11-2024

Cohort Expansion and Genotype-Phenotype Analysis of RAB11A-Associated Neurodevelopmental Disorder.

Maria Carla Borroto

Heena Patel

Siddharth Srivastava

Lindsay C. Swanson

Boris Keren

See next page for additional authors

Recommended Citation

Borroto MC, Patel H, Srivastava S, et al. Cohort Expansion and Genotype-Phenotype Analysis of RAB11A-Associated Neurodevelopmental Disorder. Pediatr Neurol. 2024;160:45-53. doi:10.1016/j.pediatrneurol.2024.07.010

Pediatric Neurology 160 (2024) 45–53

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Research Paper

Cohort Expansion and Genotype-Phenotype Analysis of *RAB11A*-Associated Neurodevelopmental Disorder

Maria Carla Borroto [a], Heena Patel, PharmD [a], Siddharth Srivastava, MD [b], Lindsay C. Swanson, MS [c], Boris Keren, MD, PhD [d], Sandra Whalen, MD [e], Cyril Mignot, MD, PhD [f], Xiaodong Wang, PhD [g], Qian Chen, MSc, PhD [h], Jill A. Rosenfeld, MS [i], Scott McLean, MD [i, j], Rebecca O. Littlejohn, MS [i, j], Undiagnosed Diseases Network, Lisa Emrick, MD [k], Lindsay C. Burrage, MD, PhD [i], Ruben Attali, MSc, PhD [l], Gaetan Lesca, MD, PhD [m], Cecile Acquaviva-Bourdain, PharmD, PhD [n], Catherine Sarret, MD, PhD [o], Laurie H. Seaver, MD [p, q], Konrad Platzer, MD [r], Tobias Bartolomaeus, MD [r], Cornelia Wünsch, MD [s], Susann Fischer, MD, PhD [s], Ana Maria Rodriguez Barreto, MD [t], Jorge L. Granadillo, MD, MSc [u], Elisabeth Schreiner, MD [v], Theresa Brunet, MD [w, x], Ulrich A. Schatz, MD [y], Isabelle Thiffault, PhD [z, aa], Sureni V. Mullegama, PhD [ab], Jacques L. Michaud, MD [a, ac], Fadi F. Hamdan, PhD, MSc [a, ad], Elsa Rossignol, MD, MSc [a], Philippe M. Campeau, MD [a, ad, *]

[a] *Centre de recherche Azrieli du CHU Sainte-Justine, Montreal, Québec, Canada*
[b] *Department of Neurology, Translational Neuroscience Center, Boston Children's Hospital, Harvard Medical School, Boston, Massachusetts*
[c] *Department of Neurology, Boston Children's Hospital, Boston, Massachusetts*
[d] *Département de génétique, APHP-Sorbonne Université, Hôpital de la Pitié-Salpêtrière, Assistance Publique des Hôpitaux de Paris, Paris, France*
[e] *UF de Génétique Clinique et Centre de Reference Anomalies du Développement et Syndromes Malformatifs, APHP, Sorbonne Université, Hôpital Trousseau, Paris, France*
[f] *Département de Génétique, Centre de Référence Déficiences Intellectuelles de Causes Rares, Groupe Hospitalier Pitié-Salpêtrière and Hôpital Trousseau, APHP, Sorbonne Université, Paris, France*
[g] *Cipher Gene Ltd., Beijing, China*
[h] *Children's Hospital, Capital Institute of Pediatrics, Beijing, China*
[i] *Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas*
[j] *Department of Pediatrics, Baylor College of Medicine, San Antonio, Texas*
[k] *Section of Neurology and Developmental Neuroscience, Department of Pediatrics, Baylor College of Medicine, Houston, Texas*
[l] *Genomic Research Department, Emedgene, an Illumina Company, Tel Aviv, Israel*
[m] *Department of Medical Genetics, Lyon University Hospital, University Claude Bernard Lyon 1, Lyon, France*
[n] *Hospices civils de Lyon, service biochimie et biologie moléculaire, UF maladies héréditaires du métabolisme, Bron, France*
[o] *CHU Estaing, Pôle Pédiatrie, Service de Génétique, Clermont-Ferrand, France*
[p] *Corewell Health Helen DeVos Children's Hospital, Grand Rapids, Michigan*
[q] *Department of Pediatrics and Human Development, Michigan State University College of Human Medicine, Grand Rapids, Michigan*
[r] *Institute of Human Genetics, University of Leipzig Medical Center, Leipzig, Germany*
[s] *Sozialpädiatrisches Zentrum Leipzig - Frühe Hilfe Leipzig e.V., Leipzig, Germany*
[t] *Division of Clinical Genetics, Nicklaus Children's Hospital, Miami, Florida*
[u] *Division of Genetics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine in St. Louis, St. Louis, Missouri*
[v] *Diagnostic and Research Institute of Human Genetics, Medical University of Graz, Graz, Austria*
[w] *Institute of Human Genetics, Klinikum rechts der Isar, School of Medicine, Technical University of Munich, Munich, Germany*

Data availability: All relevant data for this study are presented in the main text and Supplementary Files.

Author contributions: P.M.C. and M.C.B. contributed to conceptualization, data curation, investigation, and writing-original draft; P.M.C., F.F.H, and U.D.N. contributed to resources; M.C.B. contributed to software; M.C.B., P.M.C., H.P., S.S., L. C.S., B.K., S.W., X.W., Q.C., J.A.R., S.M., R.O.L., L.E., L.C.B., R.A., G.L., C.A.-B., C.S, L.H.S., K. P., T. Bartolomaeus, C.W., S.F., J.L.G., E.S., T. Brunet, U.A.S., I.T., S.V.M., J.L.M., and E.R. performed writing-review & editing.

Ethics declaration: The clinical information of the individuals included in this study was obtained through their respective physicians. An international collaboration was facilitated by the online platform GeneMatcher, which allows clinicians, patients, and researchers interested in the same gene to find and communicate with each other. Clinical information is shared according to the rules in place by institutional review boards of each institution. A consent to publish and discuss clinical data was obtained for every patient, and so was, where applicable, the consent to publish photographs.

* Communications should be addressed to: Dr. Campeau; Sainte-Justine Hospital; Room 2.17.100, 3175, Cote-Sainte-Catherine, PO# 3931; Montreal, Québec H3T 1C5, Canada.

