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Clinical and genetic characterization of *CACNA1A*-related disease

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Abstract

Pathogenic variants in the *CACNA1A* gene have been associated with episodic ataxia type 2, familial hemiplegic migraine, and spinocerebellar ataxia 6. With increasing use of clinical genetic testing, associations have expanded to include developmental delay, epilepsy, paroxysmal dystonia, and neuropsychiatric disorders. We report 47 individuals with 33 unique likely pathogenic or pathogenic *CACNA1A* variants. A machine learning method, funNCion, was used to predict loss-of-function (LoF)/gain-of-function (GoF) impact of genetic variants, and a heuristic severity score was designed to analyze genotype/phenotype correlations. Commonly reported phenotypes include developmental delay/intellectual disability (96%), hemiplegic migraines (36%), episodic ataxia type 2 (32%), epilepsy (55%), autism spectrum disorder (23%), and paroxysmal tonic upward gaze (36%). Severity score was significantly higher for predicted GoF variants, variants in the S5/S6 helices, and the recurrent p.Val1392Met variant. Seizures/status epilepticus were correlated with GoF and were more frequent in those with the p.Val1392Met variant. Our findings demonstrate a breadth of disease severity in *CACNA1A*-related disease and suggest that the clinical phenotypic heterogeneity likely reflects diverse molecular phenotypes. A better understanding of the natural history of *CACNA1A*-related disease and genotype/phenotype correlations will help inform prognosis and prepare for future clinical trials.

KEYWORDS
autism spectrum disorder, *CACNA1A*, developmental delay, epilepsy, episodic ataxia, hemiplegic migraine, paroxysmal tonic upward gaze

1 | INTRODUCTION

The *CACNA1A* gene encodes the pore-forming α_{1A} subunit of the voltage-gated $Ca_v2.1$ (P/Q-type) Ca^{2+} channel. $Ca_v2.1$ channels are found at central synapses and are particularly abundant in cerebellar granule and Purkinje cells. Ca^{2+} flux through these channels is essential for neurotransmitter release.[1]

Pathogenic variants in *CACNA1A* are associated with episodic ataxia type 2 (EA2; MIM 108500), familial hemiplegic migraine type 1 (FHM1; MIM 141500), and spinocerebellar ataxia 6 (SCA6; MIM 183086). EA2 is a paroxysmal disorder characterized by recurrent episodes of ataxia, nystagmus, and vertigo, and FHM1 is characterized by migraine episodes associated with an aura of hemiparesis.[2] SCA6 is a late-onset progressive cerebellar ataxia.[3] With increasing use of clinical exome/panel gene sequencing, pathogenic *CACNA1A* variants have been associated with a wider phenotypic spectrum including developmental delay/intellectual disability, epilepsy, developmental and epileptic encephalopathy 42 (DEE42; MIM 617106), paroxysmal dystonia, and neuropsychiatric disorders.[1,4–6]

$Ca_v2.1$ channels are heteromultimers composed of a principal α_{1A} subunit and auxiliary β and $\alpha_2\delta$ subunits. The pore-forming α_{1A} subunit is composed of four transmembrane repeats (I–IV), each with six membrane-spanning α-helices (S1–S6). The S4 helices each contain 5–6 positively charged amino acids (positions R0–R5) which are the

main voltage-sensors of the channel. The S5 and S6 helices line the channel conduction pore.[7]

Functional studies have aimed to link channel function with phenotypic features. EA2 has been associated with loss of function (LoF) variants.[8] Gain of function (GoF) missense variants have been associated with FHM1. SCA6 is due to a CAG repeat expansion in exon 47 which results in toxic polyglutamine accumulation.[3] Epilepsy has been associated with both LoF and GoF variants with varying seizure characteristics.[5]

We report the clinical and genetic features of 47 participants with likely pathogenic and pathogenic *CACNA1A* variants with the goal of further characterizing the phenotypic spectrum of *CACNA1A*-related disease and understanding genotype–phenotype correlations. We present results of a machine learning method to predict channel function which will facilitate scalable analysis of genotype–phenotype correlations as data grow in the future.

