

SHORT REPORT

Diagnostic yield of genetic testing in 324 infants with hypotonia

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Abstract

This retrospective cohort study was designed to determine the yield of genetic tests in hypotonic infants and develop a diagnostic algorithm. Out of 496 patients identified by International Classification of Diseases (ICD) 9/10 coding, 324 patients met the inclusion criteria. Diagnostic yields were 32% for karyotype, 19% for microarray, 30% for targeted genetic tests, 38% for gene panels, and 31% for exome sequencing. In addition, we considered the diagnostic contribution of ancillary tests, including neuroimaging, metabolic tests, and so forth. The combination of microarray and exome sequencing gave the highest diagnostic yield. None of the other tests added significant value in arriving at a diagnosis. Based on these results we propose that the vast majority of infants with congenital hypotonia should start with a microarray and proceed with exome sequencing, with the notable exception of infants with clearly syndromic features in whom karyotyping or targeted testing may be more appropriate.

KEYWORDS

genetic testing, hypotonia, syndrome, whole exome sequencing

1 | INTRODUCTION

Hypotonia is defined as decreased resistance to passive movement and is a common abnormal clinical finding in infants, especially during the neonatal period.¹ Studies have estimated the incidence of congenital hypotonia in term infants to be 0.8/1000 births by extrapolating the incidence of the commonest cause, hypoxic ischemic encephalopathy, to the general population.²

Hypotonia can occur in association with multiple genetic and/or acquired etiologies. Several algorithms have been suggested to help streamline this evaluation.³⁻⁵ Laugel et al. assessed the yield of the different types of testing in 144 neonates with hypotonia. They suggested that neuroimaging, karyotype, and molecular testing

should be used first line, whereas specific metabolic testing, nerve conduction study/Electromyography and muscle biopsy should be reserved as second line testing modalities.⁵

With the advent of Next Generation Sequencing (NGS) testing has markedly improved over the last decade and this has had a unique impact on the evaluation of hypotonic infants. Yet, there is a lack of information regarding the diagnostic yield of different types of genetic tests in hypotonic infants. This lack of information has resulted in a diagnostic approach largely based on expert opinion. Wang et al reported 186 neonates with hypotonia out of which 89 underwent targeted NGS. They uncovered a diagnosis in 20 (22.5%) patients,⁶ including six novel mutations. We conducted a retrospective review of 324 consecutive infants with a diagnosis of congenital hypotonia

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to report the diagnostic yield of genetic testing and propose a diagnostic algorithm in this patient population.

2 | MATERIALS AND METHODS

This is a retrospective cohort study of infants aged 0–12 months with a diagnosis of hypotonia (ICD 10 code P94.2, ICD 9 codes 781.3 and 781.99) seen at a tertiary care hospital (Children's Mercy Hospital, Kansas City, MO) between January 1, 2014 and January 1, 2019. The study protocol was approved by the hospital's institutional review board. A waiver of parental permission/child assent was granted since this was a data collection study only. Data was obtained via an electronic medical record query.

Patients who did not have genetic testing were excluded. Our primary aim was to determine and compare diagnostic yield of genetic testing (karyotype, microarray, targeted genetic testing for specific conditions, gene panels, whole exome [WES], or whole genome sequencing [WGS]). A secondary aim was to test if clinical variables (demographics, type of hypotonia [axial, appendicular, or diffuse], associated symptoms, neuroimaging results, and metabolic testing results) increased the yield of testing and if the results prompted a clinical intervention (medication, therapy, or counseling).

Targeted genetic testing consisted of single gene testing with reflex to deletion/duplication analysis for unique syndromes based on clinical suspicion, methylation and copy number analysis for Prader-Willi (PWS) or Angelman syndrome (AS), *DMPK* gene expansion for myotonic dystrophy, *FMR1* triplet repeats for Fragile X syndrome, and so forth.

