GRIN1 polymorphisms do not affect susceptibility or phenotype in NMDA receptor encephalitis

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ABSTRACT

Objective: To determine whether distinct single nucleotide polymorphisms (SNPs) within the glutamate receptor ionotropic NMDA 1 gene (*GRIN1*) are associated with NMDA receptor (NMDAR) encephalitis and whether these same variants are associated with variability in the clinical presentation and course of affected patients.

Methods: We performed clinical follow-up on 48 patients with NMDAR encephalitis and NMDAR autoantibodies detected in serum or CSF. All RefSeq *GRIN1* coding exons were sequenced in 39 Caucasian-European patients, and the frequencies of SNPs were compared with those of an ethnically similar population using a case-control study design. Predetermined clinical variables were compared between patients with and without identified SNPs.

Results: Two SNPs were identified in *GRIN1*: 24 (62%) Caucasian-European patients with NMDAR encephalitis had alternate alleles at both rs6293 (exon 6) and rs1126442 (exon 7; exon numbering according to NM_001185090). The SNPs were in complete linkage disequilibrium. The frequency of these variants did not differ between patients with NMDAR encephalitis and ethnically matched individuals in the general population. No differences in clinical presentation, measures of disease severity, clinical course, or outcomes were observed between patients with different genotypes at these SNPs.

Conclusion: Disease susceptibility or course in patients with NMDAR encephalitis was not strongly affected by SNPs in *GRIN1*. This study provides an estimate of the frequency of SNPs in *GRIN1* in patients with NMDAR encephalitis and emphasizes the need for multisite collaborative studies enrolling larger numbers of patients to identify the genetic contributions to NMDAR encephalitis. ***Neurol Neuroimmunol Neuroinflamm* 2015;2:e153; doi: 10.1212/NXI.0000000000000153**

GLOSSARY

CI = confidence interval; **EVS** = Exome Variant Server; **NMDAR** = NMDA receptor; **OR** = odds ratio; **SNP** = single nucleotide polymorphism.

NMDA receptor (NMDAR) encephalitis is a rare, life-threatening autoantibody-mediated neurologic disease characterized by profound changes in personality, psychiatric symptoms, memory loss, seizures, and autonomic dysfunction.[1,2] First characterized in 2007,[3] IgG autoantibodies against the GluN1 subunit of the CNS NMDAR are now recognized as the cause of psychiatric and neurologic dysfunction in pediatric[4,5] and adult patients with NMDAR encephalitis.[2] NMDAR encephalitis is associated with ovarian teratoma in more than 50% of cases affecting adult women,[1,6] with recent evidence suggesting that the presence of dysplastic glioneuronal cells may distinguish teratomas resected from patients with NMDAR encephalitis from teratomas resected from patients without neurologic dysfunction.[7] Dysplastic cells may provide a focus for

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immune infiltration, explaining why a minority of patients with ovarian teratomas develop NMDAR autoantibodies. The factors promoting autoantibody formation in the majority of patients without ovarian teratomas, however, remain unknown.

The glutamate receptor ionotropic NMDA 1 gene (*GRIN1*, chromosome 9q34.3) encodes the GluN1 subunit of the NMDAR. Autoantibody reactivity in NMDAR encephalitis depends on the conformation of GluN1 and is specifically dependent on the amino acid identity within a small region of the GluN1 amino-terminal domain.[8] Thus, a small change in host receptor conformation could markedly affect autoantibody binding, contributing to disease susceptibility and variability in clinical presentation and course. The study aimed to determine (1) whether single nucleotide polymorphisms (SNPs) within *GRIN1* were associated with NMDAR encephalitis compared with the general population, and (2) whether *GRIN1* genetic variability was associated with the clinical presentation or disease course.

METHODS Consecutive pediatric and adult patients with NMDAR encephalitis were enrolled from 2011 to 2014 at 3 tertiary care centers in Germany and Canada. All patients had clinical symptoms and signs of NMDAR encephalitis with NMDAR autoantibodies detected in serum or CSF. Antibodies were confirmed using a standard cell-based assay (Canadian provider: Mitogen Advanced Diagnostics Laboratory, Calgary, Alberta; German provider: Euroimmun, Lübeck).

