Comparative analysis of C9orf72 and sporadic disease in an ALS clinic population

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ABSTRACT

Objective: We investigated whether the C9orf72 expansion mutation in patients with amyotrophic lateral sclerosis (ALS) is associated with unique demographic and clinical features.

Methods: Between 2001 and 2015, approximately half of all patients attending the Emory ALS Clinic agreed to donate DNA for research. This research cohort of 781 patients was screened for the C9orf72 expansion, and demographic and clinical data were compared between those with and without the C9orf72 mutation. For mutation carriers without a family history of ALS, we sought further family history of dementia and other non-ALS neurodegenerative diseases in first-degree relatives.

Results: The C9orf72 expansion was identified in 61 patients (7.8%). Compared to those without the expansion mutation, these patients did not differ in race, age, or site of onset. As expected, C9orf72 patients were more likely to have a family history of ALS (59% vs 7.9%) and to present with comorbid frontotemporal dementia (FTD) (14.8% vs 1.7%). Survival was shorter in patients with the expansion (log-rank $\chi^2[1] = 45.323$, $p < 0.001$). Further investigation in 28 patients initially categorized as having no known family history of ALS identified a family history of dementia in 16 cases; 6 of these had characteristics suggestive of FTD.

Conclusions: Comparing the C9orf72 ALS population to the general ALS population, there were no differences in race, age at onset, or proportion of patients with bulbar onset disease. Differences identified in patients with the C9orf72 mutation included shortened survival and an equal proportion of men and women. In addition, we found that assessing family history for dementia may identify other family members likely to be carrying the C9orf72 expansion, reduce the number of sporadic cases, and thus increase our understanding of disease penetrance. ***Neurology*®** **2016;87:1024–1030**

GLOSSARY

ALS = amyotrophic lateral sclerosis; **C9Neg** = patients without the C9orf72 hexanucleotide repeat expansion; **C9Pos** = patients with the C9orf72 hexanucleotide repeat expansion; **FTD** = frontotemporal dementia.

The C9orf72 hexanucleotide repeat expansion is the most common genetic mutation identified in patients with amyotrophic lateral sclerosis (ALS). The expansion mutation is reported to be present in 40%–50% of patients with familial (hereditary) ALS, and 5%–10% of patients with sporadic ALS.[1–3] It remains unclear whether patients with the expansion (C9Pos) are phenotypically different from patients without the expansion (C9Neg).

C9Pos patients with ALS are reported to have a higher prevalence of bulbar onset, earlier age at onset, reduced survival, and a higher incidence of comorbid dementia when compared to patients with sporadic disease.[4–8] Other studies, however, describe C9Pos patients with ALS as exhibiting clinical features similar to those with sporadic disease, only with a more rapidly progressive disease course.[9,10] Table e-1 at Neurology.org summarizes previous reports comparing C9Pos to C9Neg cohorts. Additionally, the pathologic expansion has been identified in 5%–10% of patients with ALS designated as sporadic, though these analyses have

Supplemental data at Neurology.org

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excluded family histories of nonmotor manifestations that are seen in people with the C9 expansion, specifically frontotemporal dementia (FTD).[1,9,11]

There are practical advantages in defining the clinical similarities and differences between C9Pos and C9Neg patients, including whether mechanistic findings from C9 models can be generalized to C9Neg ALS, and whether ALS clinical trials should focus specifically on the C9Pos population. The Emory ALS Center maintains a large collection of DNA samples from patients with ALS along with their demographic and clinical characteristics.[12] In this large clinical cohort, we screened patients' DNA for the C9orf72 expansion and compared the baseline characteristics of C9Pos and C9Neg patients with ALS as well as their survival profiles to determine if C9Pos ALS is phenotypically distinct from C9Neg ALS.

METHODS

Standard protocol approvals, registration, and patient consents. Informed consent was obtained from all patients or their authorized legal representatives. The Emory University Institutional Review Board approved the protocols for DNA analysis and retrieval of de-identified clinical information.

Patients. From January 2001 until December 2015, all patients with a diagnosis of ALS based on a clinical phenotype of progressive upper and lower motor neuron disease, corroborated by exclusionary testing and EMG findings, were recruited for collection of DNA and clinical information for research. A total of 859 blood samples were collected, representing 50% of all eligible patients. The relative proportion of patients contributing DNA was uniformly distributed throughout the 15-year study period. Blood samples were processed for DNA extraction according to the manufacturer's protocol (Qiagen, Venlo, Netherlands) and stored at −80°C until analysis.