2 | MATERIALS AND METHODS

2.1 | Clinical data

This study was approved by the Columbia University Institutional Review Board, and informed consent was obtained from all participants or their guardians. Clinical data were collected between January 2021 and November 2021 through an online medical history questionnaire recruited through an international family support group, the CACNA1A Foundation. The questionnaire was completed by either the participant or a parent. The study recruited participants who had previously received clinical genetic testing with pathogenic/likely pathogenic *CACNA1A* variants. Eligibility was not dependent on symptoms. The medical history included questions on neurological symptoms, epilepsy, developmental milestones, and psychiatric diagnoses. Complete medical history data are included in Table S1. To summarize clinical severity, we designed a heuristic severity score using a weighted sum of common symptoms. One point was assigned for developmental delay, ataxia, hemiplegic migraines, status epilepticus, and autism spectrum disorder; one point for 1–7 seizures and two points for eight or more lifetime seizures; one point for nonambulatory (half point if the participant is between 18 and 36 months, zero points if less than 18 months); one point if nonverbal (half point if verbal but with language delay or if between 18 and 36 months, zero points if less than 18 months).

2.2 | Genetic variants

All participants had previously undergone exome sequencing or panel gene testing. Clinical genetic test reports were submitted and reviewed by a medical and molecular geneticist and complete genetic data are included in Table S1. *CACNA1A* variants were classified according to the American College of Medical Genetics (ACMG) classification guidelines,[9] and only those participants with likely pathogenic or pathogenic variants were included in the analysis. All variants reported in this study are submitted to ClinVar under accession numbers (SCV002506523-SCV002506555). We investigated genotype/phenotype correlations according to computational scores, variant type, variant location, and predicted functional impact. We collected multiple computational scores and assessed their correlation with the heuristic severity score. Computational scores included are homologous conservation (GERP++), paralogous conservation based on 10 *CACNA1A* proteins summarized using JalView, VEST,[10] MetaSVM,[11] REVEL,[12] CADD,[13] Eigen,[14] and gMVP.[15] We classified variants into one of six transmembrane segments or linker regions based on the topology data provided in UniProtKB (protein ID O00555). We also used a machine learning method, funNCion[16] to predict loss-of-function (LoF) or gain-of-function (GoF) effects for missense variants. Predicted negative and positive scores refer to LoF and GoF effects, respectively, and reflect the predicted effect on Ca^{2+} channel current. Truncating variants are assumed to result in LoF via nonsense-mediated decay. Function of in-frame variants is imputed using the mean predictive score of all possible missense variants at the overlapping positions.

2.3 | Statistical analysis

In addition to the heuristic severity score, we further performed a principal components analysis (PCA) using additional clinical data to assess participant clinical clustering. We included 17 clinical features including age, gender, developmental delay (gross motor, fine motor, language, social), age of achieving motor milestones (rolling over, sitting with support, crawling, walking), nonverbal/nonambulatory, ataxia, seizures, age of the first seizure, history of status epilepticus, hemiplegic migraines, and autism spectrum disorder. We investigated genotype–phenotype correlations using heuristic clinical severity scores, variant type, variant location, and predicted functional effect. Pearson correlation and significance level were estimated using the cor.test package in R. Differences in severity scores between groups of variants were compared using the Wilcoxon test.

3 | RESULTS

3.1 | Demographics

Forty-seven participants had likely pathogenic or pathogenic *CACNA1A* variants and completed the medical history questionnaire. The median age of participants was 6.9 years with ages ranging from 1 to 40 years. The cohort includes 15% (7/47) adult participants (over age 18 years). Participants were 66% (31/47) female. Clinical characteristics for this cohort are summarized in Table 1.

3.2 | Genetic data

Of the 47 participants with heterozygous likely pathogenic or pathogenic *CACNA1A* variants, 28 were de novo, 6 were inherited (including 2 with mosaic parents), and 13 were of unknown inheritance due to

TABLE 1 Clinical characteristics of 47 individuals with likely pathogenic or pathogenic *CACNA1A* variants

