ERN ITHACA

Webinar 2025

Rho-GTPase in intellectual disability and neurodevelopmental disorders

In-Depth Webinar - January 21, 2025

Chaired by Valeria Capra,

MD - Genomic and Clinical Genetics, IRCCS G.Gaslini, Genoa, Italy.

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

Contact PM: anne.hugon@aphp.fr

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Welcome – Technical points

- Thank you for joining us today
- We are please to be numerous + 103 registration / 75 participants
- Please follow these guidelines during the session:
  - Please mute your microphone and disable your camera.
  - Raise your hand or use the Chat during Q&A sessions
  - A satisfaction survey will be provided afterward
- Webinar is being recorded and available on ITHACA's Website
  - https://ern-ithaca.eu/documentation/educational-resources/
- Contact
  - Anne Hugon Project Manager - anne.hugon@aphp.fr

Agenda

- Welcome and Introduction
  - Speaker : Valeria Capra, MD - Genomic and Clinical Genetics, IRCCS G.Gaslini, Genoa, Italy.

1. Rho family GTPases: key players in neuronal development and synaptic function
   - Speaker : Antonio Falace, PhD Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini", Genoa, Italy.
2. RAC3-related disorders of cortical development in human neurodevelopmental phenotypes
   - Speaker : Marcello Scala, MD PhD “NeuroRacopathies – Department of Neurosciences (DINOGMI), University of Genoa, Genoa, Italy; Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy.
3. Mechanistic analysis of RAC1-related neurodevelopmental disorders
   - Speaker : Tom H. Millard, PhD - Division of Developmental Biology and Medicine, Faculty of Biology, Medicine and Health, University of Manchester M13 9PL, UK.

- Discussion & Conclusion with speakers and moderator

Welcome and Introduction

Valeria Capra, MD - Genomic and Clinical Genetics, IRCCS G.Gaslini, Genoa, Italy

Rho GTPases

V.Capra

- Rho GTPases belong to the Ras small GTPase superfamily of GTP-binding proteins (that also include Ras, Ran, Rab and Arf GTPases)
- The Rho GTPase family encompasses eight subfamilies, amongst them the RhoA, Rac1, and Cdc42 ones are the best known and more recently Rac3.
- Together with their upstream and downstream regulators, they act as efficient molecular relays to transduce extracellular signals to downstream effectors.
- Like other small classical GTPases, they can be switched between active and inactive conformational states by specific guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs), respectively.

Rho GTPases

V.Capra

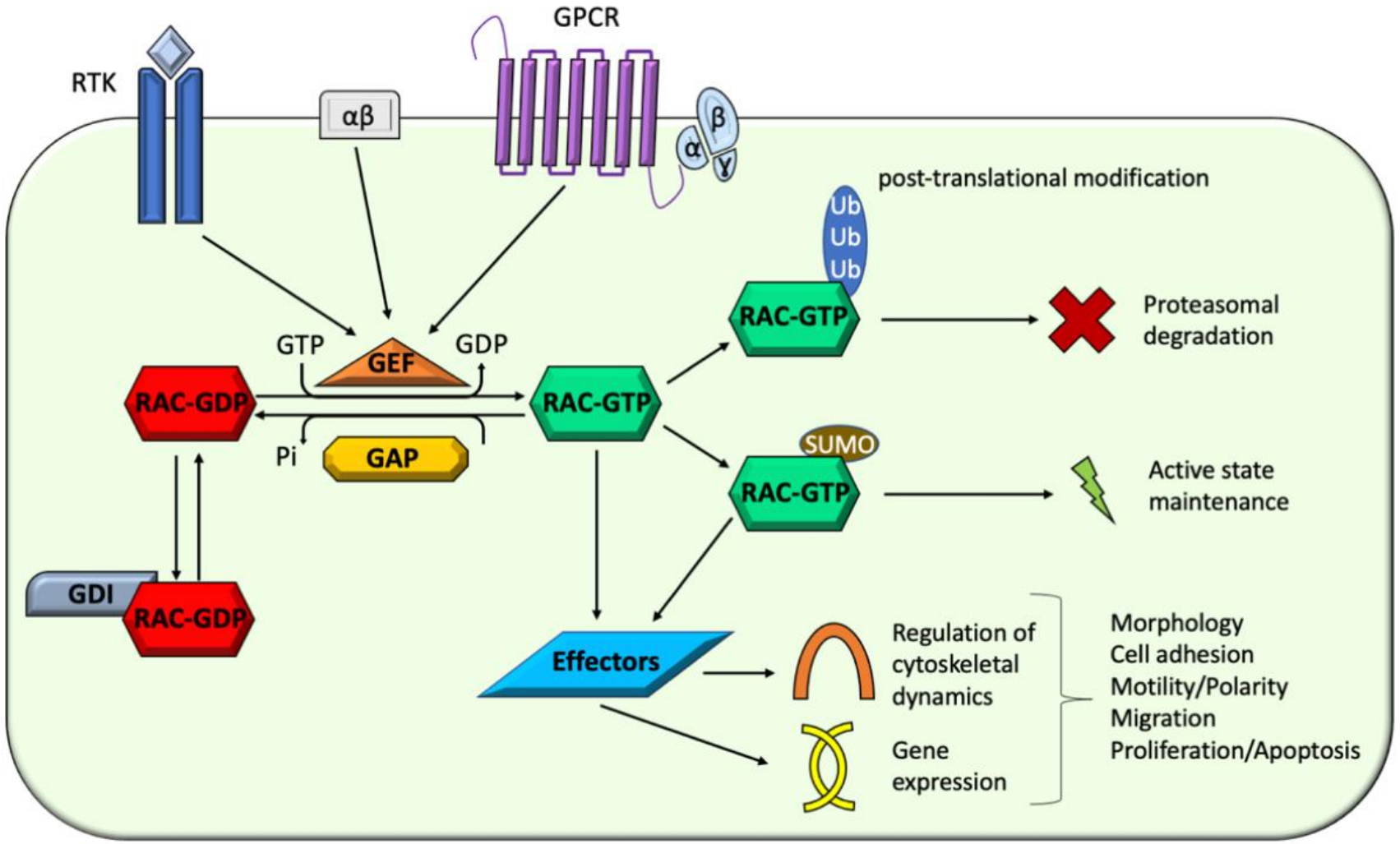


Table 1. NDD in humans associating Rho GTPases pathogenic variants and motor symptoms.

Genes	Human locus	Human pathology	References
RHO B	2p24.1	No ID but brain malformations + craniofacial anomalies, strabismus (mosaicism), CP	[73]
RHO GEF			
GEFH1 (ARHGEF2)	1q22	ID, midbrain-hindbrain malformations	[45]
TRIO/ARHGEF23	5p15.2	ID, behavioral defects. Schizophrenia, ASD, Bipolar disorder	[81–91]
C9orf72	9p21.2	ALS and FTD	[94, 95]
ARHGEF10	8p23.3	Spastic paraplegia, slow eye movements, hypomyelination, schizophrenia	[98–102]
RHO GAP			
OLIGOPHRENIN1	Xq12	ID, psychomotor retardation, ataxia, hypotonia	[46–50]
RAC1	7p22.1	ID	[42]
RAC effector			
PAK3	Xq23	ID, behavioural symptoms, oro-motor hypotonia, CP, motor delay with inability to walk, hyperreflexia, afinalistic movements	[55–57]
RAC GEF			
DOCK3	3p21.2	ID+muscle hypotonia, ataxia (and DMD)	[44, 45]
DOCK4	7q31.1	ASD, schizophrenia, dyslexia	[80]
TRIO/ARHGEF23	5p15.2	ID, ASD, schizophrenia, psychomotor defects	[81–91]
ALS2	2q33.1	IAHSP and JPLS	[106–110]
RAC1 other activator			
AUTS2	7q11.22	ID, ASD, Schizophrenia, dyslexia, hypotonia	[76, 77]
RAC1GAP			
A2-chimaerin (CHN1)	2q31.1	ASD (paralytic strabismus DRS)	[69–71]
RAC1-E3-Ubiquitin ligase			
Hace 1	6q16.3	ID with spastic paraplegia & psychomotor retardation	[64, 65]
CDC42			
AUTS2	7q11.22	ID, ASD, Schizophrenia	[76, 77]

NDD neurodevelopmental disorders, *ASD* Autism Spectrum Disorder, *ID* Intellectual deficiency, *DRS* Duane Retraction Syndrome, *ALS* Amyotrophic Lateral Sclerosis, Analogous to SMA, *FTD* Frontotemporal Demency, *HSP* hereditary spastic paraplegia, *IAHSP* Familal Infantile-onset ascending hereditary spastic paraplegia, *JPLS* Juvenile-onset primary lateral sclerosis, *CMM* Congenital Mirror Movements.

RAC1 and RAC3

- The small GTPase RAC1 belongs to Rac1–3 members of the Rho family of GTPases involved in the regulation of actin dynamics.
- Rac1 is a ubiquitous and highly conserved Rho GTPase across species that modulates numerous cellular functions that are essential for normal development.
- Individuals with distinct de novo missense RAC1 mutations present varying degrees of developmental delay and brain malformations.
- Analogously, variants in RAC3, encoding a small GTPase RAC3 which is critical for the regulation of actin cytoskeleton and intra-cellular signal transduction, are associated with a rare neurodevelopmental disorder with structural brain anomalies and facial dysmorphism.

1.Rho family GTPases: key players in neuronal development and synaptic function

Antonio Falace, PhD Pediatric Neurology and Muscular Diseases Unit, IRCCS Istituto Giannina Gaslini", Genoa, Italy.

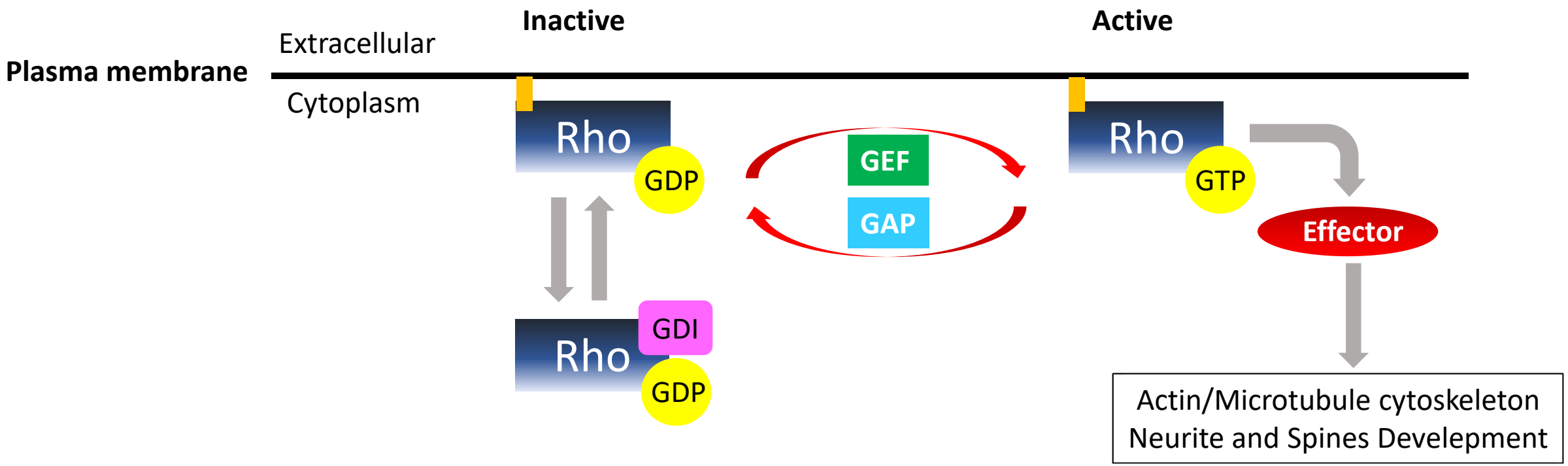
Rho family GTPases:
key players in neuronal development and synaptic function

Antonio Falace
Pediatric Neurology and Muscular Diseases Unit
IRCCS Istituto Giannina Gaslini, Genoa

Rho family GTPases: key players in neuronal development and synaptic function

CORE CONCEPTS

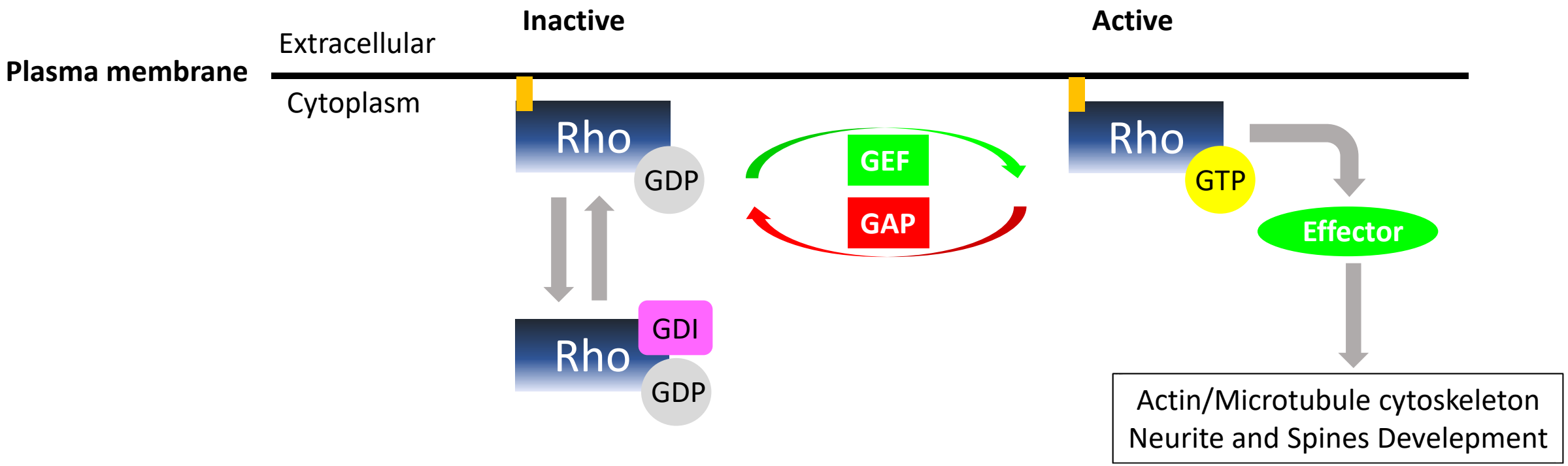
- The **Rho GTPase family** belongs to the Ras superfamily of low molecular weight **GTP binding proteins**
- These proteins act as **molecular switches**, cycling between **an active GTP**-bound state and an **inactive GDP**-bound state, thereby transducing signals from cell surface receptors to **downstream effectors**



Rho family GTPases: key players in neuronal development and synaptic function

CORE CONCEPTS

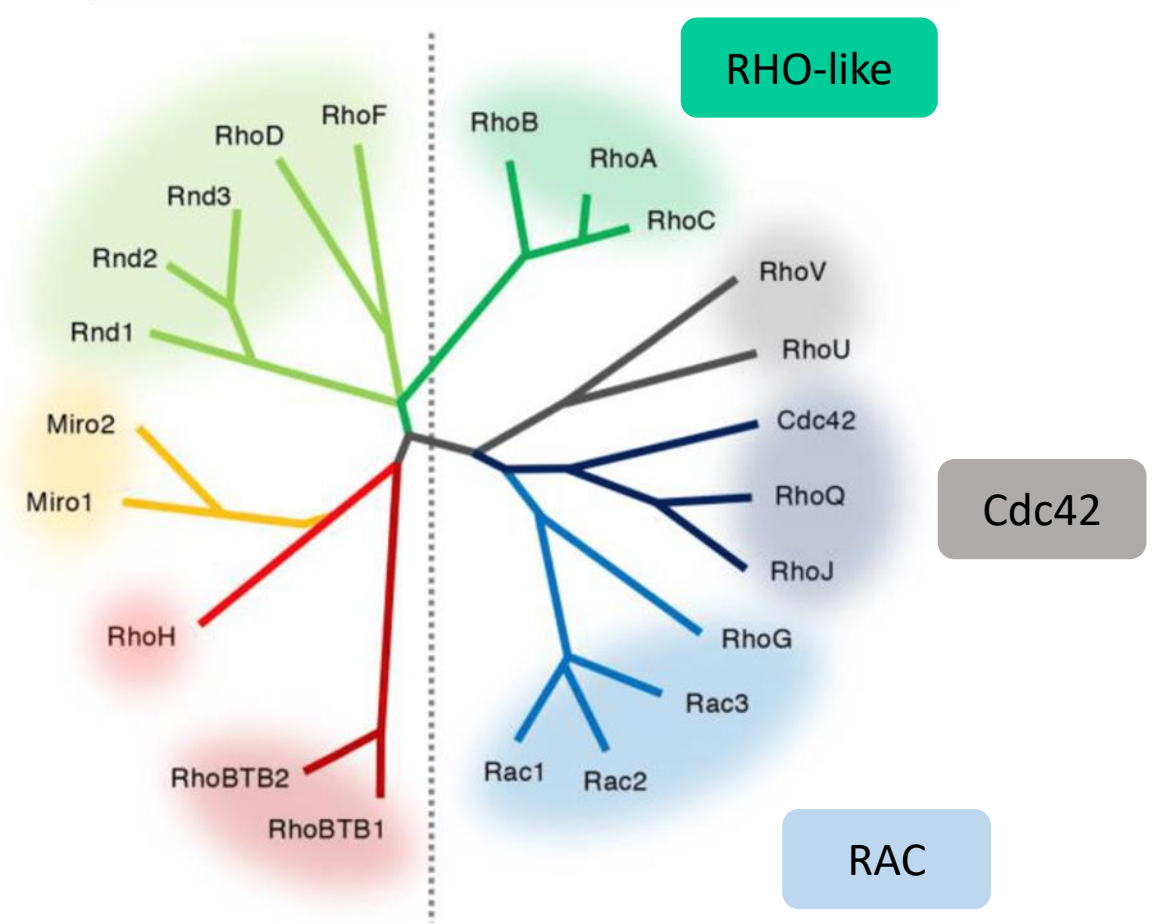
- Rho-GTPases are **activated** **by guanine nucleotide exchange factors (GEFs)**
- while are **inactivated** **by GTPase-activating proteins (GAPs)** that stimulate the GTPases' enzymatic activity



Rho family GTPases: key players in neuronal development and synaptic function

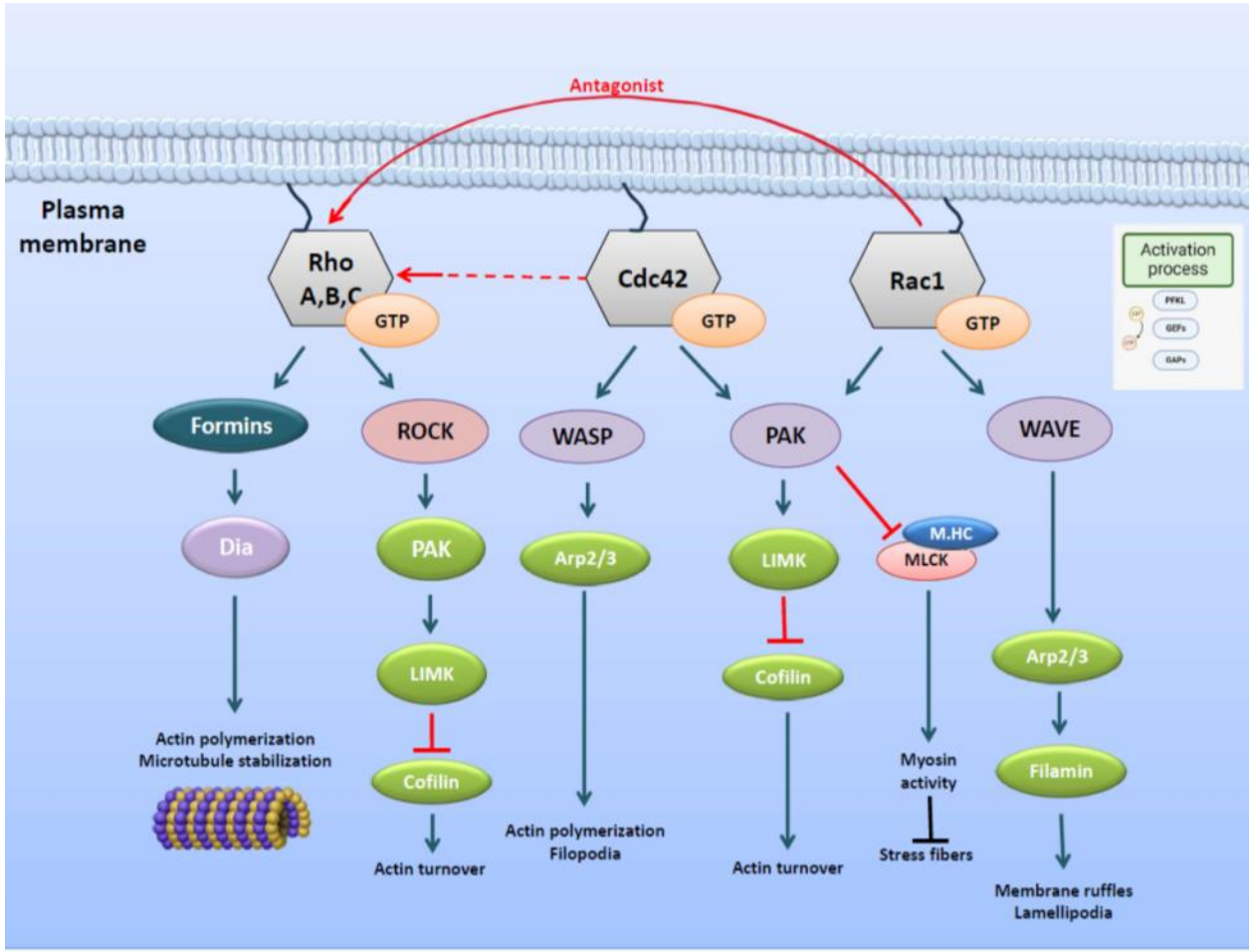
CORE CONCEPTS

- 22 Rho-GTPases divided into **8 subfamilies**
- The atypical Rho GTPases are predominantly GTP-bound
- Nearly 90 GEFs, 60 GAPs, combining with several Rho-GTPase effectors in the mammalian genome
- **RAC**, **Cdc42** and **RHO-like** subfamilies are the most studied



adapted from Uemura and Fukushima, 2021

Rho family GTPases: key players in neuronal development and synaptic function

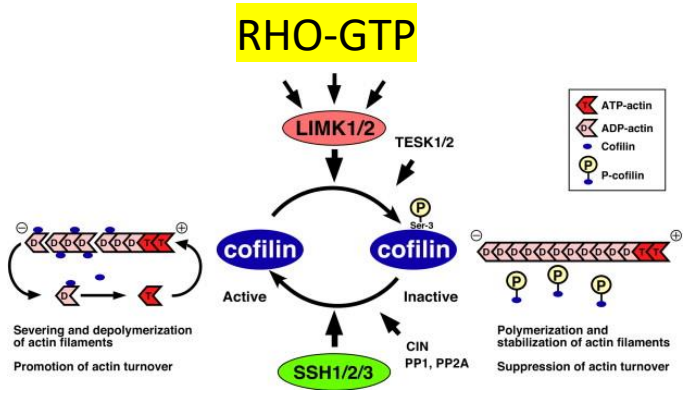


RHO FAMILY GTPases

A hub for many **signalling pathway**

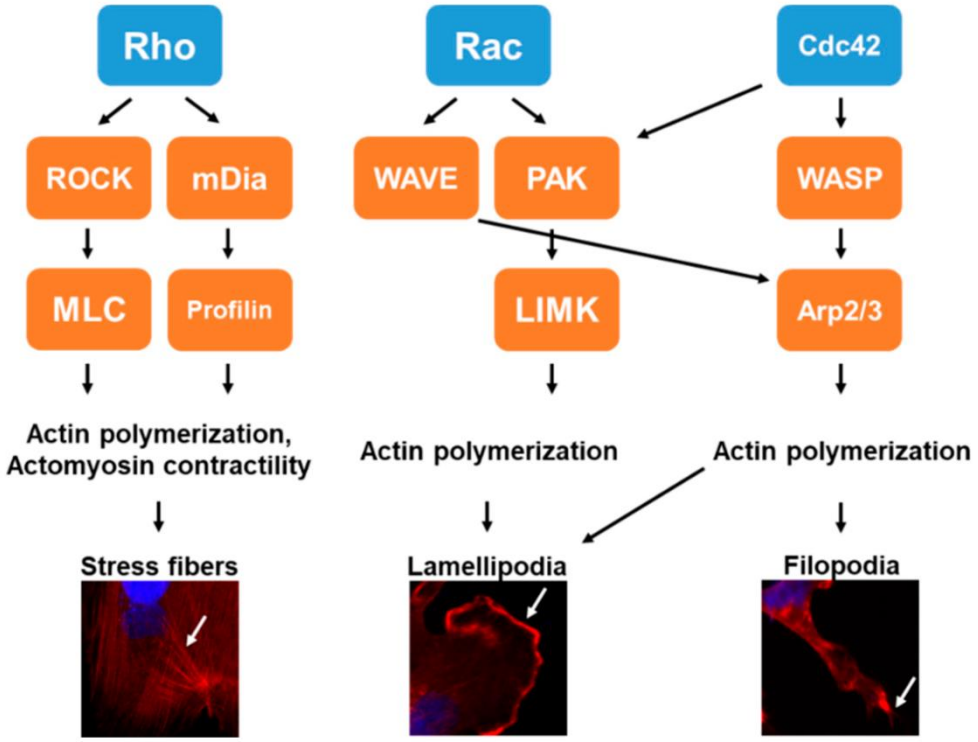
They regulates **cytoskeletal dynamics** by controlling **actin polimerization**.

Rho activation lead to the phosphorylation and inactivation of the actin **severing enzyme cofilin**.

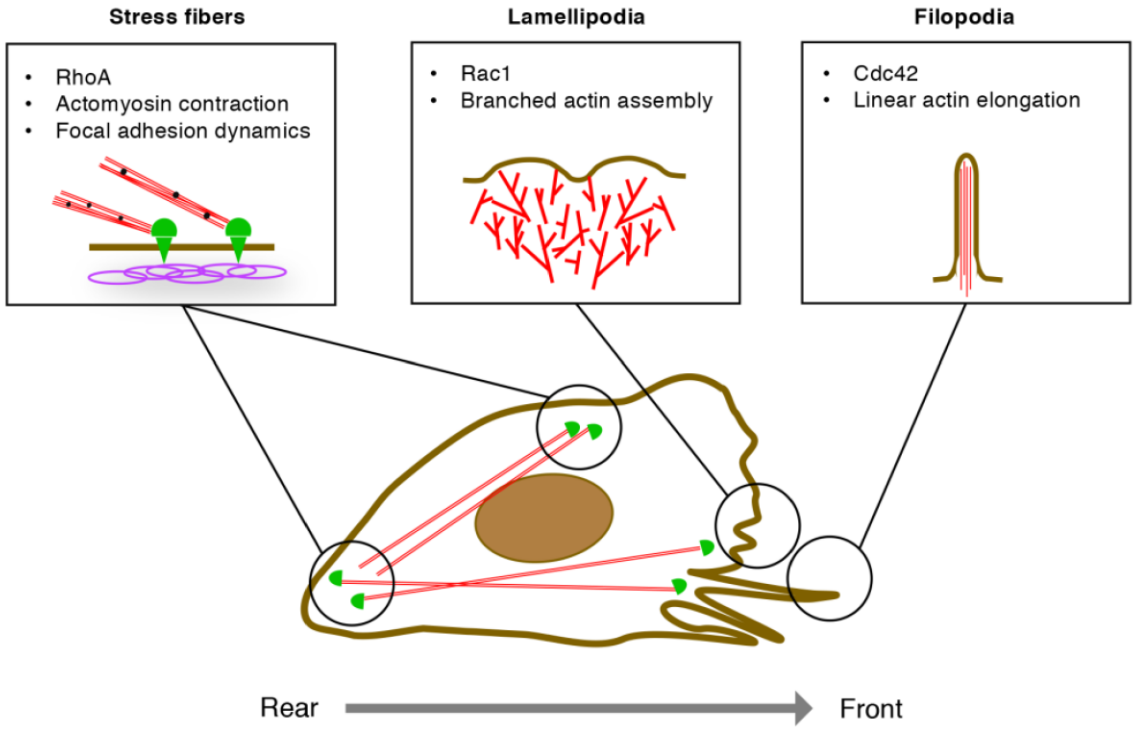


adapted from Mizuno, 2021

Rho family GTPases: key players in neuronal development and synaptic function



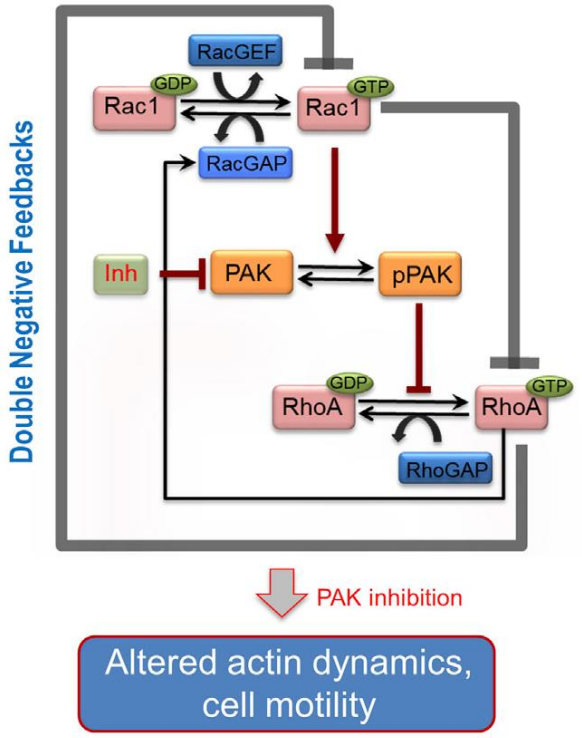
adapted from Humphries, 2020



adapted from Uemura and Fukushima, 2021

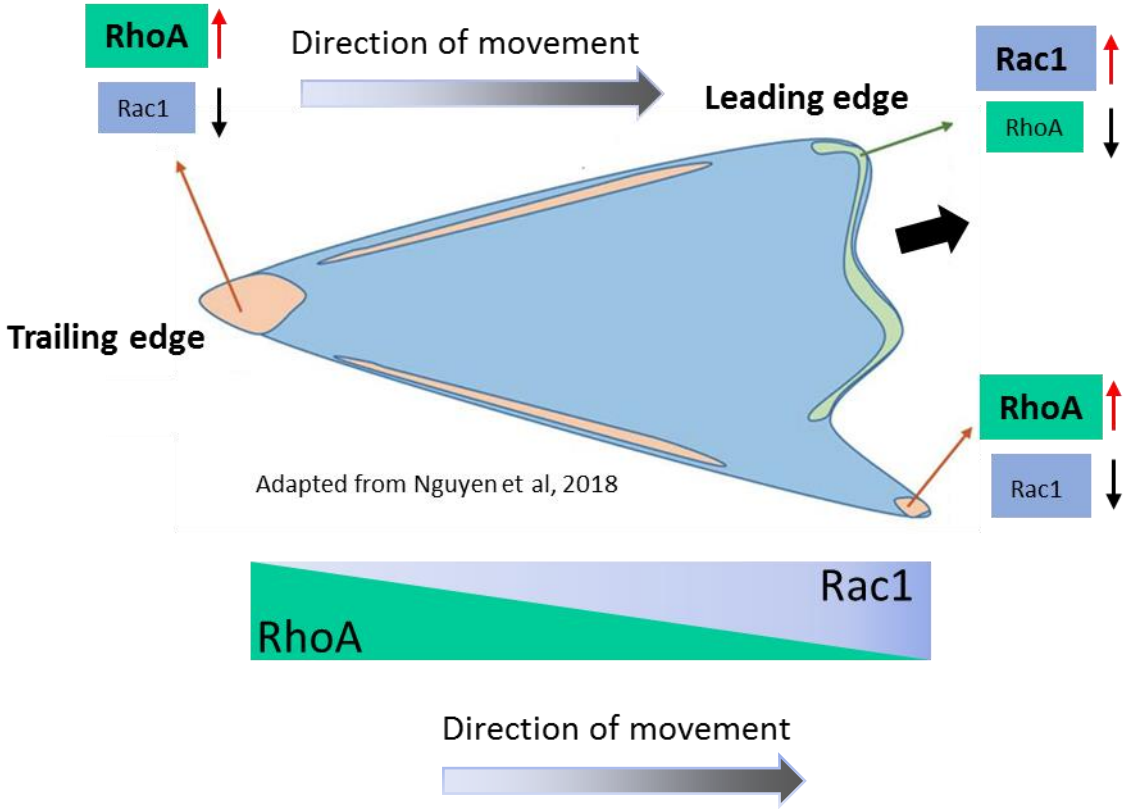
Rho family GTPases: key players in neuronal development and synaptic function