Demographic and clinical information acquisition. Demographic (sex, race, and family history of a first-degree relative with ALS) and clinical information (site and age at onset and diagnosis) were obtained at the initial visit. Onset of ALS is defined by the first recognition by the patient of weakness or spasticity. A comorbid diagnosis of FTD was determined by the treating ALS neurologist (J.D.G.). Because of the evolving consensus FTD diagnostic criteria throughout the study period, operationalized criteria proposed in 2001 were consistently applied in subsequent years.[13] Information regarding death and tracheostomy-free survival was provided by family members, caregivers, and through searches of public records, including published obituaries and the social security death index (http://FamilySearch.org).

Genetic analyses. Genotyping for the C9orf72 hexanucleotide repeat was performed using the repeat primed protocol, as described by Dejesus-Hernandez et al.[14] Briefly, DNA was PCR amplified using 3 primers, one that is fluorescently labeled and incorporates into the amplified product, which is read using capillary electrophoresis on a DNA analyzer (ABI3730; Thermo Fisher, Waltham, MA). Results are interpreted using amplified fragment length polymorphism analysis in GeneMarker software (Softgenetics, State College, PA); a cutoff of 30 repeats was used to differentiate C9Pos from C9Neg cases.[14] DNA from a C9Pos control from Coriell Institute for Medical Research (6769B1) was included in sample runs.

Investigation of family histories. Family history was obtained through a structured interview during clinic visits and recorded on a standardized form. Patients who had a first-degree relative with ALS were categorized as familial ALS; those without a family member with ALS were considered sporadic. In order to verify the number of sporadic cases in our C9Pos cohort, we telephoned family members (spouses or children) of C9Pos patients without a known family history of ALS. During these follow-up calls, we confirmed the accuracy of the absence of ALS in the family history, and also asked about diagnoses of other neurodegenerative diseases in first-degree relatives. If dementia was present in a first-degree relative, we asked about behavioral changes, since behavioral variant FTD is the most common form of dementia seen in conjunction with ALS, whether or not the C9orf72 expansion mutation is present.[2,15,16]

Statistical analyses. Statistical analysis was carried out in IBM SPSS Statistics 22 (Chicago, IL). Between-group comparisons of continuous variables (age at onset, age at diagnosis, and time to diagnosis) were performed using Student t tests, and comparisons of categorical variables (sex, race, site of onset, presence of FTD, and positive family history of ALS) were analyzed by χ^2 tests. Kaplan-Meier analysis was used to determine effect of C9orf72 repeat expansion on survival from age at onset of symptoms to death (or tracheostomy). Significance was set at $p < 0.05$ (2-tailed test). Multiple comparisons were accounted for by Bonferroni correction (p threshold after correction = 0.017). Other factors that may influence survival were adjusted for through a Cox proportional hazards model (backward stepwise likelihood ratio), including age at onset (as a continuous variable), sex (male vs female), site of onset (bulbar vs not bulbar), family history of ALS (familial vs sporadic), presence of FTD, and C9orf72 expansion status. Missing data in the analysis comparing presence of clinical FTD between the groups were handled by excluding cases from analysis as this was only assessed in 727 of the 781 cases.

RESULTS

A total of 859 patients donated DNA for research, of which 781 samples were available for screening. The expansion mutation was identified in 61 cases (7.8%). Comparing the C9Pos and C9Neg populations, there were no differences in age at onset or diagnosis, race, or site of onset (bulbar vs other) (table 1). There was a shorter time to diagnosis in the C9Pos group (1 year vs 1.5 years, $p < 0.001$). There was also a difference in the sex ratio of the 2 groups; 1.81 in the C9Neg group vs 0.97 in the C9Pos group. Clinical symptoms consistent with FTD were identified in 21 patients among 727 for whom cognitive or behavioral information was available. FTD was more common in C9Pos than C9Neg patients with ALS (9/61 vs 12/720, $p < 0.001$). This was expected given the understanding that the C9orf72 expansion is the most common known mutation found in patients with familial FTD.[17]

A family history of ALS was originally recorded for 90 of the 781 patients examined (11.5%); 33 of these