3 | RESULTS

We identified 496 patients out of which 172 were excluded due to absence of genetic testing and 324 met inclusion criteria (Figure 1). Among them, 171 (52.77%) were male and 153 (47.22%) were female. Mean age at presentation was 5.17 months (SD \pm 4.01 months) with a median age of 5.33 months (IQR 0.87, 8.92). Overall, 176 out of 324 (54%) patients achieved a diagnosis by one or more of the genetic testing methods. The diagnostic yield of different genetic tests is detailed in Figure 1. Note that some patients underwent more than one genetic test and therefore the sum of percentages of yield exceeds 100% (Table S1). Of 176 patients with a diagnosis, 20% were found to have a chromosomal abnormality, 26% were found to have a copy number change, 45% were found to have single nucleotide variants, and 9% were diagnosed through one of the targeted genetic testing methods, which included methylation studies, trinucleotide repeats, and so forth (Figure 1). Of the 48 patients with diagnostic karyotypes, 35 (73%) had trisomies. One patient had a negative microarray but a diagnostic karyotype that showed a mosaic duplication of the distal long arm of Chromosome 17. Using Cohen's kappa of McNemar's test, we found substantial concordance between the diagnostic yield of karyotype and microarray (kappa = 0.6303 [95% CI

0.4353, 0.8252]). There was no significant association between the diagnostic yield of microarray and targeted genetic tests (Fisher's exact test, p -value = 0.012) or that of microarray and gene panels (p value = 1.00). Of the 41 diagnoses obtained by targeted genetic testing, nine (22%) had PWS, seven (17%) had SMA, three (7%) had myotonic dystrophy, one (2%) had AS, and 21 (54%) had another recognizable syndrome. Two patients who had a nondiagnostic or non-informative gene panel but a diagnostic or positive microarray, were found to have chromosomal microdeletions (Table S2). Five patients, who had a nondiagnostic WES but diagnostic microarray, were found to have chromosomal deletion, duplication, or unbalanced translocation (Table S3).

The most common clinical features in this patient population were developmental delay (82%), followed by gastrointestinal disorders (48%), dysmorphism (33%), respiratory disorders (26%), congenital heart disease (24%), and epilepsy (20%). In most of the cases developmental delay was an a posteriori observation gathered from chart review. Of the 29 patients with hypotonia whose sole other clinical feature was developmental delay, eight (28%) were eventually diagnosed through genetic testing. We attempted to identify symptoms that might increase the yield of genetic tests. Karyotype was the only test, when comparing diagnostic versus nondiagnostic results that flagged clinical symptoms as candidate variables. A stepwise logistic regression model applied to 148 individuals who underwent karyotype revealed that dysmorphism increases the odds of obtaining a diagnostic karyotype by 15-fold (95% OR; CI: 6.1, 36; p -value = <0.0001). The most common type of hypotonia noted in 212 out of 324 patients (65%) was diffuse hypotonia, that is, axial and appendicular low tone on physical exam. There was a significant association between the presence of diffuse hypotonia and a diagnostic karyotype result (p -value = <0.001 , Table S4).

Two hundred fifty-five patients had a brain magnetic resonance imaging (MRI), of those 126 (49%) were abnormal. No MRI abnormality was found to associate with an increased yield of any genetic test (Fisher's exact p -value = 0.9558, Table S5). We also looked at the yield of metabolic testing in this patient population. Of the 209 patients who underwent some combination of metabolic testing (serum amino acids, acylcarnitine profile, urine organic acids, very long chain fatty acids, etc.) seven (3%) patients were diagnostic (Table S6). Of those, four were confirmed by NGS. Among the study population we identified novel pathogenic or likely pathogenic variants in 18 patients (6%) out of which 13 remain unpublished (Table 1). Finally, we evaluated for any intervention following the genetic diagnoses. Of 176 patients with a molecular diagnosis, 172 (98%) received some combination of physical, occupational, and/or speech therapy, 161 patients (91%) received genetic counseling, and 10 patients (6%) received a disease altering drug including two SMA patients who received avxs-101 gene therapy (Table S7).

