Longitudinal Phenotypic Trajectories in *GNAO1*-Related Disorders: Defining Disease Progression and Clinical Profiles

Jana Domínguez-Carral, MD,[1] Ana María Domínguez Cobo, MD,[2] Sol Balsells, MSc,[3] Anna Aguilar-Ros, BS,[4,5] Chu-Ting Chang, MSc,[6,7] William G. Ludlam, PhD,[6] Kathryn Yang, MD,[8] Katerina Bernardi, MD,[8] Micaela Chinigioli, MD,[9] Ainara Salazar-Villacorta, MD,[10] Veronica Di Pisa, MD,[11] Nuria Lamagrande-Casanova, MD,[12] Elena González-Alguacil, MD,[12] Beatriz De la Casa-Fages, MD, PhD,[13,14,15] Akihisa Okumura, MD,[16] Josefina Rodríguez, MD,[17] Ayush Agarwal, MD,[18] Daniela Muñoz-Chesta, MD,[19,20] Carolina Reynoso-Osnayo, MD,[21] Amy Lin, MD,[22] Brahim Tabarki, MD,[23] Jobaida Parvin, MD,[24] Adolfo Alberto Gallo, MD,[25] Andreia Forno, MD,[26] Fabian Maass, MD,[27] Johnny Montiel Blanco, MD,[28] Salomé Nasif, MD,[29] Elizabeth Jennions, MD,[30] Jorge Luis Ramón-Gómez, MD,[31] Helene Verhelst, MD, PhD,[32,33] Juan José Nieto Barceló, MD,[34] Dunja Čokolić Petrović, MD,[35] Luz Victoria García Ruiz, MD,[28] Christoph van Riesen, MD,[27] Paulo Rego Sousa, MD,[26] Maria del Pilar Massaro Sanchez, MD,[25] Husnea Ara Khan, MD,[24] Wejdan Hakami, MD,[23] Jennifer Friedman, MD,[22] Iván Espinoza-Quinteros, MD,[21,36] Monica Troncoso, MD,[37] Divyani Garg, MD,[18] Micaela Pauni, MD,[17] Hirokazu Kurahashi, MD,[16] María Concepción Miranda-Herrero, MD, PhD,[14,15,38] Anna Duat-Rodriguez, MD,[12] Luca Soliani, MD,[11] Manju A. Kurian, MD, PhD,[10] Angeles Schteinschnaider, MD,[9] Siddharth Srivastava, MD,[8] Darius Ebrahimi-Fakhari, MD, PhD,[8] Kirill A. Martemyanov, PhD,[6,7] and Juan Darío Ortigoza-Escobar, MD, PhD[39,40,41]

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Address correspondence to Dr Ortigoza-Escobar, Movement Disorders Unit, Pediatric Neurology Department, Institut de Recerca Hospital Sant Joan de Déu Barcelona, Passeig Sant Joan de Déu, 2, 08950, Barcelona, Spain. E-mail: juandario.ortigoza@sjd.es; Prof Martemyanov, Department of Neuroscience, The Scripps Research Institute, Jupiter, FL 33458, USA. E-mail: kmartemyanov@ufl.edu

From the [1]Epilepsy Unit, Department of Child Neurology, Coordinating member of the ERN EpiCARE, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain; [2]Department of Rehabilitation, Hospital Sant Joan de Déu, Barcelona, Spain; [3]Statistical Advising Service, Fundació de

Objective: Pathogenic variants in *GNAO1* cause a spectrum of epilepsy, movement disorders, and developmental impairment. Clinical heterogeneity complicates prognosis and therapeutic development. We present the first longitudinal natural history study of *GNAO1*-related disorders (*GNAO1*-RD) to delineate phenotypic trajectories.
Methods: Sixty-six individuals with *GNAO1*-RD were included in a cross-sectional analysis. Of these, 21 were enrolled in a prospective natural history arm (March 2021–December 2024), undergoing annual standardized evaluations with validated clinical scales to monitor phenotypic progression.
Results: Our cohort exhibited broad phenotypic and severity variability. *GNAO1*-RD severity scores ranged from 0.5 to 13. Neurodevelopmental impairment varied: 45.5% lacked head control, whereas 22.7% achieved independent walking; and 65% had no expressive language. Movement disorders were nearly universal (95.5%), with dyskinetic crises in 54.5%. Epilepsy affected 51.5%, with different seizure types. Individuals carrying recurrent variants showed consistent phenotypes and severity, supporting a genotype–phenotype correlation reinforced by molecular functional data. Molecular functional analysis for 20 of 31 missense variants correlated with severity scores. Longitudinal data from 21 patients in the natural history cohort showed overall stability or mild improvement across most functional domains. No significant deterioration was observed in global severity, motor function, cognition, or quality of life. However, severe patients experienced progressive worsening of movement disorder.
Interpretation: This largest *GNAO1*-RD cohort and first longitudinal natural history study provide insights into disease progression. *GNAO1*-RD generally follows a non-degenerative course, showing stability or mild improvements over time in cognition, language, adaptive skills, and motor function. Importantly, although global severity scores remained stable overall, severe cases showed cumulative functional burden driven by progressive movement disorder, rather than global neurodegeneration. Mortality occurred in a subset of patients because of complications from dyskinetic crises, infections, and epilepsy-related events. Genotype–phenotype data and the *GNAO1*-RD severity score support early risk stratification and personalized treatment development.

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GNAO1 gene was first related to developmental and epileptic encephalopathy (DEE) 17 (Online Mendelian Inheritance in Man [OMIM] 615473) by Nakamura et al[1] in 2013. Individuals with DEE-17 typically exhibit pronounced neurological impairments with severe psychomotor developmental delay and profound intellectual disability (ID), frequent movement disorders, epilepsy, and the need for nutritional support.[1–3] Nevertheless, a subgroup of individuals with variants in *GNAO1* displays a phenotype in which the movement disorder, particularly dystonia and dyskinetic crises, take precedence over the epilepsy, accompanied by a broader spectrum of ID.[2,4–7] Recognizing this variability, OMIM distinguished the following phenotypes related to *GNAO1* variants: (1) DEE-17 (OMIM 615473) and (2) neurodevelopmental disorder with involuntary movements (NEDIM) (OMIM 617493). The expanding clinical case descriptions have revealed a spectrum of disease severity, ranging across intermediate or even mild clinical phenotypes,[8–18] ultimately leading to the umbrella term *GNAO1*-related disorders (*GNAO1*-RD). To date, approximately 282 individuals have been reported worldwide and more than 150 distinct variants—predominantly

Recerca Sant Joan de Déu, Esplugues de Llobregat, Spain; [4]Pediatric Neurology Department, Hospital Sant Joan de Déu, Full Member of ERN EpiCare, Barcelona University, Barcelona, Spain; [5]Institut de Recerca Sant Joan de Déu, Barcelona, Spain; [6]The Herbert Wertheim UF Scripps Institute for Biomedical Innovation and Technology, University of Florida, Jupiter, FL, USA; [7]Skaggs Graduate School, The Scripps Research Institute, Jupiter, FL, USA; [8]Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, USA; [9]Department of Pediatric Neurology, FLENI, Buenos Aires, Argentina; [10]Developmental Neurosciences Programme, UCL-Institute of Child Health, London, UK; [11]IRCCS Istituto delle Scienze Neurologiche di Bologna, UOC Neuropsichiatria dell'età Pediatrica, Bologna, Italy; [12]Pediatric Neurology Unit, Hospital Universitario Niño Jesús, Madrid, Spain; [13]Movement Disorders Unit, Neurology Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain; [14]European Reference Network-Rare Neurological Diseases (ERN-RND), Madrid, Spain; [15]Instituto de Investigación Sanitaria Gregorio Marañón, Madrid, Spain; [16]Department of Pediatrics, Aichi Medical University, Nagakute, Japan; [17]Department of Pediatrics, Neuropediatric Division, Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; [18]Department of Neurology, All India Institute of Medical Sciences, New Delhi, India; [19]Department of Pediatric Neurology, San Borja Arriaran Hospital, University of Chile, Santiago, Chile; [20]Department of Neurology, Neuroscience Center, Clinica Universidad de los Andes, Santiago, Chile; [21]Unidad de Investigación Clínica y Epidemiológica en Pediatría, Universidad Peruana Cayetano Heredia, Lima, Peru; [22]Department of Neurosciences, University of California, San Diego, La Jolla, CA, USA; [23]Pediatric Neurology, Department of Pediatrics, Prince Sultan Military Medical City, Riyadh, Saudi Arabia; [24]Department of Pediatric Neurology, National Institute of Neurosciences and Hospital, Sher-E-Bangla Nagar, Dhaka, Bangladesh; [25]Department of Neurology, Paediatric Hospital Dr. Juan P. Garrahan, Buenos Aires, Argentina; [26]Pediatric Neurology Unit, Hospital Central de Funchal, Funchal, Portugal; [27]University Medical Center Göttingen, Göttingen, Germany; [28]National Institute of children's Health in Breña, Lima, Peru; [29]Pedro de Elizalde Children's Hospital, Buenos Aires, Argentina; [30]Department of Paediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg, Sweden; [31]Department of Child Neurology, Instituto Roosevelt, Bogotá, Colombia; [32]Department of Pediatrics, Division of Pediatric Neurology, Ghent University Hospital, Ghent, Belgium; [33]Faculty of Medicine and Health Sciences, Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium; [34]Department of Pediatrics, Hospital Rafael Méndez, Lorca, Spain; [35]Osijek University Hospital Centre, Department of Pediatrics, Osijek, Croatia; [36]Escuela de Posgrado, Universidad Continental, Lima, Peru; [37]Pediatric Neuropsychiatry Service, Hospital Clínico San Borja Arriarán, Pediatric Department, Universidad de Chile, Santiago, Chile; [38]Pediatric Neurology Unit, Pediatrics Department, Hospital General Universitario Gregorio Marañón, Madrid, Spain; [39]Movement Disorders Unit, Department of Child Neurology, Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain; [40]U-703 Centre for Biomedical Research on Rare Diseases (CIBER-ER), Barcelona, Spain; and [41]European Reference Network for Rare Neurological Diseases (ERN-RND), Barcelona, Spain