Standard protocol approvals, registrations, and patient consents. Study protocols were approved by the institutional research ethics boards at the University of Toronto and affiliated hospitals (University Health Network and The Hospital for Sick Children) and Universitätsmedizin Berlin Charité hospital. Written informed consent was obtained from all patients or their substitute decision makers.

Clinical information, illness features, disease course, and outcome. Pediatric (28 days old to ≤17 years old) and adult (≥18 years old) patients diagnosed with NMDAR encephalitis were invited to participate. For all participants, data were collected regarding clinical information (demographic details, health history, family history), illness features (associated teratoma, psychiatric symptoms, seizures), disease course (requirement for intensive care admission and/or mechanical ventilation as a consequence of NMDAR encephalitis, provision of first- and/or second-line treatments), and outcome (return to prior level of vocational function at work or school, clinically diagnosed disease relapse). Seizures were classified as "difficult to treat" when multiple simultaneous anticonvulsant medications were required to prevent seizure recurrence. Patients who required temporary mechanical ventilation for the performance of a diagnostic procedure (e.g., neuroimaging, lumbar puncture) were not included in the cohort of patients requiring intubation due to the direct consequences of NMDAR encephalitis (e.g., central hypoventilation, impending airway compromise). First-line therapies were defined as high-dose pulse steroids, IV immunoglobulin, and/or plasma exchange. Second-line therapies were defined as IV rituximab and/or cyclophosphamide. The length of hospital admission (weeks) and duration of follow-up post discharge from hospital (months) were calculated for each patient.

DNA extraction and genetic analysis. Genomic DNA was extracted from blood samples using Gentra Puregene kits (Qiagen N.V., Venlo, the Netherlands) following the manufacturer's protocol in a Clinical Laboratory Improvement Amendments and College of American Pathologists certified laboratory (Molecular Genetics Laboratory, The Hospital for Sick Children, Toronto, Ontario, Canada). PCR primers targeting *GRIN1* exons and exon/intron junctions were designed from GRCh37 assembly sequence using Primer3,[9] avoiding annotated variants from public SNP databases (http://www.ncbi.nlm.nih.gov/projects/SNP/ build 137). The PCR primer sequences used are detailed in table e-1 at Neurology.org/nn. PCR and Sanger sequencing was performed by The Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Ontario, Canada. Fifty ng of genomic DNA was used to PCR amplify each exonic region, and PCR products were purified using DNA Clean & Concentrator-5 kits (Zymo Research, Irvine, CA). Sanger sequencing was performed on a 3730XL DNA Analyzer (Life Technologies, Grand Island, NY).

Analysis. Differences in genotype counts between patients of declared Caucasian-European ancestry were compared against those in the publicly available general population using genotype data from a large group of people with European ancestry (Exome Variant Server [EVS]).[10] Clinical information, illness features, disease course, and outcomes were compared between patients with different genotypes at the *GRIN1* SNPs. Descriptive analysis was performed, reporting medians and ranges for continuous measures and percentages for categorical measures. Comparisons were tested for significance using the Fisher exact test for categorical measures and the Mann-Whitney *U* test for continuous measures. Statistical significance was defined as $p < 0.05$. A 2-sample frequency calculation was performed to determine the number of participants that would need to be studied to detect an effect of *GRIN1* SNP on disease susceptibility with 80% power, assuming SNPs were observed in patients with NMDAR encephalitis at the frequency reported in the study results (odds ratio [OR] = 1.42). Statistical analyses were completed using Statistical Analysis Software version 9.3 (Cary, NC).

RESULTS Forty-eight patients with NMDAR encephalitis were enrolled from tertiary care centers in Germany (31) and Canada (17), including 13 pediatric and 35 adult patients. Overall, 85% (41/48) of patients were female, with a median age at diagnosis of 24.0 years (range 3.1–77.5). The majority of patients were of self-declared Caucasian-European ancestry (81%, 39/48, including 1 patient of mixed African/Caucasian-European heritage). Five patients (10%) were of Asian descent, 3 (6%) were of Indian descent, and 1 (2%) was of Jamaican descent.