E-mail address: p.campeau@umontreal.ca (P.M. Campeau).

https://doi.org/10.1016/j.pediatrneurol.2024.07.010

[x] Department of Pediatric Neurology and Developmental Medicine, Dr. v. Hauner Children's Hospital, LMU - University of Munich, Munich, Germany
[y] Institute of Human Genetics, Technical University of Munich, Munich, Germany
[z] Department of Pediatrics, Children's Mercy Kansas City, Kansas City, Missouri
[aa] Department of Pathology and Laboratory Medicine, Children's Mercy Kansas City, Kansas City, Missouri
[ab] GeneDx, Gaithersburg, Maryland
[ac] Departments of Pediatrics and Neurosciences, Université de Montréal, Montreal, Québec, Canada
[ad] Department of Pediatrics, University of Montreal, Montreal, Québec, Canada

ARTICLE INFO

Article history:
Received 16 May 2023
Accepted 13 July 2024
Available online 20 July 2024

Keywords:
RAB11
GTPase
Neurodevelopmental disorder
Epileptic encephalopathy

ABSTRACT

Background: GTPases of the Rab family are important orchestrators of membrane trafficking, and their dysregulation has been linked to a variety of neuropathologies. In 2017, we established a causal link between *RAB11A* variants and developmental and epileptic encephalopathy. In this study, we expand the phenotype of *RAB11A*-associated neurodevelopmental disorder and explore genotype-phenotype correlations.
Methods: We assessed 16 patients with pathogenic or likely pathogenic *RAB11A* variants, generally *de novo*, heterozygous missense variants. One individual had a homozygous nonsense variant, although concomitant with a pathogenic *LAMA2* variant, which made their respective contributions to the phenotype difficult to discriminate.
Results: We reinforce the finding that certain *RAB11A* missense variants lead to intellectual disability and developmental delays. Other clinical features might include gait disturbances, hypotonia, magnetic resonance imaging abnormalities, visual anomalies, dysmorphisms, early adrenarche, and obesity. Epilepsy seems to be less common and linked to variants outside the binding sites. Individuals with variants in the binding sites seem to have a more multisystemic, nonepileptic phenotype.
Conclusions: Similar to other Rab-related disorders, *RAB11A*-associated neurodevelopmental disorder can also impact gait, tonus, brain anatomy and physiology, vision, adrenarche, and body weight and structure. Epilepsy seems to affect the minority of patients with variants outside the binding sites.

Introduction

Membrane trafficking is crucial for a cell's survival and adequate functioning, and the Ras superfamily of guanosine triphosphatases (GTPases) are major regulators of this system.[1] GTPases act as molecular "switches" in the cycle involving guanosine diphosphate (GDP) and guanosine triphosphate (GTP). More specifically, GDP binds to a guanine nucleotide-binding (G) protein until signal reception, when subsequent replacement by GTP results in conformational change and activation of the G protein. The GTP-bound protein is now able to influence various steps in signal transduction, eventually going back to its inactive state once a GTPase hydrolyzes the bound GTP into GDP and inorganic phosphate.[2]

The Ras GTPase superfamily comprises several branches. One of the largest and best studied among them is the main orchestrator of vesicle and organelle identification as well as cargo recognition and active transportation along the cytoskeleton: the Rab (Ras-related proteins in brain) family. Rab was first isolated from cerebral tissue, where it was noted to be abundant, diverse, and adaptable. These characteristics reflect Rab's significance, namely, within neurons, and the pathologic potential of its dysregulation.[1,3,4]

Variants affecting Rab GTPases have been associated with intellectual disability (ID). *RAB39B* variants are known to cause X-linked ID featuring autism, epilepsy, macrocephaly, and early-onset Parkinson disease (PD).[5] Biallelic *RAB18* variants are implicated in Warburg micro syndrome, associated notably with severe to profound ID, occasional epilepsy, and ocular and endocrine anomalies, as well as in Martsolf syndrome, a milder form of the disease.[6] It should be noted that biallelic *RAB3GAP1* or biallelic *RAB3GAP2* variants can also cause Warburg micro syndrome or Martsolf syndrome, and biallelic *TBC1D20* variants can cause Warburg micro syndrome. RAB3GAP1, RAB3GAP2, and TBC1D20 are GTPase-activating proteins (GAPs) that assist hydrolysis of bound GTP.[7,8] Another Rab-related syndrome that features ID, in addition to skeletal anomalies, is Carpenter syndrome, caused by biallelic *RAB23* null alleles.[9] Last, there is evidence that heterozygous variants of *RAB11B* cause neurodevelopmental disorder with ataxic gait, absent speech, and decreased cortical white matter (NDAGSCW).[10]

In 2017 we established a causal link between *de novo* variants in *RAB11A* and developmental and epileptic encephalopathy, which encompasses conditions where ID and epilepsy co-occur.[11] In this study, we sought to better characterize the clinical consequences of *RAB11A* variants and to assess whether the localization of the variants could inform us on their clinical repercussions. We thus describe 16 individuals with pathogenic or likely pathogenic *RAB11A* variants and assess their potential consequences. In a study submitted back-to-back with this one, colleagues Ahmad et al. expanded the cohort with *RAB11B*-associated neurodevelopmental disorder. We found striking parallels between their patients and ours in terms of phenotypes and genotype-phenotype correlations.

Materials and Methods

We established a cohort with 16 individuals who have been diagnosed with a pathogenic or likely pathogenic *RAB11A* variant. Their clinical information was obtained through their respective physicians. An international collaboration was facilitated by the online platform GeneMatcher,[12] which allows clinicians and researchers interested in the same gene to find and communicate with each other. Clinical information is shared according to the rules in place by institutional review boards of each institution, and a consent to publish photographs was obtained where applicable. We also reported Individuals 1 to 4 in a previous publication.[11]

Results

We identified 16 individuals aged from one to 20 years (average 6.5, median 5) with rare missense or nonsense pathogenic or likely pathogenic variants in *RAB11A* (see Table 1).