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missense mutations—have been implicated in *GNAO1*-RD, underscoring its genetic and phenotypic heterogeneity.[19]

The *GNAO1* gene encodes the Gαo subunit of neuronal heterotrimeric G proteins, involved in transducing signals from a wide range of G protein-coupled receptors (GPCRs), including those for dopamine, GABA, acetylcholine, and opioids.[20,21] On GPCR activation, Gαo binds to GTP and dissociates from the Gβγ subunits to regulate downstream effectors, until it deactivates on hydrolyzing GTP. Pathogenic variants in *GNAO1* disrupt this signaling through diverse mechanisms: loss-of-function, dominant-negative, reduced expression and trimer formation, and altered receptor interactions with GPCRs, and other proteins, which contribute to variability in clinical outcomes.[9,20,22,23] Notably, recent studies show that although many variants reduce Gαo expression, only the impairments in its ability to localize to the plasma membrane (PM) correlates with disease severity and with the presence of seizures, suggesting that this parameter could be used as a predictive biomarker for phenotypic stratification.[14] Moreover, disruptions in interactions with the regulators of G protein signaling (RGS)—proteins that speed up GTPase activity of Gαo and Gβγ subunits—vary among mutants and may also align with clinical severity.[9] These mechanistic insights underscore the complexity of *GNAO1*-RD and the importance of variant-level molecular characterization for prognosis and therapy.[14,16,24,25]

Although a few studies examined genotype–phenotype correlation,[4,9,14,16–18,26–28] no comprehensive natural history data of *GNAO1*-RD is available. In this study, we analyzed the clinical characteristics of 66 individuals with *de novo GNAO1* pathogenic variants, 21 of whom underwent a standardized, in-depth longitudinal assessment over a 4-year follow-up period. We characterized their clinical and molecular features, incorporating the recently developed *GNAO1*-RD severity score,[9] which permitted categorizing patients into severity-based subgroups, considering the diverse clinical presentations of epilepsy, movement disorders, motor and language development, and feeding difficulties.

Our study provides insights into the natural course and phenotypic trajectories of *GNAO1*-RD. By integrating the *GNAO1*-RD severity score, we aim to precisely delineate the nuances in severity among these individuals, thereby enhancing our understanding of the heterogeneity inherent to this disorder.

Patients and Methods

Ethical Approvals, Registrations, and Patient Consent

This study was reviewed and approved by the Ethics Committee of the Sant Joan de Déu Research Institute in Barcelona, Spain (PIC-77-21 and PIC-150-23). Written informed consent was obtained from the legal guardians of all participants, in accordance with the principles of the Declaration of Helsinki.

Study Design and Participant Ascertainment

A cross-sectional analysis was conducted using combined data from the main study "Prospective and retrospective study of phenotypic and genotypic characterization of patients affected by *GNAO1*-related disorders" (PIC-77-21) and the sub-study "Development and validation of a severity score for *GNAO1*-related disorders and its correlation with genotype and molecular mechanisms" (PIC-150-23). A subset of participants was enrolled in the prospective arm, designed to longitudinally assess phenotypic trajectories through a standardized protocol with annual clinical evaluations. The cross-sectional cohort was partly used to develop the *GNAO1*-RD severity score,[9] and selected cases were previously reported,[14,15] or included in the caregiver survey by Axeen et al.[15]

Clinical Data Collection

Clinical data were collected for all individuals through medical record review, supplemented with structured interviews with families or caregivers when needed. Prospective standardized evaluations were incorporated for the longitudinal subset. Data included age at symptom onset and diagnosis, neurodevelopmental milestones, epilepsy and movement disorder characteristics, feeding and nutritional status, current and past treatments, hospitalizations, and neurophysiological and neuroimaging findings. Prolonged video-electroencephalography (EEG) monitoring was performed in a subset of children and analyzed separately for biomarker identification in a dedicated study.[29] Likewise, brain magnetic resonance imaging (MRI) data are the subject of an independent study on volumetric and connectome alterations. Medication history was obtained through detailed chart review and caregiver and clinician reports. Although medication use was documented, specific information on positive or negative responses to individual drugs was not systematically collected.

Genetic Analysis

All individuals received a molecular diagnosis of *GNAO1* variants as part of their routine clinical evaluation. The genetic findings were obtained using 1 of the following approaches: (1) targeted sequencing of the *GNAO1* gene, (2) a multi-gene panel specific for DEEs, or (3) whole exome sequencing.

Molecular Functional Analysis and Experimental Measurement Score

Missense *GNAO1* variants underwent molecular characterization using bioluminescence resonance energy transfer (BRET)-based assays, as previously described by Domínguez-Carral et al.[9] Further details on these molecular assays, as reported in that study, are provided in the Supplementary Material. This methodology enabled the evaluation of 5 functional parameters: Gαo expression, heterotrimer formation, loss-of-function, dominant-negative activity, and receptor interaction. Each parameter was scored from 0 to 3, where 0 represented values comparable to wild-type and 3 indicated the most pronounced deviation. These scores were integrated into a composite experimental measurement score, ranging from 0 (no molecular impact) to 15 (maximum functional alteration). This molecular severity scoring approach contributed to the genotype–phenotype analysis presented in this study.

Statistical Analysis

Descriptive analysis summarized categorical variables as frequencies and percentages, and numerical variables as mean and standard deviation (SD) or median and interquartile range, depending on distribution assessed QQ plots. Natural history was studied with linear mixed models, including age as independent variable and random effects associated to subject ID to control for repeated measures. Group differences (according to *GNAO1*-RD severity score) and its interaction with age were also studied. Model assumptions (normality and homoscedasticity) were evaluated using robust methods when the fit of the models was inadequate. Significance was set at $p < 0.05$. The analysis was performed in R v4.3., using *lme4*, *lmerTest*, and *robustlmm* libraries. For graphical representation only, selected continuous variables were discretized using data binning, grouping values into predefined intervals to simplify the data, reduce noise, and facilitate visualization while improving interpretability. These discretized variables were not used in inferential analyses.

Longitudinal Clinical Assessments

Global Disease Severity

The recently developed *GNAO1*-RD severity score[9] was applied annually throughout the follow-up period. This score evaluates 5 domains: epilepsy, movement disorders, gross motor development, language development, and feeding. The epilepsy and movement disorder components consider frequency, intensity, associated falls or injuries, and treatment. Total scores range from 0 to 13 and are categorized into mild (0–3.9), moderate (4–7.9), or severe (>8) disease severity. For individuals in the prospective natural history cohort, mean scores across multiple time points were used to determine overall severity category.

Movement Disorder Severity

Movement disorder severity was assessed longitudinally using the Burke-Fahn-Marsden Dystonia Rating Scale (BFMDRS), the Abnormal Involuntary Movement Scale (AIMS), and the movement disorders domain of the *GNAO1*-RD severity score. Assessments combined video recordings and direct clinical observations performed by a single experienced movement disorder specialist, without altering ongoing treatments, and included evaluations during rest and voluntary movement. Potential confounders (eg, hypotonia or speech limitations) were considered when assigning regional BFMDRS scores. The most severe abnormal movements observed were scored. Dyskinetic crises were explicitly incorporated into the movement disorder domain of the *GNAO1*-RD severity score and rated according to frequency, intensity and duration, associated injuries, and treatment requirements. Therefore, dyskinetic crises contributed directly and substantially to the overall severity score rather than being analyzed as a separate feature. These crises were defined as sudden, paroxysmal exacerbations of abnormal involuntary movements, differing in pattern, or intensity from baseline, with preserved awareness and environmental responsiveness, and easily identified by caregivers. This definition follows the guidelines provided by the recently published Delphi consensus.[30]

Epilepsy Severity

Epilepsy severity was measured using the epilepsy domain of the *GNAO1*-RD severity score. Seizure types were classified in accordance with the International League Against Epilesy (ILAE) guidelines.[31]

Motor Function

Motor development and function were assessed longitudinally using 2 complementary tools: the Gross Motor Function Measure (GMFM-88) and the Motor Skills subdomain of the Vineland Adaptive Behavior Scales (VABS).

Adaptive Functioning

Adaptive functioning was measured using the Vineland Adaptive Behavior Composite (VABC). Both standard scores and raw scores were analyzed. Although standard scores provided an age-normed overview of adaptive functioning, raw scores were used to track intra-individual changes over time. The VABC raw score was derived by summing the raw scores from the communication, daily living skills, socialization, and motor skills subdomains to generate a composite measure.