Table 1 Demographic and clinical characteristics of patients with amyotrophic lateral sclerosis (ALS) with (C9Pos) and without (C9Neg) the pathogenic C9orf72 expansion

	C9Pos (n = 61)	C9Neg (n = 720)	*p* Value
Sex, n (%)			0.027[a]
Male	30 (49.2)	457 (63.5)	
Female	31 (50.8)	263 (36.5)	
Race, n (%)			0.572
Asian	0	3 (0.4)	
Black	2 (3.3)	69 (9.6)	
Caucasian	59 (96.7)	630 (87.5)	
Other	0	5 (0.7)	
Other–East India	0	1 (0.1)	
Pacific Islander/Native Hawaiian	0	4 (0.6)	
Unknown	0	8 (1.1)	
Family history of ALS, n (%)			<0.001[a]
True	33 (54.1)	57 (7.9)	
False	28 (45.9)	663 (92.1)	
Site of onset, n (%)			0.742
Bulbar	15 (24.6)	191 (26.5)	
LE	19 (31.1)	235 (32.6)	
UE	25 (41.0)	279 (38.8)	
LE + UE	1 (1.6)	5 (0.7)	
Diaphragm	0	9 (1.3)	
Head drop	0	1 (0.1)	
Unknown	1 (1.6)	0	
Age at onset, y, mean (SD)	57.9 (8.7)	56.3 (13.5)	0.177
Age at diagnosis, y, mean (SD)	58.9 (8.6)	57.8 (13.4)	0.387
Time to diagnosis, y, mean (SD)	0.9 (0.8)	1.6 (2.1)	<0.001[a]
Survival, y, median (95% CI)	2.4 (1.9–2.9)	4.3 (3.8–4.7)	<0.001[a]
Survival range, y	0.1–7.0	0.3–37.7	
Clinical FTD, n (%)			<0.001[a]
False	52 (85.2)	654 (90.8)	
True	9 (14.8)	12 (1.7)	
Data not collected		54 (7.5)	

Abbreviations: CI = confidence interval; FTD = frontotemporal dementia; LE = lower extremity; UE = upper extremity.
p Values listed are from *t* test, Pearson χ^2 test, or Kaplan-Meier survival log-rank analysis; see Statistics in Methods. Chi-square analysis for site of onset compared bulbar onset to nonbulbar onset.
[a] Significant.

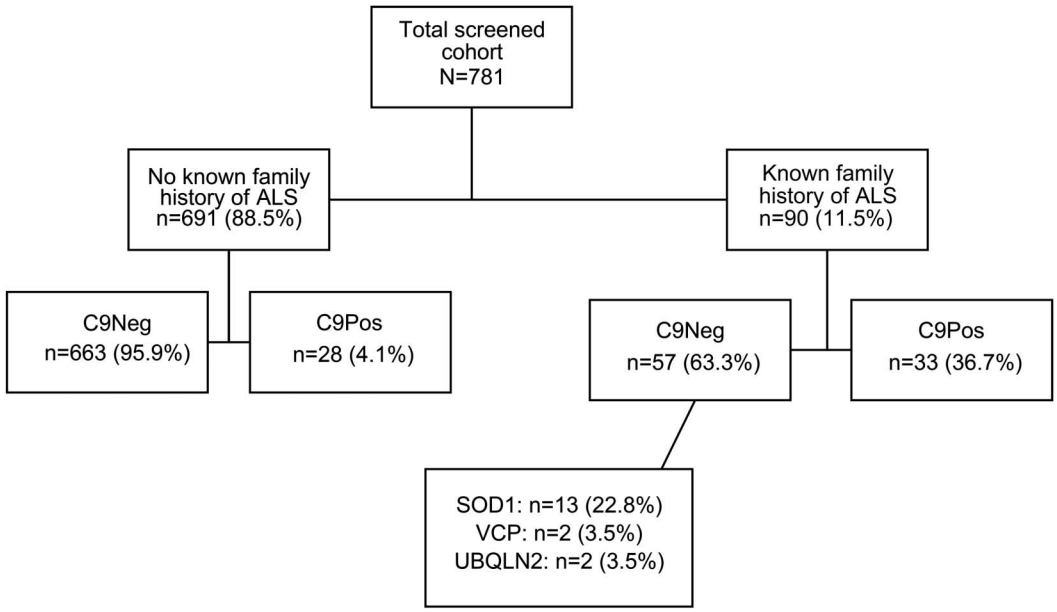
patients with a family history had the C9orf72 expansion (36.7%, figure 1). Other disease-causing mutations accounted for an additional 19% of patients with familial disease, including 13 patients with *SOD1*, 2 patients with *VCP*, and 2 patients with *UBQLN2* mutations. As expected, a family history of ALS was more common in C9Pos patients (33/61, 54%) than C9Neg patients (57/720, 7.9%) ($\chi^2[1] = 117.63$, $p < 0.001$). Twenty-eight of the 61 C9Pos patients had no family history of ALS (45.9%), indicating that 4.1% (28/691) of our sporadic population carried the C9orf72 expansion.