Clinical findings

Impaired neurodevelopment

Affecting all patients except for one (94% [15 of 16]), the most common clinical feature observed in our cohort was ID and/or developmental delay (DD). Note that the term DD is usually reserved for children younger than five years, but both DD and ID refer to a deficit in intelligence and/or adaptive behaviors. Only one patient in our cohort did not present with this phenotype, Individual 9 (p.Trp65Arg), who had displayed normal development until her early death after a fatal seizure at age five years. Seizures were reported in a minority of patients (25% [four of 16]) (see how seizures were medically treated in *Supplementary Information*). More frequently observed were magnetic resonance imaging (MRI) and electroencephalography (EEG) abnormalities (67% [eight of 12] and 36% [four of 11], respectively). Some MRI observations were enlargement of cerebrospinal fluid spaces and subarachnoid spaces, brain atrophy, myelination delay, and partial agenesis or dysplasia of the corpus callosum (see *Supplementary Information* for complete clinical data). A slim brainstem was noted in Individual 7 (p.Val22Asp), who also presented with a motor phenotype, cardiac anomalies, and visual problems. Some EEG observations were abnormal background activity, diffuse changes, and West syndrome features. Individuals 1 (p. Lys24Arg) and 6 (p.Gly21Arg) had EEG anomalies without clinical evidence of seizures so far, whereas Individual 7 presented with abnormal movements at age two years yet her EEG was unremarkable.

Microcephaly (see "OFC" row in *Supplementary Information*) and autistic traits were noted (25% [four of 16] and 36% [five of 14], respectively), as well as high distractibility, behavioral concerns, and gait disturbance. The latter was, in fact, the second most predominant clinical feature in our cohort (62% [eight of 13]), often accompanied by hypotonia and/or hypertonia (57% [eight of 14] and 21% [three of 14], respectively) and other motor phenotypes (57% [eight of 14]), such as spastic diplegic cerebral palsy, muscular dystrophy (although mainly attributed to another pathogenic variant in the patient), hyperreflexia, ataxia, and coordination problems. In some cases, improvement was observed throughout the years, whereas in others regression was observed, namely, in Individuals 10 (p.Asp66Tyr) and 14 (p.Ser154Leu). The former presented with ID and autistic features since childhood, but developed progressive gait disturbance, ataxia, and hypertonia in his teenage years. He is now mostly reliant on a wheelchair and presents with an apparently degenerative phenotype. In the case of Individual 13, he initially had spastic diplegic cerebral palsy but over time developed worsening spasticity in lower extremities. Individual 5 (p.Tyr10Cys), who did not have DD, started regressing in terms of autistic traits at age 18 months.

Dysmorphisms

Dysmorphisms were also present (47% [seven of 15]), and included flat occiput, frontal upsweep of hair, coarse facial features, hypertelorism, prominent cheeks, bulbous nose, broad nasal bridge, abnormal earlobes, high and narrow palate, downturned corners of mouth, long and flat philtrum, micrognathia, abnormal skin folds, and inverted nipples. These were all seen once in only one individual. Epicanthus was seen in four patients, abnormal palmar creases and a thin upper lip in three, and deep-set eyes in two patients (see *Supplementary Information* for all clinical data).

We also obtained two facial photographs of Individual 15 (p.Ser154Leu), who presented with deep-set eyes, broad nasal bridge, downturned corners of mouth, and thick, round, and uplifted ear lobes (see Fig 1). As discussed above, this morphology is not necessarily representative of the rest of the cohort.

Perinatal complications

Perinatal complications were a relatively common occurrence (56% [nine of 16]), including oligohydramnios, neonatal hypotonia, jaundice and anemia, and gestational diabetes in two mothers. In addition, Individual 11 (p.Thr67Ile) had history of *in utero* alcohol exposure.

Involvement of other systems

Half of the assessed patients featured some visual problems (50% [seven of 14]) including hypermetropia, myopia, astigmatism, strabismus, and visual inattention. Other noted clinical features were early adrenarche (29% [two of seven]), obesity (13% [two of 16]), one skeletal condition (6% [one of 16]) of advanced bone age noncomorbid with early adrenarche, and three gastrointestinal conditions (19% [three of 16]): gastroesophageal reflux disease, constipation, and aversion to meat. There was one patient with a genitourinary malformation (6% [one of 16]), a micropenis, and two patients with cardiac malformations (13% [two of 16]). These cardiac malformations were arterial septal defect and acleistocardia in the case of Individual 7 (p.Val22Asp), whereas Individual 8 (p.Thr43Ala) had several anomalies: ventricular septal defect, atrial septal defect, patent ductus arteriosus, coarctation of aorta, aortic stenosis, and heart failure at six weeks of life secondary to these congenital heart defects. The phenotype of Individual 8 might suggest the possibility of *RAB11A* pathogenic variants being associated with severe heart malformations.

Variant description

A total of 12 different *RAB11A* variants were identified among the 16 patients (see Fig 2A). Inheritance was determined for 10 patients: nine were *de novo* variants and one was a homozygous variant inherited from heterozygous parents. The CADD scores varied from 25.5 to 32 (average of 29.5, median of 28.5). All residues involved are highly conserved across vertebrates (see Fig 2B).

Heterozygous missense variants

All patients but Individual 16 had heterozygous missense variants (see Fig 1A). The same p.Ser154Leu variant was seen in four patients (Individuals 3, 4, 14, and 15), three of whom had corpus callosum anomalies.

Neighboring variants do not necessarily lead to similar phenotypes. As seen in 3D models (see Fig 1D and E), most of the altered residues are inside the active, nucleotide-binding site or near it, thus suggesting that virtually all variants in this cohort—and not only those directly in the binding sites—could lead to conformational changes in the RAB11A GTPase, which would affect the main molecular function of this GTPase: binding GTP or GDP.

Homozygous nonsense variant

Individual 16 had the only nonsense and homozygous variant, p.Arg33*. His phenotype was not as multisystemic as others, featuring ID, seizures, and motor disorders. Besides hypotonia and gait disturbance, frequently seen in the rest of the cohort, this individual also had autosomal recessive congenital muscular dystrophy, attributed to a pathogenic, homozygous variant (NM_000426.3:c.8244+1G>A) in *LAMA2*, which encodes for the laminin-211 α2 subunit of the muscular extracellular matrix.[13] Regarding the ID and seizures, it is unsure if or how the *LAMA2*