Clinical characteristics	
Developmental delay	96% (45/47)
Global developmental delay	68% (32/47)
Nonverbal (age 3+)	33% (13/39)
Nonambulatory (age 3+)	36% (14/39)
Hypotonia	75% (35/47)
Ataxia	75% (35/47)
Paroxysmal tonic upward gaze	36% (17/47)
Ophthalmologic findings	
Nystagmus	55% (26/47)
Eye movement abnormalities	53% (25/47)
Astigmatism	28% (13/47)
Depth perception problems	23% (11/47)
Strabismus	38% (18/47)
Hemiplegic migraine	36% (17/47)
Episodic ataxia type 2	32% (15/47)
Epilepsy	55% (26/47)
Status epilepticus	69% (20/29)
Generalized tonic clonic	69% (20/29)
Absence	48% (14/29)
Atonic	34% (10/29)
Complex partial	52% (15/29)
Simple partial	55% (16/29)
Infantile spasms	28% (8/29)
Head injury/loss of consciousness	38% (18/47)
Severe neurologic events	
Coma	21% (10/47)
Cerebral edema	11% (5/47)
Stroke	6% (3/47)
Behavioral and psychiatric diagnoses	
Autism spectrum disorder	23% (11/47)
Depression (age 18+)	57% (4/7)
Anxiety (age 18+)	86% (6/7)
ADHD (age 18+)	43% (3/7)
OCD (age 18+)	29% (2/7)

lack of parental testing. Thirty-three unique variants were reported including 27 missense, 3 nonsense, 2 frameshift, and 1 in-frame deletion (Table 2). All missense variants were rare (minor allele frequency < 10^{-5} in gnomAD) with a CADD score greater than 23. FunNCion predicted 11 LoF and 17 GoF missense/in-frame variants. The 5 nonsense/frameshift variants are presumed to be LoF. Of the 28 missense/in-frame variants, 9 were located in the voltage-sensing S4 helix, 10 in the pore-forming S5 and S6 helices, and 9 in the S3 helix or linker regions (Figure 1). Eighteen of the variants are reported in ClinVar, and one participant (p.Arg1672Pro) has been previously published.[17] The cohort includes two familial pairs: one sibling pair (p.Arg1779Ter) and one parent and child pair (p.Arg582Gln).

3.3 | Early manifestations and developmental delay

One of the earliest symptoms of *CACNA1A*-related disease is hypotonia, reported by 75% (35/47) of participants. Forty-three percent (15/35) reported onset of hypotonia in the newborn period with the remaining noting hypotonia onset within the first year of life. Seventy-five percent (35/47) reported some form of ataxia (either nonepisodic or episodic). Thirty-six percent (17/47) of participants noted early-onset dystonia in the form of paroxysmal tonic upward gaze. Ophthalmologic findings were often early manifestations with 55% (26/47) of participants reporting nystagmus and 53% (25/47) reporting eye movement abnormalities. Other vision issues include astigmatism in 28% (13/47), depth perception problems in 23% (11/47), and strabismus in 38% (18/47).

Almost all participants (96%) reported developmental delay/intellectual disability with 68% (32/47) reporting global developmental delay defined by delay in gross motor, fine motor, language, and social skills. The median ages to achieve major developmental milestones were 9 months for rolling over ($n = 45$), 14 months for sitting without support ($n = 44$), 16 months for crawling ($n = 39$), 26 months for walking ($n = 28$), and 13.5 months for first word ($n = 30$). Of participants ages 3 years and older, 36% (14/39) are nonambulatory, 33% (13/39) are nonverbal, and 23% (9/39) are both nonverbal and nonambulatory.

3.4 | Hemiplegic migraine, episodic ataxia type 2, and seizures

Thirty-six percent (17/47) of participants reported hemiplegic migraines and 32% (15/47) have been diagnosed with episodic ataxia type 2. Only four participants reported both HM and EA2. Sixty-two percent (29/47) of participants have had at least one seizure with 55% (26/47) reporting epilepsy as defined by two or more unprovoked seizures. Seizure frequency ranges from one total lifetime seizure to 150 seizures per month. The average age of seizure onset was 1.9 years (median: 1.5 years; total range: 0–12 years). Seventy-two percent (21/29) reported that seizure frequency has decreased with treatment. Sixty-nine percent (20/29) of the participants with seizures have been diagnosed with status epilepticus. Forty-eight percent (14/29) reported intractable seizures. The majority have experienced multiple seizure types: 69% (20/29) generalized tonic clonic, 48% (14/29) absence, 34% (10/29) atonic, 52% (15/29) complex partial, and 55% (16/29) simple partial. Twenty-eight percent (8/29) reported infantile spasms.

3.5 | Severe neurological events and skill regression

Head injury or loss of consciousness is common, with 38% (18/47) having at least one episode. Twenty-one percent (10/47) of participants have been in a coma, 11% (5/47) have experienced cerebral edema and 6% (3/47) reported stroke. Thirty percent (14/47) of participants reported regression of skills with the most common triggers

TABLE 2 Variant characteristics for the 33 unique variants observed in the cohort.