Clinical information, illness features, disease course, and outcome. Clinical data including initial presentation and inpatient management were prospectively obtained in 85% (41/48) of patients and retrospectively

Table 1 Clinical information, illness features, disease course, and outcomes in patients with NMDAR encephalitis, stratified by *GRIN1* genotype

	Clinical information				Illness features				Disease course				Outcome	
		Autoimmune disease							Treatment					
Patient/age, y, sex	Ancestry	Personal history	Family history	Ovarian teratoma	Psychosis	Seizures	ICU	Ventilation	First line	Second line	Hospital admission, wk	Returned to baseline	Relapse	Follow-up, mo
rs6293-A/A, rs1126442-G/G														
A/10F	Caucasian	N	N	N	N	N	Y	N	Y	N	6	Y	N	22.3
B/16F	Caucasian	N	N	N	Y	Y	Y	Y	Y	Y	32	Y	N	56.4
C/16F	Caucasian	N	N	N	Y	Y	Y	Y	Y	Y	7	Y	N	6.0
D/21F	Caucasian	N	N	N	N	N	Y	Y	N	N	5	Y	Y	67.4
E/23F	Caucasian	N	N	Y	Y	N	N	N	Y	N	8	Y	N	64.2
F/24F	Caucasian	N	N	N	Y	Y[a]	Y	Y	Y	Y	5	N	Y	17.0
G/25F	Caucasian	N	N	N	Y	Y	N	N	N	N	7	Y	N	94.1
H/26F	Caucasian	N	N	N	Y	Y[a]	Y	Y	Y	N	10	Y	Y	103.3
I/27F	Caucasian	N	N	Y	Y	Y	Y	Y	Y	Y	15	N	N	9.0
J/29F	Caucasian	Y, HT	N	N	N	Y[a]	Y	Y	Y	Y	16	N	N	34.2
K/32M	Caucasian	N	N	N	Y	Y	Y	N	Y	N	14	N	N	40.3
L/33F	Caucasian	N	N	N	Y	Y[a]	N	N	Y	N	7	Y	N	28.4
M/34F	Caucasian	N	N	N	Y	Y[a]	Y	N	Y	Y	32	N	N	42.0
N/36F	Caucasian	N	N	Y	N	N	N	N	Y	N	6	Y	N	22.3
O/62M	Caucasian	N	N	N	Y	Y	N	N	Y	Y	7	Y	N	7.0
R/24F	Chinese	N	N	Y	Y	Y	Y	Y	Y	N	16	Y	Y	190.3
P/3F	Filipina	N	N	N	Y	Y[a]	Y	Y	Y	Y	3	N	N	6.2
S/25F	Filipina	N	N	N	N	N	N	N	Y	N	3	N	N	78.5
Q/20M	Korean	N	Y, AS	N	Y	Y	Y	Y	Y	N	6	Y	N	16.0
T/40M	Vietnamese	N	N	N	Y	N	Y	Y	Y	N	11	N	Y	25.0
U/36F	Indian	N	N	Y	Y	Y[a]	Y	Y	Y	N	24	N	N	36.0
V/38F	Jamaican	N	N	Y	Y	Y[a]	Y	Y	Y	Y	33	N	N	24.5
rs6293-A/G, rs1126442-A/G														
Av/3F	Caucasian	N	N	N	Y	Y[a]	Y	N	Y	Y	4	Y	N	50.3
Bv/8M	Caucasian	N	N	N	Y	Y[a]	Y	Y	Y	Y	4	Y	N	16.3
Cv/12F	Caucasian	N	N	N	Y	Y	N	N	Y	Y	18	N	Y	82.1
Dv/14F	Caucasian	N	N	N	Y	Y[a]	Y	N	Y	Y	3.5	Y	Y	14.5
Ev/14F	Caucasian	N	N	N	Y	Y	Y	N	Y	Y	7	Y	N	19.8