TABLE 1.
Clinical Summary

Individual	Previously Reported				Newly Reported													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16		
Gender	M	F	M	F	M	F	M	F	F	M	M	NA	F	M	M	F		
Age (years)	5.5	9.5	NA	NA	3.5	6	1.6	4.4	NA	20	13	NA	1	3	7	2		
Variant	p.K24R	p.R82C	p.S154L	p.S154L	p.Y10C	p.G21R	p.V22D	p.T43A	p.W65R	p.D66Y	p.T67I	p.H112R	p.N124D	p.S154L	p.S154L	p.R33*		
Coding impact	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Missense	Nonsense		
CADD score	26.9	32	32	32	25.6	27.9	28.0	25.8	28.5	29.2	27.7	25.5	27.5	32	32	36		
Inheritance	Dn	dn	Dn	dn	dn	dn	dn	NA	dn	NA	NA	dn	dn	NA	NA	hmz		
Identification method	TES	ES	TES	TES	TES	TES	TES	DES	TES	ES	ES	ES	TES	DES	DES	TES		
Clinical Information																	Affected/ Assessed	%
Microcephaly	+	+	–	–	–	–	–	–	–	–	+	+	–	–	–	–	4/16	25
ID/DD	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	15/16	94
Autistic features	–	+	–	–	+	NA	–	+	–	–	+	–	NA	–	+	–	5/14	36
Seizures	–	+	–	–	–	–	–	–	+	–	–	+	–	–	–	+	4/16	25
Hypotonia	+	+	–	+	–	+	+	–	NA	–	–	+	NA	–	+	+	8/14	57
Hypertonia	–	–	–	–	–	–	+	–	NA	+	–	–	NA	+	–	–	3/14	21
Gait disturbance	+	NA	–	–	–	+	+	–	NA	+	+	–	NA	+	+	+	8/13	62
Other motor phenotype	+	–	–	–	–	+	+	+	NA	+	–	+	NA	+	–	+	8/14	57
MRI abnormalities	+	+	+	NA	–	NA	+	NA	NA	+	–	–	+	+	+	–	8/12	67
EEG abnormalities	+	+	–	–	–	+	–	–	NA	–	NA	+	NA	NA	NA	–	4/11	36
Dysmorphisms	+	+	–	–	–	–	+	+	–	–	+	+	+	–	+	–	7/15	47
Cardiac anomalies	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–	–	2/16	13
Genitourinary malformation	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	1/16	6
Early adrenarche	NA	–	NA	NA	–	–	NA	NA	–	+	NA	+	NA	NA	–	NA	2/7	29
Vision problem	+	+	–	–	–	+	+	–	NA	+	+	–	NA	–	+	–	7/14	50
Obesity	–	–	–	+	–	–	–	–	–	–	–	–	–	–	+	–	2/16	13
Gastrointestinal conditions	–	–	–	–	–	–	–	–	–	+	–	–	+	–	+	–	3/16	19
Skeletal conditions	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	1/16	6
Perinatal complications	–	+	–	+	+	–	+	+	–	+	–	–	+	+	+	–	9/16	56
Death in childhood	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	1/16	6
Other pathogenic variants	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	1/16	6

Abbreviations:
+ = Yes
– = No
CADD = Combined Annotation Dependent Depletion
DD = Developmental delay
DES = Duo ES
dn = *De novo*
EEG = Electroencephalography
ES = Exome sequencing
F = Female
hmz = Homozygous
ID = Intellectual deficiency
M = Male
MRI = Magnetic resonance inmging
NA = Not available
OFC = Occipitofrontal circumference
TES = Trio ES

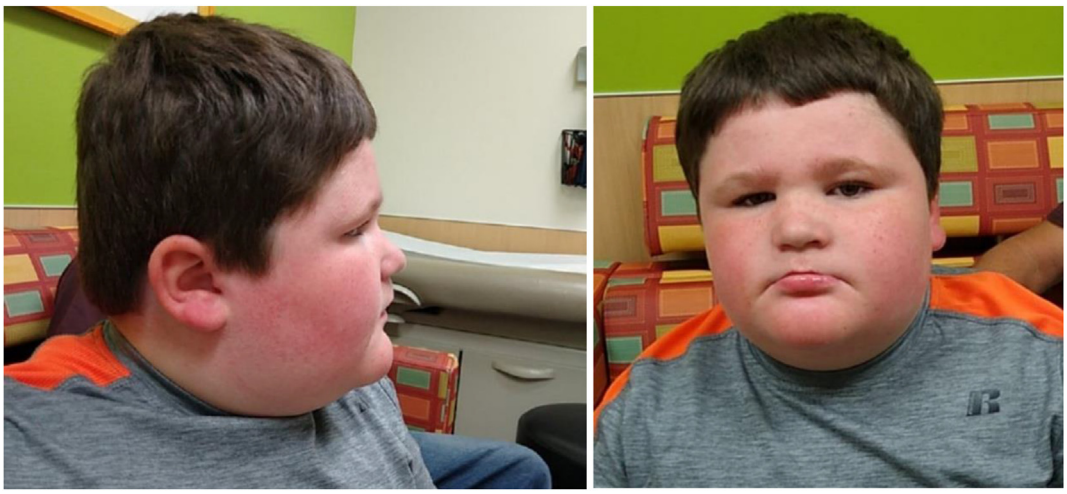

FIGURE 1. Facial photographs of Individual 15 (p.Ser154Leu). The color version of this figure is available in the online edition.

variant could have contributed to this phenotype, as these are sometimes observed with biallelic pathogenic *LAMA2* variants.[14]

Discussion

Genotype-phenotype analysis of our cohort

Rab proteins are highly conserved across species (see Fig 1B). Even the most rudimentary eukaryotes bear no less than 10, whereas 66 have been identified in humans,[15] among which 24 are enriched or specifically found in the central nervous system.[3] In their active form, Rabs are found on membranes, bound to both GTP and effector proteins associated with the different steps of vesicular trafficking. Meanwhile, in their inactive form, Rabs distribute across the cytosol, bound to GDP and GDP dissociation inhibitors (GDIs). The former also act as chaperones in Rabs' membrane-cytosol bidirectional displacement as well as membrane extrication after their inactivation.[16] Indeed, most of these GTPases have significantly low intrinsic rates of nucleotide exchange and, thus, need multiple mediators that insure a painstaking spatiotemporal regulation, including guanine nucleotide exchange factors (GEFs), which assist activation at the membrane, and the previously discussed GAPs and GDIs.[4,15,16] Prenylation is another crucial mechanism in Rab's regulation, especially membrane anchoring. A Rab escort protein (REP) first presents newly synthesized Rab GTPases to a Rab-specific protein prenyl transferase, leading to the transfer of lipid prenyl groups onto two cysteines in Rab's C terminus. The REP then escorts prenylated Rab to the target donor compartment, where it can insert its hydrophobic, lipidized amino acids into the membrane.[16]

