al., 2011). The remaining ~5% of patients harbor an intragenic *SMN1* variant (hypomorphic or inactivating) in -trans with a *SMN1* deleted or converted allele. Regardless of mutation type, SMA disease severity is modulated by *SMN2* (OMIM: 601627), a paralogous gene differing from *SMN1* by a single synonymous variant, c.840C>T (Campbell et al., 1997; Mailman et al., 2002; McAndrew et al., 1997; Prior et al., 2004; Wirth et al., 1999). Although this change does not affect the protein, it disrupts a critical exon splice enhancer site in *SMN2* that reduces its splicing efficiency by ~90% compared with *SMN1* (Burghes & Beattie, 2009). The residual full-length *SMN2* transcript (~10%) partially rescues the cellular phenotype in SMA patients, with the severity of the disease ameliorated by each additional copy of *SMN2* present.

As one of the most prevalent autosomal recessive disorders (Lazarin et al., 2013), the American College of Obstetricians and Gynecologists recommends pan-ethnic SMA carrier testing for all women who are pregnant or considering pregnancy in the future (American College of Obstetricians and Gynecologists' Committee Opinion, 2017). In nearly all cases, carriers for SMA have a single copy of *SMN1* (~96%), which is readily detectable by standard quantitative approaches (e.g., quantitative PCR, multiplex ligation-dependent probe amplification, digital droplet PCR) (Prior et al., 2011). A small subset of carriers (~4%) have two copies of *SMN1* duplicated on the same chromosome with no copies on the other chromosome [2 + 0], a configuration that is missed by standard testing methodologies and causes these individuals to be misclassified as noncarriers.

To increase the detection of [2 + 0] carriers for SMA, Luo et al. (2014) performed haplotype analysis and *SMN1* gene-specific sequencing in a cohort of Ashkenazi Jewish individuals to identify genetic variants that segregate with *SMN1* duplication alleles. Using this approach, a set of six haplotype-defining variants, including the NC_000005.10(NM_000344.3):c.*3+80T>G substitution in intron 7 of the *SMN1* gene, were in linkage disequilibrium with a subset of *SMN1* duplication alleles. With *SMN1* population frequency data, this group used Bayesian statistics to approximate the residual risk of being a [2 + 0] carrier for SMA based on the detection of the c.*3+80T>G haplotype-defining SNP. This SNP is present in other populations and linked to *SMN1* duplication alleles at varying degrees; therefore, the utility of this risk-modifying SNP is applicable to additional populations.

To detect carriers for SMA with greater sensitivity, our laboratory routinely quantifies *SMN1* and *SMN2* copy numbers and genotypes for the [2 + 0] risk-modifying SNP (c.*3+80T>G). We reported the clinical impact of this enhanced screening protocol in our laboratory over the last year. The workflow we adopted can be implemented in any clinical molecular diagnostics laboratory in a cost-effective manner without delays in turnaround time.

2 | MATERIALS AND METHODS

2.1 | Case selection and data collection

This study is comprised of adults (≥18 years of age) that were referred to The Ohio State University James Molecular Pathology Laboratory for spinal muscular atrophy carrier testing over the course of 1 year (July 2020–July 2021). In all cases, the 1275 individuals meeting the specified inclusion criteria sought routine prenatal care at The Ohio State University Wexner Medical Center and elected to pursue SMA carrier testing. Of these individuals, a retrospective chart review was conducted to collect demographic data (biological sex, age, and self-described race/population) and genetic data (*SMN1* copy number and risk-modifier status). Race/population data were consolidated into the following six categories: Caucasian, African (African American, African other), Asian (east Asian, south Asian, Asian other), Latino/Hispanic, Mexican/Mexican-American, and Other/Not Specified/Unknown.

2.2 | *SMN1* copy number determination

To determine *SMN1* copy number, a competitive multiplex PCR strategy was used to coamplify *SMN1*, *SMN2*, and *CFTR* (McAndrew et al., 1997). A *Dra*I restriction digest was performed to distinguish *SMN1* and *SMN2* products followed by capillary electrophoresis to size the digestion products (ABI 3130 DNA Analyzer). ABI capillary electrophoresis data were analyzed using GeneMarker v.2.6.3 (SoftGenetics, State College, PA), and relative *SMN1* and *SMN2* copy numbers were determined by expressing the peak areas (*SMN1* and *SMN2*) as a ratio to the two-copy reference control (*CFTR*).