- RhoA and Rac1 are linked by a double-negative feedback loop



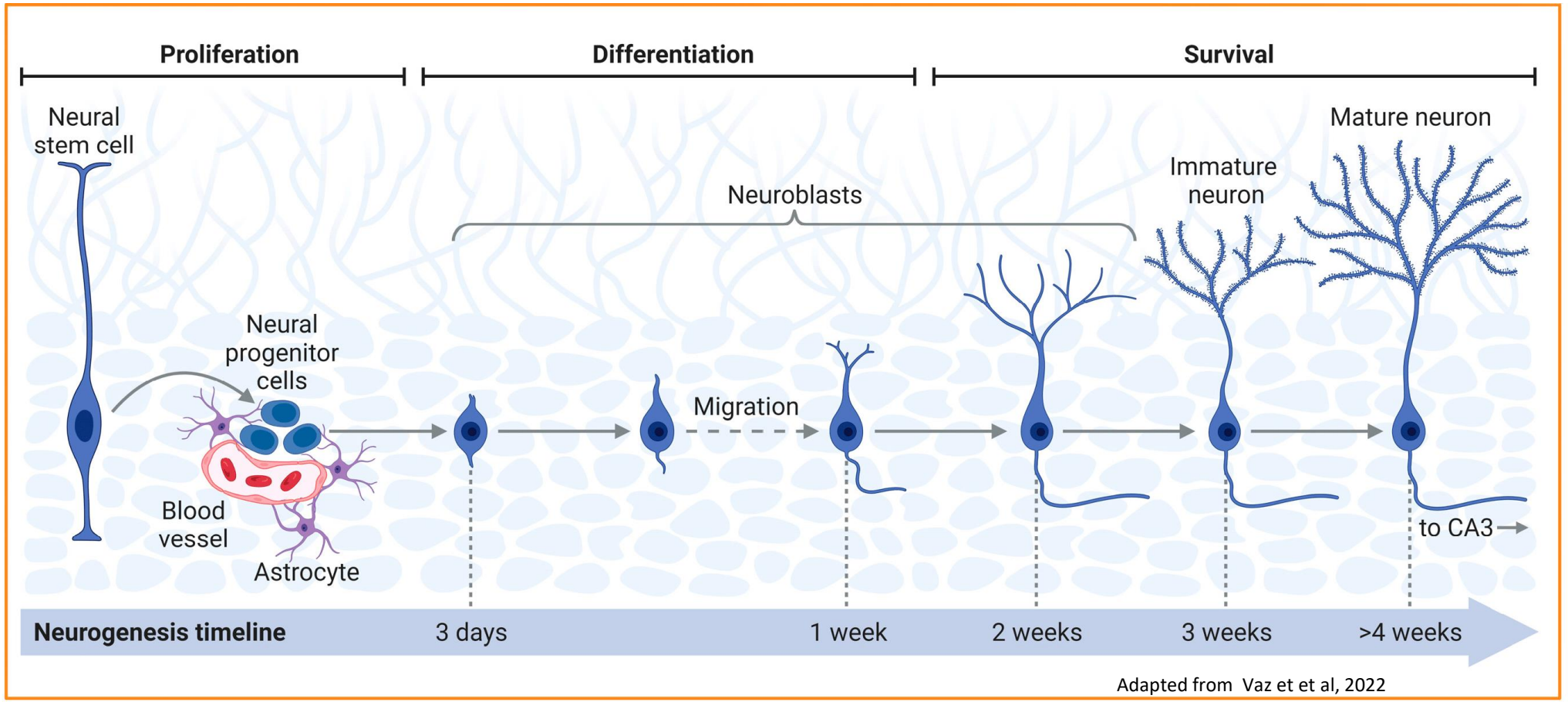
Adapted from Byrne et al, 2016

Dynamic interactions between **RhoA** and **Rac1**, play a vital role in the control of cell migration



Rho family GTPases: key players in neuronal development and synaptic function

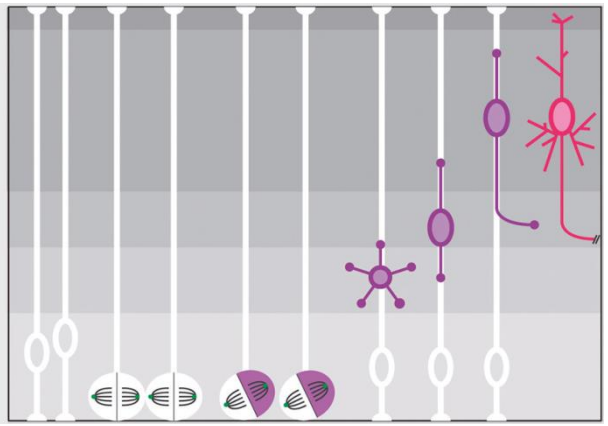
- **RHO family GTPases in the brain development and function**



Adapted from Vaz et et al, 2022

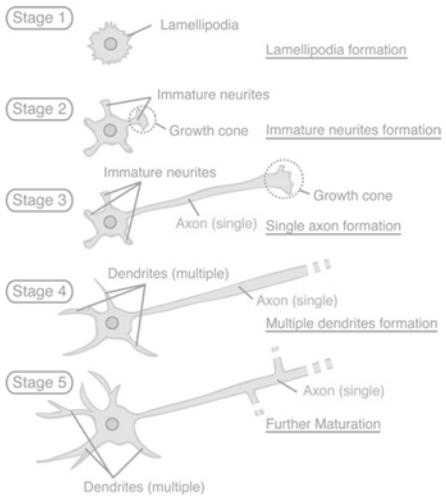
Rho family GTPases: key players in neuronal development and synaptic function

Neuronal migration



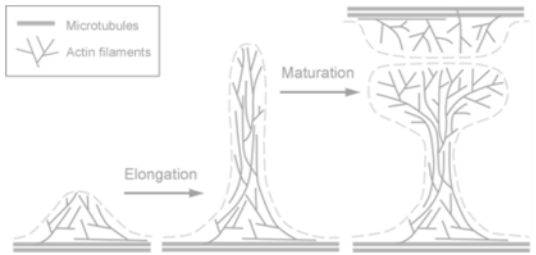
Adapted from Guarnieri et et al, 2018

Neuronal polarity



Adapted from Yoshimura et al, 2006

Synaptogenesis

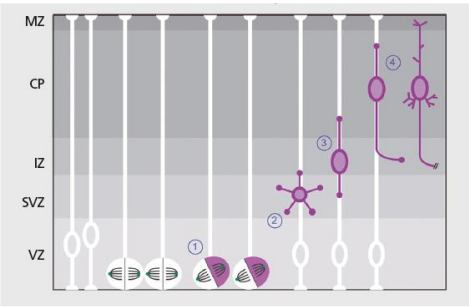


Adapted from Korobova et al, 2009

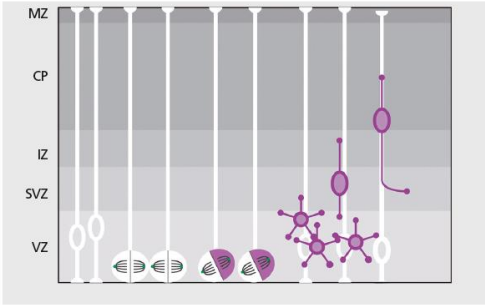
Rho family GTPases: key players in neuronal development and synaptic function

- **RAC1/RAC3** deregulation impairs neuronal migration

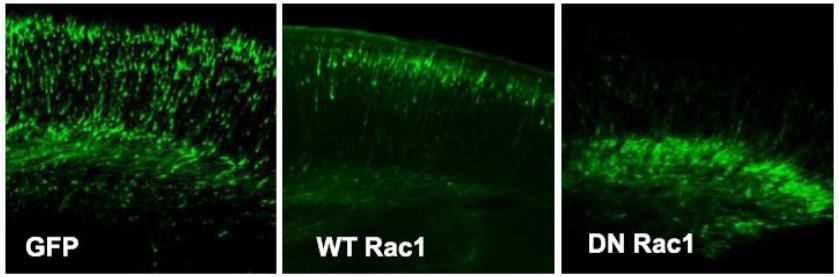
Wild type

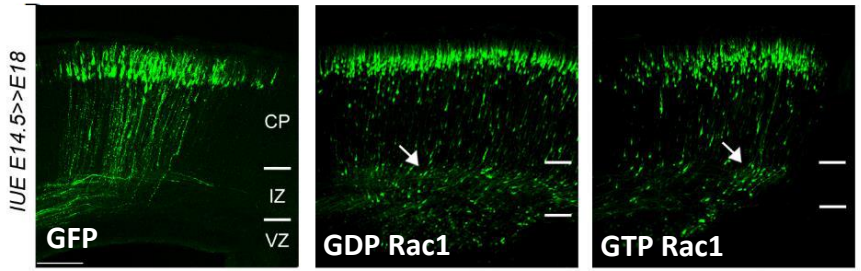


RAC1 or RAC3 dysfunction



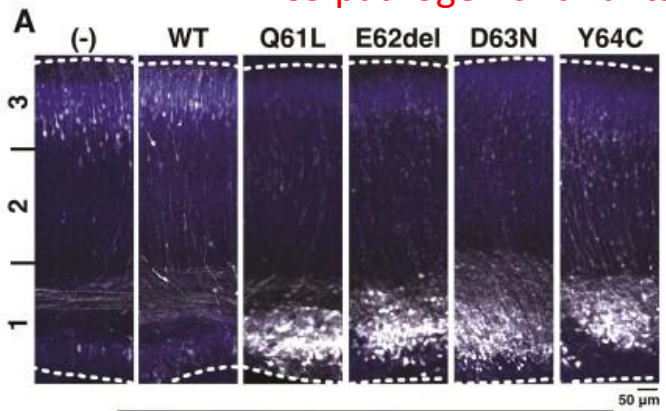
Adapted from Guarnieri et et al, 2018

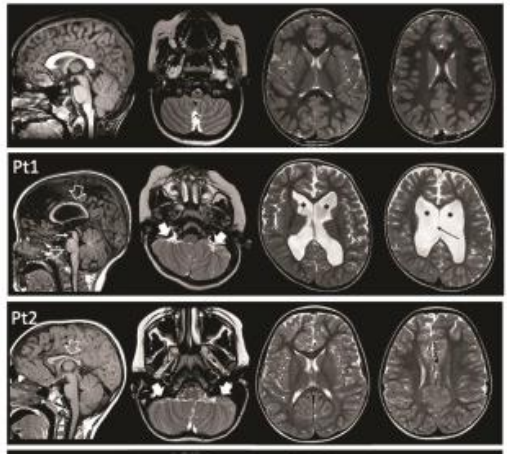




Adapted from Yang et al, 2012 and from Falace et al, 2024

RAC3 pathogenic variants





Adapted from Scala et al, 2022

Cell autonomous role for RAC1 and RAC3 in neuronal migration

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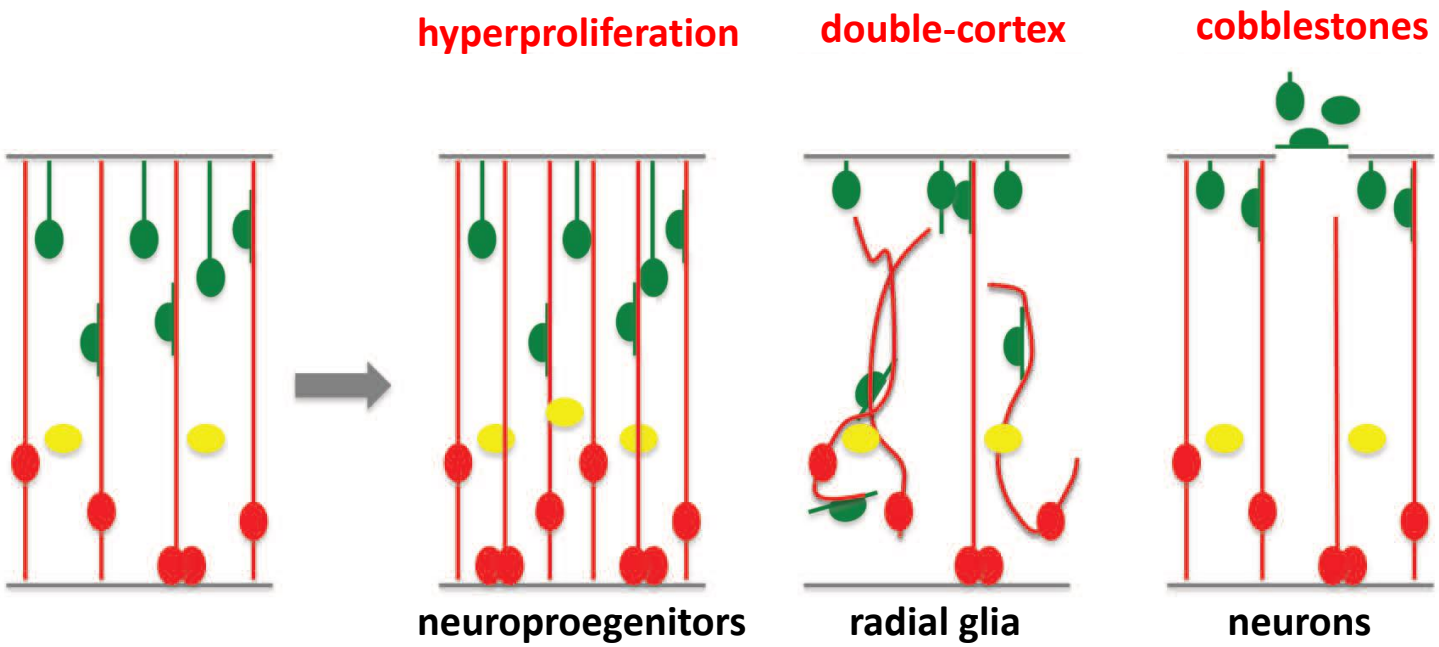
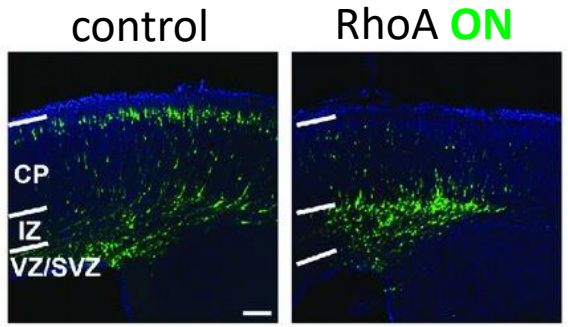
Rho family GTPases: key players in neuronal development and synaptic function

- genetic deletion of **RhoA** in the developing mouse cerebral cortex results in three distinct cortical malformations

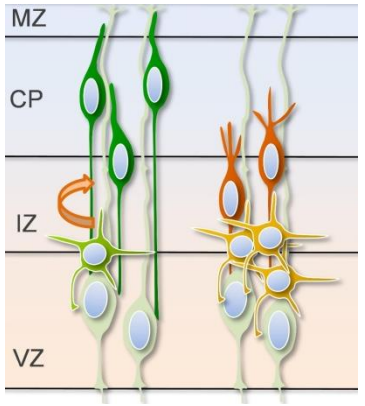


Adapted from Cappello, 2013

- genetic activation of **RhoA** impairs neuronal migration



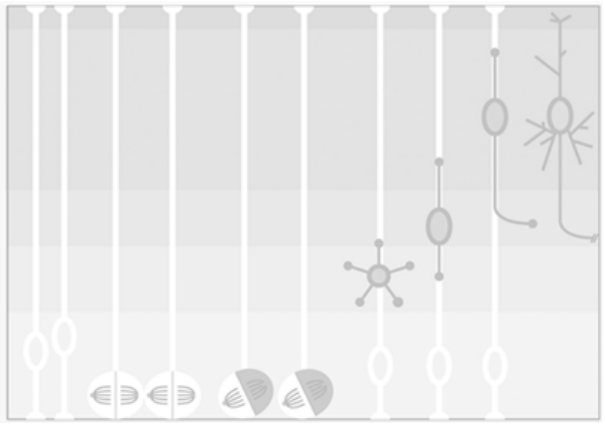
Adapted from Qin et al, 2017



Adapted from Chen et al, 2018

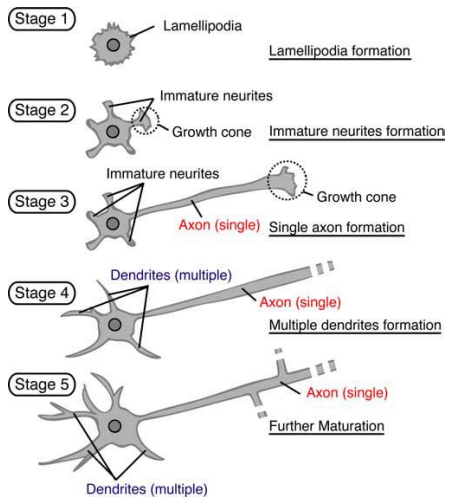
Rho family GTPases: key players in neuronal development and synaptic function

Neuronal migration



Adapted from Guarnieri et et al, 2018

Neuronal polarity



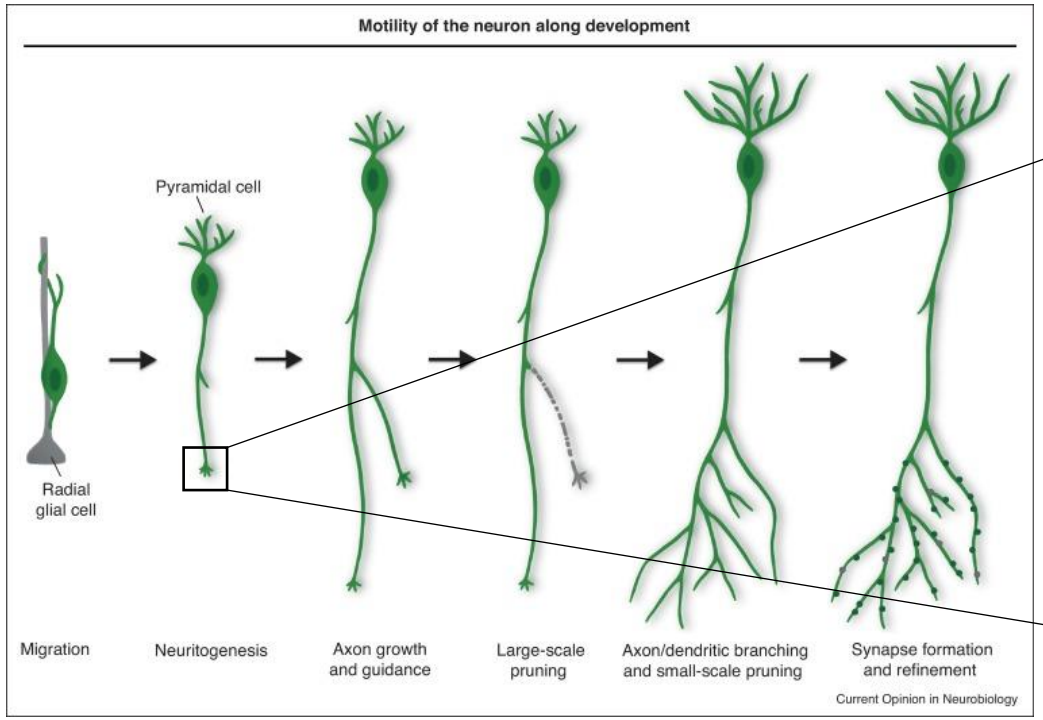
Adapted from Yoshimura et al, 2006

Synaptogenesis

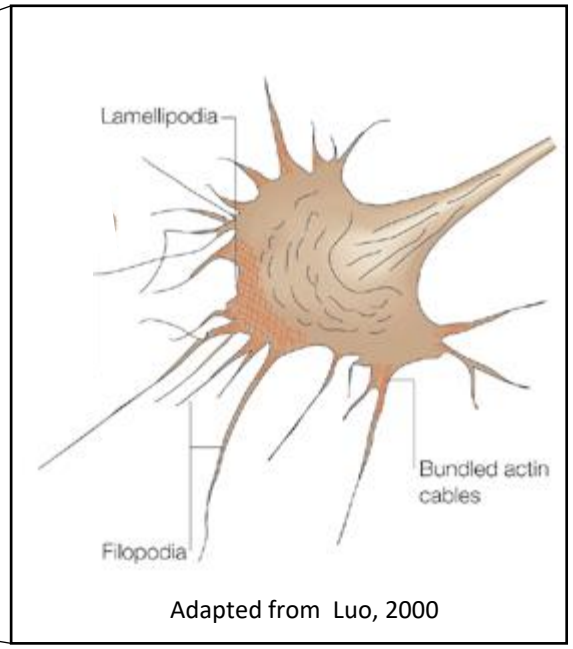
Adapted from Korobova et al, 2009

Rho family GTPases: key players in neuronal development and synaptic function

- **RHO family GTPases in axonogenesis**



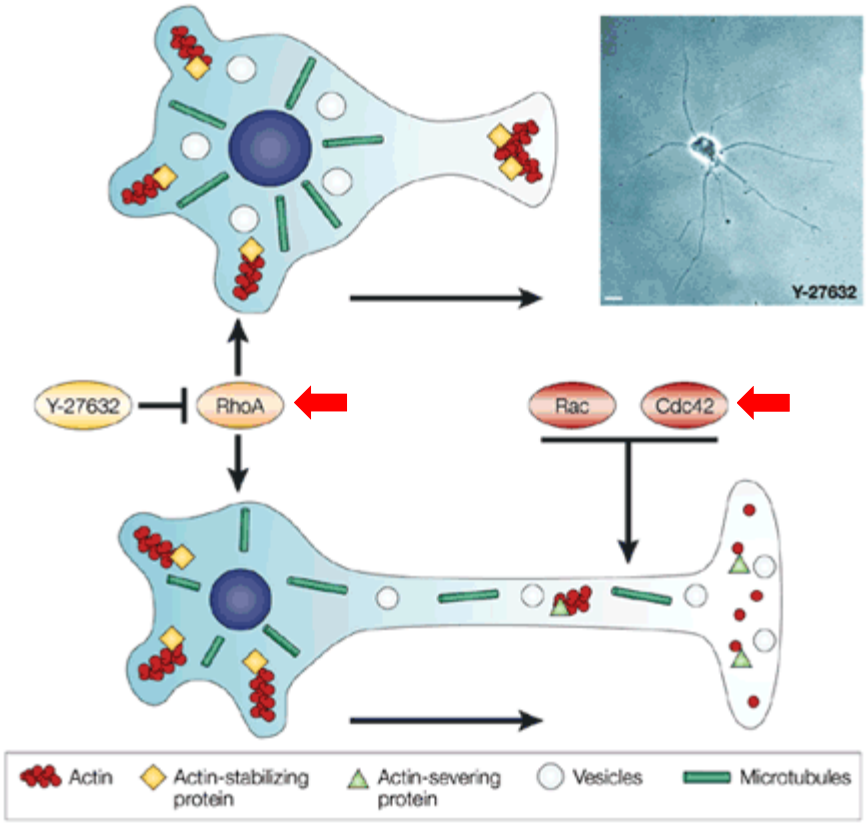
Neuron growth cone



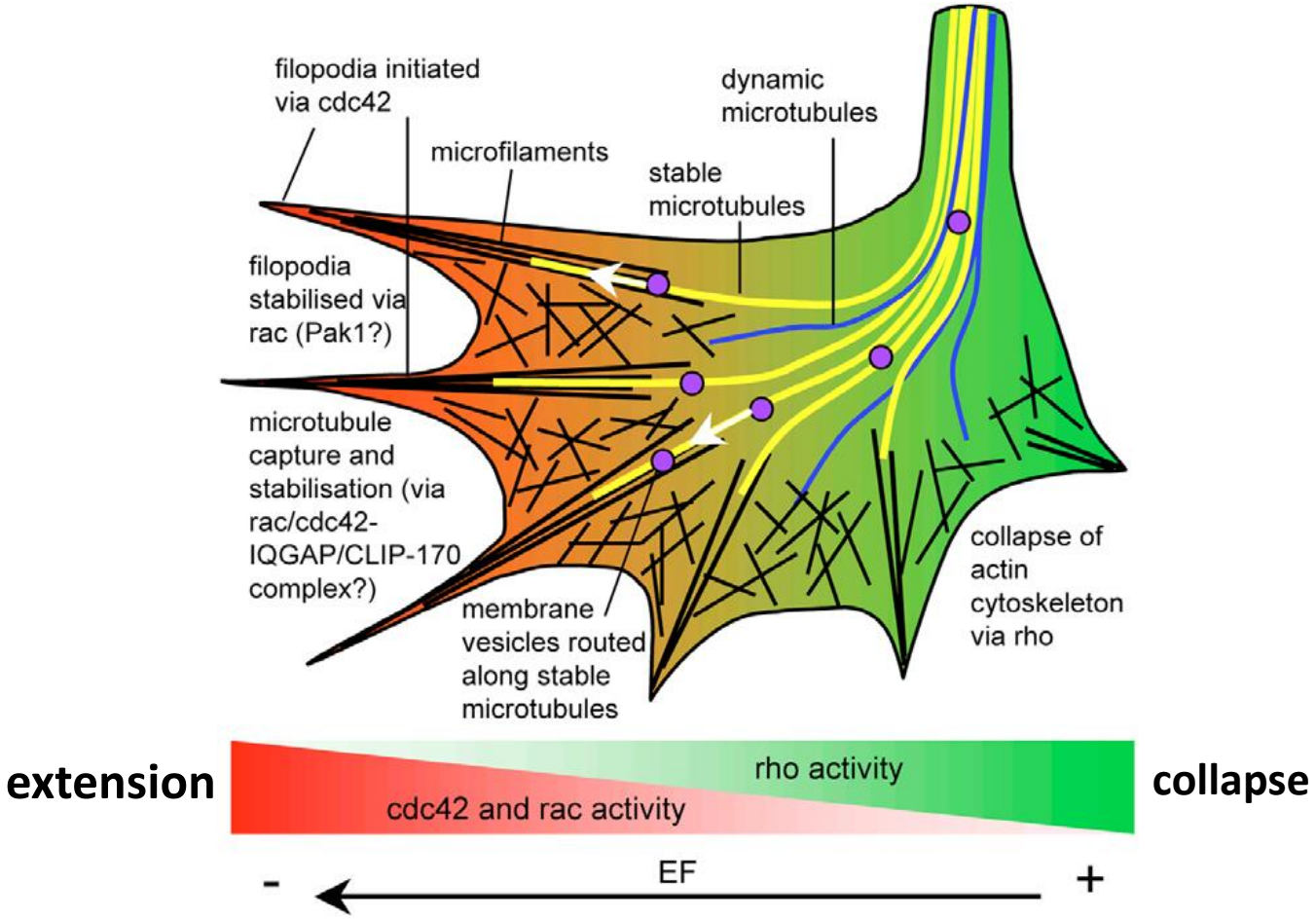
Adapted from Luo, 2000

Rho family GTPases: key players in neuronal development and synaptic function

- **actin-based motility is regulated by Rho family GTPases interplay at the growth cone**