Consequently, any disruption in the multilayered cycle of Rab proteins can easily affect cell regulation and translate into systemic dysfunction.[17] For instance, an X-linked form of hereditary retinal degeneration is caused by variants in the gene encoding REP-1, which result in underprenylation of RAB27A.[18] Additionally, GDI1 loss of function underlies an X-linked cognitive impairment.[19] Altered membrane trafficking has also emerged as a major pathway in various neurodegenerative conditions, especially in PD. *RAB29* variants have been identified as significant contributors to PD risk.[20] Correlations have also been found between modified expression levels of different Rab GTPases and cancer metabolism, migration, and drug resistance.[21]

The RAB11 subfamily consists of the isoforms RAB11A and RAB11B, both ubiquitously expressed, as well as RAB11C, also known as RAB25, which is expressed in epithelial tissues of the gastrointestinal mucosa, kidney, and lung.[22] As GTPases, their activation and inactivation depend on the binding of either GTP or GDP. In the case of RAB11A, the binding sites include two guanine base-binding motifs (SAK and NKxD), a phosphate-binding loop (P-loop), and switches I and II, which interact with the phosphates of GTPs.[23] Switch I activates the Rab GTPase by binding to a GTP that will later be hydrolyzed by switch II, thus inactivating the protein. A Mg^{2+} ion acts as a catalytic cofactor, also interacting with phosphates of the nucleotide and residues of the two switch regions.[23,24] Overall, the catalytic machinery constituted by the binding sites, especially the switch I and switch II regions, are key elements in the pathogenesis of Rab variants. For instance, oncogenic variants decrease the rate at which switch II hydrolyzes GTP, thus hindering the downstream transmission of cellular signal.[23] *In vitro* studies have confirmed that variants in the active site sterically disrupt nucleotide binding. The degree varied depending on the residue, but there was, at minimum, decreased nucleotide affinity. Mutations outside the binding sites had similar or slighter impacts.[4]

Phenotypical comparisons between subgroups can be drawn (see Table 2), but without disregarding the limited size of our cohort and the disproportional subgroup distribution, which could potentially diminish the statistical accuracy of these comparisons.

In our cohort, 11 individuals (Individuals 1, 3 to 7, 10, 11, 13 to 15) had a variant in one of the binding sites, whereas only five (Individuals 2, 8, 9, 12, and 16) had variants elsewhere along the protein. We noticed that the subgroup of patients with variants in the binding sites had a more diverse phenotype. Unlike the other subgroup, the former featured cardiac anomalies (18%), genitourinary anomalies (9%), vision problems (60%), hypertonia (30%), obesity (18%), and gastrointestinal and skeletal conditions (27% and 9%, respectively). This finding aligns with the fact that *RAB11A* is ubiquitously expressed.[22] Interestingly, the subgroup of patients with variants outside binding sites was the only one to feature epilepsy (four of five), including Individual 9 (p.Trp65Arg) who passed away in early childhood due to a status epilepticus. This finding suggests perhaps a correlation between epilepsy and variants outside RAB11A's binding sites. However, it should be noted that the subgroup of patients with variants in the binding sites included Individuals 1 and 7, who featured EEG anomalies, and that