Variant	Amino acid change	dbSNP	Function	Domain	gnomAD	CADD	FunNCion
c.652T > C	p.Ser218Pro	-	Missense	Linker	0	28.2	−0.7
c.835C > T	p.Arg279Cys	rs1555773764	Missense	Linker	0	26.1	−0.77
c.841del	p.Cys281AlafsTer29	-	Frameshift	Linker	0	-	−1
c.1635C > A	p.Tyr545Ter	rs1427473572	Stop-gain	Linker	0	40	−1
c.1745G > A	p.Arg582Gln	rs121908217	Missense	S4	4.0E−06	29.7	−0.58
c.1843A > C	p.Ser615Arg	rs2057947681	Missense	Linker	0	28.4	−0.63
c.1850T > C	p.Leu617Ser	-	Missense	S5	0	28.7	0.7
c.2019_2033del	p.Met674_Ile678del	-	In-frame	Linker	0	-	−0.84
c.2099G > A	p.Gly700Glu	-	Missense	S6	0	24.8	0.65
c.2133C > G	p.Ile711Met	rs764839814	Missense	S6	0	27.7	0.93
c.2134G > A	p.Ala712Thr	-	Missense	S6	0	27.5	0.84
c.2137G > A	p.Val713Met	-	Missense	S6	0	23.3	0.93
c.2311A > T	p.Lys771Ter	-	Stop-gain	Linker	0	-	−1
c.3948C > A	p.Asp1316Glu	-	Missense	S3	0	24.4	−0.76
c.4028C > A	p.Ser1343Tyr	rs2056767982	Missense	S4	0	29.6	−0.66
c.4031T > C	p.Leu1344Pro	-	Missense	S4	0	28	−0.64
c.4043G > A	p.Arg1348Gln	rs1057520918	Missense	S4	0	33	0.69
c.4052G > A	p.Arg1351Gln	rs1555745467	Missense	S4	0	29.8	0.87
c.4055C > T	p.Pro1352Leu	rs1064794808	Missense	S4	0	32	0.87
c.4064C > A	p.Thr1355Asn	rs2056767062	Missense	S4	0	28	0.53
c.4064C > T	p.Thr1355Ile	rs2056767062	Missense	S4	0	28	0.62
c.4174G > A	p.Val1392Met	rs794727411	Missense	S5	0	24.6	0.63
c.4519G > A	p.Ala1507Thr	-	Missense	S6	0	28.4	0.74
c.4897G > A	p.Asp1633Asn	rs1555740805	Missense	S3	0	29.7	−0.77
c.4927G > A	p.Asp1643Asn	rs1064795531	Missense	S3	0	27.1	−0.73
c.4997G > C	p.Arg1666Pro	rs1568447650	Missense	S4	0	33	0.76
c.5015G > C	p.Arg1672Pro	rs1057519429	Missense	Linker	0	33	0.67
c.5014dup	p.Gln1673SerfsTer43	-	Frameshift	Linker	0	-	−1
c.5120T > C	p.Ile1707Thr	rs121909326	Missense	S5	0	29.4	−0.58
c.5335C > T	p.Arg1779Ter	-	Stop-gain	Linker	0	35	−1
c.5393C > T	p.Ser1798Leu	rs1064794261	Missense	S6	0	33	0.59
c.5417T > C	p.Val1806Ala	-	Missense	S5	0	29.6	0.91
c.5422G > A	p.Val1808Ile	-	Missense	Linker	7.0E−06	23.8	0.84

Note: Transcript ENST00000360228 (NM_001127222) is used in the HGVS notations. The maximum allele frequency in gnomAD2 and gnomAD3 is given in the gnomAD column. Further information is available in Table S1.

being severe hemiplegic migraines and seizures, particularly status epilepticus. Half reported the regression was temporary with the other half reporting permanent losses in speech or motor abilities.

3.6 | Behavioral diagnoses

The most common behavioral diagnosis was autism spectrum disorder, reported in 23% (11/47). Eleven percent (5/47) of participants have been diagnosed with depression, 17% (8/47) with anxiety, 13% (6/47) with attention deficit hyperactivity disorder (ADHD), and 4% (2/47) with obsessive compulsive disorder (OCD). While the prevalence of these disorders is low in the overall sample, the median age of participants is only 6.9 years, well below the average age of diagnosis for many of these conditions. Among adult participants, 57% (4/7) have been diagnosed with depression, 86% (6/7) with anxiety, 43% (3/7) with ADHD, and 29% (2/7) with OCD.