4 | DISCUSSION

The list of conditions that cause congenital hypotonia is extensive and the approach to the diagnostic workup is complex. We attempted to clarify this

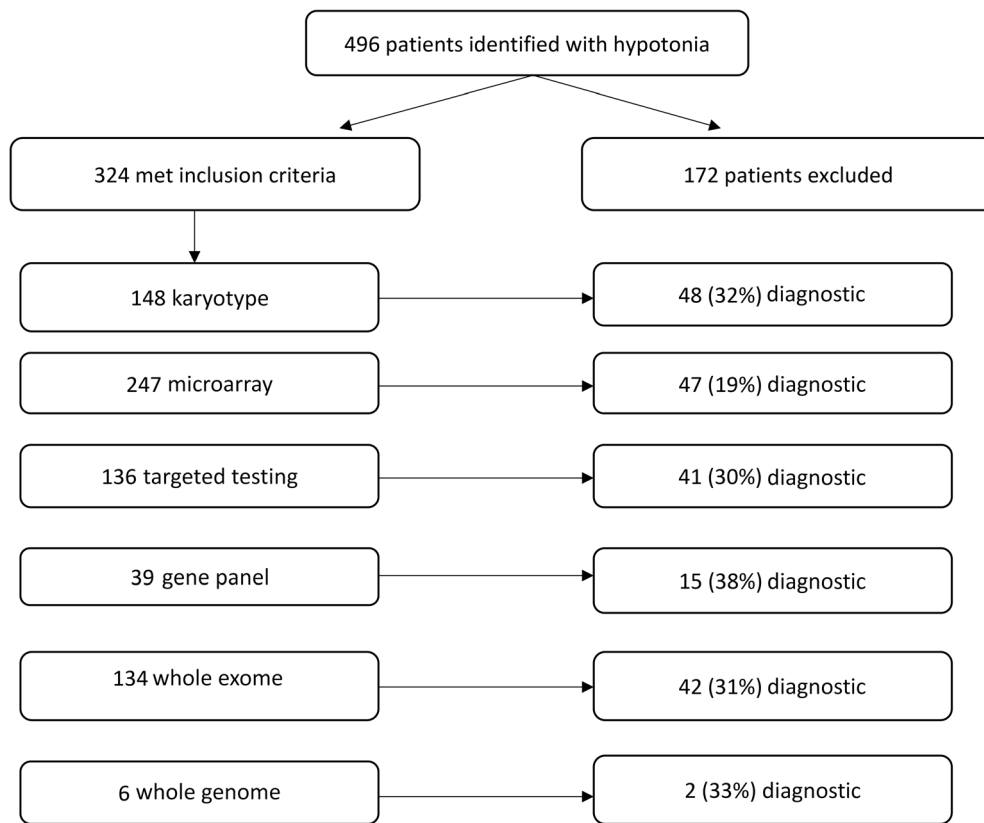
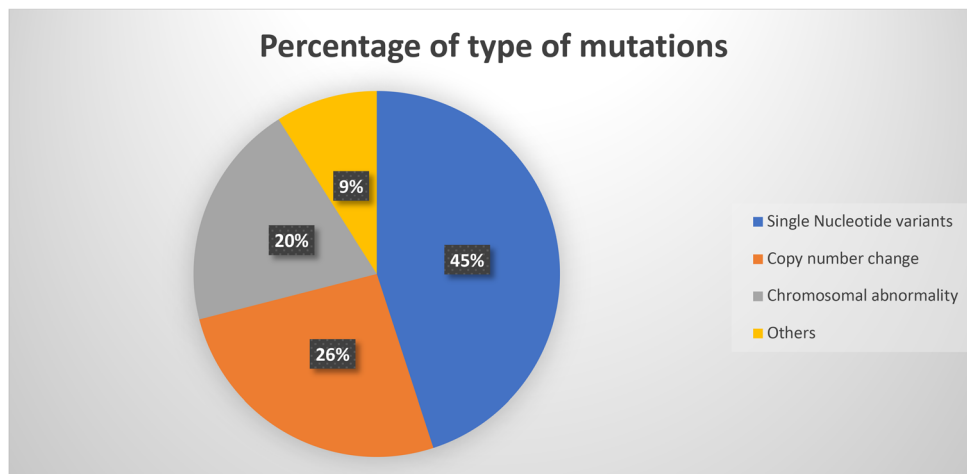