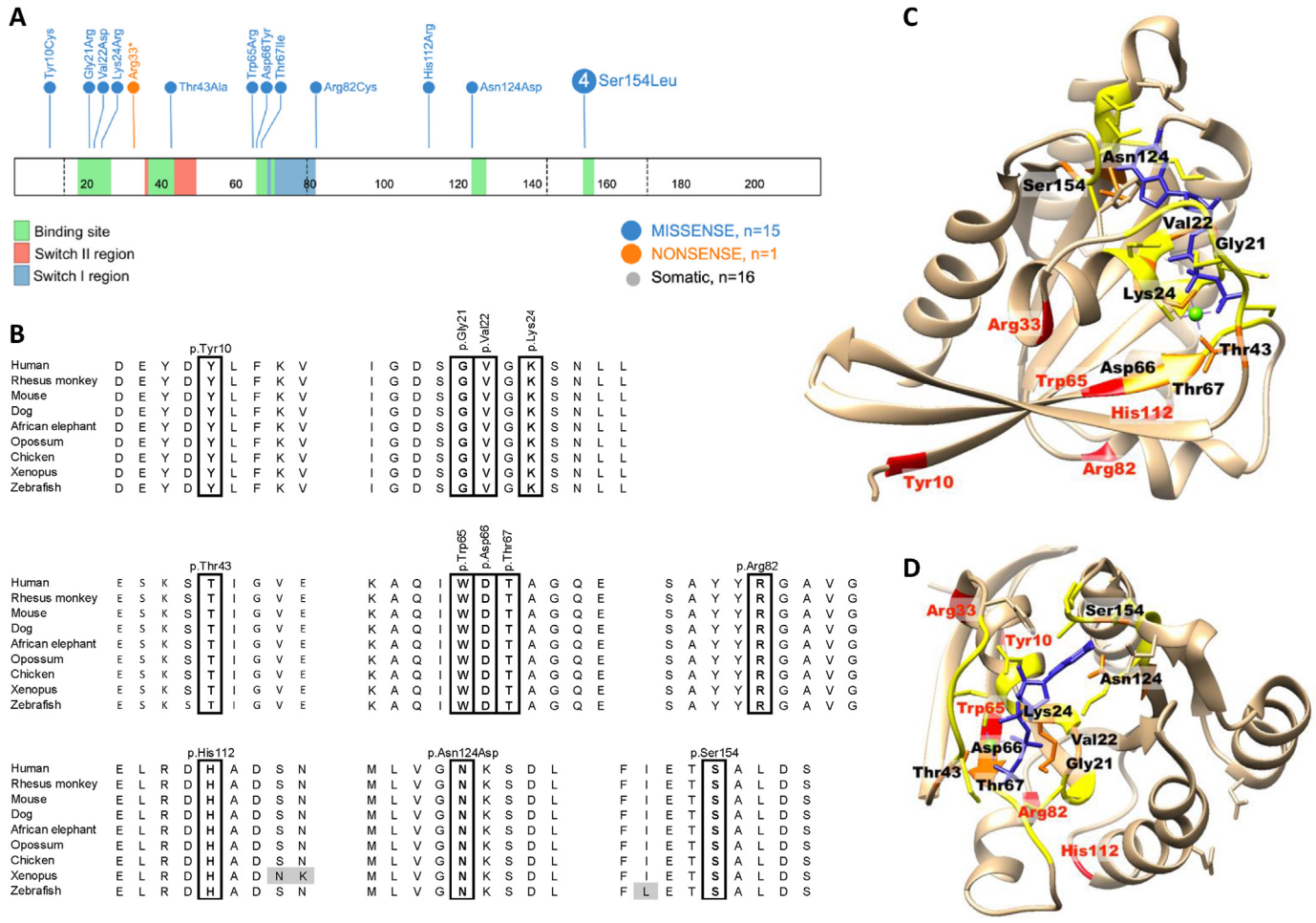

FIGURE 2. Variant location along RAB11A. (A) The variants studied in our cohort are represented on the RAB11A protein. If the same variant was shared by multiple patients, their total number is in a circle. Adapted from ProteinPaint. (B) Amino acid conservation of each missense variant. (C) Homology model of human RAB11A (GenBank: NM_004663.5) predicted by PHYRE2 Protein Fold Recognition Server and represented by UCSF Chimera. The binding sites are in yellow, whereas variants on the binding sites are in orange, although labeled in black. Variants outside the binding sites are in red and labeled in red. The GTP is in violet-blue, interacting with the yellow or orange structures as well as with the green Mg^{2+} ion. (D) A different view of the same 3D homology model of human RAB11A. The color version of this figure is available in the online edition.

brain abnormalities were confirmed through MRI in 78% of patients in this subgroup, although there was no clinical evidence of seizures. Individual 8 also had abnormal movements at age two years, yet an unremarkable EEG. It should be noted that this patient also had severe heart malformations and that her variant (p.Thr43Ala) is the only one in our cohort that coincides with the switch II domain of RAB11A, which might suggest a correlation between the two.

As discussed in our previous study reporting Individuals 1 to 4, *RAB11A* variants can cause developmental and epileptic encephalopathy or ID phenotype, notably because of its role in the regulation of synaptic plasticity through the endocytic recycling of postsynaptic receptors.[11] The larger cohort of this current study might suggest that although ID/DD is predominant in all *RAB11A* variants, epilepsy is mainly attributed to alterations of residues outside the binding domain, whereas more multisystemic phenotypes are displayed by patients with variants in the active sites. Larger cohorts might show whether this observation is statistically supported or not, and further *in vitro* studies might help shed light on the physiological mechanisms underlying it.

Motor phenotypes were abundant in our cohort, even more so than other neurological features such as seizures, microcephaly, and autistic traits. Hypotonia and gait disturbance were the most predominant features in the entire cohort, after ID/DD, followed by MRI abnormalities. This observation suggests that, overall, *RAB11A* variants are generally associated with ID/DD and motor impairments more than they are with ID/DD and epilepsy. The motor phenotypes seen in our cohort (ataxia, spasticity, hypertonia, etc.) suggest a spinal and/or cerebellar involvement despite normal brain imaging of the cerebellum and spine, although Individual 7 did feature a slim brainstem. It is important to note that spasticity, unlike ataxia, is also seen in patients with Warburg micro and Martsolf syndromes (see section *Comparison with other Rab-related disorders*).

Last, concerning the one nonsense variant in our cohort (p.Arg33*, Individual 16), it should be noted that, due to the occurrence of this variant in the second of five exons, nonsense-mediated RNA decay is more probable than the production of a truncated protein; this would mean that this variant results in loss of protein expression. If ever some truncated protein is expressed, it would be small and nonfunctional. Besides ID/DD, seizures, and motor impairment, also seen elsewhere in our cohort, this patient did not present any additional clinical feature. Considering that the concomitant *LAMA2* variant contributes to the motor impairment and that MRI anomalies, visual problems, early adrenarche, perinatal complications, and neuromotor impairment were seen

TABLE 2.
Variant Comparison According to Position Along RAB11A and Coding Impact

Clinical Information	On Binding Site (%) n = 11	Not on Binding Site (%) n = 5	Heterozygous Missense (%) n = 15	Homozygous Nonsense (%) n = 1
Microcephaly	18	25	27	–
ID or DD	100	75	93	+
Autistic features	33	50	38	–
Seizures	0	75	20	+
Hypotonia	50	67	54	+
Hypertonia	30	0	23	–
Gait disturbance	70	50	58	+
Other motor phenotype	60	33	54	+
MRI abnormalities	88	33	73	–
EEG abnormalities	29	33	40	–
Dysmorphisms	50	25	50	–
Cardiac anomalies	18	0	13	–
Genitourinary malformation	9	0	7	–
Early adrenarche	33	0	29	NA
Vision problem	60	33	54	–
Obesity	18	0	13	–
Gastrointestinal conditions	27	0	20	–
Skeletal conditions	9	0	7	–
Perinatal complications	64	50	60	–
Death in infancy	0	25	7	–
Other pathogenic variants	0	25	0	+

Abbreviations:
+ = Yes
– = No
DD = Developmental delay
EEG = Electroencephalography
ID = Intellectual disability
MRI = Magnetic resonance imaging
NA = Not available

among the other patients with seizures, we cannot conclude that this homozygous, nonsense *RAB11A* variant correlates with a more severe phenotype than do heterozygous, missense *RAB11A* variants.

In a study from 2014, *Rab11A* global knockout resulted in murine embryonic lethality. In both mouse and *Drosophila* midgut, the *Rab11A* inactivation caused epithelial cell-intrinsic cytokine production, inflammatory bowel phenotype, and early mortality. RAB11A depletion caused abnormal lumen formation in cultured human colonic epithelial cells, which is interesting considering that *RAB11A* is adjacent to a Crohn disease risk locus.[25] Other studies have shown that in mouse, fly, and human gut epithelium, loss of this GTPase has led to hyperproliferation, increased tumorigenic activity, and progression of colon cancer, thus highlighting the importance of *RAB11A* in epithelial homeostasis.[26,27] However, it has also been demonstrated that Rab11A and Rab11B can function redundantly, for instance, in the case of cyst formation during epithelial development of canine kidney tissue,[28] although their respective functions are not completely redundant.[22]

On the grounds of this literature, we would expect Individual 16 to present perhaps with a multisystemic or, at least, gastrointestinal phenotype, but this was not the case. It is possible that the loss of RAB11A expression in humans is compensated by some unelucidated mechanism, likely involving other Rab GTPases; this could either lead to a milder phenotype or no phenotype at all. Based on this single individual and the fact that there is a concurrent *LAMA2* pathogenic variant, it is not possible to distinguish the two possibilities from a medical genetics' perspective. This family's clinical data support, however, the hypothesis that missense variants cause dominant-negative (DN) or dominant gain-of-function effects, rather than simply a loss of function. Indeed, the fact that the parents of this child are clinically unaffected tells us that haploinsufficiency for *RAB11A* is benign, which is also supported by the fact that there are three individuals with loss-of-function variants in the gnomAD non-neuro cohort.