Cognitive, Communication, and Socialization Development

Cognition was assessed using the cognitive domain of the Bayley Scales of Infant and Toddler Development, third edition (Bayley-III). Communication was evaluated using both the communication subdomain of the VABS and the expressive and receptive language subscales of the Bayley-III. Socialization was assessed through the socialization subdomain of the VABS. It is recognized that some of these developmental instruments have floor effects in individuals with very low developmental functioning, which may reduce sensitivity to detect further decline in profoundly affected individuals. The Bayley-III was administered across a broad age range. Scores obtained beyond the instrument's normative age limits were interpreted descriptively to support intra-individual longitudinal comparison rather than normative developmental benchmarking.

Daily Life Function

Daily functioning in everyday activities was evaluated using the daily living skills subdomain of the VABS.

Quality of Life

Quality of life was assessed using the Pediatric Neuro-Quality of Life upper and lower extremity function scales (NeuroQL) and the total score of the Caregiver Priorities and Child Health Index of Life with Disabilities (CPCHILD).

Results

Data from 66 individuals with *GNAO1*-RD were evaluated and analyzed, of whom 21 participated in the prospective natural history study. Among the 21 patients, 9 underwent a single evaluation, 2 had 2 evaluations, 4 had 3 evaluations, and 6 had 4 evaluations. Over the course of the study, 6 of 66 patients (9%) died because of infections (3 of 6, 50%), exacerbations of dyskinetic crises or status dyskineticus (2/6, 33%), or sudden unexpected death in epilepsy (SUDEP) (1 of 6.17%), at a median age of 4.2 years (range: 3–23).

Genetic Characteristics

Thirty-nine different *GNAO1* variants were identified in the cohort, including 6 novel variants: c.631_633del, c.823 T>G (p.F275V), c.352G>A (p.E118K), c.909_920dup, c.154C>A (Q52K) and c.164 T>G (I55S). Recurrent variants included p.G40R (P1, P38), p.T182I (P4, P5, P37), p.R209H (P7, P44), p.R209C (P14, P15, P35, P55, P61), p.G203R (P11, P12, P13, P20, P22, P28, P39, P40, P41, P62, P66), p.E237K (P16, P26, P29, P30, P42), c.724-8G>A (P17, P34, P51), p.E246K (P24, P32), c.723+1G>A (P36, P52), p.206Q (p43, P57), and p.N76K (P31, P64). Table 1 summarizes the characteristics of the genetic variants identified in the cohort, while Table S1 provides a detailed description for reference.

Clinical Overview

Patient ages in the whole cohort (n = 66) ranged from 3 months to 25 years (mean, 9.2 ± 5.9 years). In the natural history study subgroup (n = 21), mean age was 7.8 ± 13.6 years at baseline and 9.3 ± 5.5 years at last follow-up (range: 11 months to 21 years and 2 months).

Phenotypic characteristics demonstrated broad variability. Most patients exhibited early neurodevelopmental impairments, although 2 reached typical developmental milestones, 1 of them showing only autism spectrum traits. Motor function varied widely: 15 of 66 (22.7%) achieved independent walking and purposeful hand use, whereas others had variable gross motor deficits: 30 of 66 (45.5%) had no head control, 12 of 66 (18.2%) had acquired head control but were not able to sit, 2 of 66 (3%) were able to sit, and 7 of 66 (10.6%) were able to walk with support. Expressive language was affected in 54 of 66 (82%), with 43 of 66 (65%) having no language at all. Initial symptoms included motor delay or hypotonia (36/66, 54.5%), epilepsy (19/66, 28.8%), and movement disorders (8/66, 12.1%). Movement disorders were present in all but 3 patients, predominantly dystonia (57/66, 86%) and chorea (42/66, 63.6%). The mean age at onset of movement disorders was 2.37 ± 3.26 years (range: 0–14), with 50% of patients showing symptoms by 12 months of age. Dyskinetic crises occurred in 36 of 66 (54.5%), with a mean age at onset of 4.54 ± 3.5 years (range: 0–15.2), and 50% of cases presented by 3 years of age (Fig 1A). This latency highlights that dyskinetic crises emerge after initial movement disorder symptoms and emphasizes the need for anticipatory clinical monitoring. Pharmacological treatments had limited efficacy, and 17 of 66 (25.7%) patients underwent globus pallidus deep brain stimulation with variable outcomes. Epilepsy affected 34 of 66 (51.5%) of patients, with 28 of 34 (82.3%) being drug-resistant. The mean age at seizure onset was 2,9 ± 4.3 years (range: 0–17), with 50% of affected patients presenting seizures by 2 months of age (see Fig 1A). Seizure types were diverse and included focal motor clonic seizures (11/33, 33.3%), bilateral clonic seizures of unknown onset (11/34, 32.3%), focal non-motor autonomic seizures (10/34, 29.4%), focal motor tonic seizures (9/34, 26.4%), epileptic spasms (6/34, 17.6%), generalized non-motor or typical absence seizures (1/34, 3%), and an isolated febrile seizure (1/34, 3%). Among those with epileptic spasms and focal non-motor autonomic seizures, 3 of 6 and 6 of 10 patients, respectively, carried the

TABLE 1. Genetic Variants Identified in the Cohort

Patient	HGVS (NM_020988) cDNA change	Protein change	Variant type	ACMG classification	CADD score	REVEL
P1, P38	c.118G>C	G40R	Missense	Pathogenic	33	Deleterious (0.94)
P2	c.137A>G	K46R	Missense	Pathogenic	1.083	Deleterious (0.95)
P3	c.143C>T	T48I	Missense	Pathogenic	27	Deleterious (0.94)
P4, P5, P37	c.545C>T	T182I	Missense	Pathogenic	28,8	Deleterious (0.99)
P6	c.596 T>C	L199P	Missense	Pathogenic	28,5	Deleterious (0.97)
P7, P44	c.626G>A	R209H	Missense	Pathogenic	21	Deleterious (0.91)
P8	c.692A>G	Y231C	Missense	Pathogenic	29,1	Deleterious (0.99)
P9	c.704 T>C	L235P	Missense	Pathogenic	32	Deleterious (0.95)
P10	c.871 T>A	Y291N	Missense	Pathogenic	26	Deleterious (0.89)
P11, P13	c.607G>C	G203R	Missense	Pathogenic	32	Deleterious (0.94)
P12, P20, P22, P28, P39, P40, P41, P62, P66	c.607G>A	G203R	Missense	Pathogenic	33	Deleterious (0.94)
P14, P15, P35, P55, P61	c.625C>T	R209C	Missense	Pathogenic	32	Deleterious (0.89)
P16, P26, P29, P30, P42	c.709G>A	E237K	Missense	Pathogenic	32	Deleterious (0.92)
P17, P34, P51	c.724-8G>A		Splicing	Likely pathogenic	–	–
P18	c.412C>T	Q138*	Nonsense	Pathogenic	–	–
P19	c.1030_1032delATT	I344*	Nonsense	Pathogenic	–	–
P21	c.723+2 T>A		Splicing	Pathogenic	–	–
P23	c.644G>A	C215Y	Missense	Pathogenic	29.4	Deleterious (0.9)
P24, P32	c.736G>A	E246K	Missense	Pathogenic	22.8	Deleterious (0.84)
P25	c.631_633del	L211*	Nonsense	Pathogenic	–	–
P27	c.748C>T	L250F	Missense	Pathogenic	22.3	Deleterious (0.91)
P31, P64	c.228C>A	N76K	Missense	Pathogenic	27.1	Deleterious (0.91)
P33	c.127G>A	E43K	Missense	Pathogenic	31	Deleterious (0.91)
P36, P52	c.723+1G>A		Splicing	Pathogenic	–	–
P43, P57	c.617G>A	R206Q	Missense	Pathogenic	32	Deleterious (0.86)
P45	c.616C>G	R206G	Missense	Pathogenic	27	Deleterious (0.88)
P46	c.649G>A	E217K	Missense	Pathogenic	27.4	Deleterious (0.84)
P47	c.823 T>G	F275V	Missense	Pathogenic	22.5	Deleterious (0.95)
P48	c.119G>A	G40E	Missense	Pathogenic	32	Deleterious (1)
P49	c.352G>A	E118K	Missense	Likely pathogenic	23	Uncertain (0.46)
P50	c.610G>A	G204S	Missense	Pathogenic	28.5	Deleterious (0.9)
P53	c.980C>A	T327K	Missense	Pathogenic	27.8	Deleterious (0.93)
P54	c.622G>C	E208Q	Missense	Pathogenic	27.5	Deleterious (0.78)
P56	c.909_920dup	A306_Q307 ins HIA	In-frame duplication	Likely pathogenic	–	–
P58	c.154C>A	Q52K	Missense	Pathogenic	26.4	Deleterious (0.93)
P59	c.164 T>G	I55S	Missense	Pathogenic	27.5	Deleterious (0.94)
P60	c.1046_1055del10ins10	R349_G362delinsQGCA	In-frame indel	Pathogenic	–	–
P63	c.526C>A	L176I	Missense	Pathogenic	28.1	Deleterious (0.83)
P65	c.626G>T	R209L	Missense	Pathogenic	33	Deleterious (0.93)

Summary of the genetic characteristics of all variants detected in our cohort of patients with *GNAO1*-related disorders.
ACMG = American College of Medical Genetics and Genomics; cDNA = complementary DNA; HGVS = Human Genome Variation Society.