3.7 | Clinical data clustering

Our heuristic severity score approximates a normal distribution across all participants (Figure 2A). The first two principal components (PCs) are highly correlated ($\rho = -0.749$ and 0.866, respectively) with the

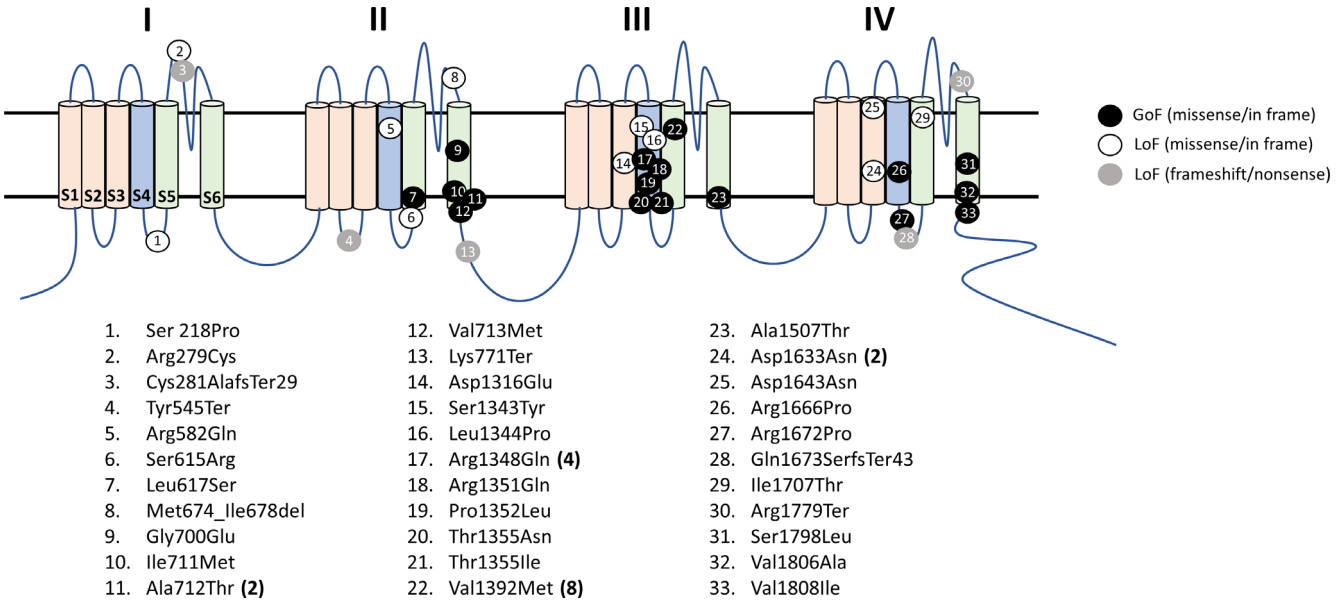

FIGURE 1 Schematic representation of the α_{1A} subunit and location of reported likely pathogenic and pathogenic *CACNA1A* variants (NM_001127222) with the color of each variant representing predicted gain-of-function (GoF) or loss-of-function (LoF) status. Each domain (I–IV) contains six membrane-spanning α-helices (S1–S6). The number of families with each unique variant is indicated in parentheses. [Colour figure can be viewed at wileyonlinelibrary.com]