For some of the specific *RAB11A* variants discussed here and initially described in previous studies, Jenkins et al. performed *in vitro* studies for the specific variants p.Lys24Arg, p.Arg82Cys, and p.Ser154Leu, notably their effects on RAB11A nucleotide binding and activation by SH3BP5.[4] SH3BP5 is a Rab11-specific GEF, thus activating it. In their experiments, p.Arg82Cys partially decreased SH3BP5 GEF activity. p.Lys24Arg and p.Ser154Leu showed abnormal deuterium exchange in hydrogen deuterium exchange mass spectrometry experiments throughout the majority of Rab11, demonstrating that the mutations destabilize RAB11A and abrogate (p.Ser154Leu) or decrease (p.Lys24Arg) nucleotide binding. When performing SH3BP5 GEF assays, p.Lys24Arg caused rapid nucleotide exchanges indicating decreased affinity for GDP even in the absence of SH3BP5. It has not yet been determined if RAB11A variants cause dominant gain-of-function or DN effects. DN variants of Rab GTPases have been extensively studied, and their impact varies depending on the protein. The DN effect can be secondary to sequestration of endogenous GEFs due to low GTP affinity, thus the aforementioned experiments could support the idea that they may act in a DN fashion. DN variants of *ubiquitous* Rab GTPases, including Rab11, cause lethality in fruit fly, unlike DN variants of endogenous *neuronal* Rab GTPases.[29] A DN Rab11 variant (p.Ser25Asn) has been specifically shown to attenuate mast cell exocytic response,[30] inhibit certain mechanisms of transferrin recycling,[31] and decrease foot-and-mouth disease virus infection by 35%, likely reducing integrin recycling and, thus, receptor availability at the cell surfaces for virus binding.[32]

Comparison with other Rab-related disorders

Rab dysregulation has been associated with relatively similar phenotypes to those observed in our cohort. Variants in these proteins are a primary cause of neurodegenerative diseases, such as PD.[20] This fact is particularly interesting because Individual 10 saw

a degeneration of his condition, which eventually led him to be wheelchair bound, whereas Individual 14's initial spastic diplegic cerebral palsy led to worsening spasticity in lower extremities (see *Supplementary Information*). In addition, variants of *RAB39B* are known to cause a rare early-onset PD (X-linked Waisman syndrome) associated with autism and epilepsy, which were seen among our patients, and also with macrocephaly, which was not, although microcephaly was. These *RAB39B* variants usually result in the loss of protein expression,[5] similar to how the p.Arg33* variant of Individual 16 would lead to loss of RAB11A expression. Similar to patients with loss of RAB39B expression, Individual 16 also suffered from seizures.

Variants in *RAB18* and other Rab-regulating proteins can cause Warburg micro syndrome or the milder Martsolf syndrome. Warburg micro syndrome is associated with ID, occasional epilepsy, and ophthalmologic and endocrine anomalies, more precisely, hypogonadism that could lead, for example, to a micropenis. Short stature has also been reported.[6,7] These clinical features were all seen in our cohort, but, in the case of endocrine anomalies, it was early adrenarche that was reported for two individuals. One individual had a micropenis. It should also be noted that well over half of the patients we assessed had some ophthalmologic problem. *RAB23* variants cause Carpenter syndrome, associated with ID and various skeletal anomalies,[9] whereas the only skeletal anomaly seen in our cohort was advanced bone age in one patient.

Variants of *RAB11B*, which encodes another isoform of the Rab11 subfamily, have been associated with NDAGSCW. Ataxic gait, absent speech, and decreased cortical white matter were interestingly all observed in our cohort. Seizures and thin corpus callosum, observed in our cohort, are also two possible features of *RAB11B*-associated NDAGSCW.[10]

In a 2023 study, submitted in parallel to this one, colleagues Ahmad et al. analyzed an expanded cohort of patients with *RAB11B*-associated neurodevelopmental disorder. The authors concluded that variants outside the nucleotide-binding sites result in milder phenotypes of speech impairment and epilepsy, which is consistent with our findings of seizure occurrence being restricted to *RAB11A* variants outside the nucleotide-binding sites, variants also associated with less multisystemic, relatively more limited phenotypes. The new study also linked *RAB11B* variants in the nucleotide-binding sites to microcephaly, ophthalmologic anomalies, and brain anomalies, all seen in our cohort. The last of these concerned mainly the corpus callosum, as was the case in our cohort; the brainstem, which we saw in only one patient (Individual 7); and the cerebellar vermis, not seen in our patients despite the predominance of motor impairments including ataxia. Ahmad et al. also report frequent muscular hypotonia and/or hypertonia. Interestingly, one of their patients had displayed normal development, besides possibly mild speech delay, until age five years, when he started having epilepsy and several episodes of status epilepticus, and his parents observed a regression. We saw not only cases of regression past a certain age in our cohort (mainly Individuals 10 and 14) but also a patient who had been developing normally until her sudden death after a status epilepticus at age five years (Individual 9).

In conclusion, the *RAB11A*-related neurodevelopmental disorder shares some clinical features with other Rab-related disorders, namely, potential neurodegenerative phenotypes, autism, epilepsy, occipitofrontal circumference anomalies, endocrine anomalies, genitourinary anomalies, and ophthalmologic problems. This disorder shows a striking overlap with the *RAB11B*-related neurodevelopmental disorder. For instance, epilepsy is seen only with variants outside the nucleotide-binding sites, also linked to less multisystemic, relatively more limited phenotypes. In both cohorts, brain abnormalities concerned mainly the corpus callosum; hypotonia and/or hypertonia were omnipresent, and there were cases of patients who displayed normal development until age five years, when they suddenly started suffering from status epilepticus.

Overall, we reinforce the existing data suggesting that pathogenic *RAB11A* variants cause ID and/or DD, while other predominant clinical features might include gait disturbances, hypotonia, MRI abnormalities, epilepsy, visual anomalies, dysmorphisms, early adrenarche, and obesity.

Declaration of competing interest

The Department of Molecular & Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing completed at Baylor Genetics Laboratories. Sureni V. Mullegama is an employee of GeneDx, LLC. Otherwise, we have no conflict of interest to disclose.

Acknowledgments

Research reported in this manuscript was supported by the NIH Common Fund, through the Office of Strategic Coordination/Office of the NIH Director under Award Number U01HG007709, as well as the National Institute of Health/National Institute of Neurological Disorders and Stroke (K23NS119666 to S.S). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.pediatrneurol.2024.07.010.