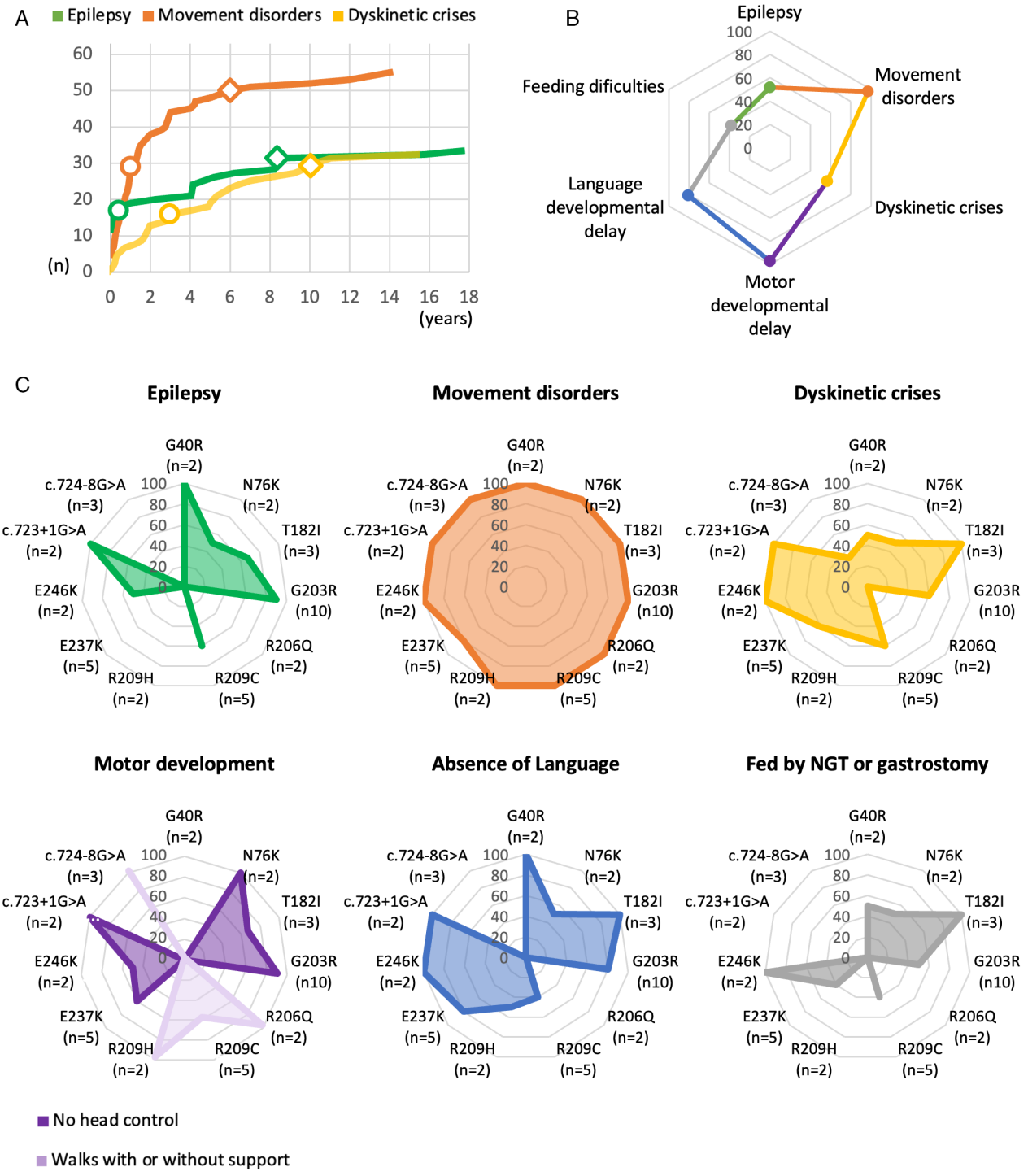

FIGURE 1: Clinical manifestations and age of onset in the full *GNAO1*-related disorders (RD) cohort and recurrent variants. (A) Cumulative incidence curves (Kaplan–Meier) showing the age at onset of epilepsy (n = 34), movement disorders (n = 63), and dyskinetic crises (n = 36). Circles indicate the age at which 50% of individuals presented the symptom (median age of onset), and diamonds indicate the age at which 90% of individuals had developed the symptom. Patients with presence of the symptom, but unknown age at onset were excluded from the analysis. (B) Prevalence of core clinical features in the full *GNAO1*-RD cohort (n = 66), including motor developmental delay, language developmental delay, movement disorders, dyskinetic crises, and epilepsy. (C) Radar plots illustrating the distribution of core clinical features (epilepsy, movement disorders, dyskinetic crises, motor developmental delay, language developmental delay, and feeding difficulties) among individuals carrying recurrent *GNAO1* variants. NGT = nasogastric tube.

G203R variant. Seizure frequency ranged from daily seizures to 6 episodes over 13 years. No anti-seizure medication was consistently reported as being more effective, although good response to sodium channel blockers was observed in 4 patients. Five patients received ketogenic diet, being the most effective treatment tried in 1 of them, and none received vagus nerve stimulation. Feeding difficulties were common, with 26 of 66 (39.4%) patients requiring gastrostomy. Additionally, uncommon features such as delayed puberty with growth hormone deficiency, pancreatitis, and a Rett-like phenotype without dystonia were observed in single individuals, highlighting the clinical heterogeneity of the disorder. Figure 1B summarizes the prevalence of core clinical features across full cohort, while Table S2 details the main symptoms for each individual. Symptom prevalence across recurrent *GNAO1* variants (n ≥ 2) varied considerably, as seen in Figure 1C.

In the full cohort of 66 patients, *GNAO1*-RD severity scores ranged from 0.5 to 13. Based on predefined thresholds, 18 of 64 patients (28%) were classified as mild, 19 of 66 (28.8%) as moderate, and 29 of 66 (43.9%) as severe (Fig 2A). For recurrent variants, mean severity scores were: c.723+1G>A (n = 2, 10.5, range: 10.25–10.75), c.724-8G>A (n = 3, 2.78, 1.1–5.75), E237K (n = 5, 6.77, 6–8.5), E246K (n = 2, 8.38, 8–8.75), G203R (n = 11, 8.2, 1.25–13), G40R (n = 2, 7.3, 5.12–9.5), R209C (n = 5, 5, 1.97–7.75), R209H (n = 2, 3.5, 2.5–4.5), N76K (n = 2, 7,75, 5,5–10), and T182I (n = 3, 10.88, 8.75–13). Differences in severity scores across variants were statistically significant ($p < 0.001$).

Functional Variant Analysis

Twenty of the 31 missense variants in the cohort were studied and evaluated using the experimental measurement score. The mean composite score was 6.5 ± 2.9 (3.5–12.5). A positive correlation was observed between the composite experimental measurement score and the *GNAO1*-RD severity score ($r = 0.51$, $p < 0.001$) (see Fig 2B). Figure 3 shows the functional study results for variants E43K, E118K, R206G, and F275V, which have not been previously reported. Additionally, Figure S1 summarizes the results of the 5 functional parameters and the composite experimental measurement score for each variant.

Phenotypic Trajectory in GNAO1*-RD*

The analysis included data from 49 *GNAO1*-RD severity score assessments across 21 individuals, 47 Bayley, Vineland, and GMFM-88 assessments across 21 individuals; 50 BFMDRS and AIMS assessments across 19 individuals; and 43 Neuro-QL and CPCHILD assessments across 17 individuals. Tables 2a and 2b presents detailed statistical results from the longitudinal models analyzing the evolution of clinical and functional scale scores with age, stratified by severity groups. Table S3 summarizes clinical

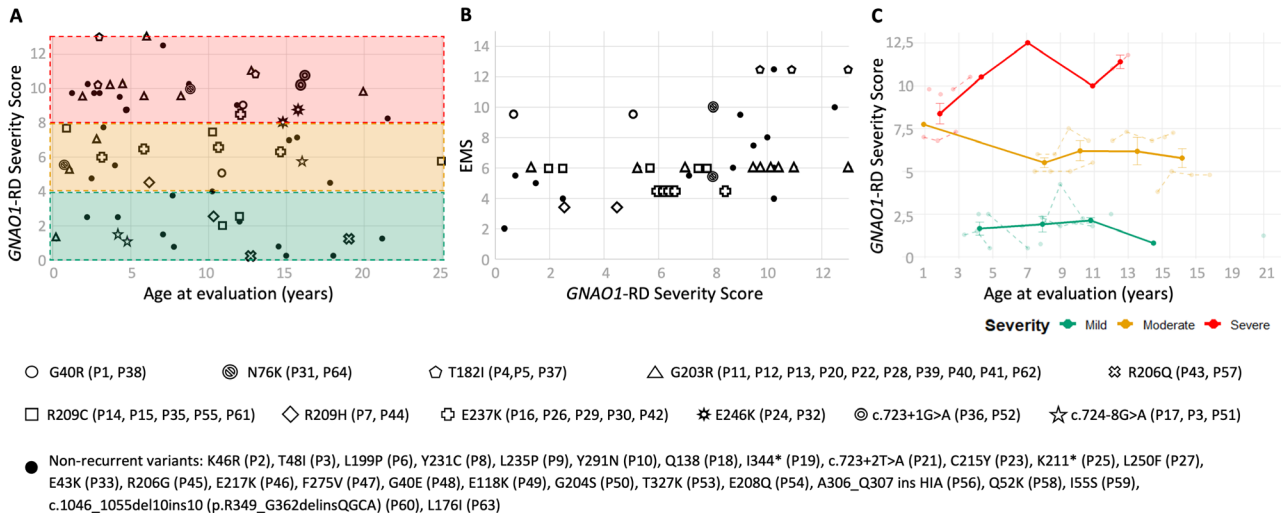

FIGURE 2: Distribution and longitudinal evolution of global disease severity in *GNAO1*-related disorders (RD). (A) Distribution of *GNAO1*-RD severity scores in the full cohort (n = 66), classified into mild, moderate, and severe groups. (B) Correlation between the experimental composite measurement score and the *GNAO1*-RD clinical severity score across all variants, showing a significant positive correlation ($r = 0.51$, $p < 0.001$). (C) Longitudinal evolution of *GNAO1*-RD severity scores by age. No significant association with age was found, indicating clinical stability over time. In A and B, each symbol represents an individual patient; recurrent *GNAO1* variants are shown using variant-specific symbols shared across patients, whereas non-recurrent variants are depicted as dots. In C, dots represent individual patient assessments, dashed lines connect repeated assessments from the same patient over time, and solid (bold) lines indicate group-level trajectories for mild, moderate, and severe phenotypic groups, calculated as mean scores within 2-year age intervals.