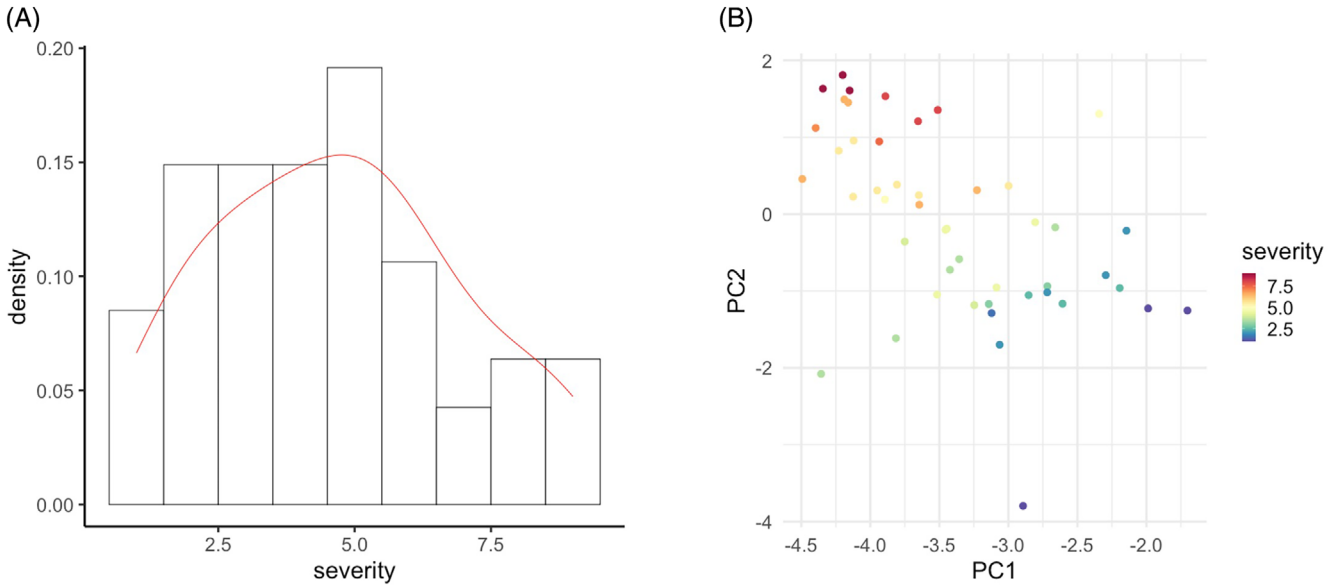

FIGURE 2 Clinical severity of 47 *CACNA1A* participants. (A) Distribution of heuristic severity score. (B) Scatterplot of principal components 1 and 2 color-coded by the heuristic severity scores. [Colour figure can be viewed at wileyonlinelibrary.com]

heuristic severity score (Figure 2B) as a good representation of *CACNA1A* phenotypic severity. PCA shows PC1 is attributed mostly to the developmental features and PC2 separates participants with status epilepticus, seizure or autism from those with developmental delays. The first three PCs explain 72.0%, 8.4%, and 4.9%, respectively, of the variance in the data. We examined the correlation across phenotypic and genotypic features. Seizure and status epilepticus are the features most correlated with the heuristic severity score (ρ = 0.771 and 0.769, respectively). The same two features are also correlated with funNCion scores (ρ = 0.333 and 0.441, respectively). Participants with GoF variants are more likely to develop seizures and status epilepticus. There was no significant correlation observed between functional status and episodic ataxia type 2 or hemiplegic migraines. We also observed variants in S4, S5 and S6 helices are more likely to be GoF, compared with variants in S3 helix and linker regions which are more likely to be LoF. Additionally, participants with variants in S5 and S6 helices have significantly higher severity scores compared with the variants in S3 helix and linker regions (p value < 0.01, Figure 3A). Participants with missense