Continued

Table 1 Continued

	Clinical information				Illness features				Disease course				Outcome	
		Autoimmune disease							Treatment					
Patient/age, y, sex	Ancestry	Personal history	Family history	Ovarian teratoma	Psychosis	Seizures	ICU	Ventilation	First line	Second line	Hospital admission, wk	Returned to baseline	Relapse	Follow-up, mo
Fv/15F	Caucasian	N	N	N	Y	N	Y	N	Y	N	8	Y	N	43.7
Gv/17F	Caucasian	N	N	N	N	Y[a]	Y	Y	Y	N	11	Y	N	29.6
Hv/17F	Caucasian	N	N	Y	Y	N	N	N	Y	N	6	Y	N	3.0
Iv/17F	Caucasian	N	N	N	Y	Y[a]	N	N	Y	Y	6	Y	N	2.0
Jv/18F	Caucasian	N	N	N	Y	Y	N	N	Y	Y	8	Y	N	4.0
Kv/19F	Caucasian	N	N	N	N	Y[a]	Y	N	Y	Y	7	Y	N	41.0
Lv/22F	Caucasian	N	N	N	N	Y[a]	Y	Y	Y	Y	44	N	N	35.0
Mv/23F	Caucasian	N	N	N	N	Y	N	N	Y	N	8	Y	N	32.8
Nv/2 M	Caucasian	N	N	N	N	Y	N	N	N	N	3	Y	Y	81.1
Ov/25F	Caucasian	N	N	N	N	Y[a]	Y	Y	Y	N	22	Y	N	90.8
Pv/29F	Caucasian	N	N	N	Y	Y[a]	Y	N	Y	N	28	Y	N	62.7
Qv/30F	Caucasian	N	N	N	Y	Y	N	N	Y	N	10	Y	Y	82.5
Rv/31F	Caucasian	N	N	N	Y	Y	Y	N	Y	N	5	Y	N	23.5
Sv/39F	Caucasian	N	N	N	N	Y[a]	Y	N	Y	Y	13	N	N	29.7
Tv/41F	Caucasian	N	N	N	N	Y	N	N	Y	N	11	N	N	67.8
Uv/10F	Indian	N	Y, SLE	N	Y	N	N	N	Y	Y	1	N	N	3.3
Vv/19F	Indian	N	N	Y	Y	Y	Y	Y	Y	N	12	N	N	10.0
rs6293-G/G, rs1126442-A/A														
Wv/21F	Caucasian	N	N	N	Y	N	Y	N	Y	N	4	Y	N	35.0
Xv/27F	Caucasian	N	Y, ADEM	Y	Y	Y[a]	Y	Y	Y	Y	52	N	N	12.0
Yv/34F	Caucasian	N	N	N	Y	Y	Y	N	Y	Y	12	Y	N	57.0
Zv/77M	Caucasian	N	N	N	Y	N	Y	Y	Y	Y	5	N	N	3.0

Abbreviations: ADEM = acute disseminated encephalomyelitis; AS = ankylosing spondylitis; HT = Hashimoto thyroiditis; ICU = intensive care unit; NMDAR = NMDA receptor; SLE = systemic lupus erythematosus.
[a] Difficult-to-treat seizures, requiring simultaneous administration of ≥2 anticonvulsant medications.

acquired through chart review in the remainder (15%, 7/48). Ninety-two percent (44/48) of study participants were prospectively followed by study authors upon discharge from the hospital.

Only 1 patient had a prior history of autoimmune disease (Hashimoto thyroiditis). Four patients (8%) reported a history of autoimmune disease affecting first-degree relatives, including ankylosing spondylitis, systemic lupus erythematosus, acute demyelinating encephalomyelitis, and Hashimoto thyroiditis. Ovarian teratomas were detected in 5 patients (10%) and resected. In a single patient (patient L), tumor surveillance led to the incidental discovery and resection of a grade 1 thyroid papillary carcinoma, which was presumed to be unrelated to NMDAR encephalitis. No other associated malignancies were identified in any patient. The median length of hospital admission was 8.0 weeks (range 3–52), with follow-up continuing for 31.3 months (range 2.0–190). Throughout this period, clinical recurrence of symptoms and signs associated with NMDAR encephalitis was documented in 9 patients (19%, 9/41). Clinical information, illness features, disease course, and outcomes for all 48 patients are summarized in table 1.