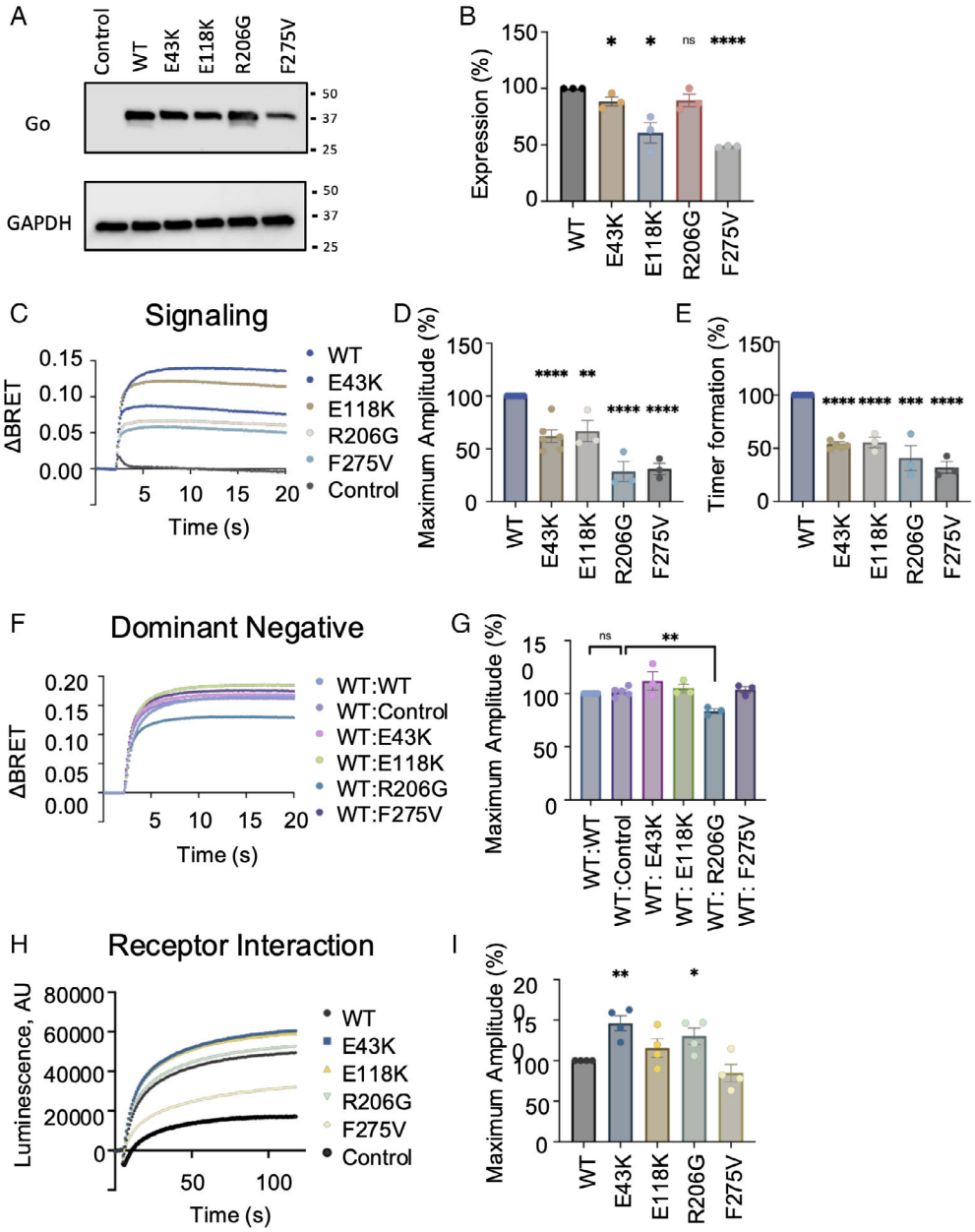

FIGURE 3: Functional characterization of *GNAO1* variants E43K, E118K, R206G, and F275V. (A) Representative western blotting of the expression level of Gαo A wild-type (WT), E43K, E118K, R206G, and F275V. (B) Quantification of the expression level. (C) Representative traces of mutations on dopamine-induced signaling. (D) The effect of mutations on dopamine-induced signaling. (E) The effect of mutations on trimer formation measured by the basal bioluminescence resonance energy transfer (BRET) ratio. The trimer formation was defined by 0% (without Gαo) to 100% (WT Gαo). (F) Representative traces of the dominant negative effect of mutations by measuring the Δ BRET in the presence of Go mutants with WT. (G) Quantification of the dominant negative effect. No significant difference between WT:WT with WT:Control indicates the saturation of the WT Gαo in the assay. WT:F275V falls below WT:Control, suggesting F275V suppresses WT Gαo coupling. (H) Representative luminescence traces measuring the D2R interaction with G protein heterotrimer. (I) Quantification of the effect of Go mutants on interactions with D2R. EMS = Experimental Measurement Score.

and functional scores at baseline and last evaluation for the whole cohort and stratified by disease severity.

Global Disease Severity. Among patients enrolled in the natural course study (n = 21), no statistically significant association was found between age and *GNAO1*-RD severity scores, indicating that disease severity remained relatively stable over time (see Fig 2C).

Adaptive Functioning. The raw VABC score showed a statistically significant increase over time across the cohort ($p < 0.001$ for age). When stratified by disease severity

TABLE 2a. Longitudinal Mixed-Effects Model Estimates for Clinical and Functional Scale Trajectories

	Coefficient	Estimate	Standard error	*t* value	*p*
GNAO1-RD severity score	Age	0.05025	0.07228	0.695	0.4908070482
BFMDRS	Age	0.4738	0.8288	0.572	0.5731929588
	Age × Severity group moderate	2.0356	1.2110	1.681	0.1058270918
	Age × Severity group severe	4.4006	2.0956	2.100	0.0474592727
AIMS	Age	−0.2751	0.1732	−1.588	0.13715
	Age × Severity group moderate	0.3940	0.2556	1.542	0.14942
	Age × Severity group severe	1.6536	0.4337	3.813	0.00268
MDSS	Age	0.02553	0.03138	0.813	0.421187
ESS	Age	−0.05232	0.03204	−1.633	0.11205
GMFM-88 (%)	Age	−0.5623	0.7792	−0.722	4.762066e-01
	Age × Severity group moderate	1.8406	1.0572	1.741	9.117942e-02
	Age × Severity group severe	−0.3808	1.6138	−0.236	8.151643e-01
VABS-motor skills (RS)	Age	2.0259	1.1571	1.751	0.09396444
	Age × Severity group moderate	−0.6807	1.5960	−0.426	0.6736901
	Age × Severity group severe	−2.3667	2.4056	−0.984	0.33679
VABS-ABC (RS)	Age	24.708	3.801	6.500	0.0000003126604
	Age × Severity group moderate	−21.915	5.202	−4.213	0.0001795889
	Age × Severity group severe	−23.936	7.949	−3.011	0.005282412
Bayley-cognitive (RS)	Age	0.585874	0.383341	1.528	0.1356922
	Age × Severity group moderate	−0.003084	0.509638	−0.006	0.9952072
	Age × Severity group severe	−0.720097	1.090380	−0.660	0.5134819
VABS-communication (RS)	Age	9.149	1.120	8.169	2.960664e-09
	Age × Severity group moderate	−8.125	1.499	−5.420	4.814578e-06
	Age × Severity group severe	−8.906	2.359	−3.775	0.0006999715
Bayley-receptive language (RS)	Age	0.7597	0.2521	3.013	4.587170e-03
	Age × Severity group moderate	−0.3111	0.3353	−0.928	3.593410e-01
	Age × Severity group severe	−0.5817	0.5663	−1.027	3.113460e-01
Bayley-expressive language (RS)	Age	2.0371	0.4237	4.808	9.018438e-05
	Age × Severity group moderate	−2.0706	0.5840	−3.546	1.978220e-03
	Age × Severity group severe	−1.9550	0.8984	−2.176	4.214194e-02
VABS-socialization (RS)	Age	6.511	1.102	5.909	0.000001220877
	Age × Severity group moderate	−6.189	1.511	−4.096	0.0002270505
	Age × Severity group severe	−5.788	2.301	−2.515	0.01704384