FIGURE 1 Schematic representation of diagnostic yield of genetic testing in study population



conundrum by proposing a sequential diagnostic approach (Figure 2). We observed a relatively high diagnostic yield of karyotype likely due to majority of these patients having trisomies that are easily recognizable clinically. Eighty-three percent (29/35) of the patients with clinically obvious dysmorphic features had a karyotype but no microarray done. Had all of these patients been tested by microarray; the yield of the microarray would have been much higher. The microarray was used almost exclusively for patients who did not have an obvious recognizable syndrome. This may account for the slightly lower yield of the microarrays (19%) in our study. Given that a microarray will pick up vast majority of copy number variations (CNV), we argue that it is no longer necessary to obtain a karyotype, except for patients with easily recognizable trisomies such as Down syndrome.

Targeted genetic tests are cheaper (\$356–\$1800) and have a faster turnaround time (7–14 days) than microarrays (\$3650, 14 days) or WES (\$5610, 27 days). We recommend pursuing these tests in recognizable clinical syndromes (PWS, AS, SMA, myotonic dystrophy, etc.). However, out of 176 patients with a diagnosis, 26% had a CNV picked up on microarray and seven patients had CNVs that were missed on gene panels or WES (Tables S2 and S3). At our institution, a microarray is significantly cheaper and has a faster turnaround time than WES (\$3650 vs. \$5610, 14 vs. 27 days). We recommend starting with a microarray for all infants with congenital hypotonia who do not have a clinically recognizable syndrome. This is in line with recommendation for first tier testing made by American College of Medical Genetics and Genomics (ACMG) targeted toward congenital anomalies and intellectual disability.⁷

TABLE 1 Pathogenic/Likely pathogenic sequence level variants identified in the cohort of studied patients, reported as novel variants at time of diagnosis

Study ID	Gene	Genotype	cDNA change	Protein change	Transcript	ClinVar ID	ACMG classification	PubMed ID
17	PHOX2B	Heterozygous	c.831dup	p.Gly278ArgfsX82	NM_003924.3	N/A	Likely pathogenic	25937001
20	CACNA1A	Heterozygous	c.1618G>A	p.Gly540Arg	NM_023035.2	N/A	Likely pathogenic	27066515
33	ALG1	Heterozygous	c.15C>A	p.Cys5*	NM_019109.4	193 419	Pathogenic	26931382
49	ARID1B	Heterozygous	c.5703dup	p.Lys1902*	NM_020732.3	280 727	Pathogenic	Unpublished
50	NDUFV2	Heterozygous	c.230_236del	p.Ala77ValfsTer7	NM_021074.4	N/A	Likely pathogenic	Unpublished
69	PURA	Heterozygous	c.458_459dup	p.Lys154Glnfs*47	NM_005859.4	N/A	Likely pathogenic	Unpublished
80	USP9X	Hemizygous	c.7218+6_7576-628del	p.Gly2407_Gln2525del	NM_001039590.2	N/A	Likely pathogenic	Unpublished
89	GNAO1	Heterozygous	c.709G>A	p.Glu237Lys	NM_020988.2	420 523	Pathogenic	29935962
116	CASK	Hemizygous	c.1033+1G>A	p.?	NM_003688.3	445 354	Likely pathogenic	Unpublished
119	GLRA1	Heterozygous	c.373G>A	p.D125N	NM_000171.3	377 302	Likely pathogenic	Unpublished
128	SOX9	Heterozygous	c.370G>C	p.Ala124Pro	NM_000346.3	392 305	Likely pathogenic	Unpublished
129	GRIN2A	Heterozygous	c.1895C>T	p.S632F	NM_000833.4	377 051	Likely pathogenic	Unpublished
151	NBAS	Heterozygous	c.2167C>T	p.Q723*	NM_015909.3	445 960	Pathogenic	Unpublished
173	ZC4H2	Heterozygous	c.199C>T	p.Arg67*	NM_018684.3	429 826	Pathogenic	31206972
201	EPG5	Heterozygous	c.3762_3789dup	p.Ala1264Tyrfs*2	NM_020964.2	466 248	Pathogenic	Unpublished
226	STIL	Heterozygous	c.2814C>G	p.Tyr938*	NM_003035.2	N/A	Pathogenic	Unpublished
233	COG5	Heterozygous	c.1828delA	p.Ile610Phefs*4	NM_006348.3	N/A	Likely pathogenic	Unpublished
235	KAT6B	Heterozygous	c.4459_4460del	p.Ser1487Ter	NM_012330.3	N/A	Pathogenic	Unpublished