2.3 | Risk-modifier detection

The genomic region harboring the NC_000005.10 (NM_000344.3):c.*3+80T>G (rs143838139) single nucleotide

polymorphism (also referred to as g.27134T>G in the literature) was coamplified along with a control to monitor digestion efficiency (F: 5′-TGG GTT TTA TTT CCA GAC TTC A-3′ and R: 5′-TGC TTT GAT GAC GCT TCT GT-3′) (Luo et al., 2014). Five microliters of the resulting PCR products were digested with 2.5 units of *HpyCH4*III (New England Biolabs, Ipswich, MA) for 1 hr at 37 °C and analyzed by capillary electrophoresis to size the digestion products (ABI 3130 DNA Analyzer). Alleles harboring the c.*3+80T>G SNP were sensitive to *HpyCH4*III digestion and resulted in a 136 bp product, whereas those that did not harbor the SNP were resistant to *HpyCH4*III digestion and remained intact (169 bp product).

2.4 | Clinical workflow

A streamlined clinical diagnostic workflow was developed for routine SMA carrier detection (Figure S1). A peripheral blood specimen was collected and accessioned into the laboratory for those patients electing to pursue SMA carrier testing. Genomic DNA was extracted using the automated EZ1 instrumentation (Qiagen, Hilden, Germany) and quantified by spectrophotometry. For maximal efficiency, samples were batched to faciliate carrying out PCR (*SMN* gene dosage and c.*3+80T>G genotyping), digestion, and capillary electrophoresis steps in parallel. Assay data were analyzed using the GeneMarker v.2.6.3 software (SoftGenetics, State College, PA) with various quantitative and qualitative analyses applied to derive *SMN1* and *SMN2* copy numbers and SNP genotype. Finally, an expert review of the data was conducted with results reported within 3–5 days of sample collection. All reports included *SMN1* and *SMN2* copy numbers; however, risk-modifier status was only reported for individuals with two copies of *SMN1*.

3 | RESULTS

Beginning in July 2020, The Ohio State University James Molecular Laboratory increased the clinical sensitivity of our standard SMA carrier screening protocol by implementing detection of the c.*3+80T>G [2 + 0] risk-modifying SNP. Using this workflow (Figure S1), 1275 individuals underwent SMA carrier testing at our institution over the course of 1 year (July 2020–July 2021). There were 1253 females (98.3%) with a median age of 29 years (range: 18–44 years) and 22 males (1.7%) with a median age of 31.5 years (range: 18–42 years) (Figure 1a). 743 individuals self-identified as Caucasian (58.3%), 319 individuals identified as African (25%), 69 identified as Asian (5.4%), 39 identified as Latino/Hispanic (3.1%), 18 identified as Mexican/Mexican-American (1.4%), and 87 individuals identified as "other" or elected not to specify a race (6.8%) (Figure 1b).

The distribution of *SMN1* copy numbers detected for this cohort includes 22 individuals with a single copy of *SMN1* (1.7%), 1016 individuals with two copies of *SMN1* (79.7%), 198 individuals with three copies of *SMN1* (15.5%), and 39 individuals with four copies of *SMN1* (3.1%) (Figure 1c). Of the 1275 individuals tested, the risk-modifying SNP was present in 181 individuals (14.2%) and absent in the remaining 1094 individuals (85.8%) (Figure 1d).

Table 1 summarizes the proportion of individuals in each demographic category that harbored the risk-modifying SNP. The SNP was most prevalent in the African subset of our cohort, being present in 46.71% of those individuals. The SNP was present at relatively low levels in the Caucasian, Latino/Hispanic, and Mexican/Mexican-American populations, 1.62%, 7.69%, and 5.56%, respectively, and was absent in the Asian population which was limited in sample size.

To understand the clinical utility of our enhanced SMA carrier screen, we performed a retrospective chart review to look for evidence that SMA carrier status was successfully communicated to the patient and whether SMA carrier testing was recommended for the father of the baby. In total, 22 females were identified with a single copy of *SMN1*. In all cases (22/22), their carrier status was communicated, and SMA carrier testing was appropriately recommended for the father of the baby (Figure 2a). Similarly, we looked for these data points in the 40 women that harbored two copies of *SMN1* and tested positive for the c.*3+80T>G risk-modifying SNP. Of these individuals, 31 (77.5%) were informed of their increased risk of being a carrier for SMA, and in 30 of the 31 cases, SMA carrier testing was recommended to the father of the baby (96.8%). In nine cases, there was no documentation of the increased risk of being a carrier for SMA communicated to the patient (22.5%).