TABLE 2b. Longitudinal Mixed-Effects Model Estimates for Clinical and Functional Scale Trajectories

	Coefficient	Estimate	Standard error	*t* value	*p*
VABS-daily living skills (RS)	Age	7.2415	0.8262	8.765	2.455124e-08
	Age × Severity group moderate	−7.1347	1.1430	−6.242	4.450757e-06
	Age × Severity group severe	−7.3688	1.7067	−4.317	0.0004003830
NeuroQL-upper extremities	Age	0.6498	0.4782	1.359	0.1835397006
	Age × Severity group moderate	−0.3009	0.6221	−0.484	0.6317276608
	Age × Severity group severe	−0.6498	1.0764	−0.604	0.5497682273
NeuroQL-lower extremities	Age	1.4677	0.7087	2.071	0.0476396612
	Age × Severity group moderate	−0.7276	0.9362	−0.777	0.4431666814
	Age × Severity group severe	−1.4677	1.6615	−0.883	0.3835562657
CPCHILD	Age	4.114	2.138	1.924	0.0670
	Age × Severity group moderate	−1.970	2.811	−0.701	0.4914
	Age × Severity group severe	−4.929	4.943	−0.997	0.3325

Estimated coefficients (Estimate), standard errors, *t*-values and *p*-values from models analyzing changes in clinical and functional scores over time with age, stratified by severity group. These results indicate the relative impact of age and disease severity on the evolution of each outcome measure.
ABC = Adaptative Behavior Composite; AIMS = Abnormal Involuntary Movement Scale; BFMDRS = Burke-Fahn-Marsden Dystonia Rating Scale; CPCHILD = Caregiver Priorities and Child Health Index of Life with Disabilities; ESS = Epilepsy Severity Score; GMFM-88 = Gross Motor Function Measure-88; *GNAO1*-RD = *GNAO1* related disorders; MDSS = Movement Disorders Severity Score; NeuroQL = Neuro-Quality of Life scale; RS = raw score; VABS = Vineland Adaptative Behavior Scale.

groups, this trend varied notably ($p < 0.001$). VABC raw scores increased with age in the mild group, showed minimal change in the moderate group, and remained unchanged in the severe group (Fig 4A).

Cognitive, Communication and Socialization Development. No statistically significant change over time was observed in Bayley-III cognitive scores across the whole cohort. However, cognitive scores differed significantly between severity groups, with patients in the moderate and severe groups showing significantly lower scores than those in the mild group ($p < 0.001$ for both comparisons). No significant interaction between age and severity group was found for cognitive scores (see Fig 4F). In contrast, VABS communication scores ($p < 0.001$), Bayley-III receptive language scores ($p = 0.005$), Bayley-III expressive language scores ($p < 0.001$), and VABS socialization scores ($p < 0.001$) all showed significant improvements over time in the whole cohort (see Fig 4B,C,G,H). When stratified by severity, the mild group showed significant improvements in VABS communication and socialization scores (both $p < 0.001$), as well as in Bayley-III expressive language scores ($p = 0.002$), whereas the moderate group showed minimal change and the severe group remained stable. No significant interaction effects between age and severity group were observed for Bayley-III receptive language scores, suggesting a comparable trajectory across groups. Bayley-III scores consistently distinguished severity groups, with mild cases showing modest gains and moderate-to-severe groups remaining low throughout follow-up.

Movement Disorder Severity. No statistically significant changes were observed over time across the cohort in the BFMDRS, AIMS, or the movement disorders subdomain of the *GNAO1*-RD severity score (Fig 5A–C). When stratified by severity, patients in the mild and moderate groups showed no statistically significant change over time in BFMDRS and AIMS. In contrast, patients classified as severe showed a significantly steeper increase in scores over time in both BFMDRS ($p = 0.047$) and AIMS ($p = 0.003$).

Epilepsy Severity. No statistically significant change in the epilepsy subdomain of the *GNAO1*-RD severity score was observed over time across the cohort (see Fig 5D).

Motor Function. There was no statistically significant change for the GMFM-88 or the motor skills domain of the VABS scores over time across the cohort (see Fig 4E

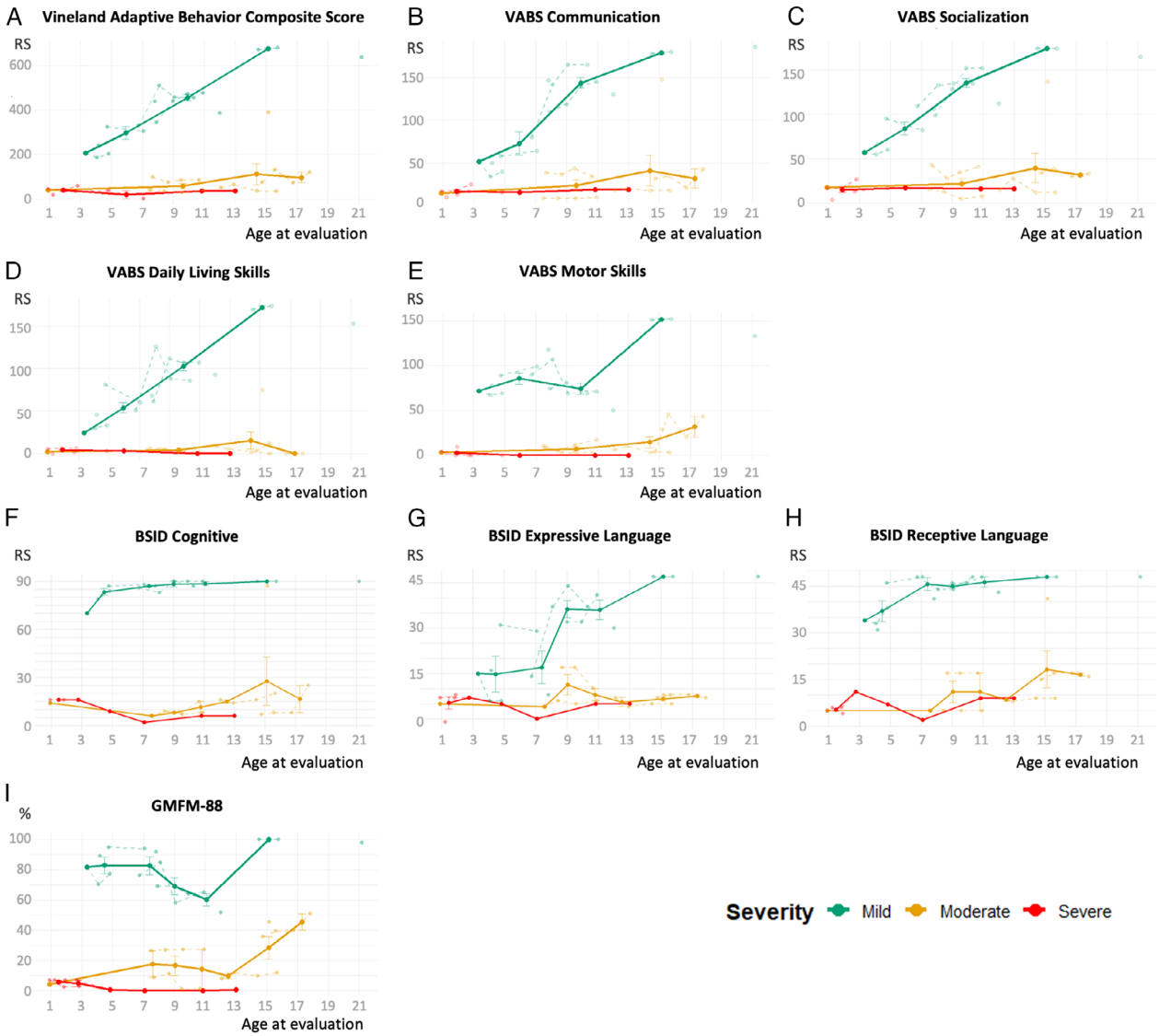

FIGURE 4: Longitudinal trajectories of adaptive and neurodevelopmental domains in individuals with *GNAO1*-related disorders. (A) Vineland Adaptive Behavior Composite (VABC) raw scores, (B–E) VABS-II domains: communication (b), socialization (c), daily living skills (d), and motor skills (E); (F–H) BSID-III domains: (F) cognitive, (g) receptive language and (h) expressive language; (I) GMFM-88 dots represent individual patient assessments. Dashed lines connect repeated assessments from the same patient over time. Solid (bold) lines indicate group-level trajectories for mild, moderate, and severe phenotypic groups, calculated as mean scores within 2-year age intervals. Significant improvements were observed in (A), (B), (C), (D), (G), and (H), mainly driven by the mild group; minimal change in moderate and stability in severe groups. No significant changes were observed in (E), (F), or (I). BSID-III = Bayley Scales of Infant and Toddler Development (third edition); GMFM-88 = Gross Motor Function Measure–88, RS = raw score; VABS-II = Vineland Adaptive Behavior Scales (second edition).

and I). Patients in the moderate and severe groups showed significantly lower motor skills scores compared to the mild group ($p < 0.001$ and $p = 0.002$, respectively). When stratified by severity groups, there was no significant difference in the trajectory of motor skills scores over time between groups.