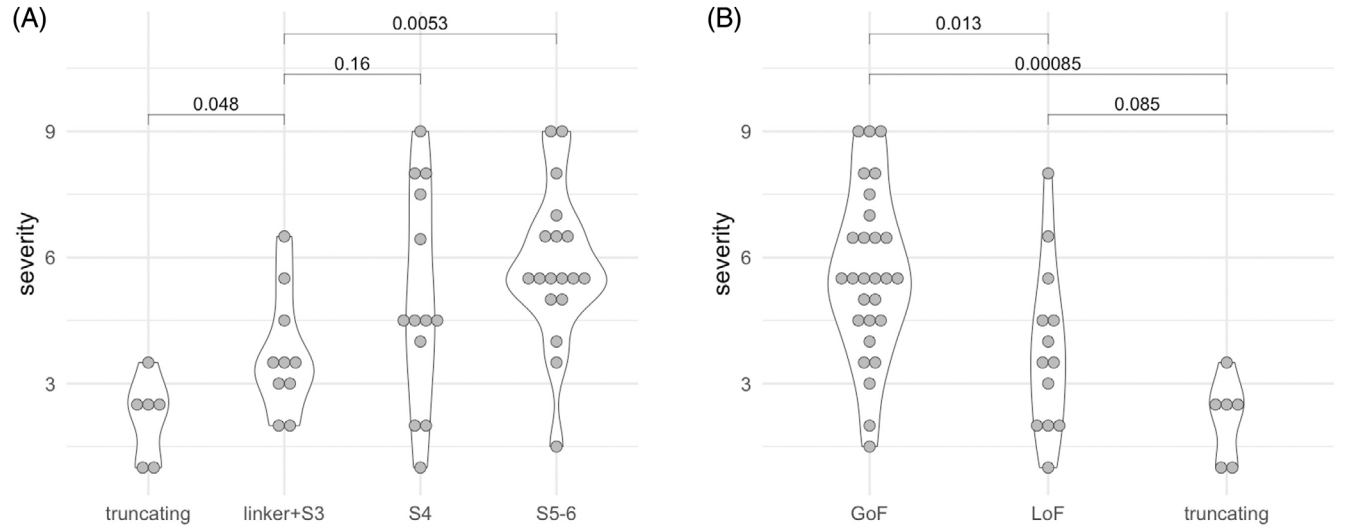

FIGURE 3 Violin plot of the heuristic severity scores for participants with variants (A) in different functional domains and (B) with different functional status predicted by funNCion score.

GoF variants were found to have significantly higher severity scores than those with missense LoF variants (p value = 0.011) as well as those with truncating variants (p value < 0.001, Figure 3B).

3.8 | Prediction of clinical severity using functional scores of variants

We investigated the correlation between the heuristic clinical severity score and predictive conservation/pathogenicity scores. Although all computational methods predict the 33 *CACNA1A* variants as highly conserved and pathogenic, their predictive scores are not correlated with the clinical severity. It suggests that we cannot directly use methods that are trained to separate conserved/pathogenic variants from nonconserved/benign variants to infer clinical severity.

3.9 | Recurrent genotypes

Clinical features of participants with the p.Val1392Met (eight participants) and p.Arg1348Gln (four participants) variants were compared to the rest of the participants. Average severity score was significantly higher in those with p.Val1392Met variant (p value 0.024) as was prevalence of seizures (p value < 0.01) and status epilepticus (p value < 0.01). Those with the p.Arg1348Gln variant did not have significantly increased severity score or correlation with any particular phenotype although we had only four participants with this variant.

4 | DISCUSSION

Our study included 47 individuals with likely pathogenic or pathogenic *CACNA1A* variants. We describe clinical features of these participants and analyze genotype–phenotype correlations based on variant type, location, and functional status predicted by the funNCion method.

The most commonly reported phenotypes were developmental delay, ataxia, and hypotonia. Nearly all our participants reported developmental delay compared to only 62% in a similar study of 47 patients with infantile onset *CACNA1A*-related disease.[6] A significant number of participants are nonverbal and/or nonambulatory. Developmental delays are common in epileptic encephalopathies associated with early onset, severe epilepsy.[4,18] Other common phenotypes included paroxysmal tonic upward gaze and ophthalmologic findings including nystagmus and eye movement abnormalities.

Hemiplegic migraines (HM) were reported in 36% of participants, episodic ataxia type 2 (EA2) in 32%, and 9% reported both. We observed a higher prevalence of HM in our series compared to that of Gur-Hartman, et al.[6] (36% vs. 9%, respectively). Prevalence of EA2 was similar in both series. We did not observe a significant correlation between EA2 and LoF or HM and GoF as has been previously reported.[3,8] Participants with both HM and EA2 had predicted GoF variants. The minimal overlap in phenotypes is consistent with previous reports that these phenotypes are generally associated with different molecular mechanisms, though symptoms of HM and EA2 have been seen to overlap.[8,19,20]

A majority of participants (62%) have had at least one seizure with most of these individuals reporting epilepsy defined as two or more unprovoked seizures. Epilepsy is more common in our series (55%) compared to 23% reported by Gur-Hartman, et al.[6] Many participants have been diagnosed with status epilepticus and most have had multiple seizure types. In general, seizures were correlated with GoF variants. A 2017 study of *CACNA1A*-associated epilepsy by Le Roux, et al.[5] further identified two main seizure presentations, status epilepticus and intractable seizures or early onset absence seizures. The first, more severe phenotype, was associated with GoF variants while those with predominantly absence seizures were associated with LoF. In our study, status epilepticus is similarly correlated with GoF.