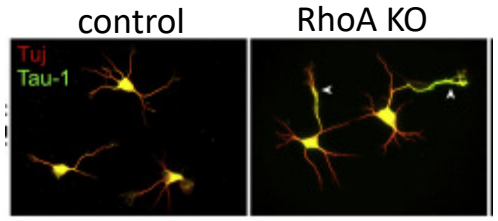
Adapted from Da Silva and Dotti, 2002

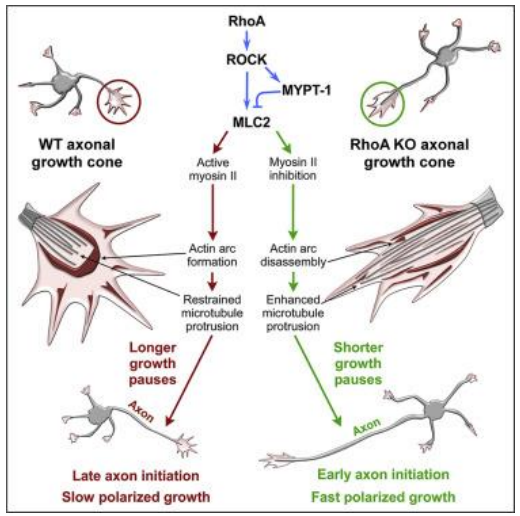


Adapted from Rajniceck et al, 2006

Rho family GTPases: key players in neuronal development and synaptic function

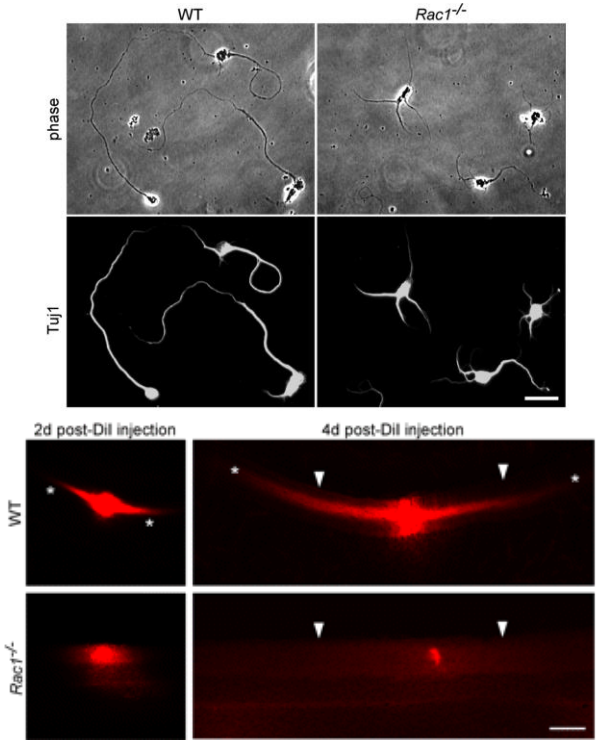
- RhoA restrains axon initiation and growth independent of specification





Adapted from Dupraz et al, 2019

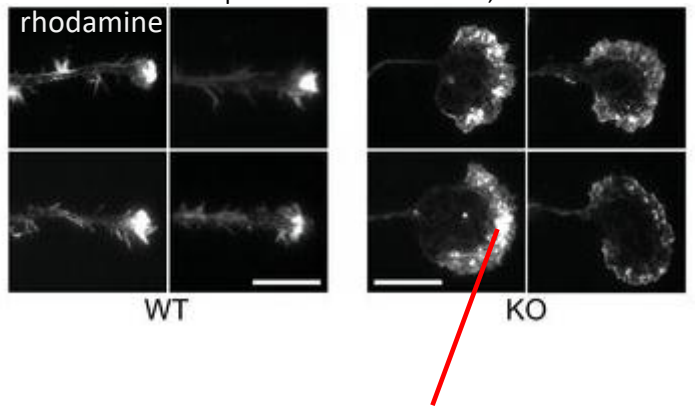
- Rac1 loss impairs axonogenesis *in vitro* and *in vivo*



Adapted from Tahirovic et al, 2010

- Cdc42 loss impairs growth cone development

Adapted from Garvalov et al, 2007



Smooth-Filopodia free growth cone

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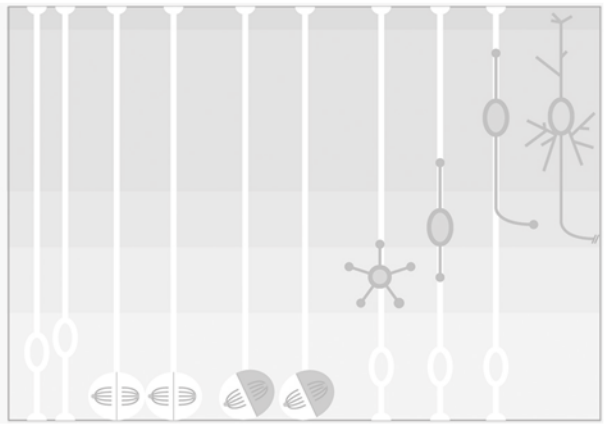
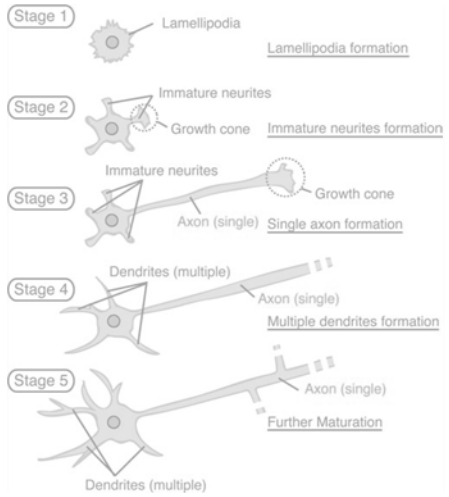
Rho family GTPases: key players in neuronal development and synaptic function

Neuronal migration



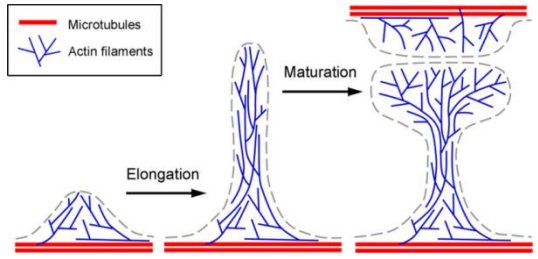
Adapted from Guarnieri et et al, 2018

Neuronal polarity



Adapted from Yoshimura et al, 2006

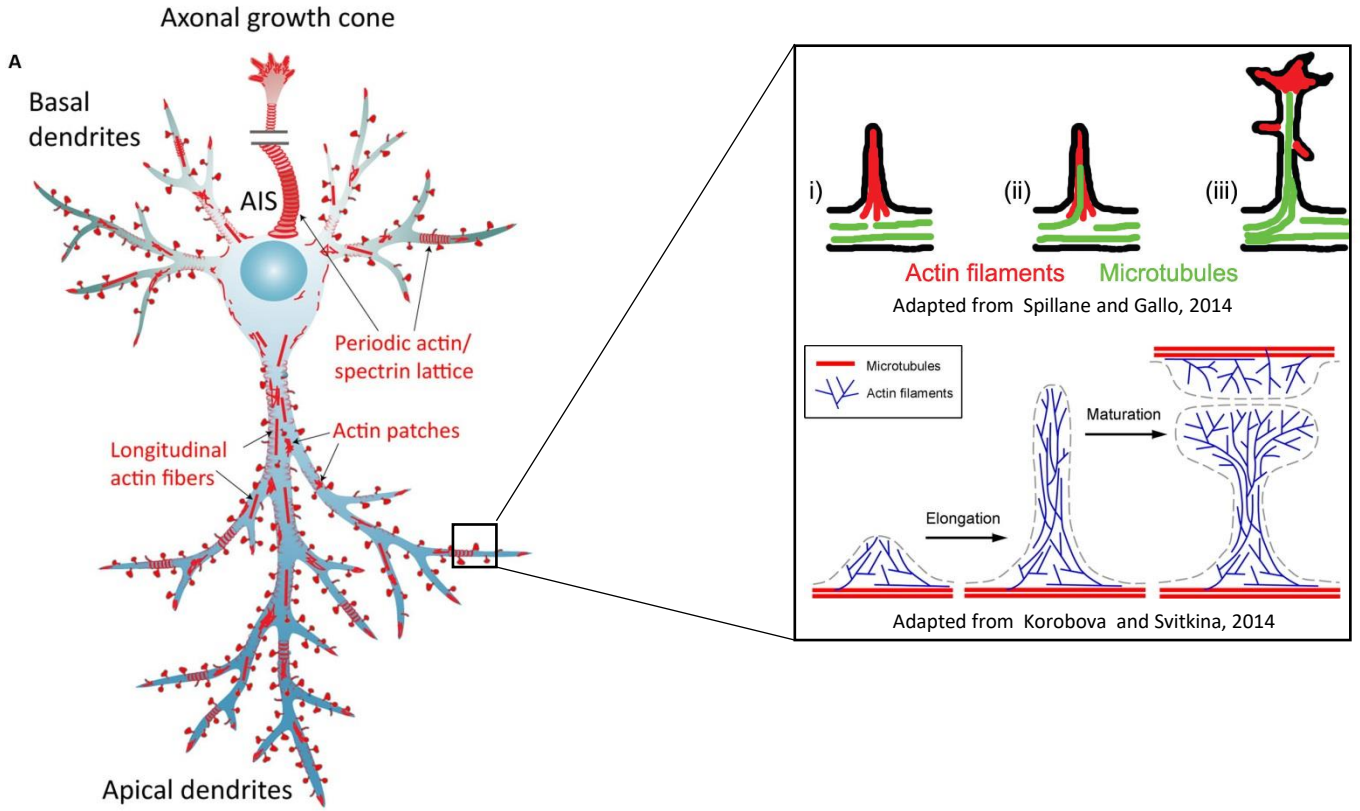
Synaptogenesis



Adapted from Korobova et, 2009

Rho family GTPases: key players in neuronal development and synaptic function

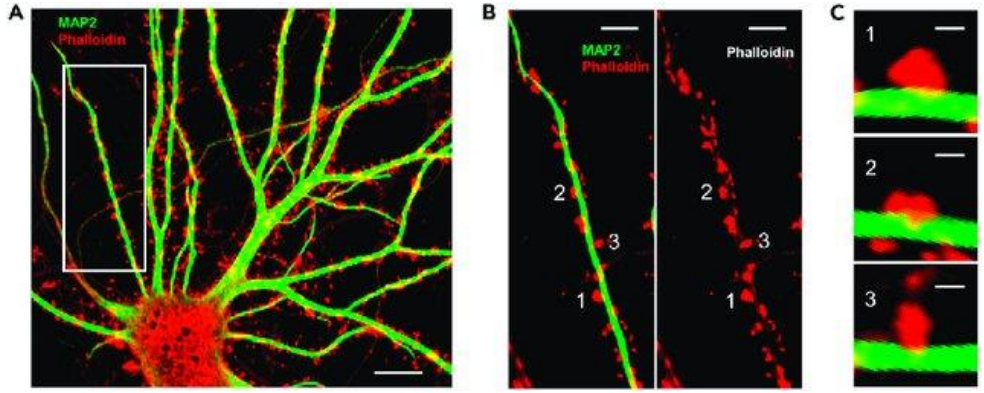
- **Dendritic spines formation, maturation, and plasticity heavily depend on the actin cytoskeleton remodeling**



Adapted from Konietzny et al, 2017

Adapted from Spillane and Gallo, 2014

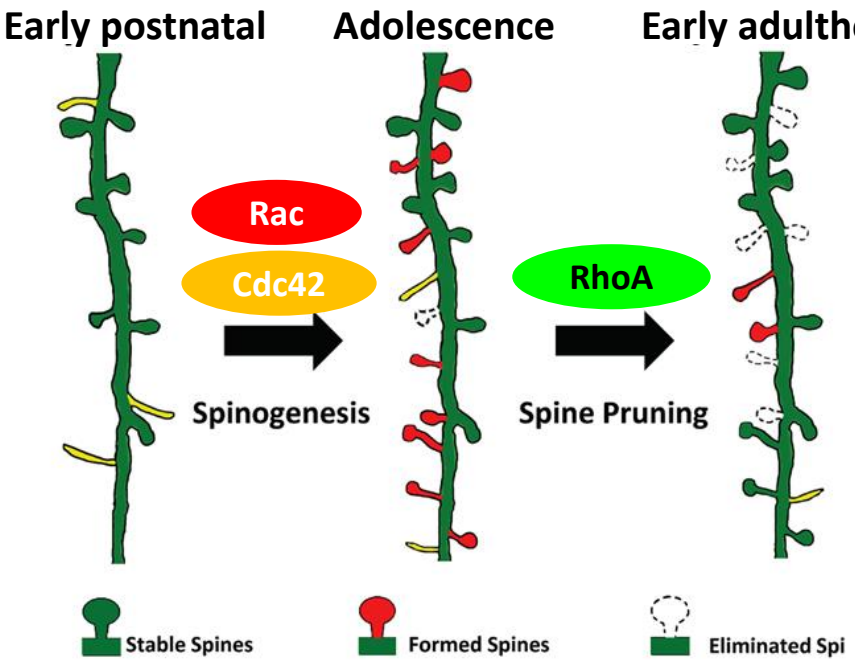
Adapted from Korobova and Svitkina, 2014



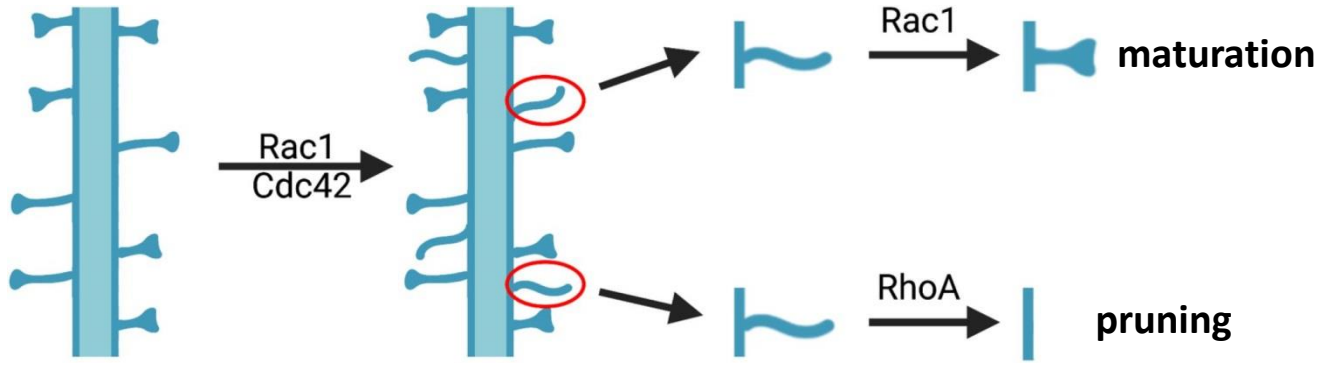
Adapted from He et al, 2022

Rho family GTPases: key players in neuronal development and synaptic function

- **The key role Rho family GTPases interplay in dendritic spines development and function**



Adapted from Chen et al, 2014



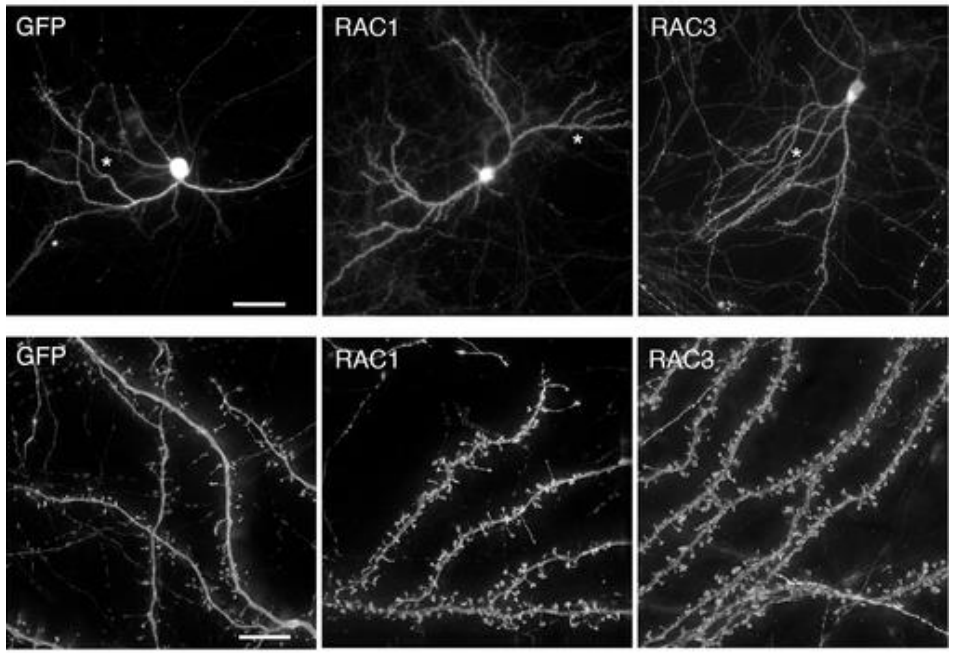
Adapted from Zhang et al, 2021

- The activation of **Rac1** and **Cdc42** leads to increased immature spines
- Mature morphology through **Rac1**-dependent mechanisms
- Pruning by **RhoA**-dependent processes

Rho family GTPases: key players in neuronal development and synaptic function

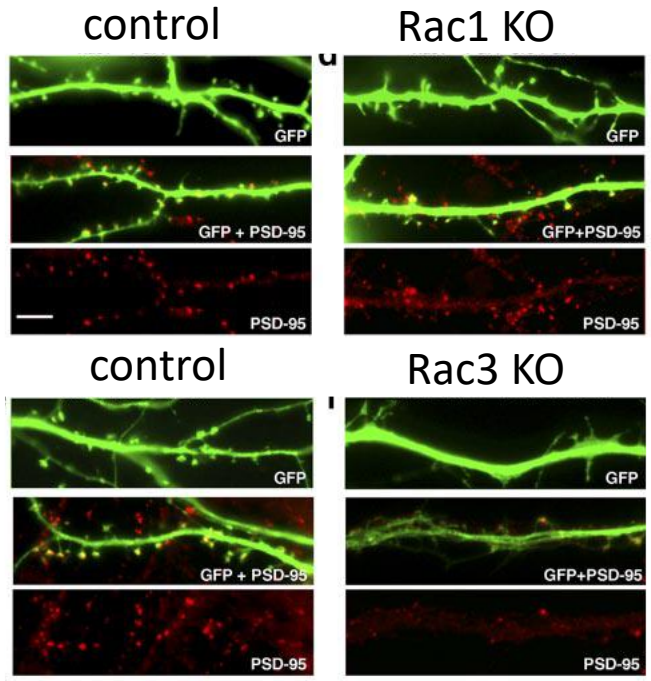
- **The key role Rho family GTPases interplay in dendritic spines development and function**

- Overexpression of RAC1 and RAC3 increases the density and size of dendritic spines



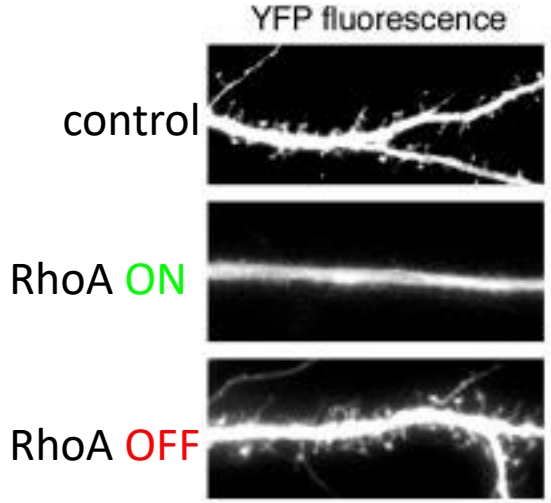
Adapted from Pennucci et al, 2019

- Deletion of both RAC1 and RAC3 deeply affects spinogenesis



Adapted from Corbetta et al, 2009

- RhoA activity inhibits spines formation

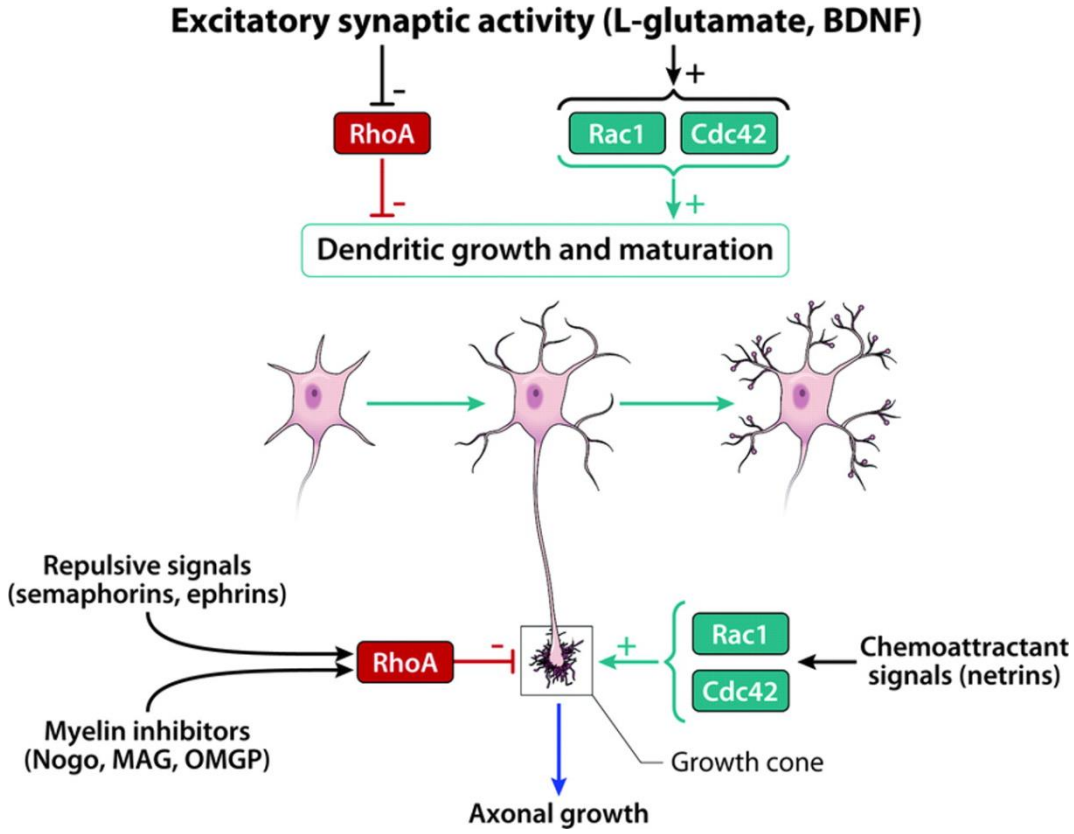


Adapted from Zhang and Macara, 2008

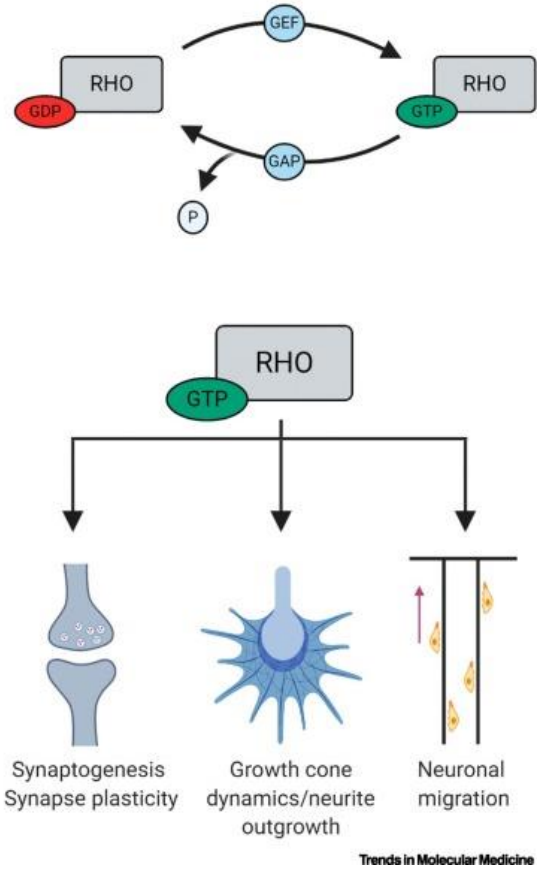
Rho family GTPases: key players in neuronal development and synaptic function

TAKE HOME MESSAGE

- **Rho family GTPases interplay regulates neuronal development and function**



Adapted from Benarroch, 2007



Adapted from Fell and Nagy, 2021

Rho family GTPases: key players in neuronal development and synaptic function

THANKS FOR YOUR ATTENTION

2.RAC3-related disorders of cortical development in human neurodevelopmental phenotypes

Marcello Scala, MD PhD “NeuroRacopathies – Department of Neurosciences (DINOGMI), University of Genoa, Genoa, Italy; Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy.

Università di Genova

DINOGMI 2018-2022 | DIPARTIMENTO DI ECCELLENZA MIUR

ISTITUTO GIANNINA GASLINI
Istituto Pediatrico di Ricovero e Cura a carattere Scientifico

RAC3-related disorders of cortical development in human neurodevelopmental phenotypes

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

Marcello Scala, MD, PhD[1,2]

Pediatrician, Researcher in Medical Genetics

[1] University of Genoa, Department of Neurosciences (DINOGMI)

[2] IRCCS Giannina Gaslini Children's Hospital and Research Institute, Department of Medical Genetics and Pediatric Neurology

European Reference Networks ITHACA

Marcello Scala, MD, PhD

European Commission

EUROPEAN REFERENCE NETWORKS

Helping patients with rare or low-prevalence complex diseases

Declaration of conflict of interest

I have no commercial disclosure

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Neurodevelopmental disorders

What are NDDs?

NDDs are a **spectrum** of disorders sharing genetic aetiology, pathophysiological mechanisms, and clinical manifestations

However, subgroups are identifiable based on distinctive biological and clinical properties

The **most common NDDs** are:

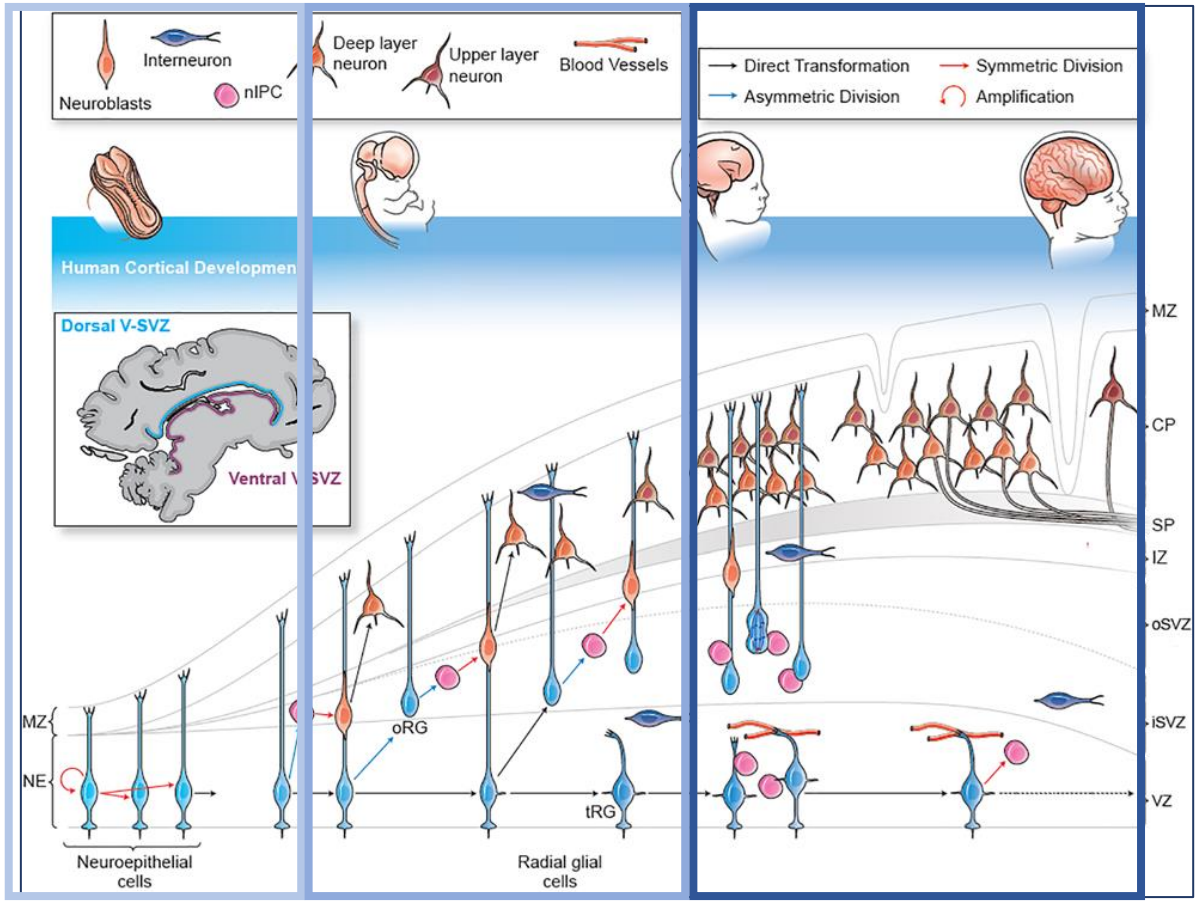
- Autism spectrum disorders (ASD)
- Attention deficit - hyperactivity disorder (ADHD)
- Intellectual disability (ID)
- Epilepsy
- *Adult-onset NDDs (Schizophrenia, depression, etc.)*

Condition	Prevalence worldwide	Common comorbidities	Commonly affected brain regions
ASD – inc. autistic disorder, Rett syndrome, childhood disintegrative disorder, pervasive developmental disorder not otherwise specified	1 % [5]	ADHD, ID, SCZ, epilepsy, developmental delay, depression, anxiety, bipolar disorder	Frontal cortex and cerebellum
ADHD – inc. predominantly inattentive, hyperactive-impulsive	2–6 % [6]	ASD, epilepsy, ID, bipolar disorder	Frontal cortex
Epilepsy – inc. partial, focal, general	0.7 % [7]	ASD, ADHD, ID, SCZ, psychosis	Frontal and temporal cortex
ID – inc. Down's syndrome	1–3 % [8]	ASD, ADHD, developmental delay	Frontal cortex
Developmental delay	1–3 % [9]	ASD, ID	Frontal cortex
SCZ – inc. schizoaffective disorder	0.3–0.7 % [10]	ASD, ADHD, epilepsy, bipolar disorder, depression	Frontal cortex
Depression – inc. major depressive disorder	3–6 % [11]	ASD, anxiety, SCZ, bipolar disorder	Frontal cortex

From development to disease

Typically, NDDs are caused by the disruption of the tightly coordinated events regulating **brain development and morphogenesis**:

- **First trimester** -> neurogenesis
- **Second trimester** -> more neurogenesis + migration -> corticogenesis (gyri formation)
- **Third trimester** -> differentiation + cortical axonogenesis -> neural networks/circuitry generation