Evaluating the effect of distinct SNPs on disease susceptibility and course. Genetic comparisons were limited to the 39 patients of Caucasian-European ancestry, given their representation within our sample and the availability of published frequency genotype data for this population (EVS). Two SNPs were identified in *GRIN1*. Fifteen Caucasian-European patients with NMDAR encephalitis were homozygous for A/A and G/G at rs6293 at chr9:14,0051,238 (hg19; exon 6) and rs1126442 at chr9:140,051,376 (hg19; exon 7; exon numbering according to NM_001185090), respectively. Twenty-four patients (62%, 24/39) had alternate alleles: 20 (51%, 20/39) had rs6293-A/G and rs1126442-A/G and 4 (10%, 4/39) had rs6293-G/G and rs1126442-A/A. SNPs were in complete linkage disequilibrium. The frequency of genotype variants observed in these patients did not differ significantly from that observed in individuals of European ancestry included in the EVS database (table 2). The risk of developing NMDAR encephalitis in patients with rs6293-A/G or -G/G and rs1126442-A/G or -A/A (vs rs6293-A/A and rs1126442-G/G) was OR 1.42 (95% confidence interval [CI] 0.75–2.73, $z = 1.080$, $p = 0.28$). Genotypes in European patients and the reference population from the EVS registry were in Hardy-Weinberg equilibrium. No other variants were identified in any patient.

Patients of Caucasian-European ancestry were stratified according to the genotype of the *GRIN1* SNPs, and predetermined demographic and clinical features were compared (table 3). Individuals with rs6293-A/G and -G/G and rs1126442-A/G and -A/A were considered together, given the small numbers of patients, and compared with the common homozygotes. No significant differences were observed between the 2 populations of patients with NMDAR encephalitis with regard to clinical presentation, measures of disease severity, clinical course, or outcomes.

A power analysis was performed to determine the number of Caucasian-European patients with NMDAR encephalitis that would be required to detect a difference in *GRIN1* SNP genotype frequencies using the reported frequencies of these 2 SNPs in our sample. This study was powered to detect a large magnitude effect (Cohen $d > 0.92$, $\alpha = 0.05$); however, no such effect was shown. Rather, we report an OR of 1.42 (95% CI 0.75–2.73), quantifying the relationship (or lack thereof) between *GRIN1* polymorphisms and NMDAR encephalitis. An estimated 507 patients would need to be recruited to detect or exclude such a small magnitude effect of *GRIN1* polymorphisms on disease susceptibility ($p < 0.05$) 80% of the time.

DISCUSSION

SNPs in coding regions of *GRIN1* were not strongly associated with NMDAR encephalitis or

Table 2 Genotype and allele frequencies of the 2 polymorphisms of *GRIN1*, observed and reported

	Genotype count (frequency)				Allele count (frequency)			
SNP	Genotype	Observed, patients	Reported, EVS	p Value	Allele	Observed, patients	Reported, EVS	p Value
rs6293	A/A	15 (0.38)	2,022 (0.47)	0.50	A	50 (0.64)	5,866 (0.68)	0.49
	A/G	20 (0.51)	1,822 (0.43)		G	28 (0.36)	2,706 (0.32)	
	G/G	4 (0.10)	442 (0.10)					
rs1126442	A/A	4 (0.10)	447 (0.10)	0.57	A	28 (0.36)	2,741 (0.32)	0.53
	A/G	20 (0.51)	1,847 (0.43)		G	50 (0.64)	5,845 (0.68)	
	G/G	15 (0.38)	1,999 (0.47)					

The frequency genotype data for single nucleotide polymorphisms (SNPs) rs6293 and rs1126442 are taken from the Exome Variant Server (EVS) public database for European Americans.[10]

Table 3 Comparison of clinical information, illness features, disease course, and outcomes between cohorts with the common homozygous *GRIN1* SNPs (rs6293-A/A, rs1126442-G/G) and alternate genotypes

Patients of Caucasian-European ancestry (N = 39)	rs6293-A/A; rs1126442-G/G (n = 15)	rs6293-A/G, -G/G; rs1126442-A/G, -A/A (n = 24)	*p* Value
Clinical information			
Age at diagnosis, y, median (range)	26.1 (3.1–62.4)	20.8 (3.2–77.5)	0.22
Females (%)	86.7	87.5	>0.99
Ovarian teratoma (%, females only)	23.1	9.5	0.35
Illness features			
Psychosis/mood disturbance (%)	73.3	66.7	0.73
Seizures (%)	73.3	83.3	0.69
ICU admission (%)	66.7	66.7	>0.99
Difficult-to-control seizures (% patients with seizures)	45.5	55.0	0.72
Requirement for mechanical ventilation (%)	46.7	25.0	0.19
Disease course			
First-line treatments (%)	86.7	95.8	0.55
Second-line treatments (%)	46.7	54.2	0.75
Duration of hospital admission, wk, median (range)	7.5 (3–33)	8.0 (3–52)	0.66
Outcomes			
Return to baseline (%)	66.7	75.0	0.72
Relapse (%)	20.0	16.7	>0.99
Length of follow-up, mo, median (range)	31.3 (6.0–190.3)	31.3 (2.0–90.8)	0.83