At least one episode of head injury or loss of consciousness was common (38%) and several participants reported coma, cerebral edema, or stroke. Trivial head trauma has been associated with seizures, hemiplegic migraine, cerebral edema, and coma in patients with pathogenic *CACNA1A* variants.[21–23]

The most common behavioral diagnosis was autism spectrum disorder in 23%, higher than the 4% reported by Gur-Hartman, et al[6] Although the number of adult participants in our cohort was low ($n = 7$), adult participants reported multiple behavioral challenges including anxiety, depression, ADHD, and OCD. ADHD was reported to have a similar prevalence in our study and by Gur-Hartman, et al.[6] Assessment of more adults with *CACNA1A* variants will be necessary to better understand the long-term challenges and help mitigate these issues earlier during childhood and adolescence.

We observed correlations between genotype and specific phenotypes and overall severity. Participants with variants in the S5 and S6 helices which line the channel pore had significantly higher severity scores compared to those in S3 helix and linker regions. Variants in the voltage-sensing S4 helix did not have a statistically significantly higher severity score when compared to those in S3 helix and linker regions Severity was also analyzed based on variant type and predicted effect on function, with missense GoF variants having significantly higher severity scores than missense LoF variants. Both missense GoF and LoF variants have significantly higher severity scores than truncating variants. The difference in severity between truncating and missense LoF variants could potentially be attributed to the different mechanisms by which they cause a loss of function as well as complex effects of missense mutations on channel function.[24–26] This heterogeneity in molecular mechanism emphasizes the need to assess individual variants to better understand the spectrum of *CACNA1A*-related disease.

In comparing the phenotypes of those with the p.Val1392Met variant with the rest of participants, those with the p.Val1392Met variant had a significantly higher severity score. These participants were also more likely to have seizures and status epilepticus.

Previous studies have reported significant phenotypic variability among relatives with the same *CACNA1A* variant.[4,27] Severity tends to be worse in children compared to their parents but similar among siblings which could be due to ascertainment bias.[6] While parental data were not reported for most participants with inherited variants, our study includes two families with two individuals each. Of two siblings (ages 2 and 3 years) with the p.Arg1779Ter variant, one has developmental delay, episodic ataxia type 2, paroxysmal tonic upward gaze, nystagmus, strabismus, and eye movement abnormalities while the other sibling reports only ataxia. A parent and child with the p.Arg582Gln variant report late-onset ataxia in the father and developmental delay, hemiplegic migraine, and ataxia in the 14-year-old child.

4.1 | Limitations

Participants were recruited among those who had previously received clinical genetic testing and were recruited from a patient support group. Participants may not be representative of all individuals with this condition and likely skewed toward more severely impacted individuals who come to clinical attention. Data were obtained by parent/self-report and have not yet been confirmed by medical record review with the exception of the genetic test report. The data presented were retrospective which may introduce bias although the study will continue collecting prospective data. The heuristic severity score was limited in that it mainly included the presence or absence of symptoms without grading the severity of each symptom. Additional measures will be added in the future to gather more detailed phenotypic information to iteratively improve the heuristic severity score. While using the funNCion machine learning method allowed for analysis of genotype–phenotype correlations on a larger scale than has previously been reported, channel function is only predicted, and future studies will require actual experimental data to support these predictions.

4.2 | Conclusions

We observe a higher than previously reported prevalence of developmental delay, hemiplegic migraine, epilepsy, and autism spectrum disorder and observe greater phenotypic severity in participants with predicted GoF variants, those with variants located in the S5 and S6 helices, and those with the p.Val1392Met variant. We find that seizures and status epilepticus are correlated with GoF and are more frequent in those with the p.Val1392Met variant. Our findings capture the breadth of disease severity in *CACNA1A*-related disease and suggest that the clinical phenotypic heterogeneity likely reflects diverse molecular phenotypes.

AUTHOR CONTRIBUTIONS

Conceptualization: Wendy K. Chung; *data curation*: Amy R. Lipman, Xiao Fan; *formal analysis and visualization*: Xiao Fan, Amy R. Lipman; *writing original draft*: Amy R. Lipman, Xiao Fan; *supervision*: Wendy K. Chung, Yufeng Shen. All authors reviewed the manuscript and approved the submission of this manuscript.

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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.14180.

DATA AVAILABILITY STATEMENT

All variants reported in this study are submitted to ClinVar (https://ncbi.nlm.nih.gov/clinvar/) under accession numbers SCV002506523–SCV002506555.

ETHICS STATEMENT

This study was approved by the Institutional Review Board of Columbia University under the protocol number IRB-AAAJ8651 approved 06/04/12. Informed consent was obtained from all participants or their guardians.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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