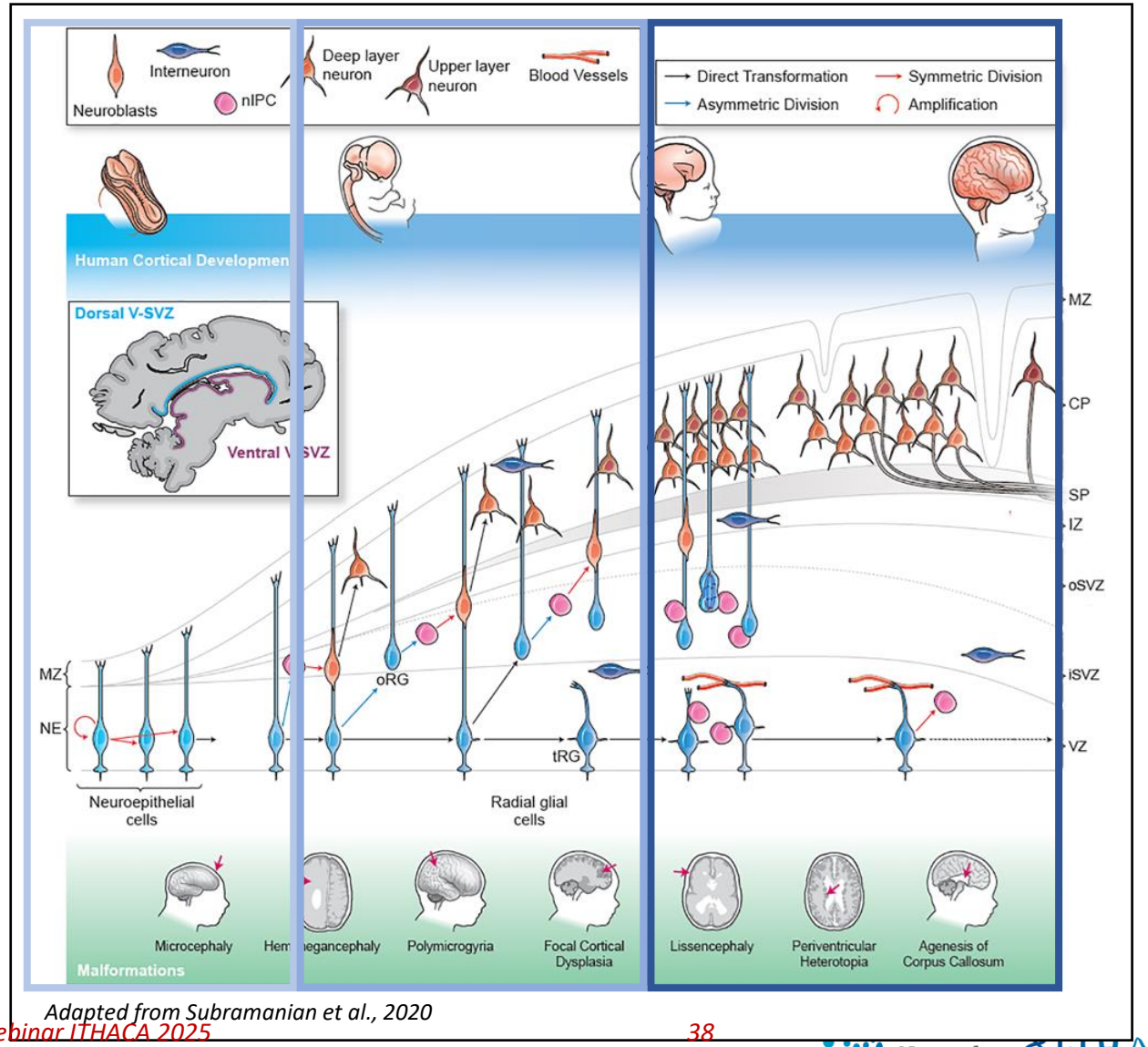
Adapted from Subramanian et al., 2020

Subramanian et al., 2020; Parenti et al., 2020

From development to disease

The disruption of each phase may result in distinctive features:

- **First trimester** ->
  - Microcephaly (OFC < -2SDs)
- **Second trimester** ->
  - Microcephaly
  - Lissencephaly (no gyri);
  - Polymicrogyria or pachygyria
  - Somatic mutation --->
    - Focal Cortical Dysplasia (FCD)
    - Hemimegalencephaly (HME)
    - Megalencephaly (ME)
- **Third trimester** ->
  - Heterotopias (grey in white)
  - Lissencephaly (milder)
  - Agenesis of corpus callosum



Adapted from Subramanian et al., 2020

Subramanian et al., 2020; Parenti et al., 2020

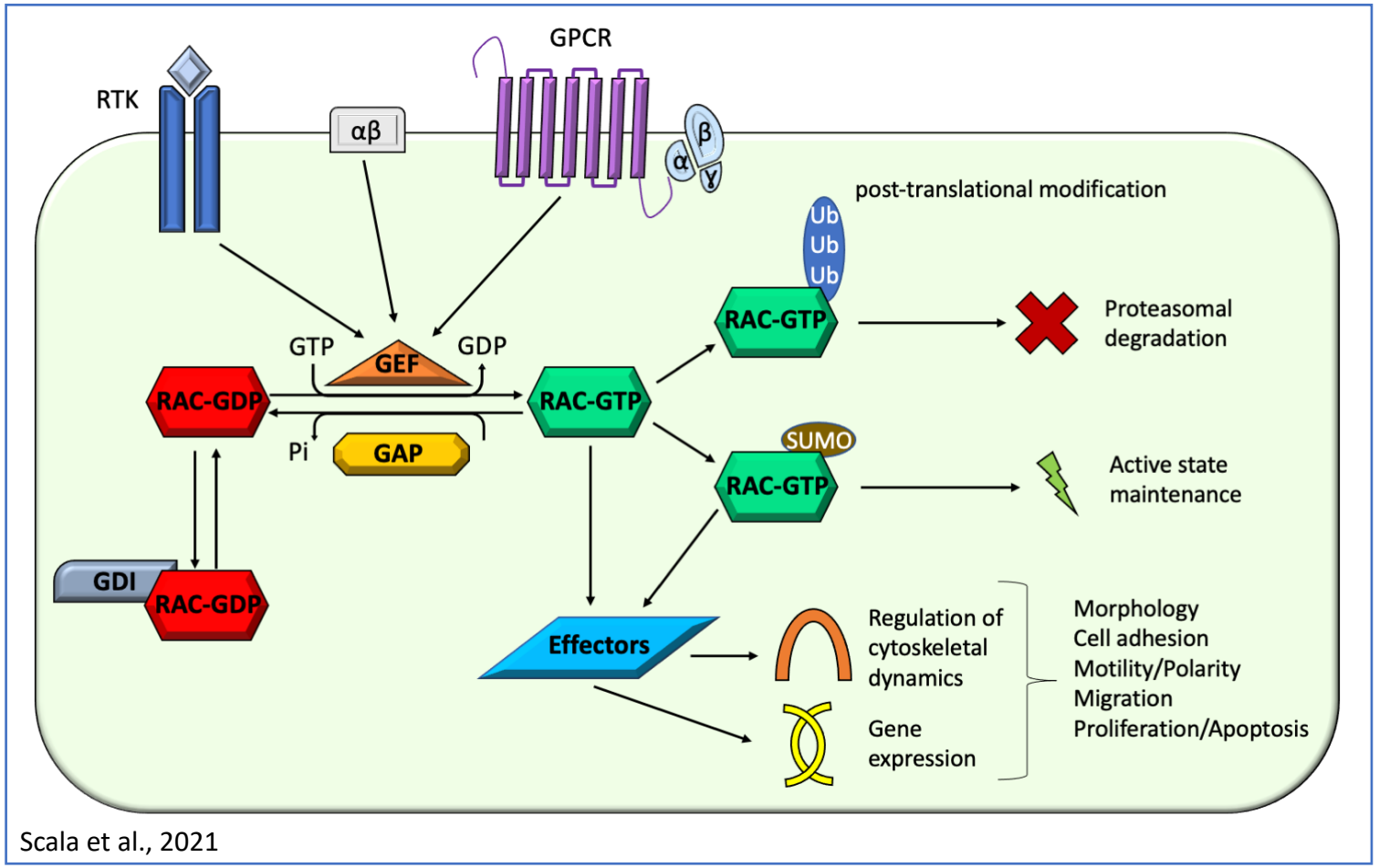
RAC3 function

Rho GTPases in brief

Twenty different Rho GTPases families are present in human, classified in 8 subfamilies based on structure and biological properties

These proteins are crucial regulators of **dynamic cytoskeletal rearrangement and intracellular signaling**:

- Cell cycle progression
- Transcription
- Cell morphology, motility, and polarity



Scala et al., 2021

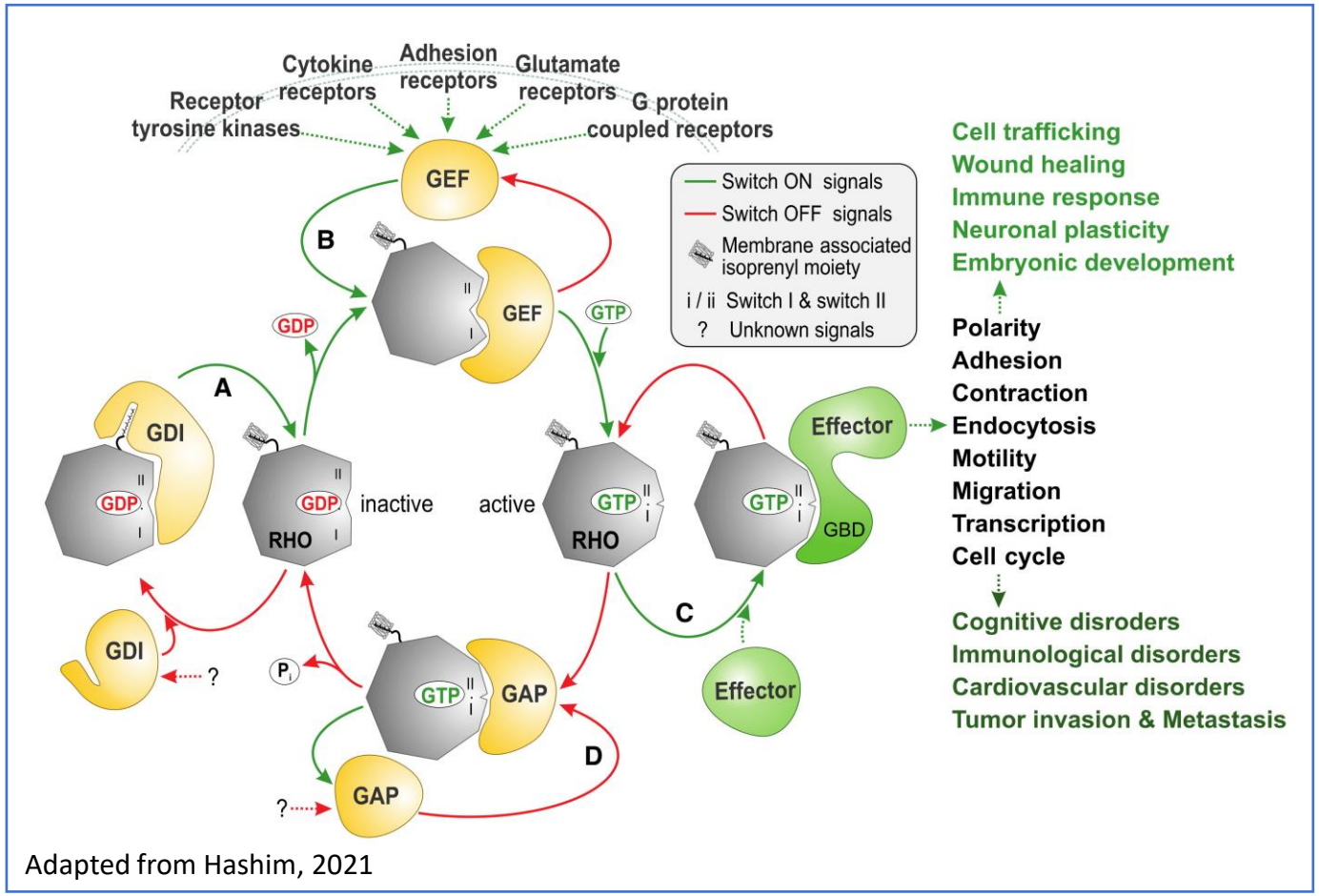
Ahmad Mokhtar, 2021

RAC subfamily

The RAC subfamily consists of four members: RAC1, RAC2, **RAC3** AND RHOG

In the classic **GTPase cycling**, they swing between a GDP-bound (inactive) and a GTP-bound (active) status

GTP hydrolysis and GDP/GTP exchange are mediated by the G domain, composed of **Switch I and Switch II regions**, under the influence of ancillary regulatory proteins (GEFs, GAPs, GDIs)



Adapted from Hashim, 2021

Aspenstrom, 2020; Hashim, 2021

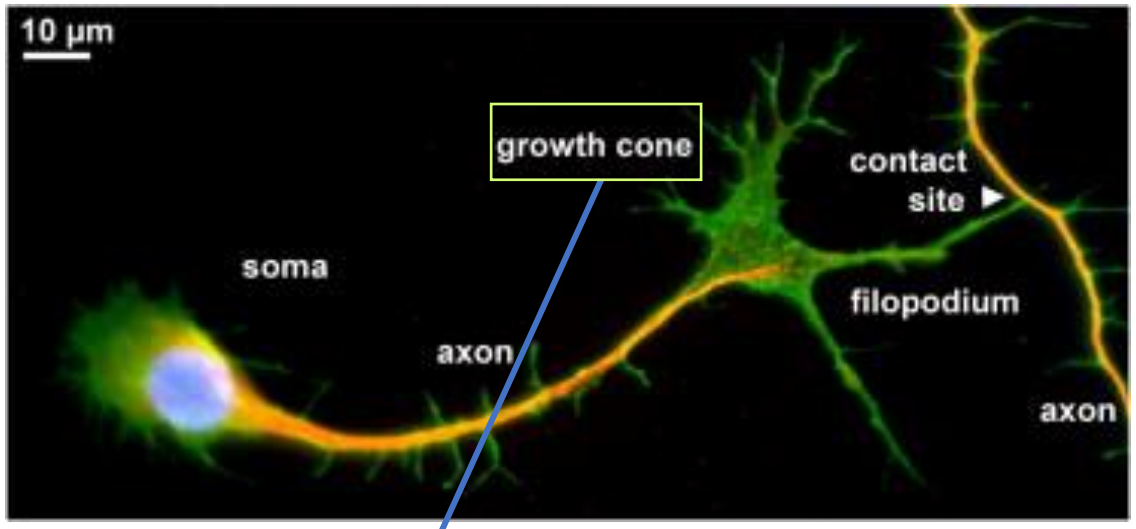
RAC3: biology and function in the brain

The ***RAC3* gene** (MIM *602050) maps to 17q25.3 and encodes a 21.4 kDa protein with 92% overlap to RAC1 and 89% overlap to RAC2

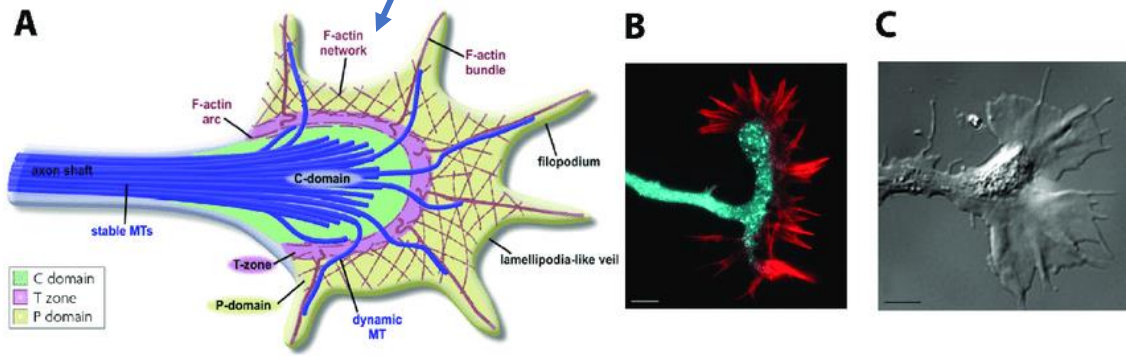
RAC3 is specifically expressed in the brain during its development and, together with RAC1, it is fundamental to regulate the formation of the **lamellipodia**, cytoskeletal structures critical for cell movement and polarity

As such, RAC3 is crucial for:

- **Neuronal migration**
- **Growth cone-mediated neuronal development/maturation**



Polleberg et al., 2013



Adapted from Tomba et al., 2014

Haataja, 1997; Corbetta, 2005

European Reference Networks ITHACA

RAC3 in human disease

NEURODEVELOPMENTAL DISORDER WITH STRUCTURAL BRAIN ANOMALIES AND DYSMORPHIC FACIES; NEDBAF, OMIM # 618577

BRIEF COMMUNICATION | Genetics inMedicine

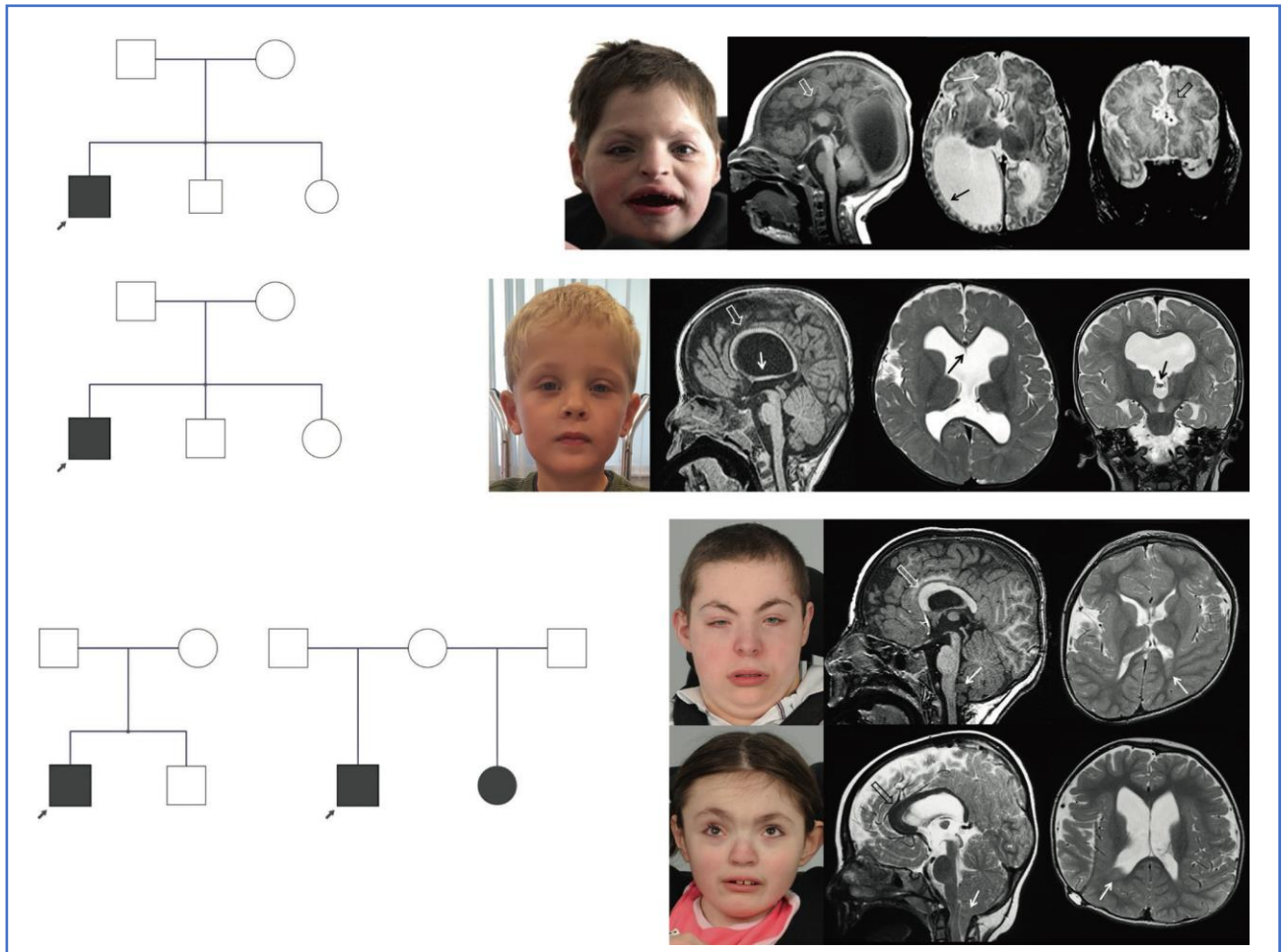
De novo missense variants in *RAC3* cause a novel neurodevelopmental syndrome

Gregory Costain, MD, PhD[1], Bert Callewaert, MD, PhD[2], Heinz Gabriel, MD[3], Tiong Y. Tan, MBBS, PhD[4], Susan Walker, PhD[5,13], John Christodoulou, MBBS, PhD[4,6], Tamas Lazar, MSc[7], Björn Menten, PhD[2], Julia Orkin, MD, MSc[8,9,10], Simon Sadedin, PhD[4], Meaghan Snell, MS[1,11], Arnaud Vanlander, MD[12], Sarah Vergult, PhD[2], Susan M. White, MBBS[4], Stephen W. Scherer, PhD[5,13,14], Robin Z. Hayeems, PhD[10,11], Susan Blaser, MD[15], Shoshana J. Wodak, PhD[7], David Chitayat, MD[1,16], Christian R. Marshall, PhD[5,11,17,18] and M. Stephen Meyn, MD, PhD[1,8,11,14,19]

In 2018, Costain et al. reported **the first four RAC3 families**, cosisting of 5 affected individuals

All subjects harbored ***de novo* missense variants** in *RAC3* and showed developmental delay, hypotonia, and **brain abnormalities**

However, no functional investigation was performed



Adapted from Costain et al., 2018

Reference Networks ITHACA

NEURODEVELOPMENTAL DISORDER WITH STRUCTURAL BRAIN ANOMALIES AND DYSMORPHIC FACIES; NEDBAF, OMIM # 618577

Journal of Human Genetics (2019) 64:1127–1132
https://doi.org/10.1038/s10038-019-0656-7

BRIEF COMMUNICATION

A *de novo* variant in *RAC3* causes severe global developmental delay and a middle interhemispheric variant of holoprosencephaly

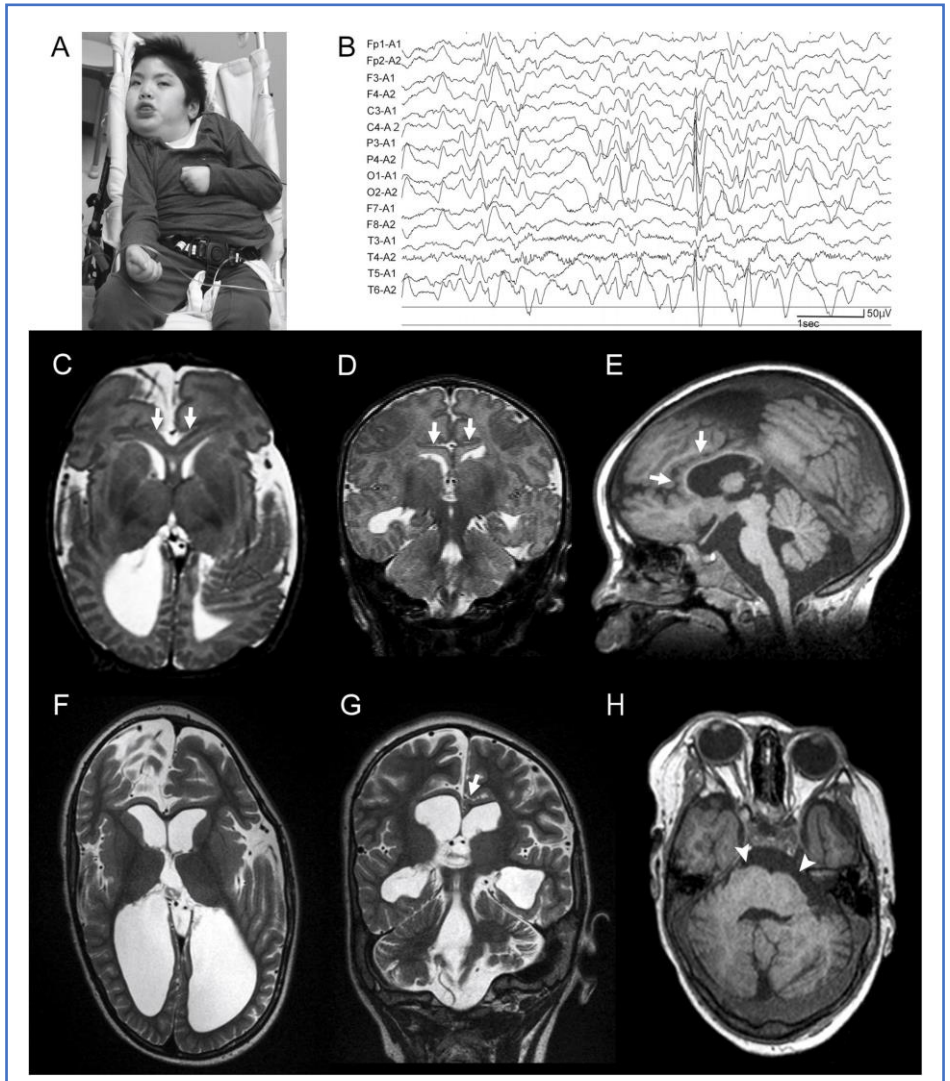
Takuya Hiraide[1] · Hikari Kaba Yasui[2] · Mitsuhiro Kato[3] · Mitsuko Nakashima[1] · Hirotomo Saitsu[1]

Received: 23 May 2019 / Revised: 22 July 2019 / Accepted: 4 August 2019 / Published online: 16 August 2019

In 2019, Hiraide et al. reported **an additional case**

This subject showed severe global **developmental delay**, intellectual disability, **epilepsy**, and laryngeal dystonia

His brain MRI showed **brain dysplasia**, including coexistence of interhemispheric variant of holoprosencephaly and brainstem dysmorphism

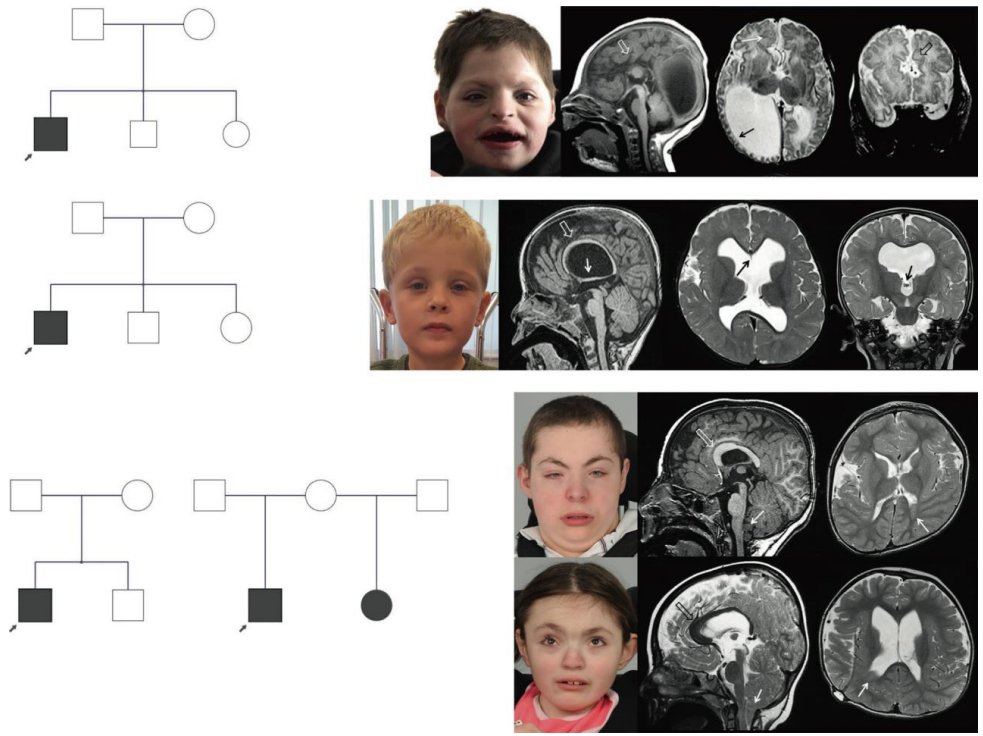


Adapted from Hiraide et al., 2019

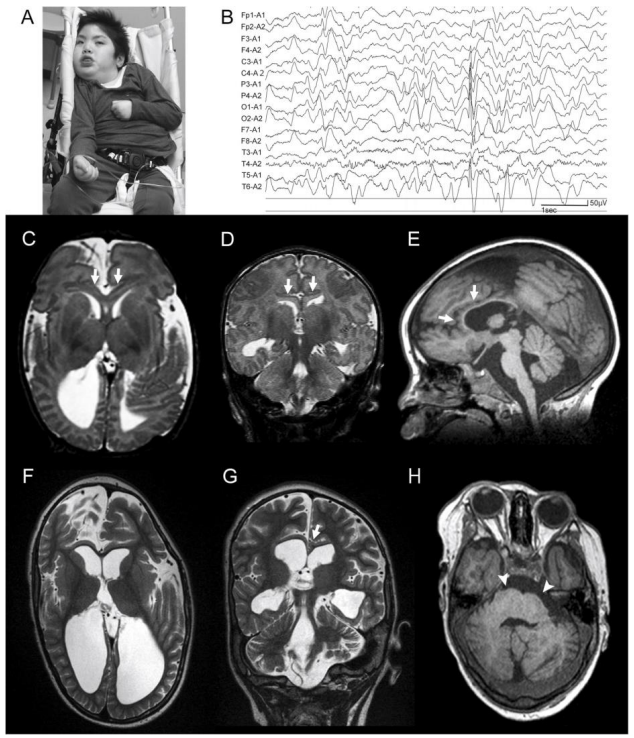
NEURODEVELOPMENTAL DISORDER WITH STRUCTURAL BRAIN ANOMALIES AND DYSMORPHIC FACIES; NEDBAF, OMIM # 618577

These reports strongly pointed to the relevance of **RAC3 variants** as the cause of a **novel NDD**

However, due to the limited clinical information and the absence of supporting functional evidence, **the actual disease spectrum and its pathophysiology remained largely elusive**



Costain et al., 2018



Hiraide et al., 2019

NEDBAF: disease spectrum and pathophysiology

NEDBAF: phenotypic spectrum and pathophysiology

In this study, we investigated a significant cohort of **ten individuals with NEDBAF**

We dissected the genetic heterogeneity of RAC3 variants, delineated the phenotypic spectrum of NEDBAF, and functionally explored the impact of RAC3 variants on corticogenesis

https://doi.org/10.1093/brain/awac106 BRAIN 2022: 00; 1–20 | 1

BRAIN
ORIGINAL ARTICLE

Variant-specific changes in RAC3 function disrupt corticogenesis in neurodevelopmental phenotypes

Marcello Scala,[1,2,3,†] Masashi Nishikawa,[3,†] Hidenori Ito,[3,†] Hidenori Tabata,[3] Tayyaba Khan,[4] Andrea Accogli,[1] Laura Davids,[5] Anna Ruiz,[6] Pietro Chiurazzi,[7,8] Gabriella Cericola,[9] Björn Schulte,[10] Kristin G. Monaghan,[11] Amber Begtrup,[11] Annalaura Torella,[12,13] Michele Pinelli,[12] Anne-Sophie Denommé-Pichon,[14,15,16] Antonio Vitobello,[14,15] Caroline Racine,[15,16] Maria Margherita Mancardi,[17] Courtney Kiss,[18] Andrea Guerin,[18] Wendy Wu,[4,19] Elisabeth Gabau Vila,[20] Bryan C. Mak,[21] Julian A. Martinez-Agosto,[21,22,23] Michael B. Gorin,[21,24,25] Bugrahan Duz,[26] Yavuz Bayram,[27,28] Claudia M. B. Carvalho,[29,30] Jaime E. Vengoechea,[5] David Chitayat,[31,32,33] Tiong Yang Tan,[34] Bert Callewaert,[35] Bernd Kruse,[9] Lynne M. Bird,[36,37] Laurence Faivre,[14,16] Marcella Zollino,[7,8] Saskia Biskup,[10,38] Undiagnosed Diseases Network, Telethon Undiagnosed Diseases Program, Pasquale Striano,[1,2] Vincenzo Nigro,[12,13] Mariasavina Severino,[39] Valeria Capra,[40] Gregory Costain[4,32,33,41] and Koh-ichi Nagata[3,42]

[†]These authors contributed equally to this work.