In contacting family members of the 28 C9Pos sporadic patients, we identified one additional case with a brother who died of ALS (the patient did not previously provide this information). During this investigation, we also identified a family history of dementia in 16 of these 28 cases (57.1%), with 6 of these cases displaying behavioral changes consistent with FTD.[18,19] These changes include withdrawal, institutionalization, aggressive behavior, irrational behavior, and suicide attempts (figure e-1). In addition, a family history of Parkinson disease was identified in 2 of the 28 cases, one of whom also had a family history of dementia.

Kaplan-Meier analysis showed a survival difference between C9Pos and C9Neg patients (log-rank $\chi^2 = 45.323$, $p < 0.001$; figure 2). Restricting the analysis to cases with survival or follow-up time less than or equal to 10 years, to more accurately represent ALS cohorts, revealed the same finding (figure e-2). A follow-up Cox proportional hazards model that adjusted for age at onset, sex, site of onset, family history of ALS, FTD, and C9orf72 expansion status confirmed that the survival difference was associated with the presence of the expansion mutation (hazard ratio 2.161, 95% confidence interval 1.596–2.927, $p < 0.001$) (table e-2).

DISCUSSION The recent discovery of the C9orf72 expansion mutation identified a distinct population of patients with ALS who many have suggested are different, clinically and pathophysiologically, from other ALS populations.[14,20] This is an important question when designing therapeutic interventions that may be specific for the C9Pos population. We approached this question by comparing baseline demographic and clinical features of C9Pos to C9Neg patients from a cohort of 781 patients with ALS to determine whether these 2 groups are clinically distinct, adding to published reports from European cohorts.[21,22] This was a clinic-based series of patients with ALS, which allowed us to avoid bias in patient selection. All patients at the Emory ALS Center are asked to donate DNA for research purposes, and thus the only criteria for inclusion were the diagnosis of ALS and the consent to donate blood for research. Analysis of demographic and clinical characteristics from the cohort of patients seen at clinic who did not donate blood during the interval of this study showed that the patients included in this study are representative of the entire population attending the ALS clinic. Previous

Figure 1 Flowchart of family history of amyotrophic lateral sclerosis (ALS) in screened cohort



A total of 781 cases were screened for the C9orf72 expansion. Of those 781, 90 cases had a known family history of ALS, while 691 had no known family history of ALS. C9orf72 hexanucleotide repeat expansion (C9Pos) cases with a known family history of ALS made up 36.7% of the cases with a known family history of ALS. In the remaining cases with known family history of ALS who did not have the C9orf72 hexanucleotide repeat expansion (C9Neg), there were 13 patients with *SOD1* mutations, 2 patients with *VCP* mutations, and 2 patients with *UBQLN2* mutations. This chart represents information prior to follow-up interviews and reanalysis of clinical charts to obtain information on family history of dementia and other neurodegenerative diseases.