To estimate compliance with the recommendation to seek carrier testing for the father of the baby, we noted that 22 male specimens were tested for SMA carrier status during this period. These 22 specimens likely represent the proportion of the 52 female patients that were appropriately counseled about their SMA carrier status; therefore, we estimate that 42% (22/52) of the parents at increased risk for a SMA pregnancy elected to have the father of the baby tested at our institution. In all cases, no SMA male carrier or males at increased risk for being a carrier for SMA were identified.

4 | DISCUSSION

Carrier screening for prevalent X-linked or recessive diseases is a critical part of prenatal care. Importantly, this

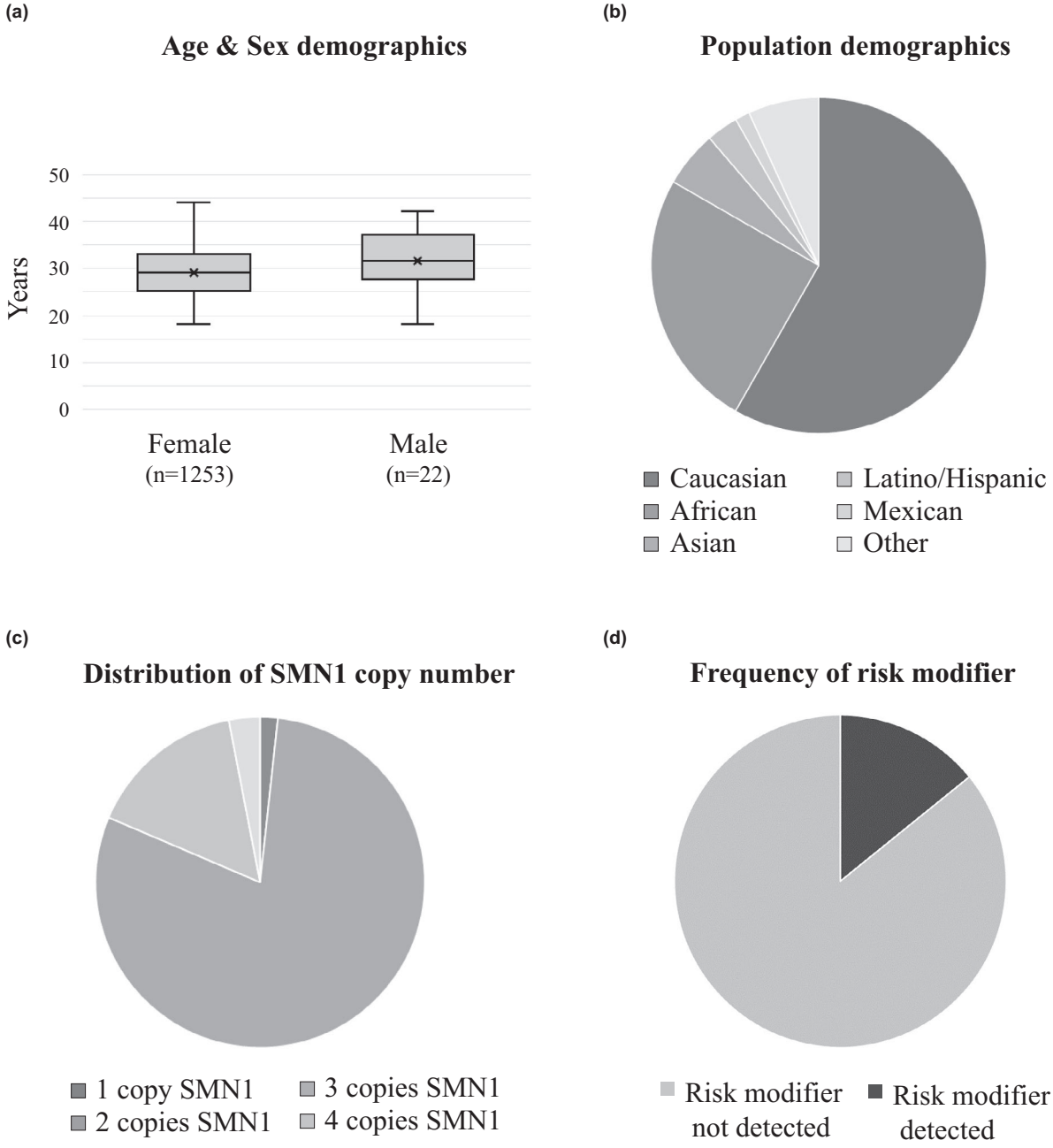

FIGURE 1 Genetic and demographic characteristics of the study cohort. Distribution of patients based on age and sex (a) and self-reported population (b). Frequency of *SMN1* copy number (c) and risk modifier (d) across the study cohort

allows individuals to understand their reproductive risk and may also be used to guide prenatal and/or preimplantation genetic testing. Disease-specific carrier testing may be influenced by ethnicity or family history; however, guidelines developed by the American College of Obstetricians and Gynecologists recommends SMA carrier testing for all patients regardless of ethnicity (American College of Obstetricians and Gynecologists' Committee Opinion, 2017). Standard SMA carrier testing is complicated by the presence of [2 + 0] duplication alleles, which are not detected by standard quantitative techniques. In this study, we describe our experience incorporating the c.