Study cohort

Affected individuals presented with **neurodevelopmental phenotypes** featuring:

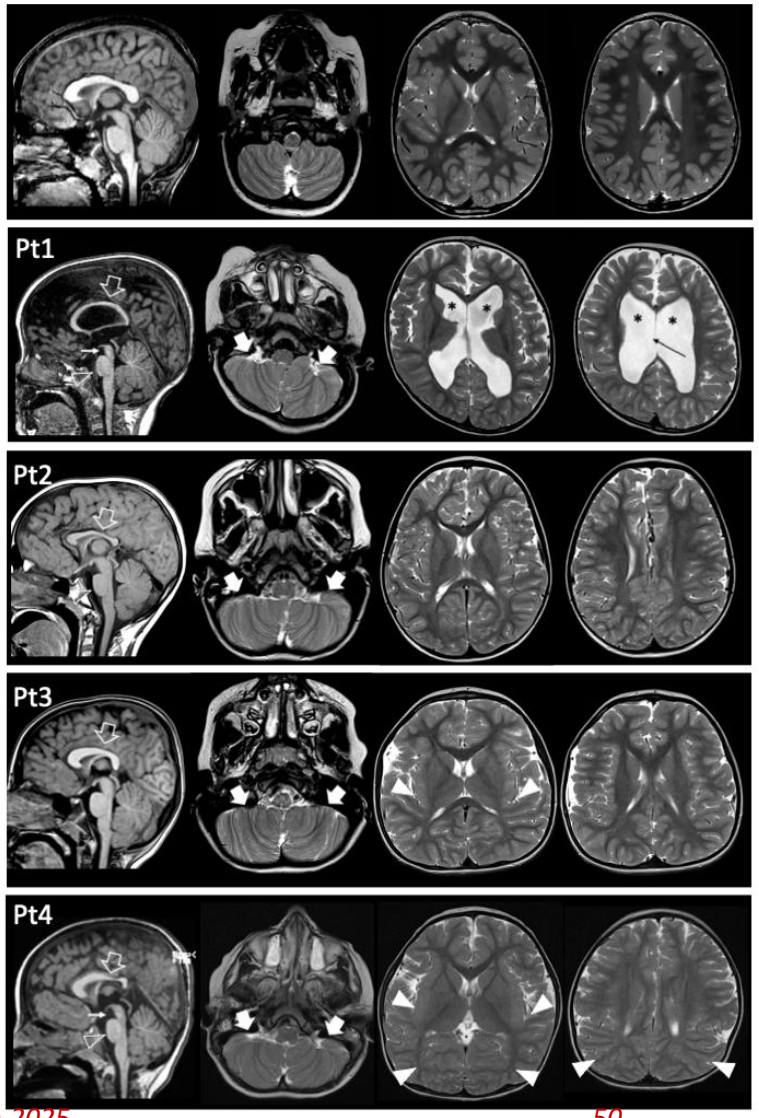
- Developmental delay (global)
- Hypotonia
- Abnormal behavior – stereotypies
- Dysmorphism
- Seizures
- Musculoskeletal defects

Scala et al., 2021

Neuroimaging

Brain MRI revealed a spectrum of brain abnormalities suggestive of **malformations of cortical development (MCDs)**:

- Corpus callosum anomalies
- White matter thinning
- Nodular heterotopia
- Dysgyria/polymicrogyria
- Cerebellar dysplasia



Scala et al., 2021

Case study

Case #9, 2 yo

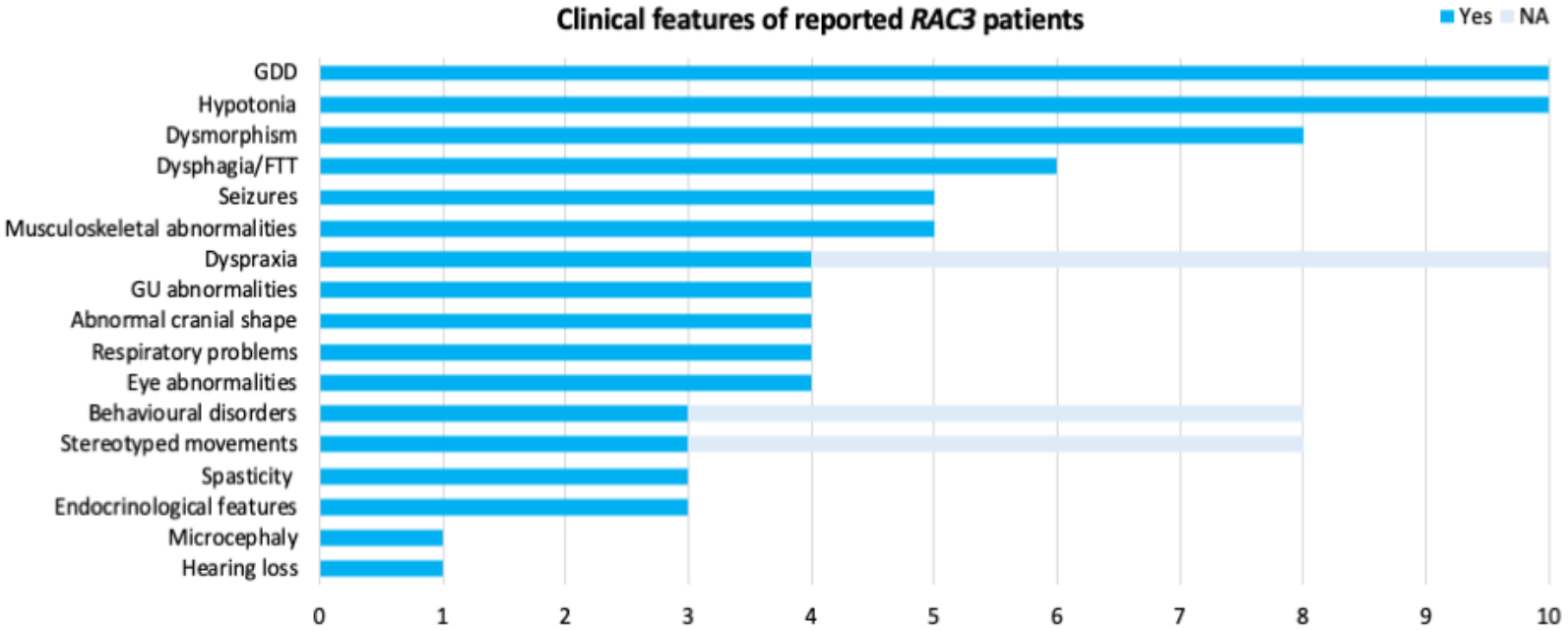
- Developmental delay
- Generalized hypotonia
- Stereotyped movements (mouth and upper limbs)
- Stereotyped deep breathing
- Stereotyped guttural sounds
- Hand mouthing
- Eyelid myoclonia

Scala et al., 2021

Phenotypic spectrum

Overall, **NEDBAF is a complex NDD** characterized by:

- Global psychomotor delay
- Cognitive deficiency (severe-profound)
- Dysmorphism
- Feeding difficulties
- Hypotonia
- Seizures
- **MCDs** (CCH, dysgyria/PMG, heterotopia, etc.)



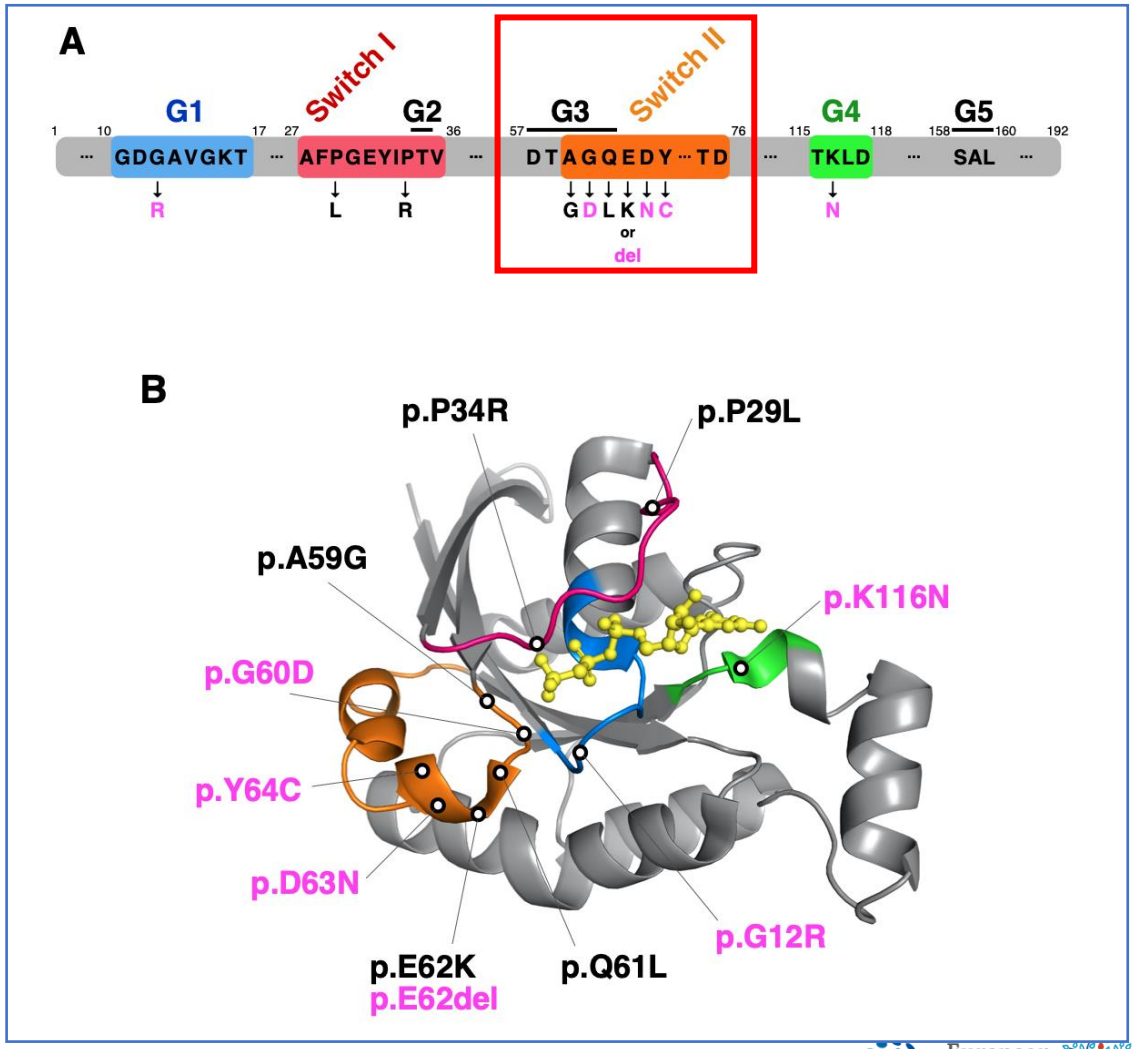
Scala et al., 2021

Genotype spectrum

Through ES, we identified **8 different *de novo RAC3* variants** (NM_005052.3), of which **6 were novel (purple in the figure)**

Seven changes affected the **Switch II region**, that is important for interactions with RAC3 effectors

This region is also a **mutational hot spot** in *RAC1*-, *RAC3*- e *CDC42*-related disorders (Reijnders *et al.*, 2017; Martinelli *et al.*, 2018)



Scala et al., 2021

Functional investigation: lamellipodia

Transfected hippocampal neurons show round shape with lamellipodia, but ↓ **neurite extension**

These effects were **variant-specific**, with P29L, -A59G, -G60D, and -K116N occasionally showing neurite extension

All variants facilitate **lamellipodia formation** and **cytoskeletal reorganization** (**gain of function – GoF**)

Structural and functional neuronal defects

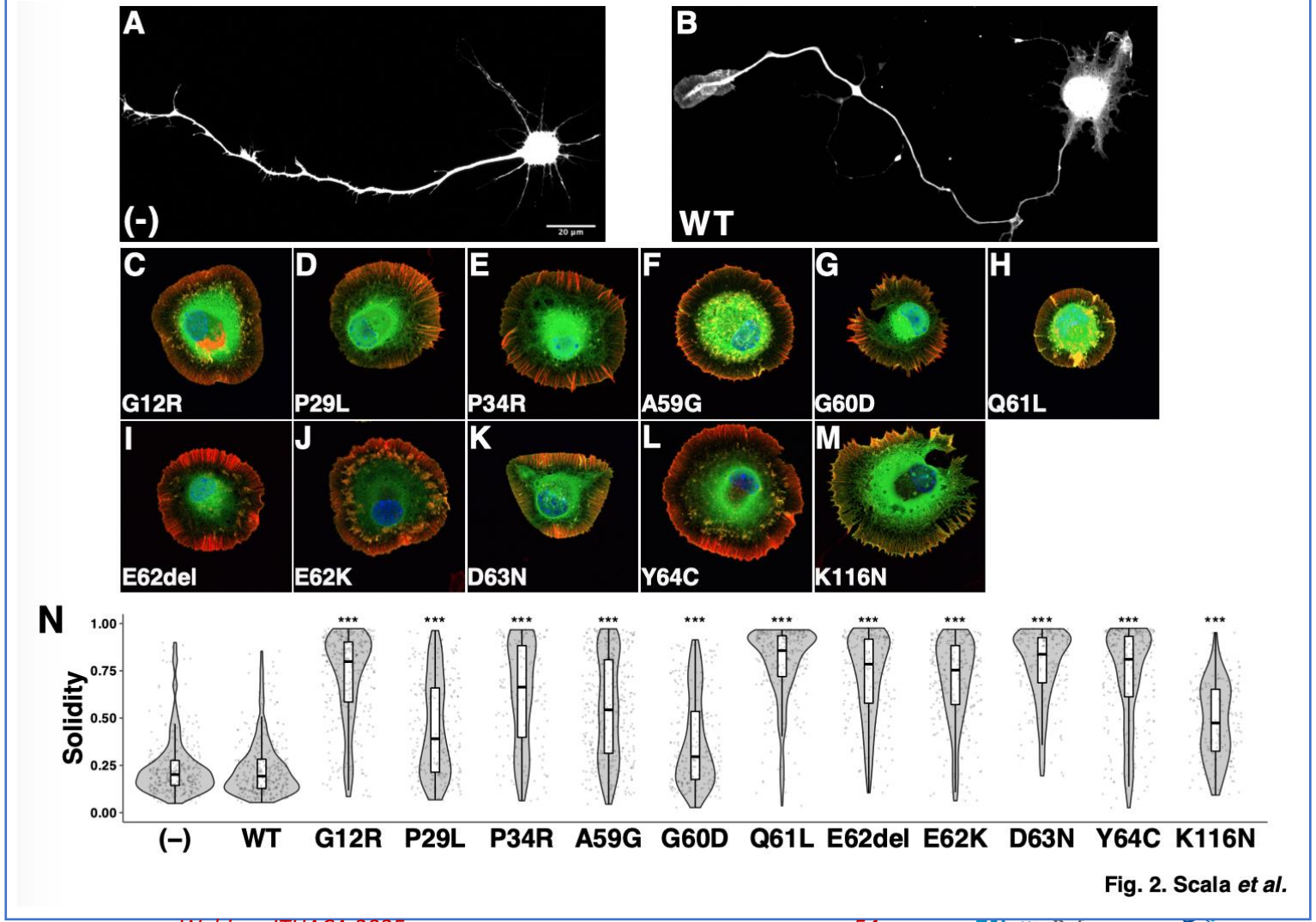


Fig. 2. Scala *et al.*

Reference Networks ITHACA

Functional investigation: GTPase activity

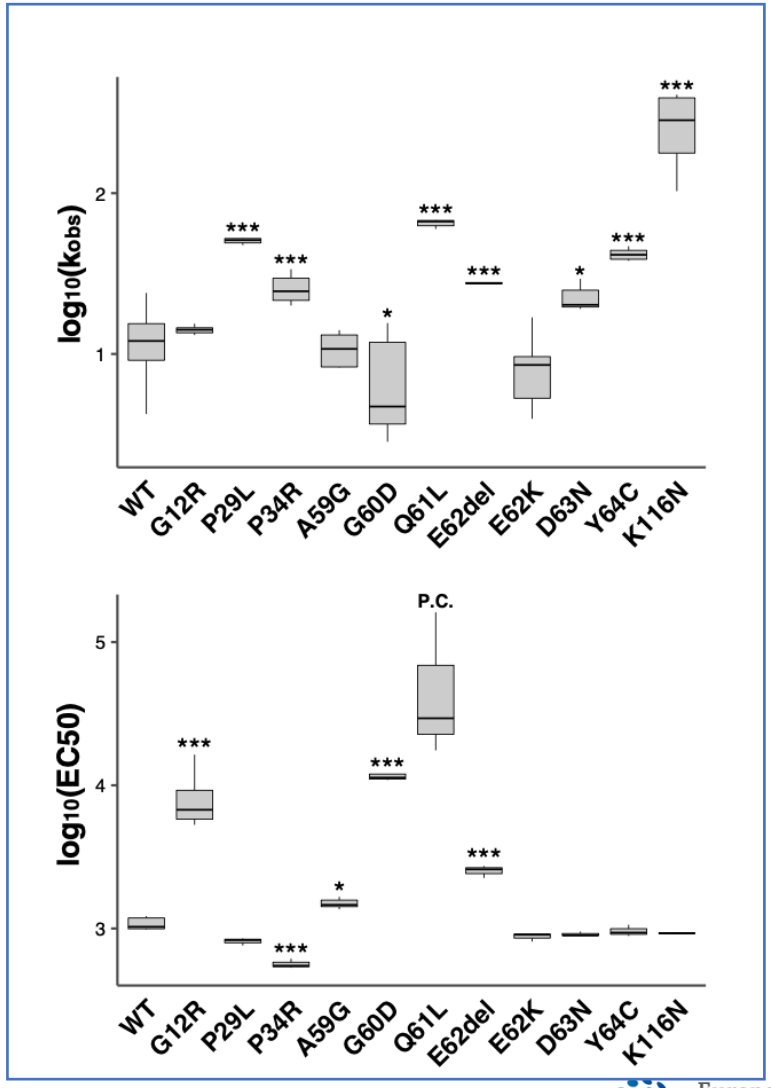
Different variants cause different defects in the **GDP/GTP exchange**, allowing us to classify them in **three subgroups**:

1) Group I (P29L, P34R, Q61L, E62del, D63N, Y64C, K116N): ↑ exchange activity

2) Group II (G12R, A59G, and E62K): normal exchange activity

3) Group III (G60D): preference for GTP binding due to ↓ GTP hydrolysis and exchange activity rate

	WT	G12R	P29L	P34R	A59G	G60D	Q61L	E62del	E62K	D63N	Y64C	K116N
GTP-loading	→	→	↑↑	↑	→	↓	↑↑	↑	→	↑	↑↑	↑↑↑
GTPase	→	↓↓	→	↑	↓	↓↓↓	↓↓↓	↓	→	→	→	→

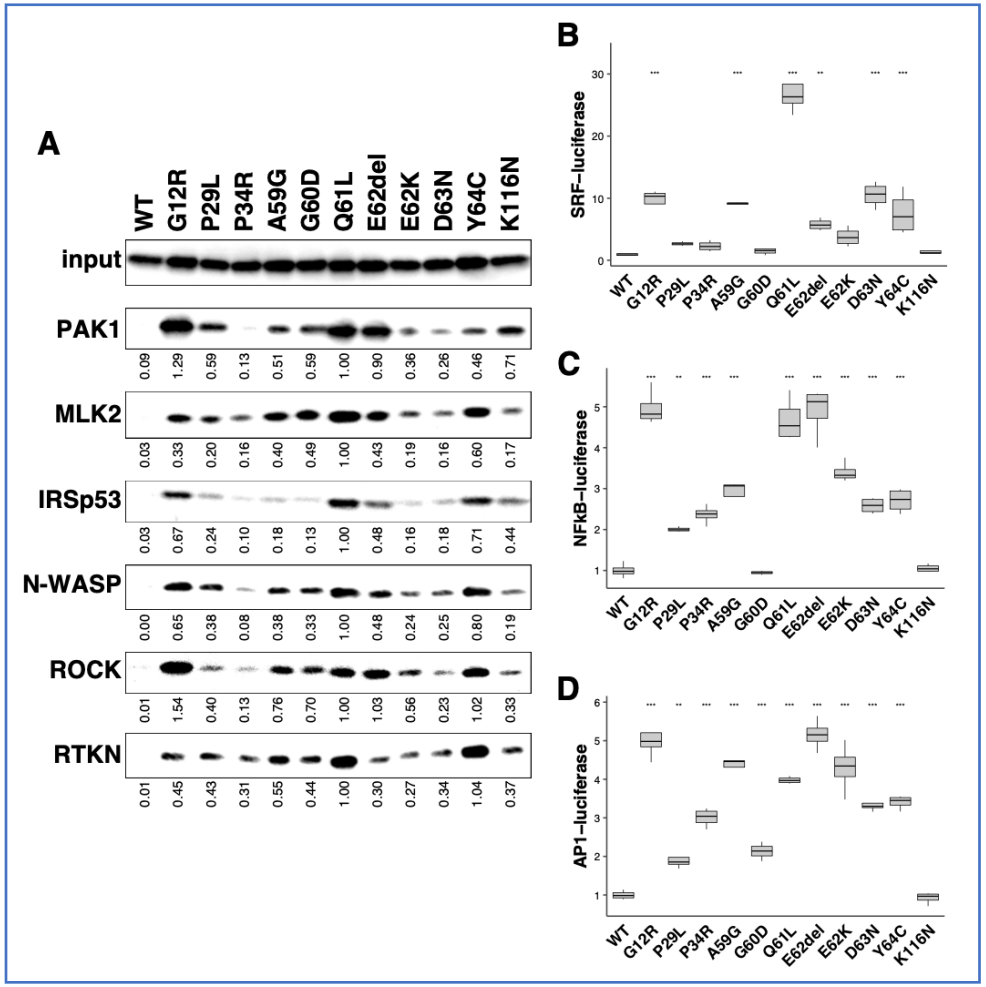


Functional investigation: downstream effectors

Clinical heterogeneity is reflected into **molecular heterogeneity**

Each variant affects the interactions with RAC3 **downstream signaling pathways** in a distinct way

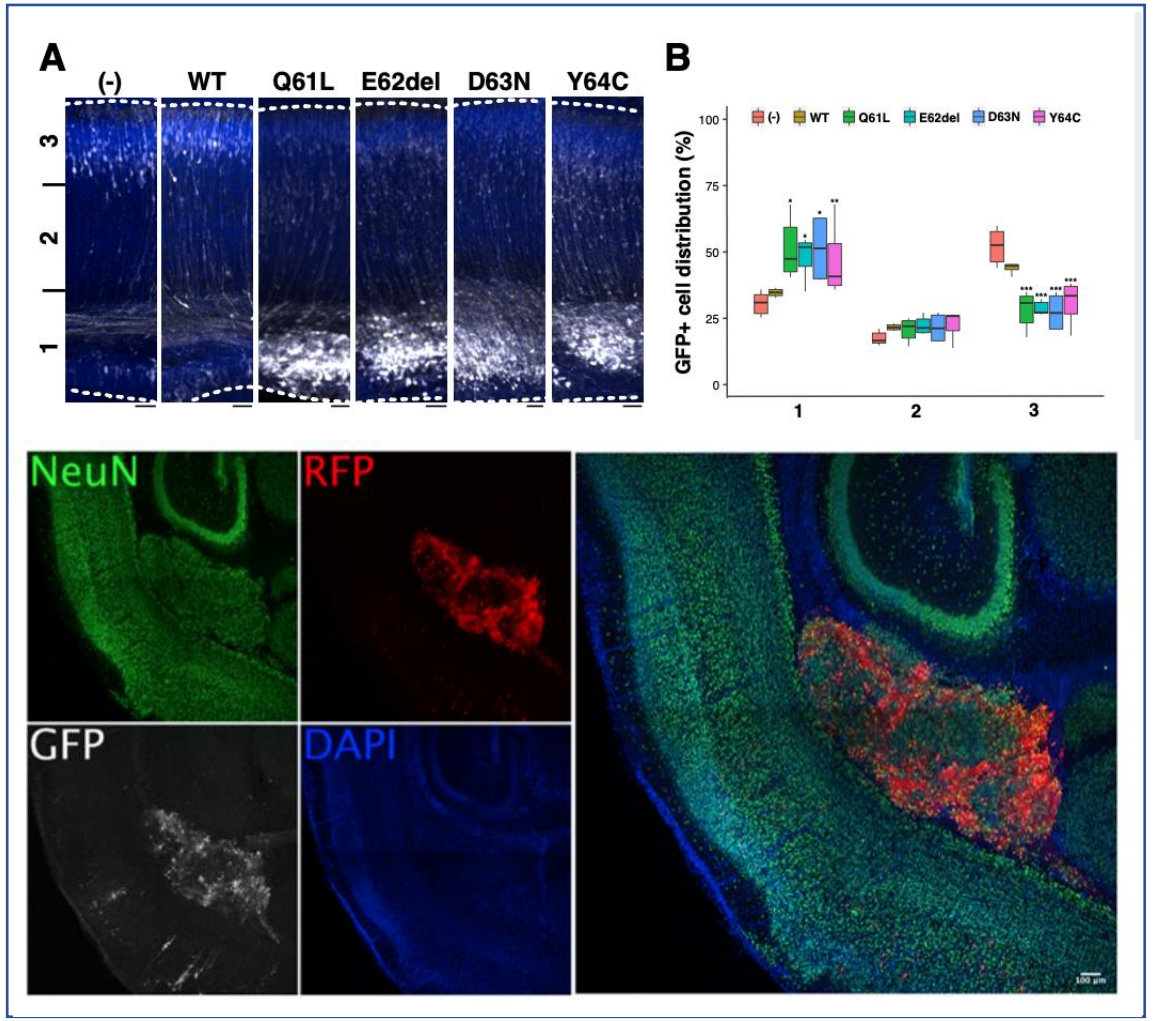
Each variant may affect **similar or different** signaling pathways



Functional investigation: cortical migration

RAC3 plays a pivotal role in the physiological **migration** of neuronal progenitors during corticogenesis, from VZ to cortical plate

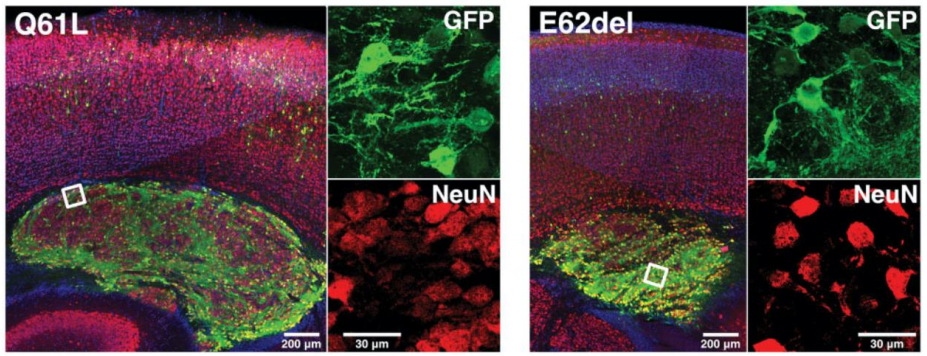
RAC3 mutant neurons are **not able to migrate properly**, leading to the formation of clusters in the VZ/SVZ and IZ



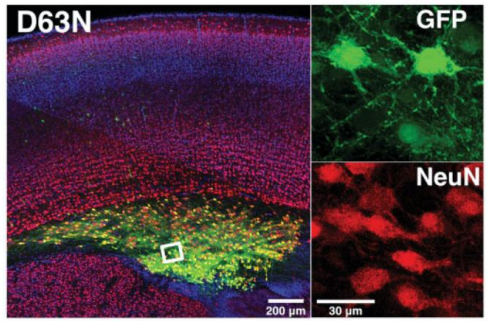
Functional investigation: cortical migration

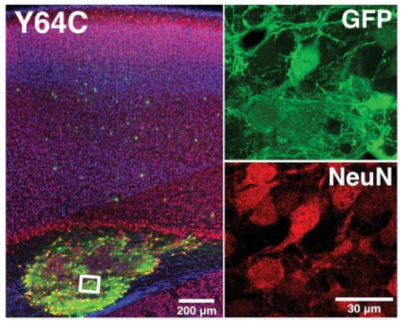
Clinically, this reflects into the spectrum of MCDs observed in NEDBAF:

- Dysgyria
- Polymicrogyria
- Nodular heterotopia

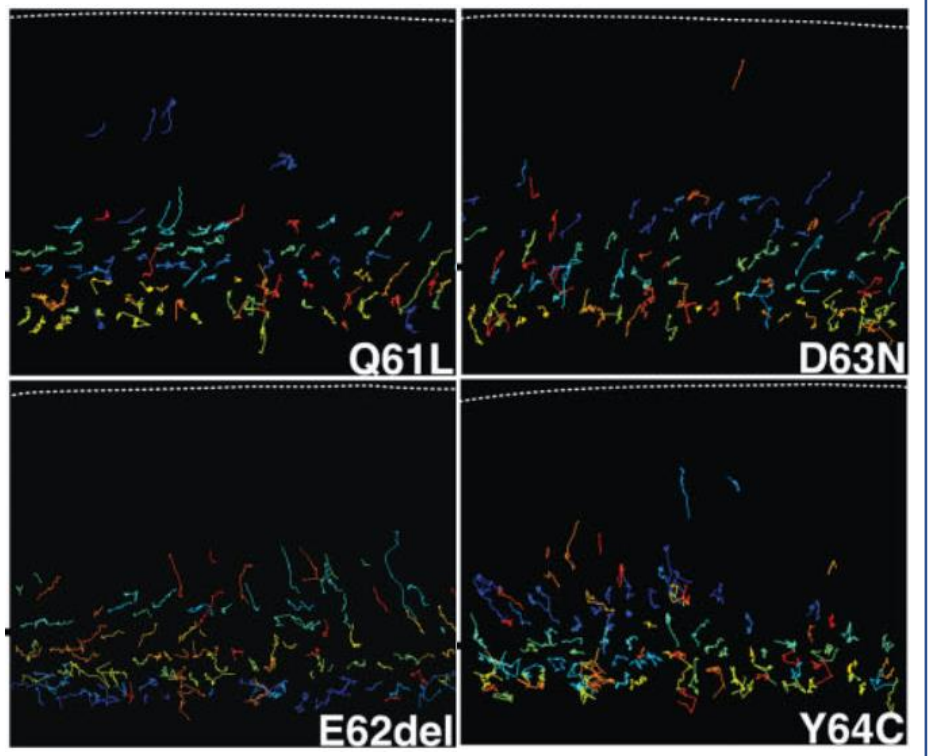


Retained islets of neuronal progenitors (heterotopia)





Time-lapse imaging analyses of migration of mutant cortical neurons

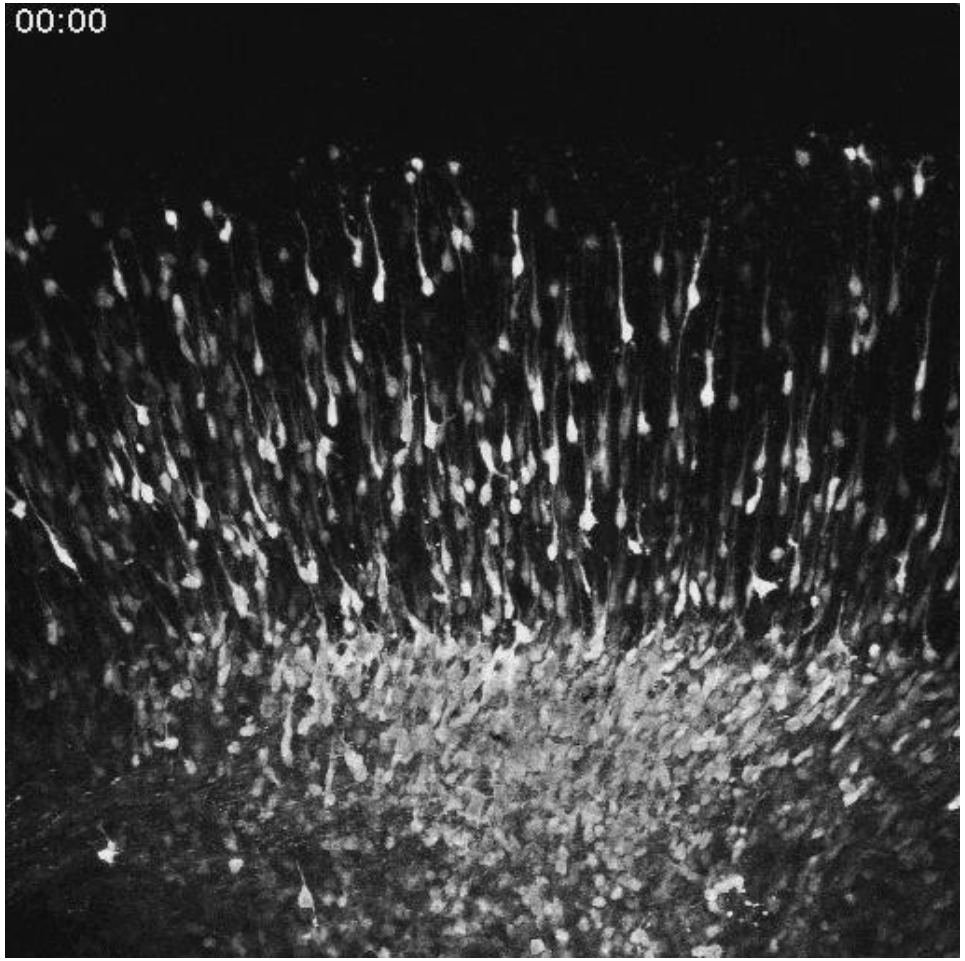


Corticogenesis: neuronal migration

Newborn **wildtype cortical neurons** in the ventricular zone (VZ) show multipolar shape in the lower intermediate zone (IZ), with:

- Slow and irregular movement (multipolar movement)
- Bipolar shaping (upper IZ)
- Radial migration with saltatory movement*

*extension of the leading process and translocation of the cell body (Tabata and Nakajima, 2003)

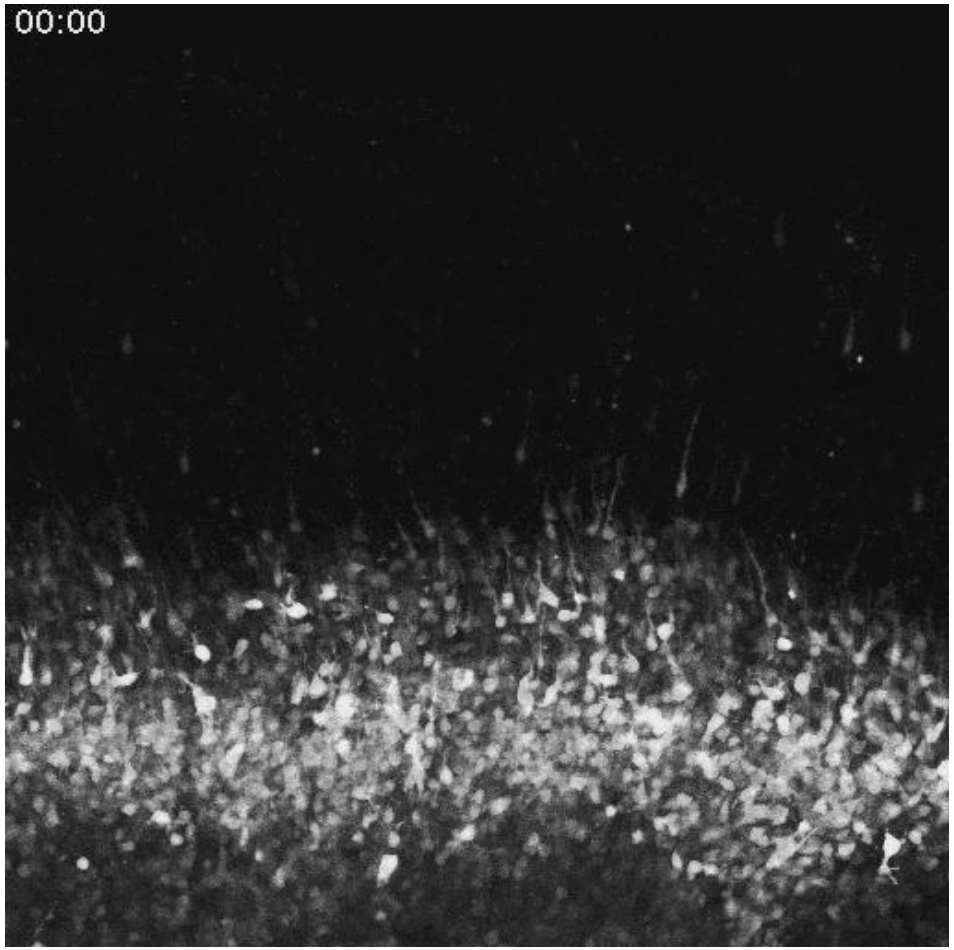


Scala et al., 2021

RAC3 variants affect neuronal maturation

Instead, *RAC3* mutant neurons show **significant abnormalities in their maturation**:

- Q61L, E62del, D63N, and Y64C mutants remain **stuck in the IZ**
- E62del and D63N mutants **fail to acquire multipolar shape** and remain round

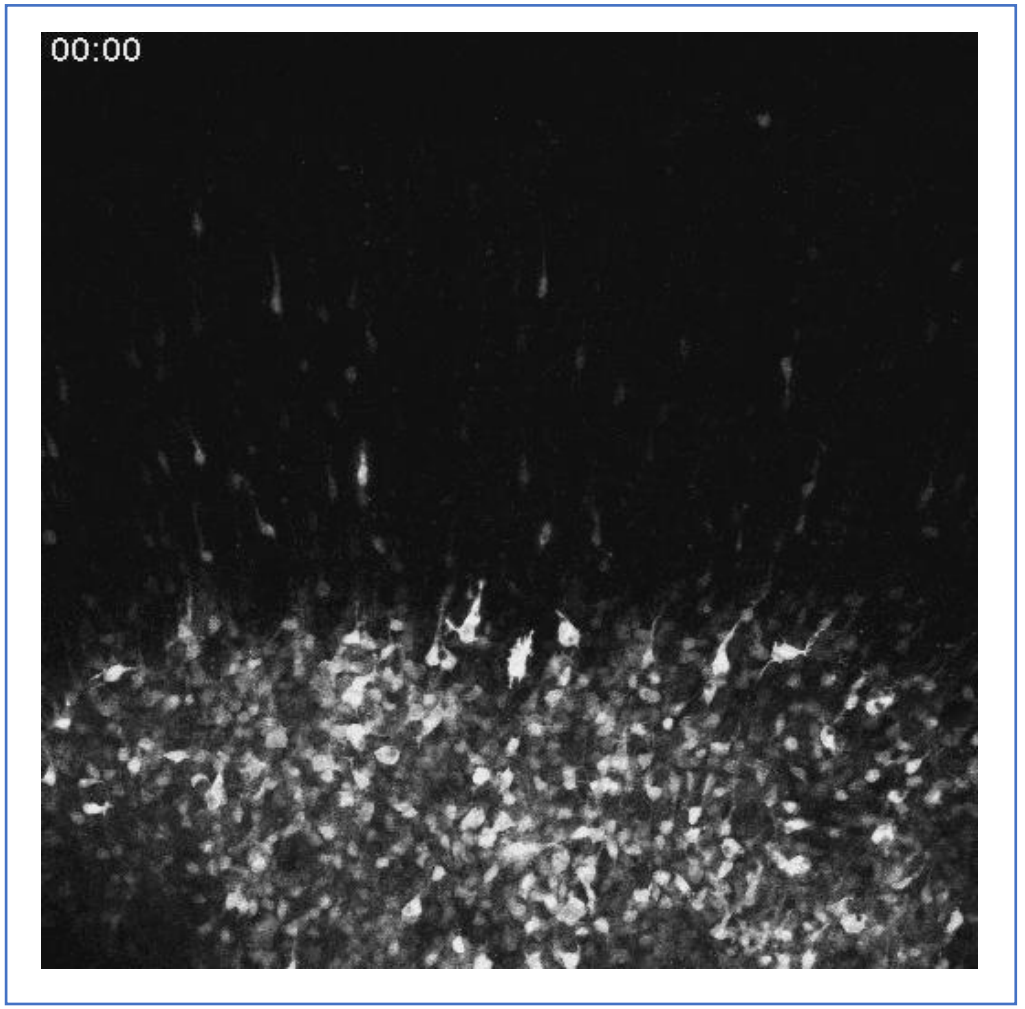


Scala et al., 2021

RAC3 variants affect neuronal maturation

The effects of RAC3 variants on neuronal maturation are also **variant-specific**

Neurons transfected with Q61L or Y64C variants become multipolar but **fail the transition to bipolar shape**



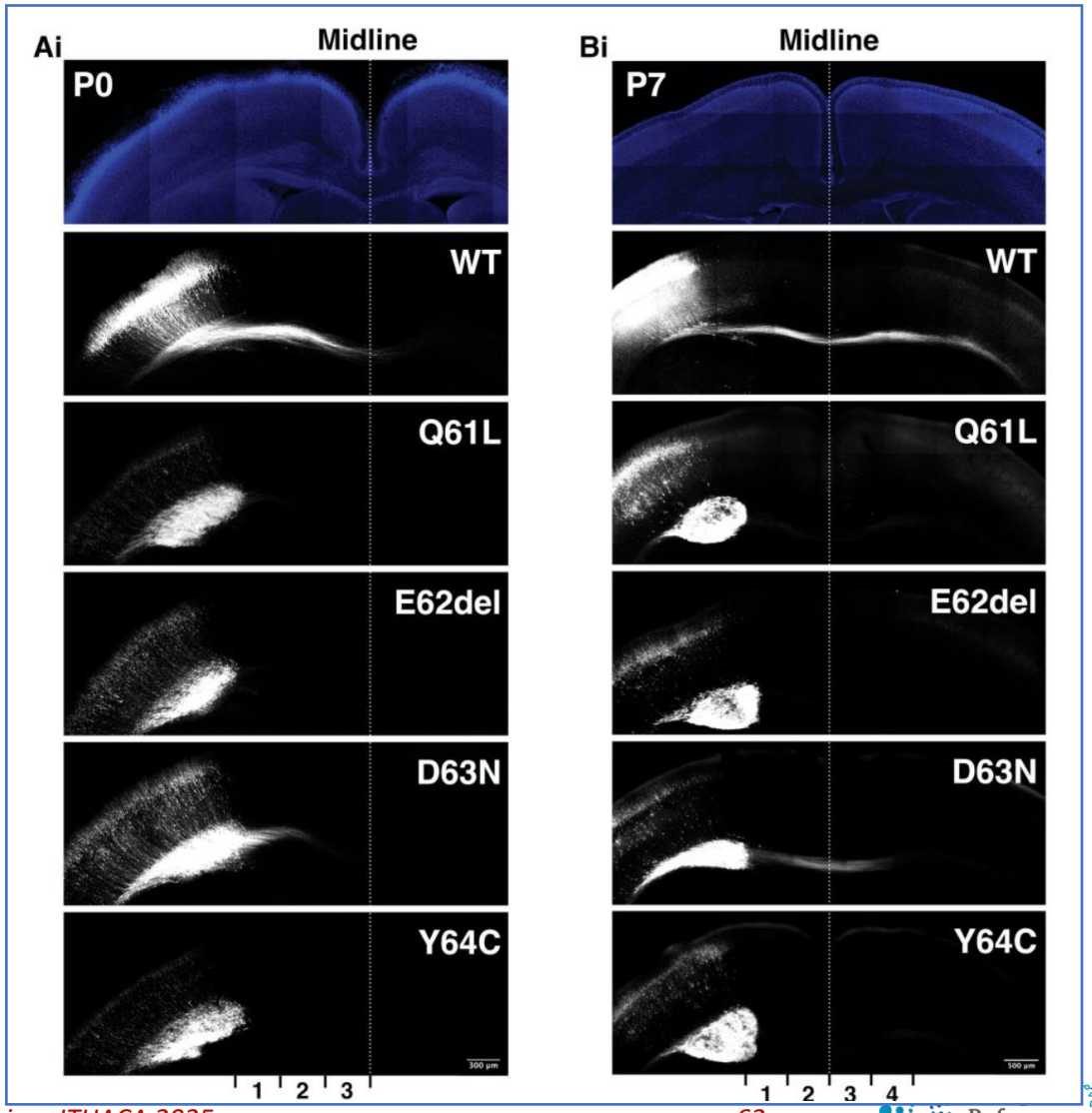
Scala et al., 2021

Functional investigation: Axonogenesis

RAC3 is important for **axonal elongation**, which underlies the development of white matter

RAC3 mutant neurons are **unable to project** their axons or can only project a thin bundle

Abornmalities in controlateral axon bundle projection explain the **white matter disorders** observed in NEDBAF, especially **corpus callosum dysgenesis**



Characterizing single variants: why?

The F28S variant

Neurogenetics

Original research

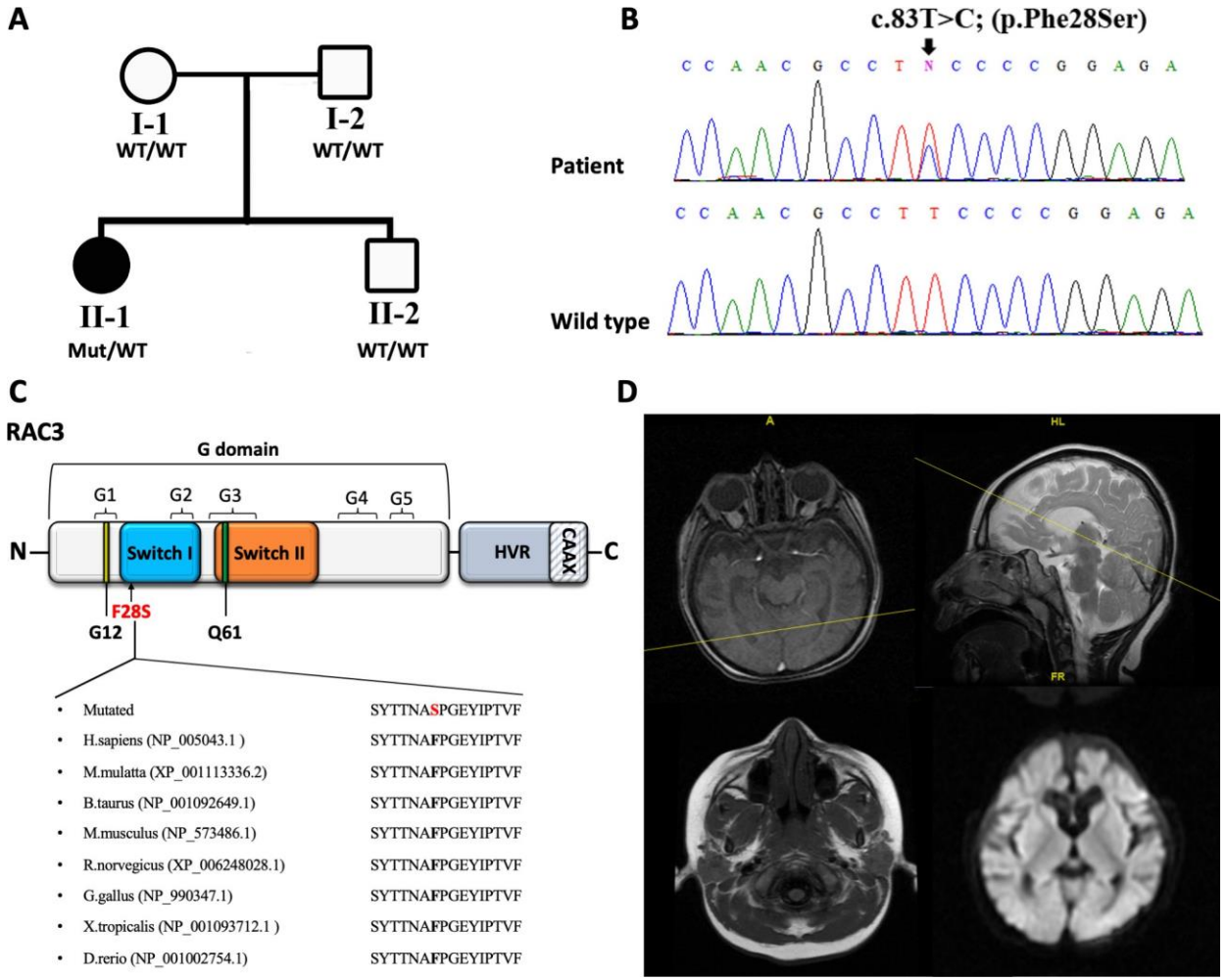
Gain-of-function p.F28S variant in *RAC3* disrupts neuronal differentiation, migration and axonogenesis during cortical development, leading to neurodevelopmental disorder

Masashi Nishikawa,[1] Marcello Scala,[2,3] Muhammad Umair ,[4,5] Hidenori Ito,[1] Ahmed Waqas,[6] Pasquale Striano ,[2,3] Federico Zara,[7] Gregory Costain ,[8] Valeria Capra,[7] Koh-ichi Nagata [1,9]

In this study, we identified and characterized a novel *RAC3* variant in the **Switch I domain**

The switch I and II domains contain the consensus binding sites for regulatory proteins and effectors

This 13yo patient had **NEDBAF** with delayed myelination, cerebral volume loss (WM++), and hypoplasia of the corpus callosum

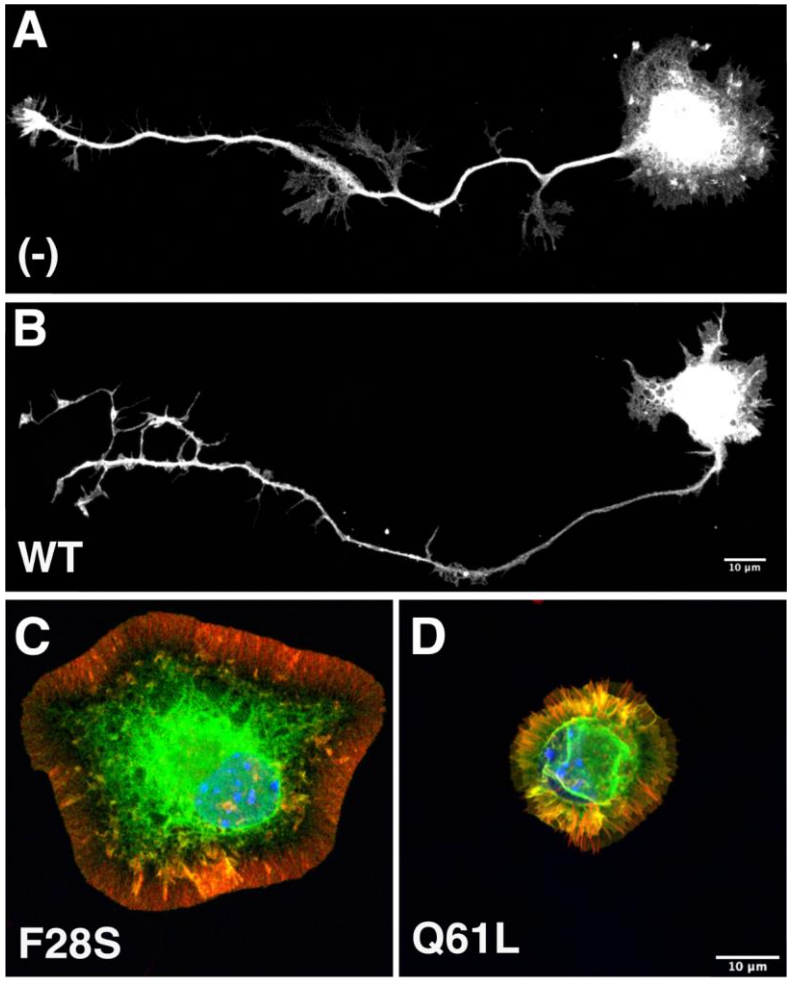


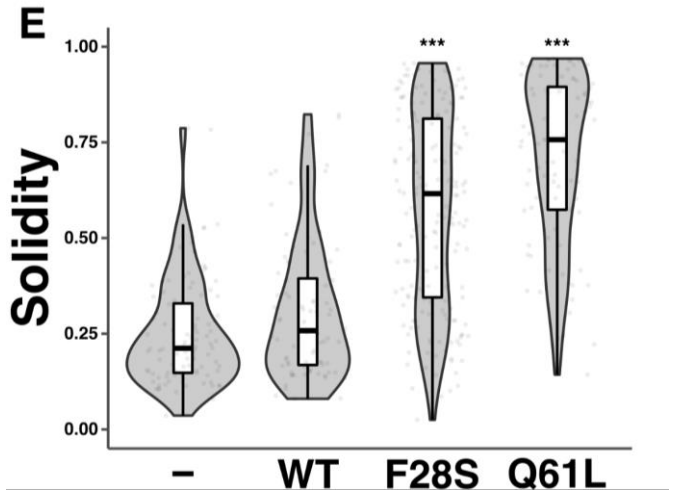
Lamellipodia formation

Hippocampal neurons electroporated with **RAC3-F28S** displayed cell rounding and lamellipodia formation, with increased solidity and defective differentiation

Compared to RAC3-Q61L, RAC3-F28S only **moderately facilitate** cytoskeletal reorganisation to form **lamellipodia**

However, **this mild impact is sufficient** to disrupt cell-signalling and impair neuronal morphology and function



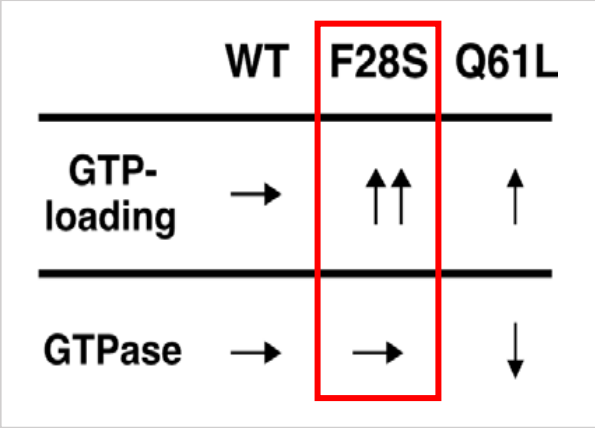


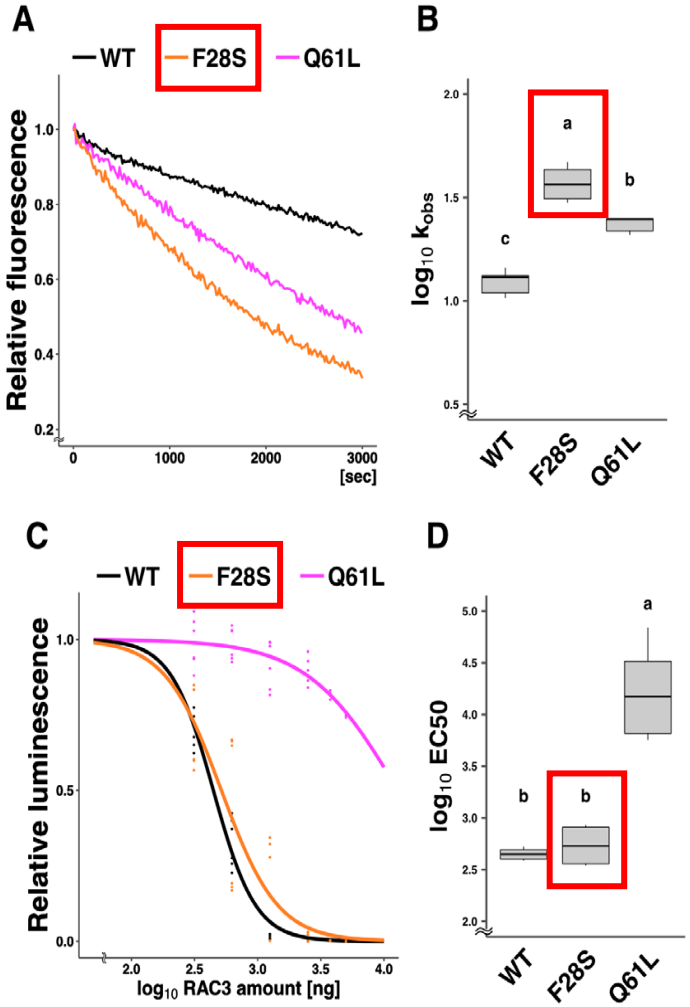
Biochemical activity

We then measured intrinsic GTP/GDP-exchange and GTP- hydrolysis activities:

- **RAC3-F28S** significantly **accelerates GDP/GTP-exchange** (> Q61L)
- RAC3-F28S slightly affects GTP hydrolisis (<< Q61L)

Thus, **RAC3-F28S acts as a GOF variant** through a higher GTP/GDP-exchange activity, despite normal intrinsic GTP-hydrolysis





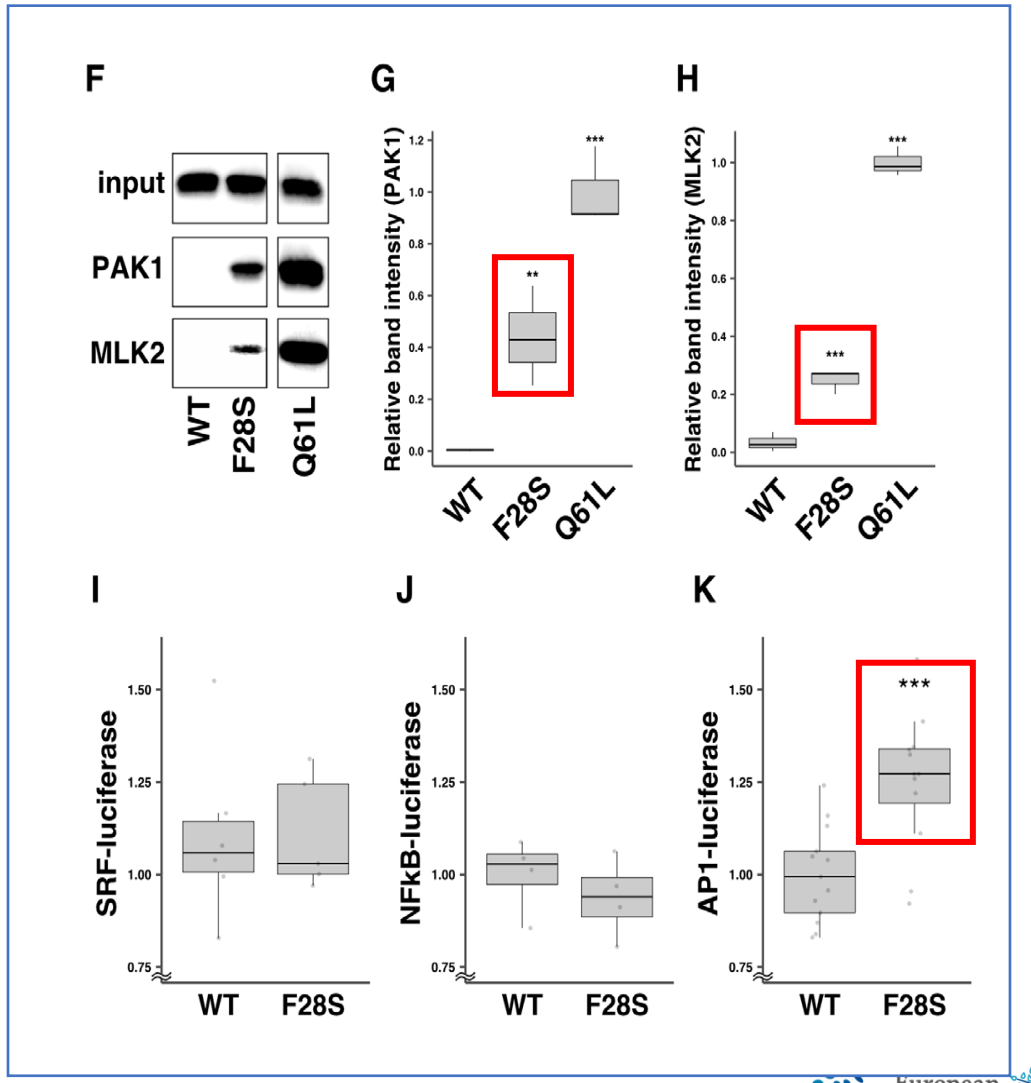
Impact on effectors

PAK1 (crucial for neuronal migration and mutated in NDDs) and **MLK2** (activator of JNK-MAP kinase downstream signalling pathways) are downstream effectors of RAC3