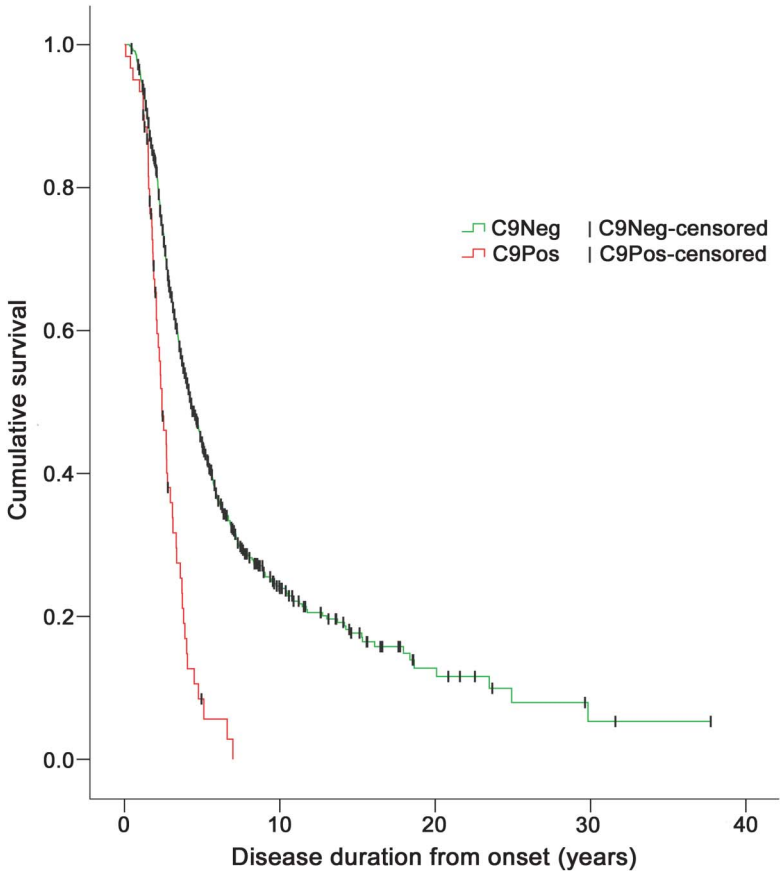
descriptions of C9Pos patients are relatively small and do not include comparisons to a large, unselected cohort of C9Neg patients (table e-1).[3,23]

Other than the presence of a family history of ALS and increased prevalence of FTD, these 2 groups were clinically similar at presentation to the clinic. The distribution of site of onset of disease in our cohort of C9Pos patients closely mirrored that of C9Neg patients and was typical of other ALS cohorts.[24] The racial makeup of both groups was largely Caucasian,[25] which reflects our patient population. The equal distribution of men and women in the C9Pos group may reflect the autosomal dominant inheritance of the C9orf72 expansion mutation. Age at disease onset and age at disease diagnosis were similar and comparable to published reports from other ALS cohorts.[26,27] Interestingly, C9Pos patients were more likely to receive a diagnosis sooner after symptom onset than C9Neg patients. This difference may be explained by a high index of suspicion in patients with a known family history of ALS. This hypothesis is supported by a shorter time to diagnosis in all patients with a family history of ALS (1.2 vs 1.6 years).

We did not identify an earlier age at disease onset or increased prevalence of bulbar onset as was previously reported for other C9Pos ALS cohorts.[4,8,28,29] Earlier age at onset in C9Pos patients has been used as a rationale to describe C9orf72-related ALS as a more aggressive form of the disease. Our inability to confirm this finding might be explained by our study design, which compared the C9Pos group to a concurrent and unselected group of patients with ALS, rather than a designated comparison cohort.[8] Additionally, the published reports of increased prevalence of bulbar onset in C9Pos patients has been used to suggest a more severe disease since patients with bulbar disease are typically thought to carry a worse prognosis.[30,31] Bulbar onset was not overrepresented in our C9pos cohort, though we acknowledge that the numbers of C9Pos compared to C9Neg patients were relatively small.

As expected, we found that C9Pos patients were more likely to report a family history of ALS, and there was an increased prevalence of clinical FTD in this group. Since formal cognitive testing was not conducted, the number of cases with comorbid FTD was low compared to reports of FTD in other ALS cohorts; our analysis of FTD within each of the groups only identified the most obvious FTD cases and likely underestimates the true presence of comorbid FTD in our population. Our finding of reduced overall survival in our C9Pos population compared to C9Neg patients is consistent with the published experience from other C9Pos cohorts.[3,4,8,22,23] In addition, we used a Cox proportional hazards model to determine the influence of variables other than the C9 mutation, such as

Figure 2 Survival analysis



Kaplan-Meier curve analysis of survival of patients with amyotrophic lateral sclerosis with (C9Pos, red line) and without (C9Neg, green line) the pathogenic C9orf72 expansion (log-rank $\chi^2 = 45.323$, $p < 0.001$). Hash marks indicate censored cases in each group. Overall, 781 cases (61 C9Pos, 720 C9Neg) were included in analysis. Median survival time for C9Pos = 2.4 years, 95% confidence interval (CI) 1.9–2.9, for C9Neg = 4.3 years, 95% CI 3.8–4.7.

comorbid FTD, that may affect survival in ALS.[30–32] Survival differences between the C9Pos and C9Neg groups persisted, indicating that the C9orf72 expansion is independently associated with survival.