*3+80T>G risk-modifying SNP to enhance our SMA carrier testing and evaluate its use in clinical practice.

The decision to add detection of the risk-modifying SNP to our standard SMA carrier screen was influenced by several factors. First, much like our approach to determining SMN copy number, we genotyped the risk-modifying SNP by fragment sizing and capillary electrophoresis. This uniform approach allowed both components of the screen to be carried out in parallel, from setup to analysis. Further, our protocol is streamlined by SNP genotyping of

TABLE 1 Population-level frequency data for *SMN1* copy number and the c.*3+80T>G risk-modifying SNP

Population (n)	*SMN1* copy number (n)	Individuals testing negative for risk modifier	Individuals testing positive for risk modifier	Proportion testing negative for risk modifier	Proportion testing positive for risk modifier
White (743)	1 (15)	15	0	0.9838	0.0162
	2 (676)	671	5		
	3 (52)	45	7		
	4 (0)	0	0		
African (319)	1 (4)	3	1	0.5329	0.4671
	2 (159)	131	28		
	3 (119)	29	90		
	4 (37)	7	30		
Asian (69)	1 (2)	2	0	1.0000	0.0000
	2 (59)	59	0		
	3 (8)	8	0		
	4 (0)	0	0		
Latino/Hispanic (39)	1 (1)	1	0	0.9231	0.0769
	2 (35)	34	1		
	3 (3)	1	2		
	4 (0)	0	0		
Mexican (18)	1 (0)	0	0	0.9444	0.0556
	2 (17)	16	1		
	3 (1)	1	0		
	4 (0)	0	0		
Other (87)	1 (0)	0	0	0.8161	0.1839
	2 (70)	64	6		
	3 (15)	5	10		
	4 (2)	2	0		