RAC3-F28S hyperactivates PAK1-mediated and/or MLK2-mediated signalling

Rho-family proteins contribute to regulate SRF-mediated, NF-κB-mediated, and AP1-mediated gene expression

RAC3-F28S dysregulates AP1-mediated signalling pathways

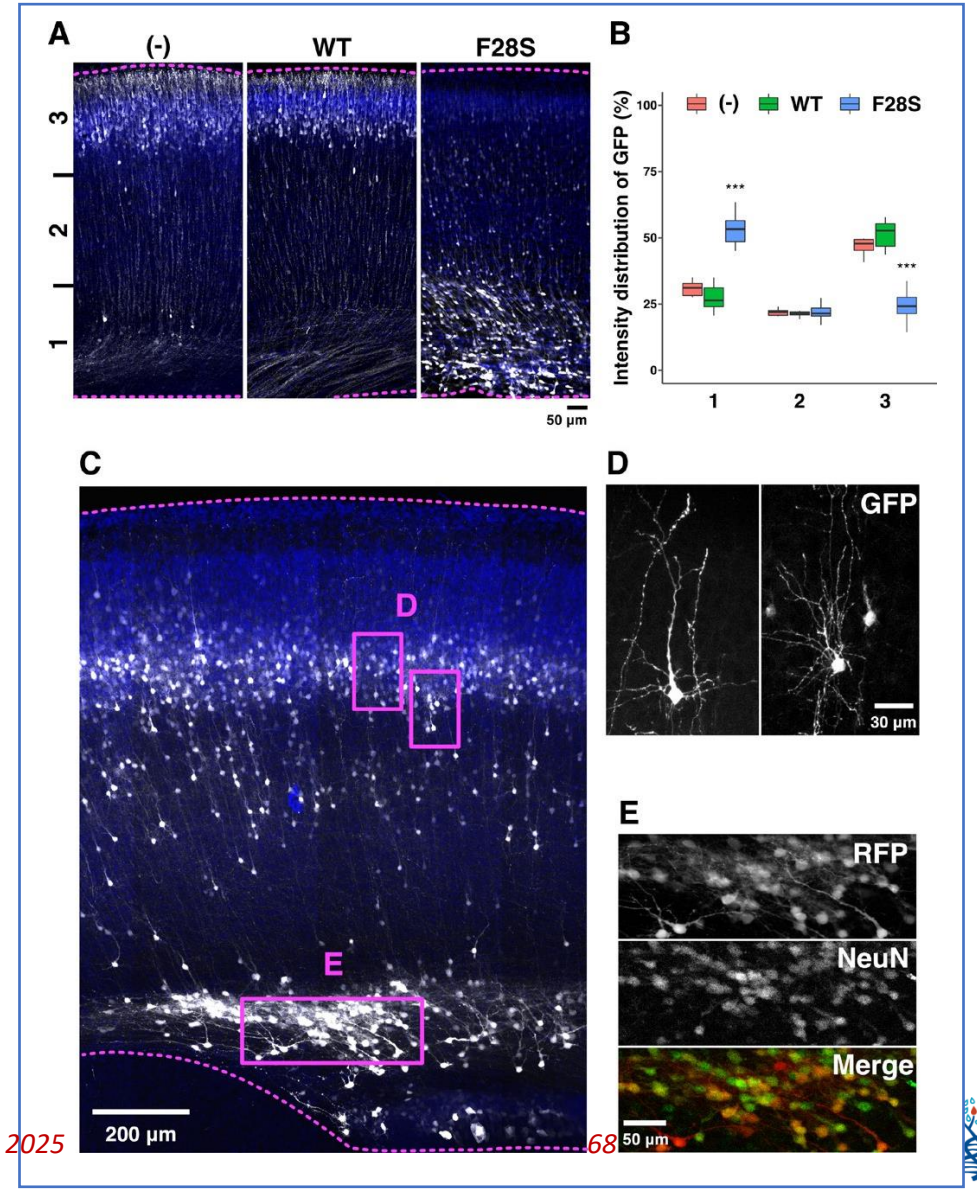


Impaired migration

The majority of RAC3-F28S-expressing cells **remain** in the ventricular and subventricular zones (VZ/SVZ) and the intermediate zone (IZ) (bin 1)

Cells incorporating high amount of the expression vector remained in the IZ and were positive for NeuN, indicating that they were **differentiate at abnormal positions and extended neurites**

These results strongly suggest that **RAC3-F28S dysregulates neuronal migration and development during corticogenesis**

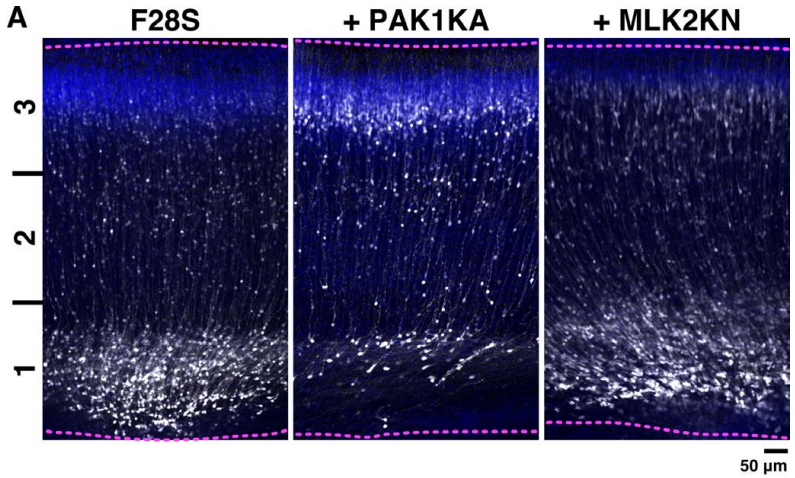


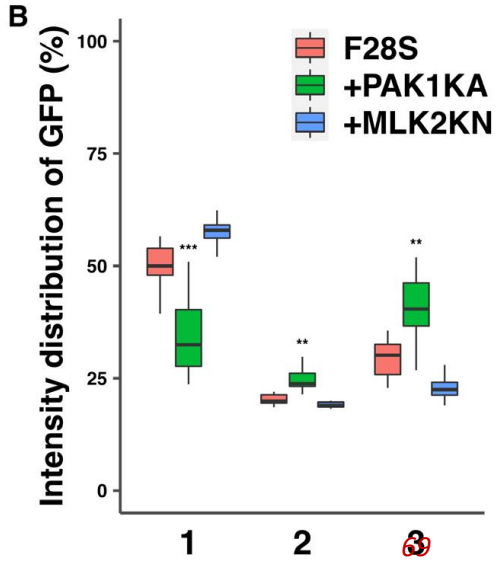
Role of PAK1 in NEDBAF

Since **RAC3-F28S** interacts with **PAK1**, which is also associated with an NDD, we analysed the possible involvement of PAK1 in the migration defects caused by RAC3-F28S

Co-electroporation with kinase-negative PAK1 (but not MLK2) **rescued** the positional defects of mutants

Thus, the **hyperactivation of PAK1 signalling** by RAC3-F28S is **responsible** for the neuronal migration defects and contributes to determine the NDD phenotype



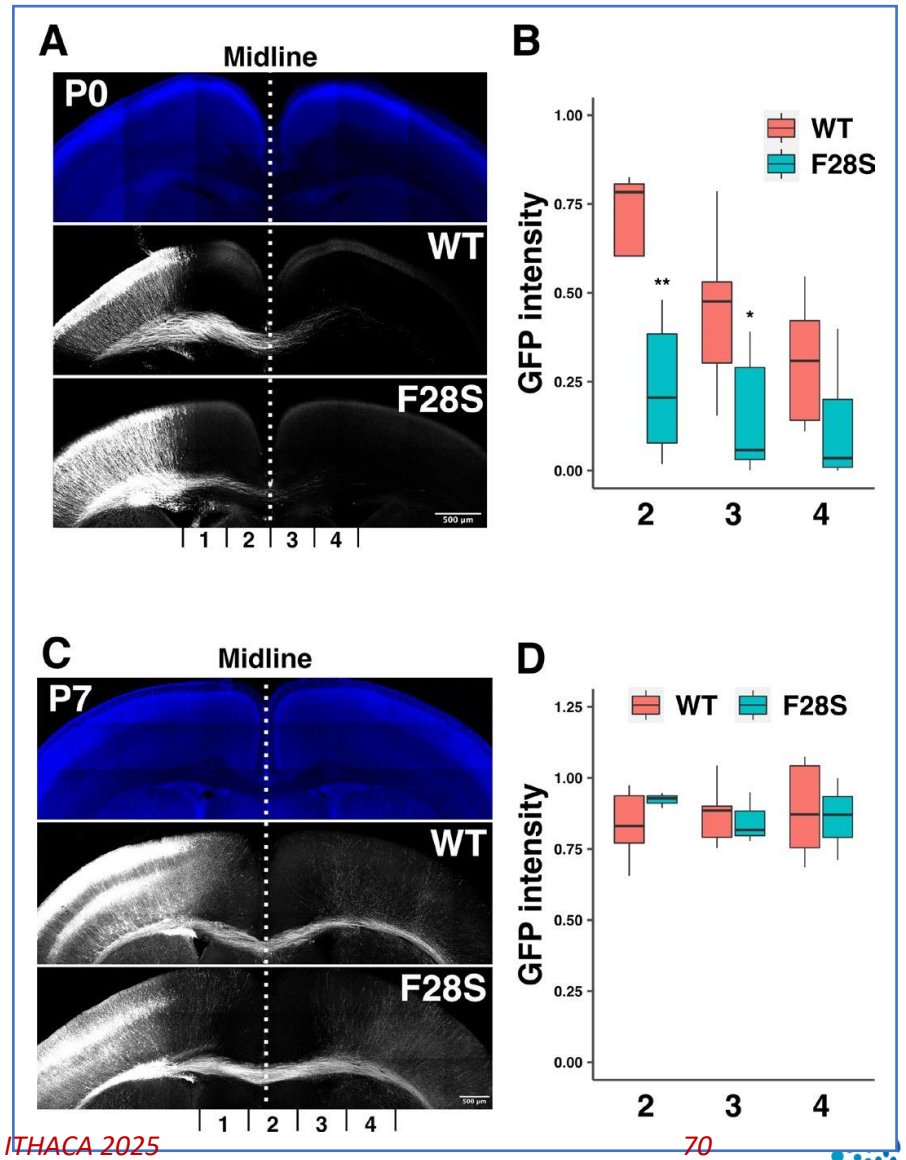


Axon elongation defect

Axon elongation was significantly delayed in neurons expressing **RAC3-F28S**

When we analysed the long-term effects at P7, control neurons as well as RAC3-F28S-expressing cells extended the axon efficiently into the contralateral cortex

These results suggest that RAC3-F28S **delayed, but not prevented**, axon elongation of cortical neurons



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Atypical NEDBAF: the current borders of RAC3 research

The N92K variant

Child's Nervous System (2024) 40:1597–1602
https://doi.org/10.1007/s00381-024-06285-z

CASE REPORT

An unusual presentation of *de novo RAC3* variation in prenatal diagnosis

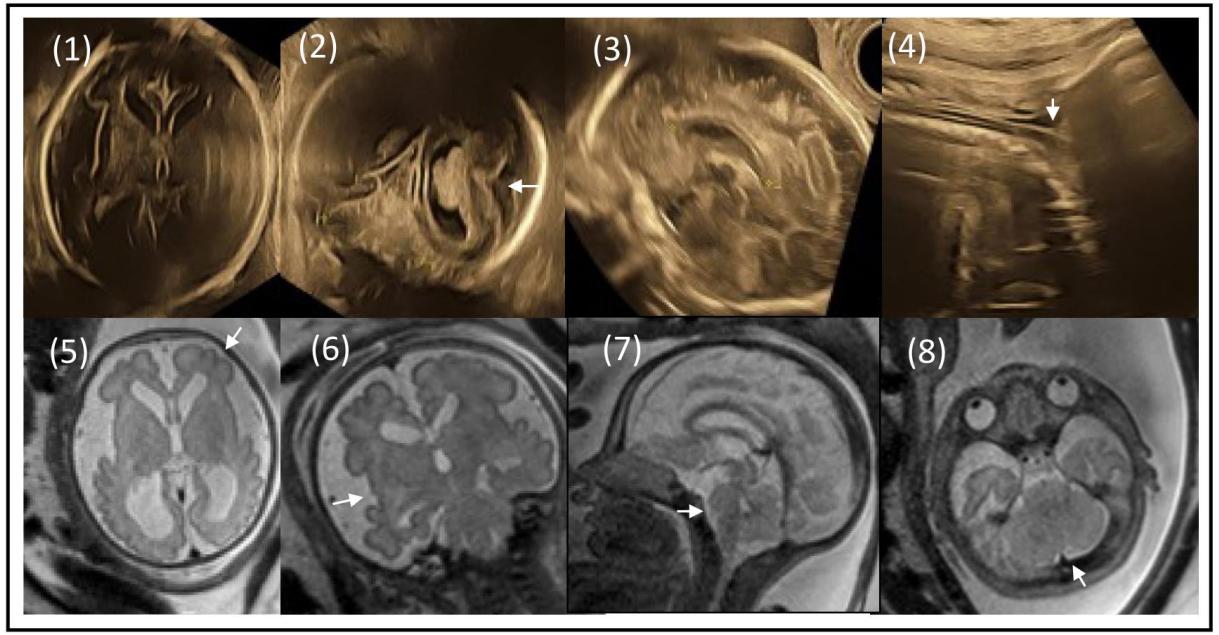
Colombine Meunier[1] · Marie Cassart[2] · Karole Kostyla[3] · Nicolas Simonis[1] · Olivier Monestier[1] · Aude Tessier[1]

Received: 5 December 2023 / Accepted: 6 January 2024 / Published online: 12 January 2024

Before this study, a single *RAC3* **prenatal case** was known, featuring midline and posterior fossa anomalies

Very recently, an **atypical clinical presentation** associated with a novel *RAC3* variant (**p.(Asn92Lys)**) was reported

This **second prenatal NEDBAF case** displayed ventriculomegaly and polymicrogyria, **without** callosal, cerebellar, or brainstem malformations



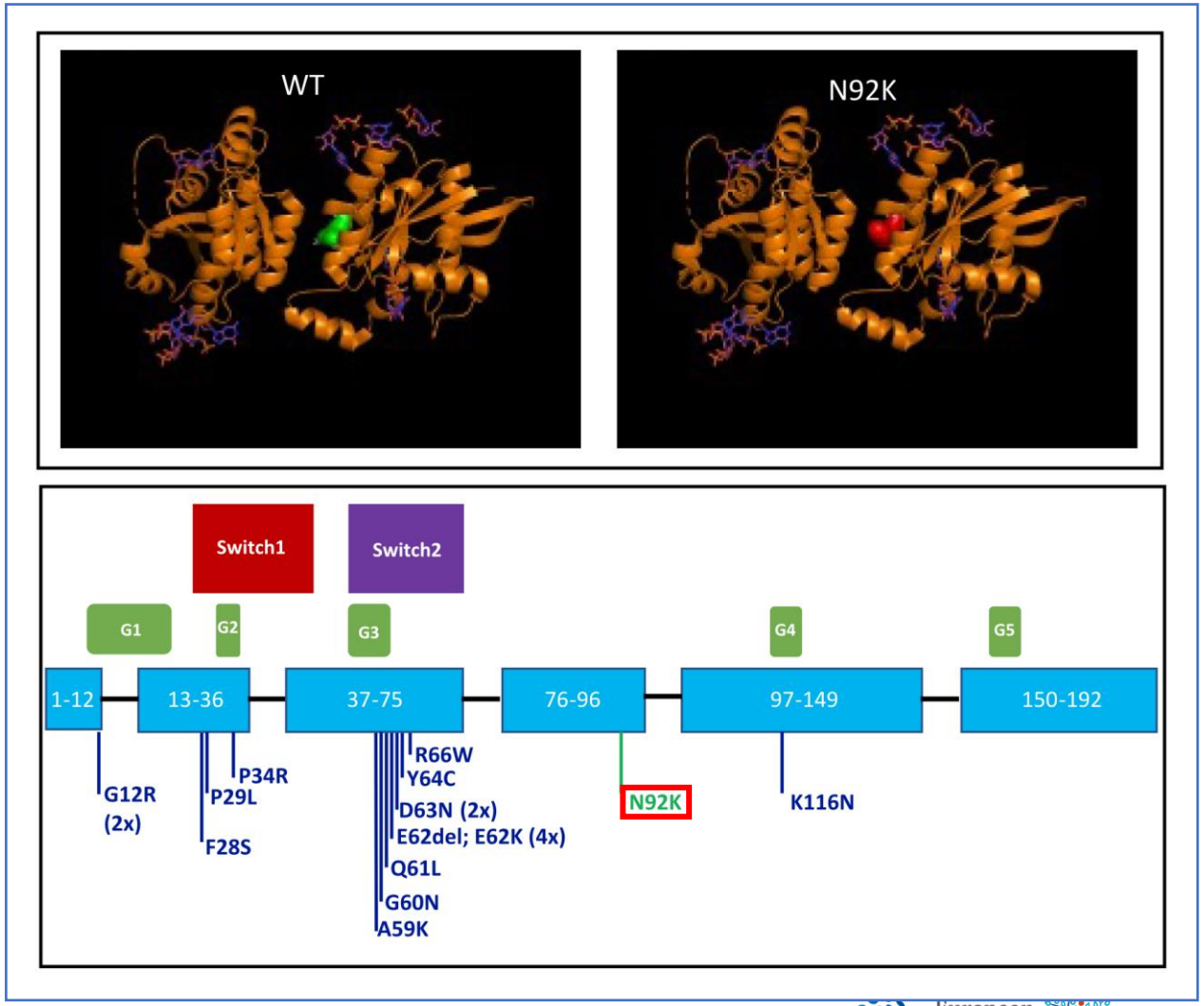
Sonographic and MRI examinations performed respectively at 28 and 31 WG showing:

- Ventriculomegaly
- Square shape of the frontal horns
- Enlarged pericerebral spaces
- Hypoplastic frontal lobes
- Abnormal Sylvian operculization

The N92K variant

The **p.(Asn92Lys)** variant affects a conserved residue within a poorly characterized region of RAC1, **outside** of the mutational hotspots for classic NEDBAF

This suggests that variants localized in different regions of the protein may lead to **atypical** clinical presentations, leading to the concept of '**NEDBAF spectrum**'



The N92K variant

Work in progress

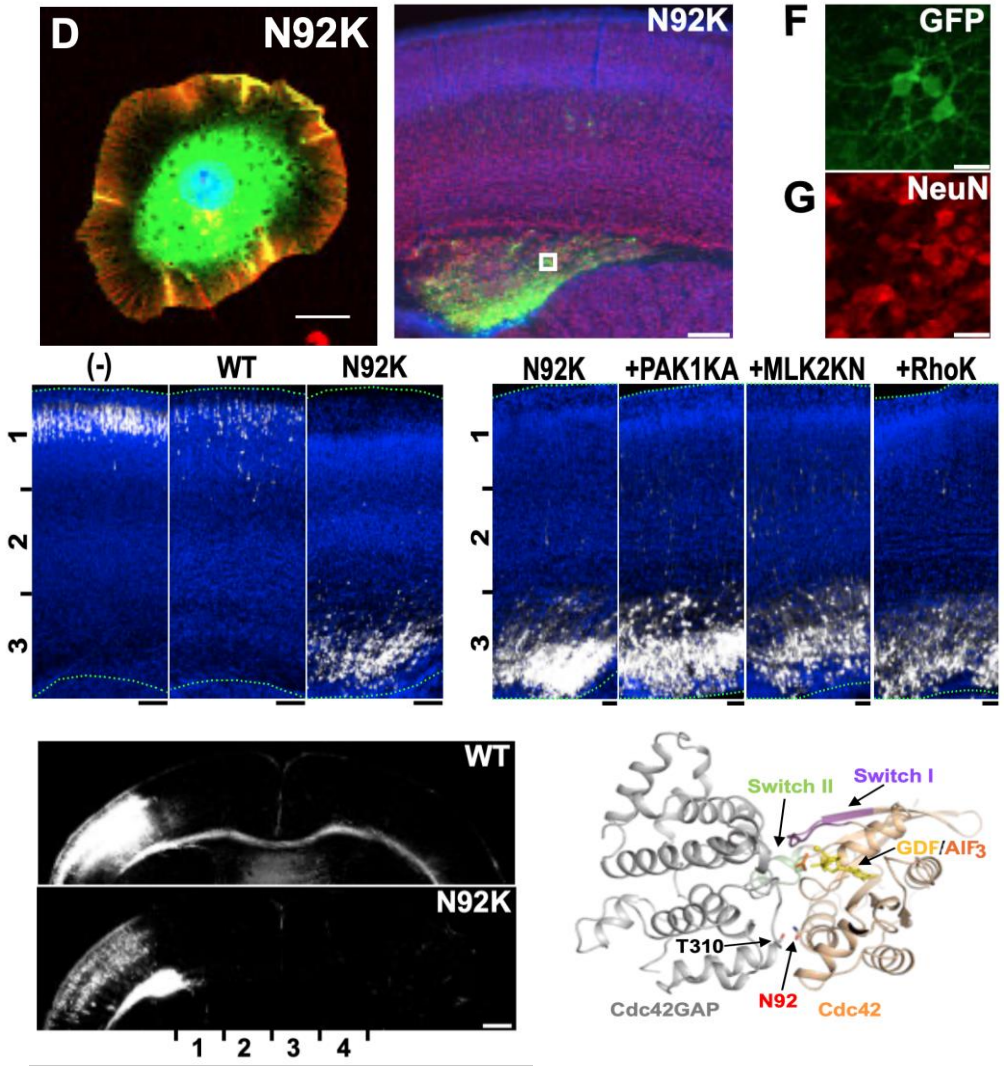
Pathophysiological significance of a neurodevelopmental disorder-causative *RAC3* p.N92K variant located outside the functional regions

Ryota Sugawara[1,2]*, Keisuke Hamada[3]*, Hidenori Ito[1], Marcello Scala[4,5], Hiroshi Ueda[2], Hidenori Tabata[1], Kazuhiro Ogata[3], and Koh-ichi Nagata[1,6]

In this study, we are exploring the functional properties of the N92K variant in search for mechanisms underlying **atypical NEDBAF**

We found that N92K:

- Is constitutively activated (**GoF**)
- Destabilize the interaction with GAPs (GoF)
- Is **independent** of PAK1, MLK2, Rho (vs Q61L!)
- Causes defects in cell migration -> heterotopia
- Impairs axon elongation



The R66W variant

Received: 15 September 2021 | Revised: 15 January 2022 | Accepted: 22 January 2022
DOI: 10.1002/pd.6106

RESEARCH NOTE

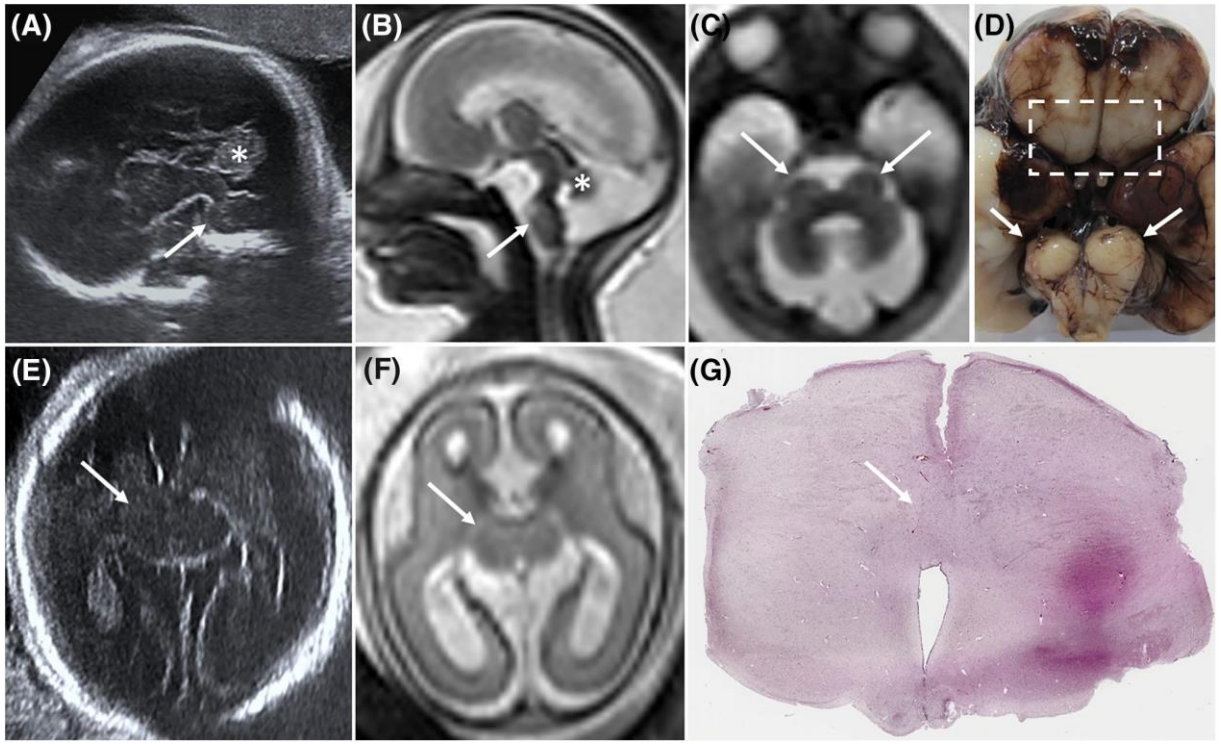
PRENATAL DIAGNOSIS WILEY

Prenatal imaging features related to *RAC3* pathogenic variant and differential diagnoses

Sara Cabet[1,2] | Alexandre Vasiljevic[3,4] | Audrey Putoux[2,5] | Audrey Labalme[6] | Damien Sanlaville[2,6] | Nicolas Chatron[2,6] | Gaetan Lesca[2,6] | Laurent Guibaud[1,4]

Aside from a variability in imaging patterns, likely reflecting a variability in NDD phenotypes, new research suggests that **atypical NEDBAF** **may embrace a much larger spectrum** of *RAC3*-related disorders

Surprisingly, a novel *RAC3* variant (**p.(Arg66Trp)**) was associated with a fetal akinesia deformation sequence, also featuring and complex brain malformations including corpus callosum agenesis, diencephalosynapsis, kinked brainstem, and vermian hypoplasia



Sonographic and MRI images at 24 WG showing:

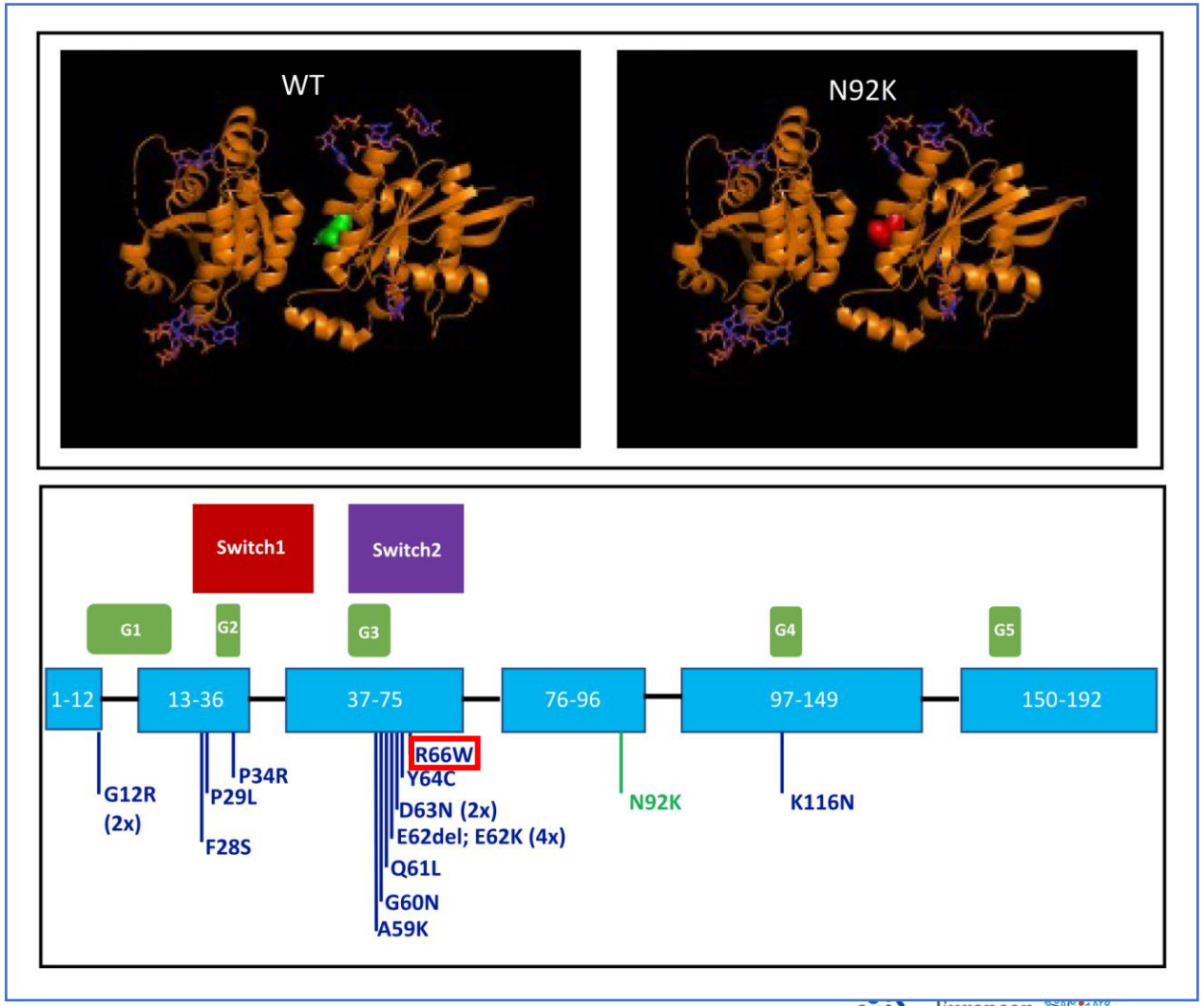
- Kinked brainstem with bifid pons
- Vermian hypoplasia
- Corpus callosum agenesis
- Arhinencephaly
- Diencephalosynapsis