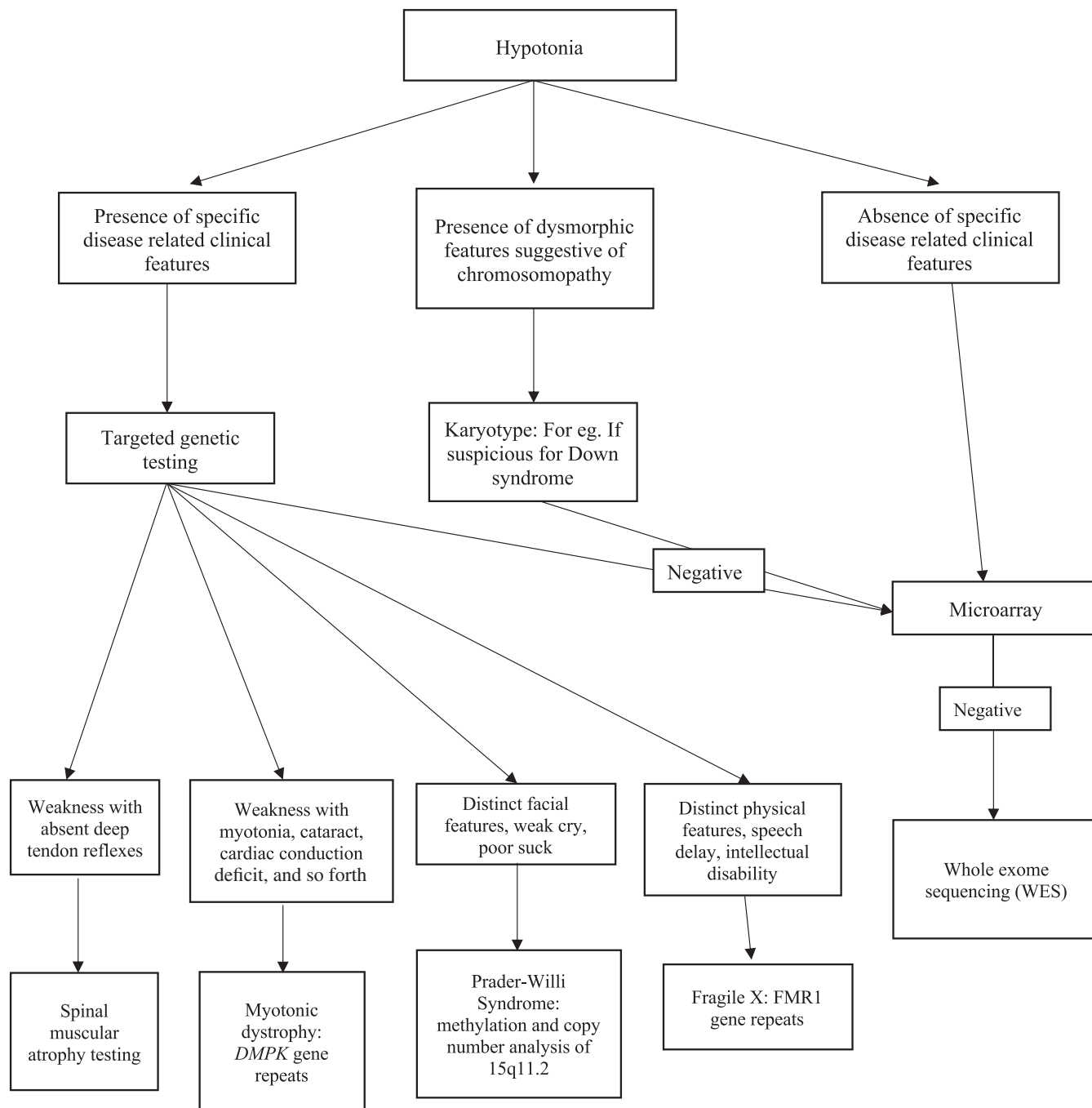


FIGURE 2 Infants with hypotonia require a step-wise approach toward diagnosis. In the absence of clinical features associated with a specific genetic disease, the highest diagnostic yield can be achieved by microarray with reflex to whole exome sequencing

It is worth noting that incorporation of deletion/duplication algorithms into WES analysis is becoming more broadly adopted and may reduce the utility of microarrays in the future.⁸

Yang et al. reported a diagnostic rate of 25% using WES in patients with suspected genetic disease, with a higher yield (36%) in children with neurologic symptoms.⁹ We observed a diagnostic yield of 31% in patients who underwent WES. Furthermore, 18 of the disease-causing variants were novel variants at the time of diagnosis that would have been missed with other genetic testing methods. In our cohort, seven of the patients who underwent gene panels required WES. This adds

unnecessary cost and time to the diagnostic process. We recommend proceeding with WES when a microarray is nondiagnostic.

In most studies, the diagnostic yield of WES (40%) is only slightly lower than that of WGS (42%).¹⁰ At this time, we do not believe that there is a strong rationale for obtaining genome sequencing over exome sequencing except for a few extraordinary cases where time is of the essence and an all-encompassing diagnostic test will allow for life altering clinical decisions.¹¹

Often when hypotonic infants present with developmental delay as their only other symptom they are labeled as “benign”

and diagnostic evaluation is withheld.¹² We identified 29 such patients, of which eight (28%) were eventually diagnosed with a genetic disease. Withholding evaluation in these patients may lead to a delay in diagnosis, potential therapeutic interventions, and genetic counseling.

A logistic regression analysis showed that dysmorphism increases the odds of a diagnostic karyotype, likely due to large number of patients with trisomies. This is in agreement with other studies that have reported high yields for karyotype testing when applied to patients with dysmorphic features.¹³

We identified a diagnostic yield of only 3% by metabolic tests, which indicate that pursuing metabolic testing in patients with hypotonia may not be necessary unless a metabolic disorder is strongly suspected and/or has an actionable outcome.

Limitations of this study include the retrospective nature of the study and absence of controls inherent to retrospective studies. The variability in provider decision making may impart a bias to the results. In addition, this is a single-center study and therefore the specific arguments regarding cost and turnaround time may vary. Nevertheless, this retrospective review of 324 hypotonic infants shows that a diagnosis will be arrived at in 54% patients via genetic testing and that for many of these patients the results will be actionable. With the exception of infants with an obviously recognizable syndrome, the best approach is to start with a microarray and proceed with exome sequencing if the microarray is nondiagnostic. Gene panels are probably not cost effective when one considers the number of patients who will need to proceed to WES. It is our hope that this approach to the infant with hypotonia will simplify the diagnostic conundrum these patients have long represented for clinicians.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/cge.14057>.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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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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