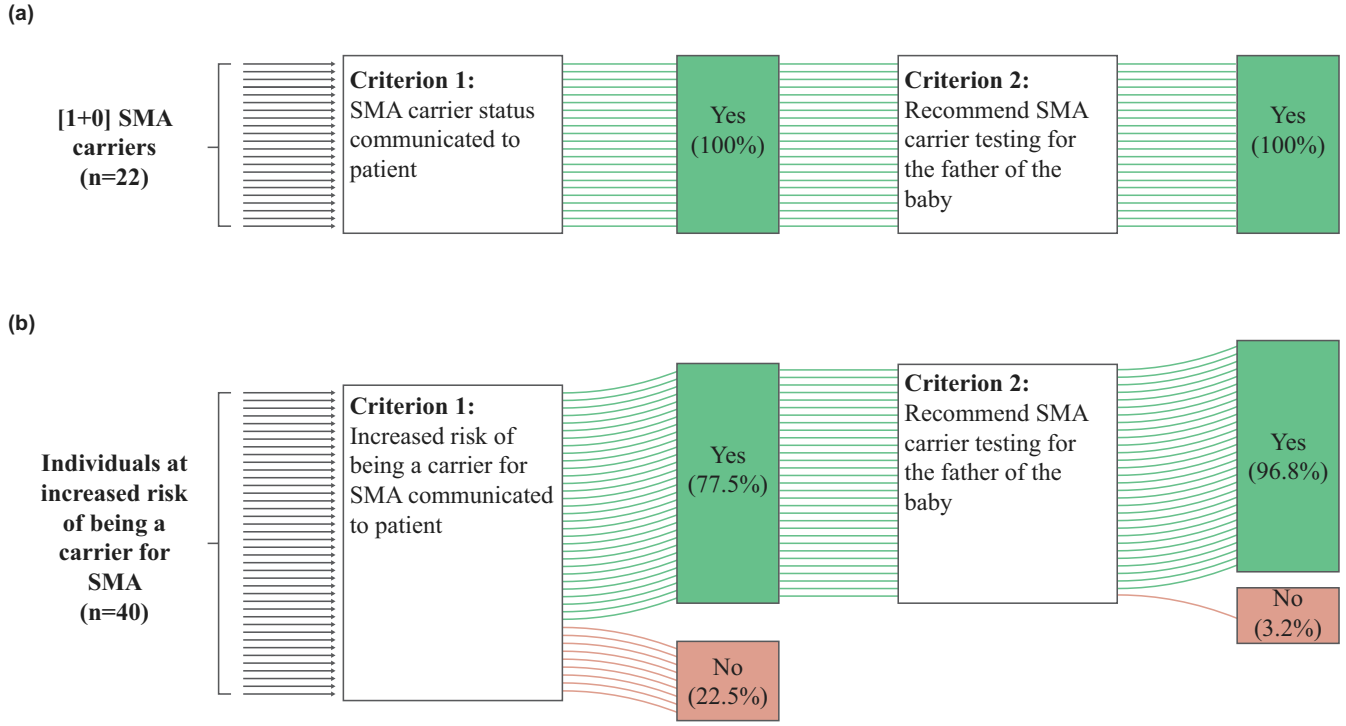

FIGURE 2 Impact of enhanced spinal muscular atrophy carrier screening on patient care. Graphical representation of chart review data to assess utilization of the risk modifier inpatient care. (a) Each horizontal black arrow represents a case testing positive for SMA carrier status with a single copy of *SMN1* detected ($n = 22$) or (b) a case at increased risk of being a carrier for SMA with two copies of *SMN1* and the risk modifier detected ($n = 40$). The chart review criteria used to evaluate the clinical utilization of the enhanced SMA carrier testing are displayed in white boxes with corresponding green (yes) or red (no) lines representing case outcomes

all samples received for SMA carrier testing. Although it is acknowledged that the utility of the risk modifier pertains only to individuals with two copies of *SMN1*, genotyping all specimens allows for uniformity and continuity in setting up the assay. Finally, to automate the reporting step, we introduced logic into the laboratory information system to only report the risk-modifier status where clinically indicated (i.e., patients with two copies of *SMN1*). This approach proved more efficient than implementing a reflex strategy to genotype only individuals testing positive for two copies of *SMN1*, which would certainly delay the turnaround time. This streamlined approach is cost-effective, can generate results quickly, and has allowed for the detection of carriers for SMA with increased sensitivity.

The distribution of *SMN1* copy number among our cohort closely mirrors that of other studies with the vast majority of individuals harboring two copies of *SMN1* (Hendrickson et al., 2009). The frequency of White and Asian individuals testing positive for the risk modifier in our cohort was also similar to others published in the literature (Luo et al., 2014). However, an approximate 5% deviation in the frequency of African, Latino/Hispanic, and Mexican individuals testing positive for the risk modifier in our cohort was noted. This difference may be due to how populations are recorded at our medical center or perhaps due to local/regional variations in immigrant populations. We also noted the occurrence of one individual testing positive for a single copy of *SMN1* and the risk modifier. This genotype has been previously reported and likely represents a recombinant chromosome derived from a duplication allele that harbored the c.*3+80T>G SNP (Alías et al., 2018; Luo et al., 2014). In this case, positive carrier status is assigned based on the detection of one copy of *SMN1* with the risk-modifying SNP bearing no clinical significance.