Abbreviations: ICU = intensive care unit; SNP = single nucleotide polymorphism.

with differences in presenting symptoms, clinical course, or outcomes in Caucasian-European patients. These findings suggest that the reported SNPs are unlikely to affect the organization of the highly conserved NMDAR autoantibody-binding antigenic domain of the GluN1 receptor subunits.[8] This supposition is further supported by the observation that the reported SNPs are located at P263 (rs6293, Chr9(GRCh37):g.140,051,238A>G) and V285 (rs1126442, Chr9(GRCh37):g.140,051,376G>A) of the *GRIN1* transcript—far removed from the immunoreactive domain at N368/G369 (numbered according to NM_000832).[8]

The frequency of SNPs within *GRIN1* has been previously evaluated in a single patient with NMDAR encephalitis[11] and in groups of patients with psychoses,[12] schizophrenia, and mood disorders,[13] recognizing the putative role of genetic variation in glutamatergic signaling pathways in the etiology of these conditions. No significant associations have been described for rs6293.[13] Variants of rs1126442, however, have been reported to contribute to genetic vulnerability to psychosis in a highly selected population of methamphetamine-dependent patients (compared with healthy controls).[12] The presence of the rs1126224-A/G or -A/A genotype exerted no discernable effect on the frequency of psychiatric symptomatology within our cohort of patients with NMDAR encephalitis: 67% of patients with and 73% without the rs1126442-A/G or -A/A genotype presented with psychoses or mood disorder as an initial feature of their illness ($p = 0.73$).

Of interest, only 1 patient (2%, 1/48) reported a personal history of autoimmune disease. This rate is lower than that reported in other populations of patients with autoimmune-mediated brain diseases (30%–75%)[14] yet remains in line with that expected from prevalence estimates of rates of autoimmune disease in the general population (3.2%–9.4%).[15,16] This finding suggests that patients with NMDAR encephalitis may not harbor an increased risk of autoimmune disease, emphasizing the potential contributions of extrinsic (i.e., infection,[1] including herpes simplex[17] and varicella-zoster viruses[18]) and additional host factors (i.e., abnormalities in teratomas[7]) to disease pathogenesis.

The scope of clinical information collected throughout the illness period and the duration of follow-up are strengths of the current work. However, the interpretation of our findings is subject to certain limitations—most notably the focused genetic analysis (considering only *GRIN1*), the number of participants recruited, and the limited generalizability to patients of Caucasian-European descent. The

hypothesis-driven nature of this project led us to specifically consider the effects of *GRIN1* SNPs on susceptibility and clinical variability in patients with NMDAR encephalitis. We acknowledge that additional genetic factors not measured in this study may affect disease course and may account for the variability observed in presentation and outcomes. In addition, because our study was powered to detect only a strong effect, a more modest effect of *GRIN1* SNPs may have been overlooked. Future studies using broader genetic analysis in a larger number of patients are required to better understand the contributions of genetic variation to NMDAR encephalitis.

Smaller cohort studies may contribute to our understanding of the contributions of single genes to disease pathogenesis, provided patient populations remain well-characterized, appropriate control groups are selected for comparison, and a sufficient number of participants are recruited.[19] Within this context, we assert that *GRIN1* SNPs do not exert a strong effect on susceptibility or clinical phenotype in patients with NMDAR encephalitis.

AUTHOR CONTRIBUTIONS

G.S. Day participated in the conception and design of the study; acquisition, statistical analysis, and interpretation of data; and drafting, revising, and finalizing the manuscript. H. Prüss participated in acquisition and interpretation of data and revised the manuscript for critical content. S.M. Benseler participated in acquisition and interpretation of data and revised the manuscript for critical content. T.A. Paton participated in analysis and interpretation of data and revised the manuscript for critical content. A.D. Paterson participated in analysis and interpretation of data and revised the manuscript for critical content. D.M. Andrade participated in the conception and design of the study; acquisition, statistical analysis, and interpretation of data; and revision and finalization of the manuscript. D.M. Andrade had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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