Daily Life Function. A statistically significant increase in daily living skills scores was observed over time across the cohort ($p < 0.001$). Analysis by severity group revealed significant improvements in the mild group ($p < 0.001$), whereas patients classified as moderate and severe showed a less marked increase over time (Fig 4D).

Quality of Life. No statistically significant association with age was observed for the Neuro-QL upper extremity function scale or the CPCHILD (Fig 5E). In contrast, a statistically significant increase in scores over time was observed for the Neuro-QL lower extremity function scale ($p = 0.048$) (see Fig 5F,G). When comparing severity

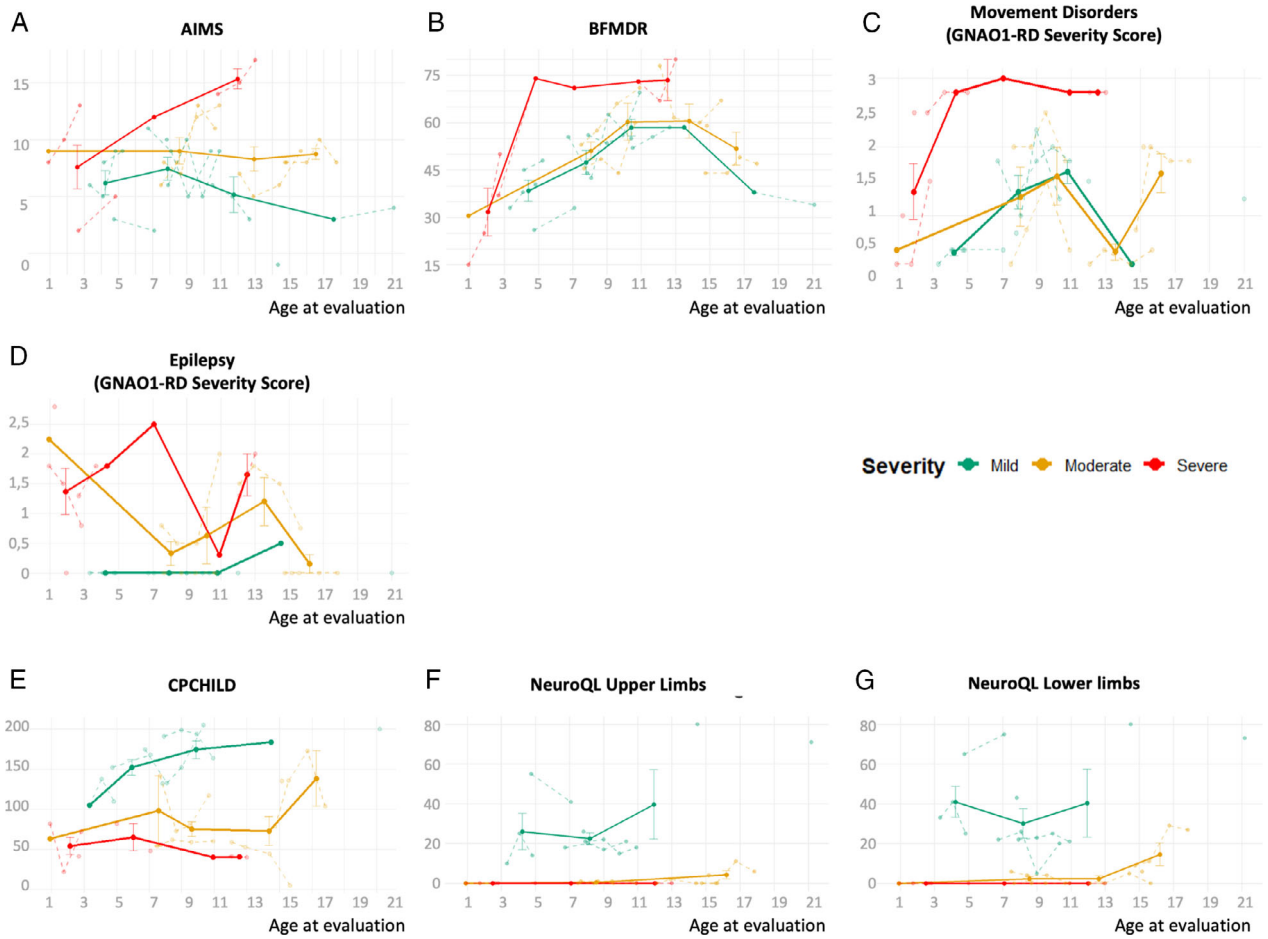

FIGURE 5: Longitudinal changes in movement disorder severity, epilepsy severity, and quality of life in *GNAO1*-related disorders. (A) BFMDRS, (B) AIMS, (C) MDSS, (D) ESS, (E) CPCHILD, and (F–G) Neuro-QL Pediatric upper and lower extremity function scores over time stratified by severity group. Dots represent individual patient assessments; dashed lines connect repeated assessments from the same patient over time. Solid (bold) lines indicate group-level trajectories for mild, moderate, and severe phenotypic groups, calculated as mean scores within 2-year age intervals. For BFMDRS, AIMS, MDSS, and ESS higher scores indicate greater severity (worse outcome), whereas for the QoL scales, higher scores indicate better outcome. No significant changes over time were observed in A–E, although the severe group showed a significant worsening in A and B. A significant improvement over time was observed in F, but not in G. Significant group differences were observed in F and G, with lower scores in the moderate and severe groups compared to the mild group. AIMS = Abnormal Involuntary Movement Scale; BFMDRS = Burke-Fahn-Marsden Dystonia Rating Scale; CPCHILD = Caregiver Priorities and Child Health Index of Life with Disabilities; ESS = Epilepsy Severity Score; MDSS = Movement Disorders Severity Score; NeuroQL = Neuro-Quality of Life scale; RS = raw score; QoL = quality of life.

groups, scores for both the Neuro-QL upper and lower extremity function scales differed significantly, with patients in the moderate and severe groups showing significantly lower values compared to those in the mild group (upper extremities: $p = 0.004$ and $p = 0.038$, respectively; lower extremities: $p = 0.004$ and $p = 0.045$, respectively).

Discussion

This study provides valuable insights into the natural history and phenotypic variability of *GNAO1*-RD through a cohort of 66 individuals, with a subset of 21 undergoing prospective longitudinal assessment. Patients exhibited a broad spectrum of neurodevelopmental impairments, movement disorders, and epilepsy, with a significant mortality rate. Six patients died because of infections and other complications, mainly associated with exacerbations of dyskinetic crises or epilepsy, further underscoring the potentially life-threatening nature of *GNAO1*-RD. This aligns with previous cohorts reporting mortality associated with dyskinetic crisis-related complications, reinforcing the need for proactive identification of high-risk patients. Longitudinal analyses suggest stability in overall disease severity, with milder patients showing improvements in adaptive functioning over time, whereas severe cases exhibit little functional gain and progressive worsening of movement disorder, underscoring the variable prognosis of

GNAO1-RD. Among the most severely affected patients, clinical deterioration appears to be driven primarily by the progressive burden of movement disorder rather than global neurodegeneration, indicating a cumulative increase in functional disability over time.

Clinical Heterogeneity in GNAO1*-RD*

The phenotypic presentation of *GNAO1*-RD varies widely across the cohort. Although a minority of individuals achieved key developmental milestones or displayed only mild features such as autism spectrum traits, the majority experienced early neurodevelopmental impairments. Motor outcomes ranged from independent walking and purposeful hand use to severe hypotonia and gross motor deficits. Movement disorders were nearly universal, typically manifesting as early-onset generalized chorea or dystonia. Dyskinetic crises emerged as a distinctive and well-recognized feature, characterized by severe, paroxysmal, involuntary movements affecting multiple body regions.[30] Previous studies have characterized these episodes as highly variable in frequency, duration, and intensity, often necessitating emergency medical intervention and imposing a considerable burden on patients and families.[7] They can cause significant morbidity, including dehydration, rhabdomyolysis, decreased consciousness, and multisystem failure, with some cases resulting in incomplete neurological recovery, increased hypotonia, or loss of previously acquired motor skills.[2,3,11,32–38] Epilepsy presented with diverse manifestations, ranging from infrequent, well-controlled seizures to drug-resistant forms, with considerable variability in age at onset and frequency, spanning from the neonatal period to adolescence and from daily episodes to isolated events. Seizure types included both motor and non-motor, with epileptic spasms, tonic seizures, and focal non-motor autonomic seizures being particularly prominent. No single anti-seizure medication proved consistently effective, although some patients responded to sodium channel blockers,[39] and 1 case showed good response to ketogenic diet.[40] Notably, a substantial proportion of patients with epileptic spasms and focal non-motor autonomic seizures carried the G203R variant, underscoring the need for careful identification of these subtle seizure types, which may be overlooked, especially in individuals with significant neurodevelopmental impairment and movement disorders.[22,27] Feeding difficulties were common. These observations align with a previous caregiver-reported survey by Axeen et al,[15] which described a large cohort with symptom onset typically before 6 months of age, universal developmental delay, and diverse movement disorder and epilepsy phenotypes. Although hypotonia and developmental delay were the most frequent initial concerns, that study also highlighted milder epilepsy cases and 1 patient without epilepsy or movement disorder at age 4, reinforcing the importance of including *GNAO1* in early diagnostic gene panels. This study expands on these findings by providing physician-confirmed clinical data. A wide range of medications was used across the cohort, often in sequential or combined strategies, with variable and frequently transient benefit. Advanced electrophysiological and neuroimaging data from this cohort will be reported separately to allow detailed modality-specific analysis. A subset of the EEG data from this cohort has already been analyzed and published, demonstrating neuronal oscillatory imbalances associated with disease severity.[29]

Phenotypic Trajectories in GNAO1*-RD: A Non-degenerative Course with Worsening Movement Disorders in Severe Cases*

Unlike previous reports, which have largely relied on cross-sectional or case-based descriptions, this study provides novel insights into disease progression by systematically evaluating global disease severity, movement disorder and epilepsy burden, motor function, adaptive behavior, cognitive and social development, daily life functioning, and quality of life over time.[13–15] This longitudinal approach enhances our understanding of phenotypic trajectories, contributing valuable data to guide clinical care and future therapeutic interventions.