The link between ALS and dementia has been established for some time, with reports many decades ago of patients with sporadic and familial ALS with comorbid FTD.[33,34] The discovery in recent years of genetic mutations underlying the coexistence of ALS and FTD in families, the most common of which is the C9orf72 repeat expansion, has advanced our knowledge of ALS as a multisystem disease.[35] Yet our clinical definitions for family history of disease have changed very little, and clearly should include questions about dementia, especially with behavioral changes, as well as other neurodegenerative phenotypes. Our re-analysis of family history in our C9Pos cohort with no known family history of ALS suggests that relatively few of these patients lacked any family history of neurodegenerative disease (figure e-1).

Consideration of these new data reduced the prevalence of C9orf72 mutations in the sporadic ALS population to less than 2%, which is on par with reports of *SOD1* mutations in the sporadic ALS population.[36,37] When *SOD1* mutations were first identified as causative in ALS, a number of cases were considered to be sporadic.[38] With further consideration of family histories, some of these cases were reclassified as familial, leading to our current understanding that an exceedingly small percentage of sporadic cases carry *SOD1* mutations. Our results and knowledge of standard methods of defining familial disease cautions us to be more expansive in classifying ALS as sporadic or familial, especially when using historical cohorts.

The C9orf72 expansion phenotype may be different even within families. In individual kindreds, some relatives have dementia, while others have ALS or both ALS and dementia.[14] Age at disease onset and survival within a single family with the C9 expansion is also very heterogeneous.[28] It has been suggested that C9orf72 expansions are fully penetrant by age 80, though there are reports of C9Pos individuals over age 80 without a clinical phenotype.[1,39] Within our C9Pos cohort, age at onset ranged from 34 to 73 years, showing the wide heterogeneity even within this population. Also, though reduced survival is present in our C9Pos group compared to the C9Neg group, the variability within the C9Pos cohort suggests that previous assumptions of expansion carriers being drastically different than other patients with ALS are open to question.

A limitation of this study was our inability to conduct follow-up telephone interviews with our C9Neg patient cohort, as this would have allowed for a comparison of how our definition of sporadic vs familial may have changed in that group as well. Also, the diagnosis of comorbid FTD was based on clinical judgment and not formal cognitive testing, likely resulting in an underestimation of comorbid FTD in each of the groups. Another limitation is the retrospective nature of our analysis. Due to this, we were unable to assess the presence of specific psychiatric manifestations in our C9orf72 cohort. Nevertheless, a strength of this work is that all of the patients included in this study are from a single clinic with a consistent provider, such that comparisons between patient presentations and clinical observations are consistent and any inherent bias is equally distributed between the 2 groups.

Overall, the similarities between C9Pos and C9Neg patients are the most interesting outcome from this work. It begs us to ask the question of why this disease clinically looks so similar in both groups, and whether common pathophysiology underlies disease expression in C9Pos and C9Neg patients. To elucidate common pathways implicated in ALS such that genetic and sporadic forms produce similar clinical phenotypes will require deeper genetic, molecular, and proteomic

analyses. This report presents characteristics of C9Pos patients with ALS and C9Neg patients with ALS side by side and identifies remarkable demographic and clinical similarities that exist in the context of substantial survival differences. These results are important for screening patients and incorporation into clinical trials.

AUTHOR CONTRIBUTIONS

M.E.U.: experimental procedures, data analysis, original and final draft of manuscript. C.F., M.P.: patient care, data collection for database, original and final draft of manuscript. Y.L., L.S.: sample processing, database entry/maintenance. J.E.L.: data acquisition, analysis, and final draft of manuscript. W.H.: patient care, original and final draft of manuscript. M.G.: intellectual contribution, original and final draft of manuscript. J.D.G.: experimental procedures, patient care, original and final draft of manuscript. All authors contributed to and have approved the final manuscript.

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DISCLOSURE

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