References

1. Mizuno-Yamasaki E, Rivera-Molina F, Novick P. GTPase networks in membrane traffic. Annu Rev Biochem. 2012;81:637–659.
2. Chakraborty A, Halder S, Kishore P, et al. The structure-function analysis of Obg-like GTPase proteins along the evolutionary tree from bacteria to humans. Genes Cells. 2022;27:469–481.
3. Kiral FR, Kohrs FE, Jin EJ, Hiesinger PR. Rab GTPases and membrane trafficking in neurodegeneration. Curr Biol. 2018;28:R471–R486.
4. Jenkins ML, Margaria JP, Stariha JTB, et al. Structural determinants of Rab11 activation by the guanine nucleotide exchange factor SH3BP5. Nat Commun. 2018;9:3772.
5. Lara Ordonez AJ, Fasiczka R, Naaldijk Y, Hilfiker S. Rab GTPases in Parkinson's disease: a primer. Essays Biochem. 2021;65:961–974.
6. Mirzaa G, Graham Jr JM, Keppler-Noreuil K. PIK3CA-related overgrowth spectrum. 2013 Aug 15 [Updated 2023 Apr 6]. In: Adam MP, Mirzaa GM, Pagon RA, eds. GeneReviews®[Internet]. 1993:2023.
7. Handley MT, Carpanini SM, Mali GR, et al. Warburg Micro syndrome is caused by RAB18 deficiency or dysregulation. Open Biol. 2015;5:150047.
8. Sidjanin DJ, Park AK, Ronchetti A, Martins J, Jackson WT. TBC1D20 mediates autophagy as a key regulator of autophagosome maturation. Autophagy. 2016;12:1759–1775.
9. Hor CHH, Tang BL, Goh ELK. Rab23 and developmental disorders. Rev Neurosci. 2018;29:849–860.
10. Lamers IJC, Reijnders MRF, Venselaar H, et al. Recurrent de novo mutations disturbing the GTP/GDP binding pocket of RAB11B cause intellectual disability and a distinctive brain phenotype. Am J Hum Genet. 2017;101:824–832.
11. Hamdan FF, Myers CT, Cossette P, et al. High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. Am J Hum Genet. 2017;101:664–685.
12. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015;36:928–930.
13. Gavassini BF, Carboni N, Nielsen JE, et al. Clinical and molecular characterization of limb-girdle muscular dystrophy due to LAMA2 mutations. Muscle Nerve. 2011;44:703–709.
14. Salvati A, Bonaventura E, Sesso G, Pasquariello R, Sicca F. Epilepsy in LAMA2-related muscular dystrophy: a systematic review of the literature. Seizure. 2021;91:425–436.
15. Klopper TH, Kienle N, Fasshauer D, Munro S. Untangling the evolution of Rab G proteins: implications of a comprehensive genomic analysis. BMC Biol. 2012;10:71.
16. Shinde SR, Maddika S. Post translational modifications of Rab GTPases. Small GTPases. 2018;9:49–56.

17. Le NQK, Ho QT, Ou YY. Using two-dimensional convolutional neural networks for identifying GTP binding sites in Rab proteins. J Bioinform Comput Biol. 2019;17:1950005.
18. Fry LE, Patricio MI, Jolly JK, Xue K, MacLaren RE. Expression of rab prenylation pathway genes and relation to disease progression in choroideremia. Transl Vis Sci Technol. 2021;10:12.
19. More L, Kunnecke B, Yekhlef L, et al. Altered fronto-striatal functions in the Gdi1-null mouse model of X-linked intellectual disability. Neuroscience. 2017;344:346–359.
20. Bonet-Ponce L, Cookson MR. The role of Rab GTPases in the pathobiology of Parkinson' disease. Curr Opin Cell Biol. 2019;59:73–80.
21. Jin H, Tang Y, Yang L, et al. Rab GTPases: central coordinators of membrane trafficking in cancer. Front Cell Dev Biol. 2021;9:648384.
22. Escrevente C, Bento-Lopes L, Ramalho JS, Barral DC. Rab11 is required for lysosome exocytosis through the interaction with Rab3a, Sec15 and GRAB. J Cell Sci. 2021;134:jcs246694.
23. Ligeti E, Welti S, Scheffzek K. Inhibition and termination of physiological responses by GTPase activating proteins. Physiol Rev. 2012;92:237–272.
24. Shin YC, Kim CM, Choi JY, Jeon JH, Park HH. Occupation of nucleotide in the binding pocket is critical to the stability of Rab11A. Protein Expr Purif. 2016;120:153–159.
25. Yu S, Nie Y, Knowles B, et al. TLR sorting by Rab11 endosomes maintains intestinal epithelial-microbial homeostasis. EMBO J. 2014;33:1882–1895.
26. Goswami S, Balasubramanian I, D'Agostino L, et al. RAB11A-mediated YAP localization to adherens and tight junctions is essential for colonic epithelial integrity. J Biol Chem. 2021;297:100848.
27. Rathan-Kumar S, Roland JT, Momoh M, et al. Rab11FIP1-deficient mice develop spontaneous inflammation and show increased susceptibility to colon damage. Am J Physiol Gastrointest Liver Physiol. 2022;323:G239–G254.
28. Homma Y, Kinoshita R, Kuchitsu Y, et al. Comprehensive knockout analysis of the Rab family GTPases in epithelial cells. J Cell Biol. 2019;218:2035–2050.
29. Chan CC, Scoggin S, Wang D, et al. Systematic discovery of Rab GTPases with synaptic functions in Drosophila. Curr Biol. 2011;21:1704–1715.
30. Wilson JD, Shelby SA, Holowka D, Baird B. Rab11 regulates the mast cell exocytic response. Traffic. 2016;17:1027–1041.
31. Ren M, Xu G, Zeng J, De Lemos-Chiarandini C, Adesnik M, Sabatini DD. Hydrolysis of GTP on rab11 is required for the direct delivery of transferrin from the pericentriolar recycling compartment to the cell surface but not from sorting endosomes. Proc Natl Acad Sci U S A. 1998;95:6187–6192.
32. Johns HL, Berryman S, Monaghan P, Belsham GJ, Jackson T. A dominant-negative mutant of rab5 inhibits infection of cells by foot-and-mouth disease virus: implications for virus entry. J Virol. 2009;83:6247–6256.