In the prenatal clinic, the utility of SMA carrier testing centers on risk assessment. Counseling for individuals with a single copy of *SMN1* or individuals with two copies of *SMN1* and the risk modifier includes recommending SMA carrier testing for the father of the baby. For cases where the father also tests positive, a referral to genetic counseling is made to discuss risk and further prenatal testing options. The follow-up component of our study assessed how enhanced SMA carrier screening translated to patient care. We noted that classic carriers for SMA, those with a single copy of *SMN1*, were counseled about their carrier status 100% of the time (Figure 2a). In contrast, individuals with two copies of *SMN1* and the risk-modifying SNP were counseled

only 77.5% of the time ($n = 31$). It is unclear why the remaining 22.5% of individuals were not appropriately counseled. We acknowledge the result may have been discussed by the provider but not documented in the electronic medical record. However, several other factors could explain this discrepancy including the recent introduction of this test, difficulties in a provider explaining the meaning of a risk-modifying SNP to patients, or the possibility that the patient did not identify with one of the characterized populations.

For the individuals appropriately counseled about their SMA carrier status, we found that 100% of classic carriers for SMA and 96.8% (30/31) of individuals with two copies of *SMN1* and the risk modifier were recommended to follow up by testing the father of the baby (Figure 2). In the only case noted where testing the father was not recommended, the modified risk based on the ethnicities of the couple was not believed to be high enough to warrant testing the partner, based on review of the chart. Following counseling, 42% (22/52) of couples at increased risk for an SMA pregnancy elected to have the father of the baby tested at our institution. There are several possible reasons why follow-up partner testing was low, including testing done at an alternative laboratory, out-of-pocket testing costs, limited parental understanding of the significance or rationale for added testing, uncertain paternity, or a judgment that the increased risk was not significant. The latter reason may be pertinent to those of Hispanic descent where the residual risk of being a carrier remains comparatively low (1 in 139.6).

In that regard, the impact of risk-modifier testing remains mainly concentrated in a few demographic populations. As *SMN1* copy number and risk-modifier status are further characterized, this modifier SNP may become more informative as the risk calculation in specific subpopulations is better defined. Nonetheless, this single site testing will never detect all *SMN1* duplication alleles. In the absence of other [2 + 0] haplotypes, the means to detect at-risk individuals primarily remains limited to pedigree and linkage analysis. Indeed, this highlights an opportunity to uncover other SNPs in linkage disequilibrium with *SMN1* duplication alleles such that carriers for SMA may be detected with even greater sensitivity. Further, as long-read sequencing technologies become more commonplace in the clinical diagnostic laboratory, this technology may also be used to facilitate the direct identification of *SMN1* duplication alleles.

Another consideration limiting the detection of [2 + 0] carriers for SMA is testing for only a single SNP in the haplotype block identified by Luo and colleagues. At this time, a single case of a confirmed [2 + 0] carrier for SMA testing negative for the c.*3+80T>G variant and positive for another SNP in the haplotype block (NM_000344.3:c.*211_*212del) has been described in the literature (Alías et al., 2018). As more is learned about the frequency of these alleles in the population, it may become advantageous to genotype multiple SNPs in the haplotype block to achieve the highest clinical sensitivity.

In this study, we present a streamlined approach for implementing the detection of a risk-modifying SNP to enhance the clinical sensitivity of our standard SMA carrier screen. Over the course of 1 year, our laboratory identified an additional 40 individuals at increased risk of being a carrier for SMA (Figure 2b). Based on our chart review, providers overwhelmingly embraced the enhanced testing with nearly 80% of two-copy *SMN1* risk-modifier-positive patients appropriately counseled about their increased risk of being a carrier for SMA (31/40). As other risk-modifying SNPs are uncovered and their utilization in clinical practice becomes more commonplace, the rate of SMA carriers that go undetected will continue to diminish.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Study conception and design: Gardenier Ware, Cecelia Miller, Dan Jones, Matthew Avenarius; *Data curation*: Gardenier Ware, Matthew Avenarius; *Analysis and interpretation of results*: Gardenier Ware, Cecelia Miller, Matthew Avenarius; *Draft manuscript preparation*: Gardenier Ware, Cecelia Miller, Dan Jones, Matthew Avenarius. All authors reviewed the results and approved the final version of the manuscript.

ETHICAL APPROVAL

This study was reviewed and approved by the Ohio State Biomedical Sciences Institutional Review Board.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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