Our findings indicate that *GNAO1*-RD does not follow a neurodegenerative course. Disease progression generally followed a stable trajectory, with *GNAO1*-RD severity scores remaining consistent across evaluations and no significant association with patient age. Longitudinal analyses revealed heterogeneous, but notable developmental gains, particularly in individuals with milder phenotypes, while patients with moderate phenotypes showed limited progression, and those with severe presentations remained largely stable. Adaptive functioning, as measured by the VABC raw score, as well as communication, socialization, expressive language, and daily living skills, showed a significant increase over time in the mild group, reflecting their potential for functional gains and greater autonomy, with important implications for educational and rehabilitative strategies. Cognitive development, assessed via Bayley-III cognitive scores, showed no significant change over time, although differences between severity groups were evident, with moderate and severe groups scoring lower than the mild group.[41] Consistently low Bayley-III scores in moderate and severe phenotypes further demonstrate limited developmental progression in these subgroups. A key exception to this stability was worsening of movement disorder severity in the severe group, as evidenced by increasing BFMDRS and AIMS

scores, whereas mild and moderate phenotypes showed no progression. This asymmetry suggests that movement disorder burden may evolve specifically in the most affected individuals, and reinforces the importance of early severity stratification for predicting clinical outcome. Importantly, because dyskinetic crises directly contribute to the severity score, their emergence and recurrence were captured analytically, supporting their role as key drivers of disability progression in the most severely affected patients. Such observations align with prior reports describing a fluctuating but progressive movement disorder phenotype, particularly in severe cases.[2,6,37] Epilepsy severity remained stable throughout the follow-up period across all severity groups, offering reassurance regarding the stability of this component, which is critical for clinical management. Motor function, as assessed by the GMFM-88 and the motor skills domain of the VABS, showed no significant changes over time across the cohort.[9] This stability informs about the functional ceiling and the potential need for intensive interventions to alter trajectories. A slight, but significant improvement was observed in Neuro-QL lower extremity function scores, however, no improvements were noted in Neuro-QL upper extremity function or CPCHIL, indicating persistent limitations and the need for ongoing support.

Genotype–Phenotype Correlations in GNAO1*-RD*

Our findings reinforce previous evidence linking specific *GNAO1* variants to distinct clinical phenotypes.[4,6,10,14,42] Individuals carrying the same variant often exhibited consistent patterns in age at onset, symptom profiles, and functional outcomes, particularly regarding core features such as epilepsy, movement disorders, and motor milestones. For instance, epilepsy was present in all individuals with the c.723+1G>A and G40R variants,[11] and in 90% of those with G203R, but absent in all with c.724-8G>A,[43] E237K, and R206Q. Movement disorders were nearly universal. Dyskinetic crises were most frequent in individuals with E246K, T182I, and c.723+1G>A variants, and less so in others. Motor outcomes showed genotype-specific trends: head control and ambulation were preserved in all individuals with c.724-8G>A and R206Q, and in most with R209C and R209H variants, whereas those with G203R and E246K variants typically lacked these abilities. These patterns align with Axeen et al[15] findings, where R209H was associated with movement disorders without epilepsy, and E237K and E246K with prominent hyperkinetic features, whereas G203R correlated with severe epileptic encephalopathy. Overall, our findings support the presence of genotype–phenotype correlation in *GNAO1*-RD, particularly for recurrent variants with consistent clinical profiles and molecular alterations. Intra-genotypic heterogeneity was also noted, suggesting broader variability than previously recognized. Functional studies further support these correlations, indicating that variants affecting plasma membrane localization and Gβγ binding (eg, G40R, L199P, and Y231C)[12,13] are linked to severe DEE phenotypes, whereas those with near-normal localization (eg, R209C, R209H, and E237K) tend to produce milder or moderate NEDIM presentations.

The GNAO1*-RD Severity Score as a Prognostic Tool*

The *GNAO1*-RD severity score proved effective in capturing clinical burden across domains and quantifying inter-individual variability.[9] Individuals with the same variant exhibited similar scores and severity categories, with significant differences between recurrent variant groups, supporting genotype–phenotype correlations. For example, variants G203R and T182I were consistently associated with high severity scores, suggesting a strong association with more severe clinical phenotypes.[9,44] In contrast, E237K was associated with a moderate phenotype, with all carriers falling within a narrow range of moderate severity. Interestingly, variants R209C and R209H, both affecting the same amino acid, displayed greater intragenotypic variability. Although a tendency toward milder phenotypes can be observed,[22] scores among the 7 children carrying these variants ranged from 1.975 to 7.75. This lack of phenotypic consistency indicates that, for these variants, genotype alone may not be a reliable predictor of clinical severity. Altogether, these findings highlight both the value and limitations of genotype-based predictions and underscore the importance of integrating detailed phenotypic characterization with longitudinal follow-up to better capture the full clinical spectrum of *GNAO1*-RD. The ability of the *GNAO1*-RD severity score to stratify patients is increasingly relevant with emerging targeted therapies, such as zinc salts restoring GTPase activity in preclinical models,[44] or antisense oligonucleotides (ASO) reducing expression of the mutated *GNAO1* allele in patient-derived models.[45] These advances underscore the need for integrated clinical-genetic phenotyping to guide prognosis, trial design, and therapeutic responses, especially for emerging targeted therapies.

Although the positive longitudinal findings observed in this cohort provide valuable insights for disease monitoring and management, they require validation in future studies. The prospective longitudinal data for one-third of the cohort and the retrospective/cross-sectional nature of the remaining data limit long-term inference and progression in all individuals cannot be

excluded. Similarly, although the *GNAO1*-RD severity score is promising, it requires validation in larger cohorts, including inter-rater reliability and longitudinal consistency assessments. Several developmental and functional scales used in this study have floor effects, particularly in the severe group. This may limit the ability to detect further decline in individuals with profound impairment. Age was used for longitudinal alignment in this study, but it is not a perfect proxy for disease duration. To address this limitation, we additionally stratified by severity phenotype and analyzed within-individual longitudinal trajectories. Future work will incorporate disease duration and age at onset into progression models as larger, prospective multicenter datasets become available. Pharmacologic treatments were not systematically evaluated in this study, and dedicated studies will be required to assess their effectiveness, including medications and neuromodulation.

GNAO1-RD exhibit marked clinical heterogeneity, necessitating tailored monitoring and management. Our study combination of retrospective and prospective data offers the most comprehensive longitudinal analysis of this ultra-rare condition to date. Overall, most domains remained stable over the observation period, supporting a generally non-degenerative course, although individuals with severe phenotypes showed progressive worsening of movement disorders. Although individuals with *GNAO1*-RD are often described as fitting into seizure-predominant or movement-predominant phenotypes, clinical overlap and frequent evolution between phenotypes make strict categorical separation challenging. For this reason, we adopted a severity-based stratification, anchored by the *GNAO1*-RD severity score, which captures the full phenotypic spectrum while preserving power in this rare-disease cohort. The severity score proved particularly valuable in capturing disease burden, guide severity-based stratification, informing prognosis, and enabling early identification of high-risk patients for individualized care planning. Future prospective datasets with larger sample size will enable formal phenotype-based trajectory models (eg, seizure-first, movement-first, or mixed evolution patterns) and further clarify phenotype-specific natural history.

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Author Contributions

J.D.C. and J.D.O.E. contributed to the conception and design of the study; J.D.C., A.M.D.C., S.B., C.T.C., W. G.L., K.Y., K.B., M.C., A.S.V., V.D.P., N.L.C., E.G.A., B.D.L.C.F., A.O., J.R., A.A., D.M.C., C.R.O., A.L., B. T., J.P., A.A.G., A.F., F.M., J.M.B., S.N., E.J., J.L.R.G., H.V., J.J.N.B., D.Č.P., L.V.G.R., C.V.R., P.R.S., M.P. M.S., H.A.K., W.H., J.F., I.E.Q., M.T., D.G., M.P., H. K., C.M., A.D.R., L.S., M.A.K., A.S., S.S., D.E.F., K.A. M., and J.D.O.E. contributed to data acquisition and analysis; J.D.C., J.D.O.E., C.T.C., W.G.L., and K.A.M. contributed to drafting a significant portion of the manuscript and preparing figures. The GNAO1-RD Study Group of site investigators and data contributors is acknowledged; the full list of members and their affiliated institutions is provided in Supplementary Table S4.

Potential Conflicts of Interest

Nothing to report.

Data Availability

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

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