The R66W variant

The **p.(Arg66Trp)** variant affects a conserved residue within the Switch II domain, crucial to mediate RAC3 interactions

Switch II is a **mutational hotspot** in classic NEDBAF

Despite this, the phenotype associated with R66W is pretty **unique**



The R66W variant

cells

MDPI

Article

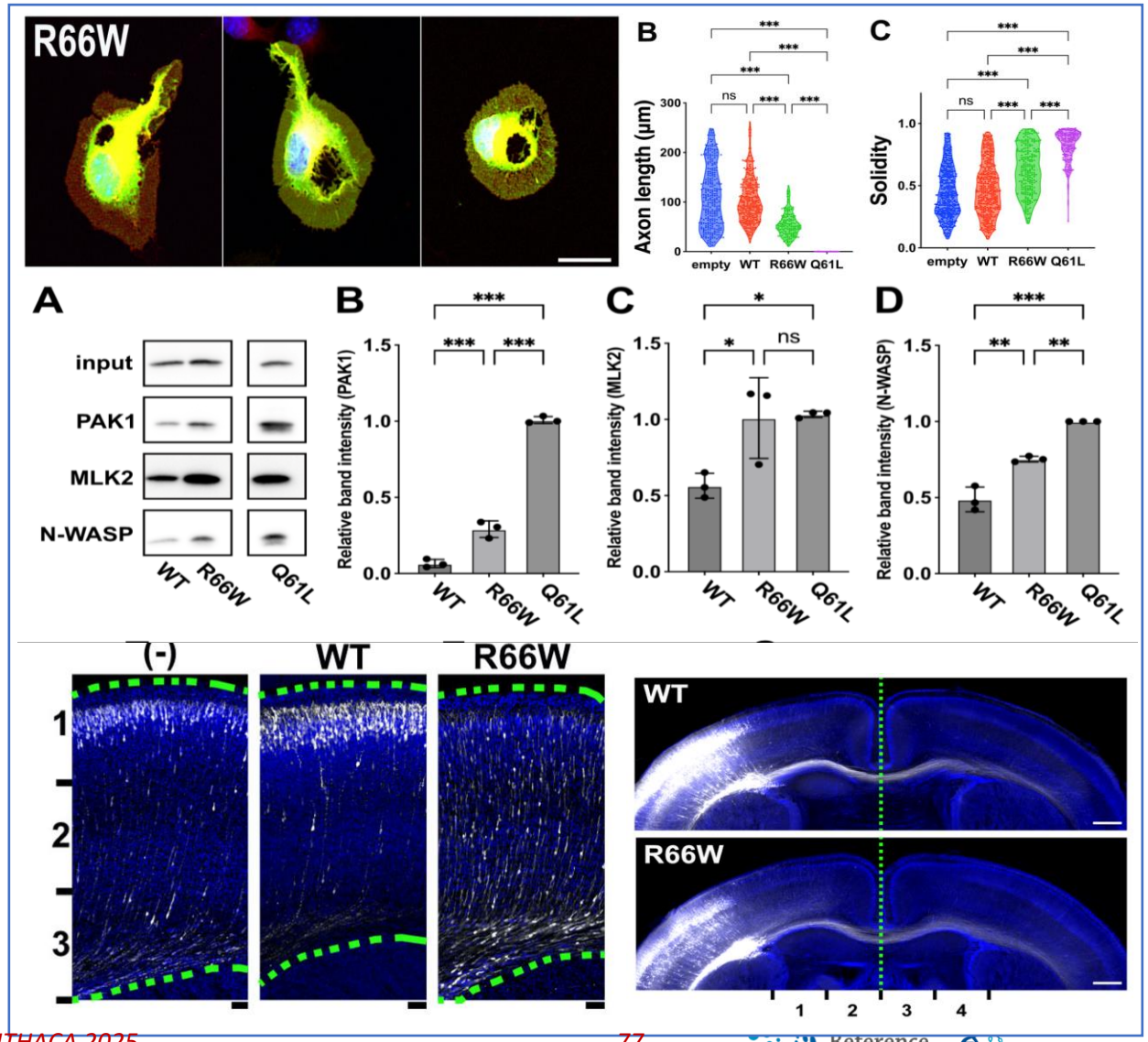
The p.R66W Variant in *RAC3* Causes Severe Fetopathy Through Variant-Specific Mechanisms

Ryota Sugawara [1,2], Hidenori Ito [1], Hidenori Tabata [1], Hiroshi Ueda [2,3], Marcello Scala [4] and Koh-ichi Nagata [1,5,*]

In this study, we delved into the **pathophysiology of R66W**, showing that this variant:

- Is only mildly activated (**mild GoF**)
- Preferentially interact with MLK2 vs PAK1
- Does not activate transcription (SRF, AP1, NFkB)
- Impairs neuronal differentiation
- Impairs cortical migration
- Impairs axon elongation

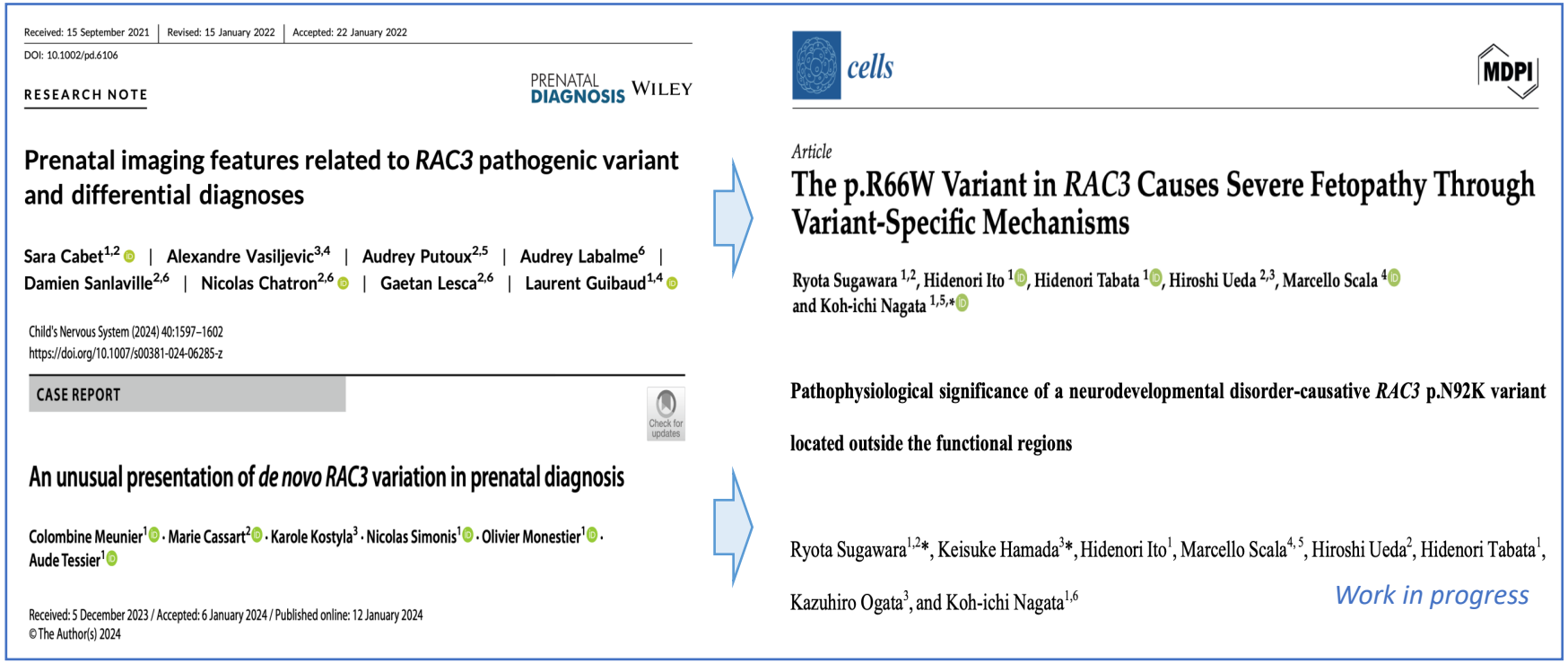
R66W acts as **GoF in specific signaling pathways**



The R66W variant

Taken together, the atypical findings observed in prenatal cases suggest the existence of a severe '**RAC3 fetopathy**'

This phenotype is part of the extended **NEDBAF spectrum** and underlain by **variant-specific molecular mechanisms**



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RESEARCH NOTE

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Child's Nervous System (2024) 40:1597–1602
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CASE REPORT

An unusual presentation of *de novo RAC3* variation in prenatal diagnosis

Colombine Meunier[1] · Marie Cassart[2] · Karole Kostyla[3] · Nicolas Simonis[1] · Olivier Monestier[1] · Aude Tessier[1]

Received: 5 December 2023 / Accepted: 6 January 2024 / Published online: 12 January 2024

cells MDPI

Article

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Ryota Sugawara [1,2], Hidenori Ito [1], Hidenori Tabata [1], Hiroshi Ueda [2,3], Marcello Scala [4] and Koh-ichi Nagata [1,5,*]

Pathophysiological significance of a neurodevelopmental disorder-causative *RAC3* p.N92K variant located outside the functional regions

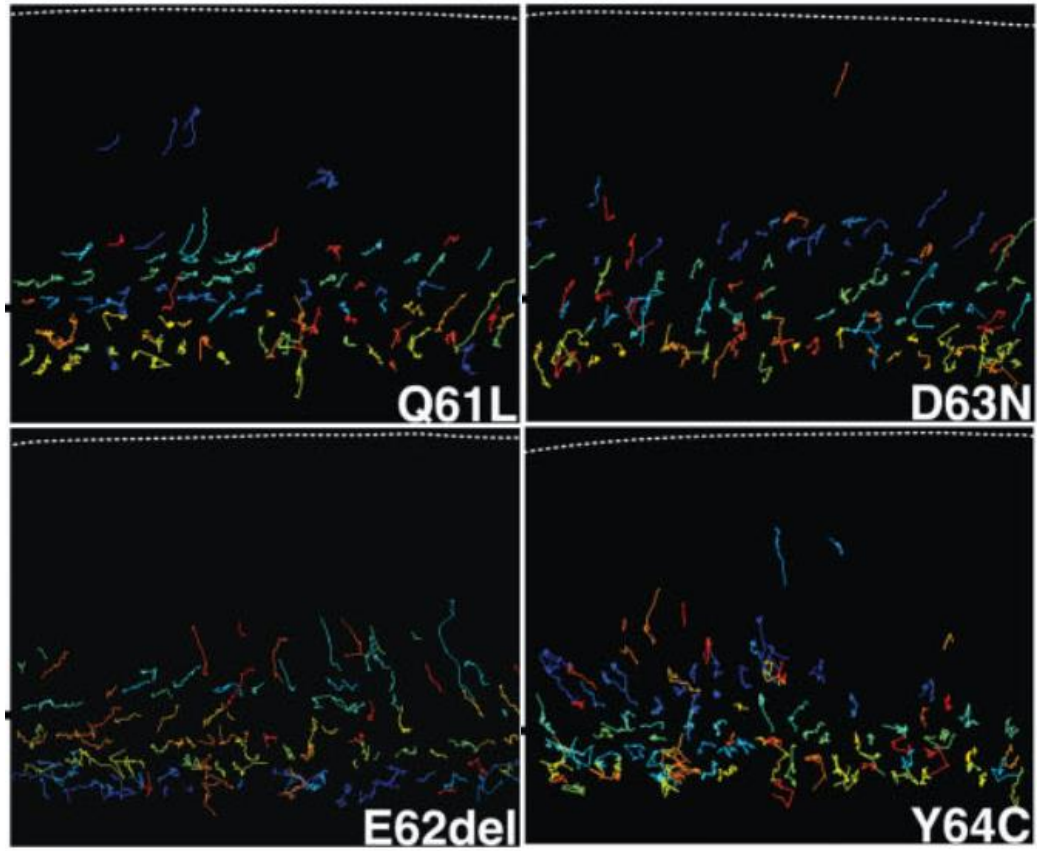
Ryota Sugawara[1,2]*, Keisuke Hamada[3]*, Hidenori Ito[1], Marcello Scala[4,5], Hiroshi Ueda[2], Hidenori Tabata[1], Kazuhiro Ogata[3], and Koh-ichi Nagata[1,6]

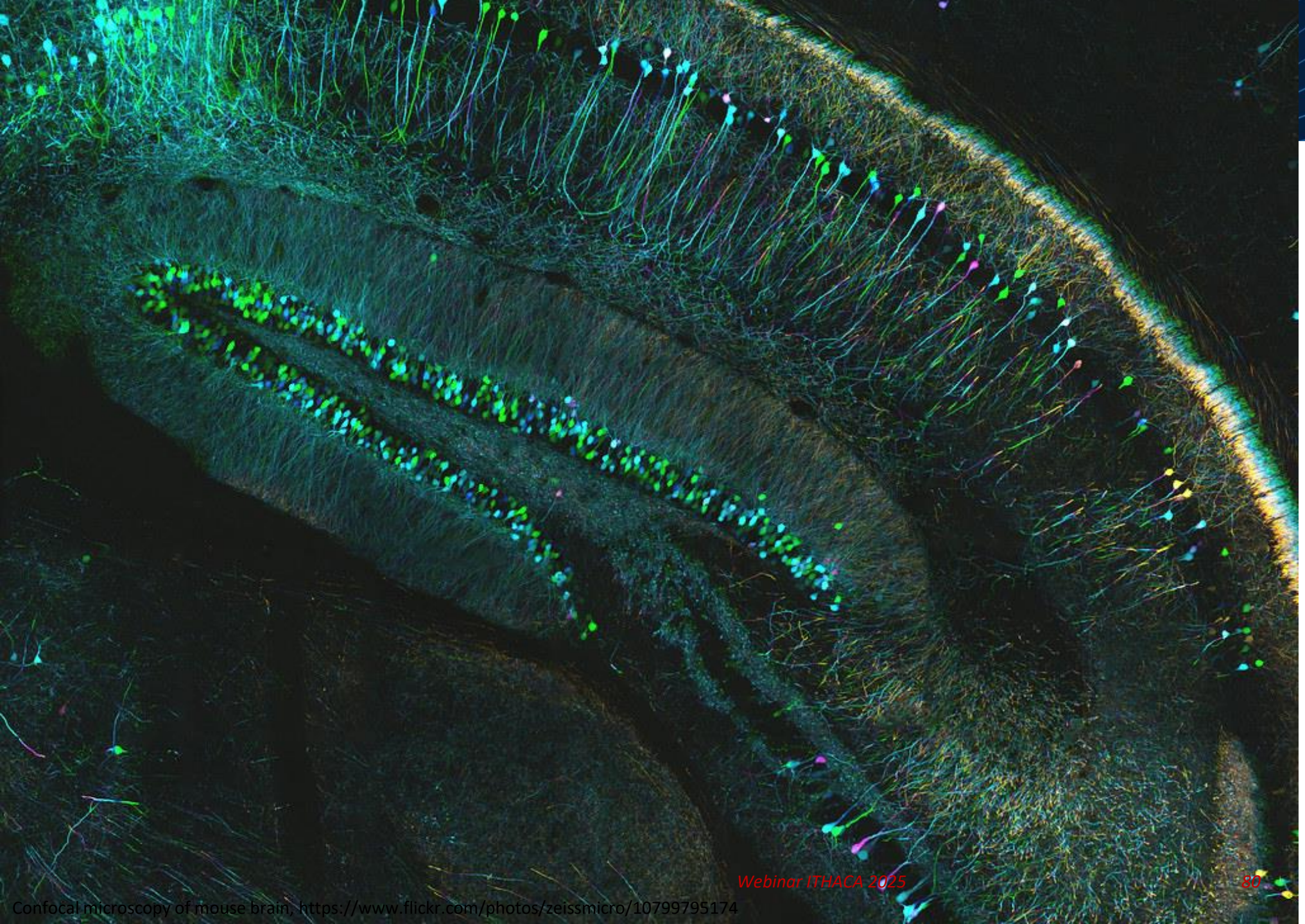
Work in progress

European Reference Networks ITHACA

Conclusive remarks

- **RAC3** is a crucial for **brain development**, regulating:
  - Neuronal maturation
  - Neuronal migration
  - Axon elongation
- *De novo* variants in *RAC3* cause a severe NDD (**NEDBAF**) featuring autistic features, cognitive deficiency, and a spectrum of brain malformations
- Generally, disease-causing variants cause RAC3 hyperactivation (**GoF**)
- However, recent research suggests the existence of **variant-specific mechanisms** -> **NEDBAF spectrum**
- This has significant impact on the **diagnosis** and **management** of affected individuals





Confocal microscopy of mouse brain, https://www.flickr.com/photos/zeissmicro/10799795174

Thanks for your attention!

European Reference Networks ITHACA

3.Mechanistic analysis of RAC1 related neurodevelopmental disorders

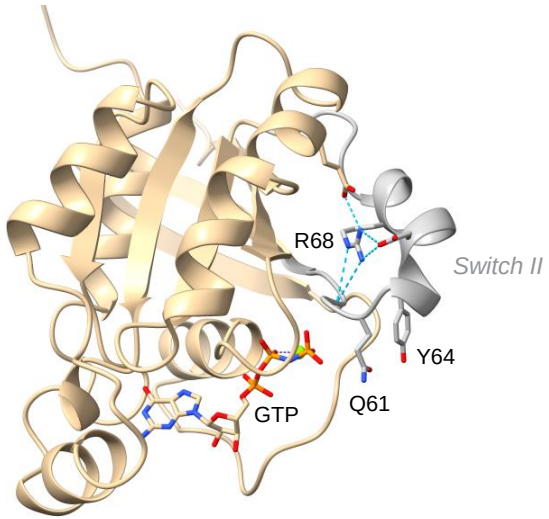
Tom H. Millard, PhD - Division of Developmental Biology and Medicine, Faculty of Biology, Medicine and Health, University of Manchester M13 9PL, UK.

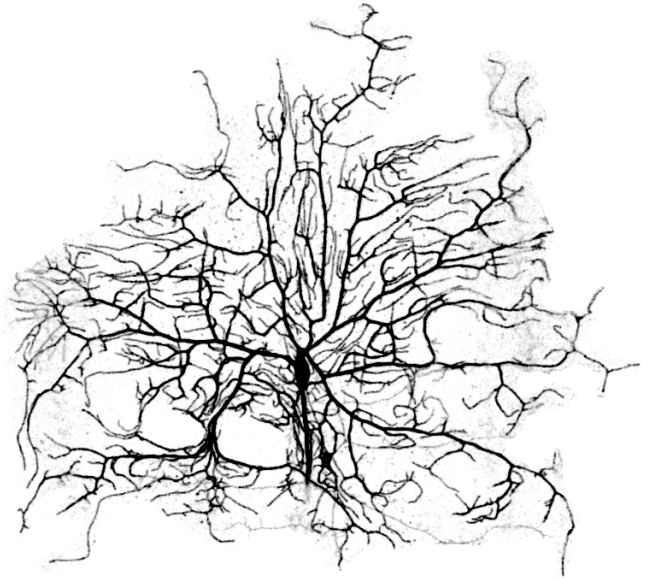
Mechanistic analysis of RAC1-related neurodevelopmental disorders

Tom Millard

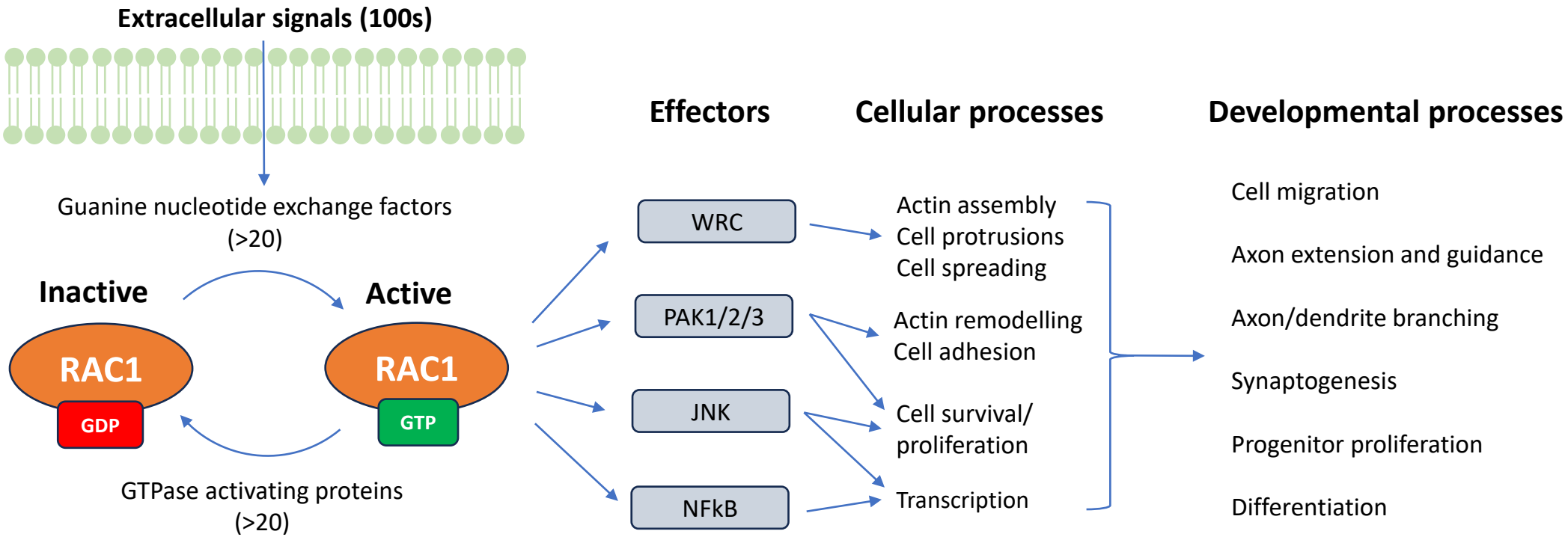
University of Manchester, U.K.

Siddharth Banka





RAC1: A key regulator of diverse cellular and developmental processes



2017: *De novo* missense variants in *RAC1* cause a developmental disorder with diverse phenotypes


Margot Reijnders


Han Brunner


Nicholas Katsanis

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	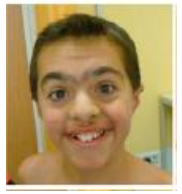					
Variant	C18Y	N39S	P73L	Y64D	V51M	V51L
Head circumference	**-2.5 SD**	**-3 SD**	**-5 SD**	**+1 SD**	**+4.2 SD**	**+4.5 SD**
Intellectual disability	Yes	Yes	Yes	Yes	Yes	Yes
Neurological/ behavioural	Epilepsy Hypotonia	Hyperactivity	Not known	Hypotonia Stereotypies	Not known	Epilepsy, hypotonia Stereotypies, ASD
Brain MRI abnormalities	Yes	Yes	Not known	Yes	Yes	Yes
Cardiovascular	NS,LVC,IV	No	Not known	VSD	Not known	No

Reijnders *et al.* 2017 AJHG

2017: *De novo* missense variants in *RAC1* cause a developmental disorder with diverse phenotypes


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Nicholas Katsanis

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	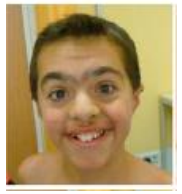					
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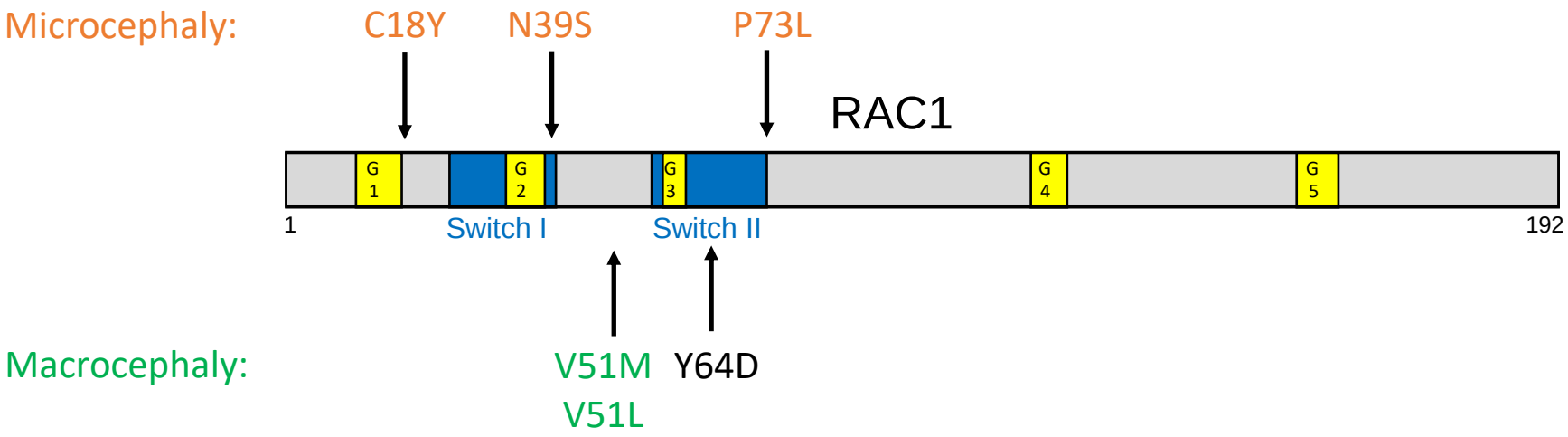
Brain MRI abnormalities	Yes	Yes	Not known	Yes	Yes	Yes
Cardiovascular	NS,LVC,IV	No	Not known	VSD	Not known	No

Microcephaly

Macrocephaly

Reijnders *et al.* 2017 AJHG

Does head circumference correlate with location of variant in RAC1 protein?

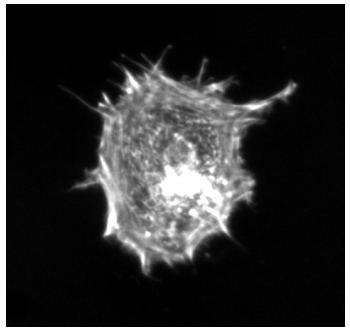


Switch domains: Different conformations in GTP and GDP bound states.
Main interaction sites for upstream regulators and downstream effectors

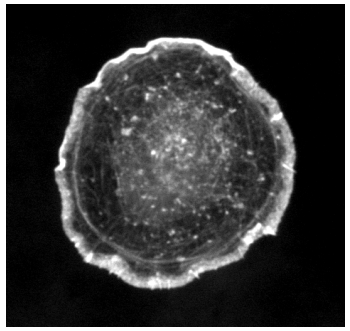
G1-5 motifs: Collectively form the GTP/GDP binding site

Does head circumference correlate with effect on RAC1 activity?

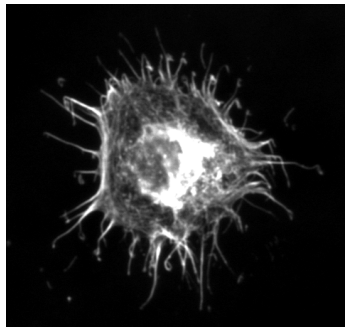
Fibroblast spreading – a simple cellular assay for RAC1 activity



Wild-type RAC1

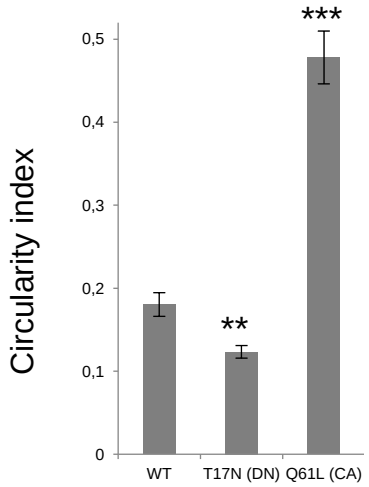


Constitutively active RAC1 (Q61L)



Dominant negative RAC1 (T17N)

Circularity index = 4π x Area / Perimeter^2



RAC1-GTP

↓

WRC

↓

Arp2/3 complex

↓

Branched actin assembly

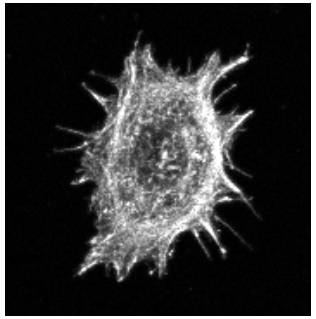
↓

Lamellipodia

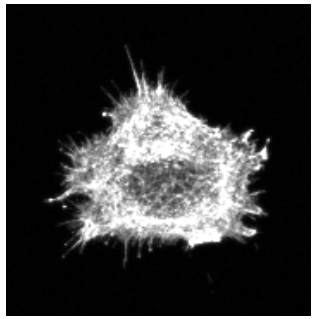
Nurhuda Ansor

Reijnders *et al.* 2017 AJHG

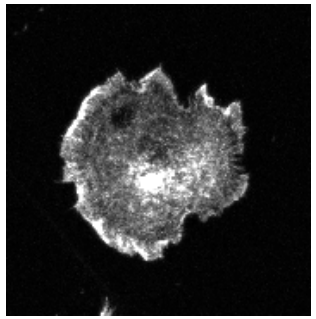
Fibroblast spreading – a simple cellular assay for RAC1 activity



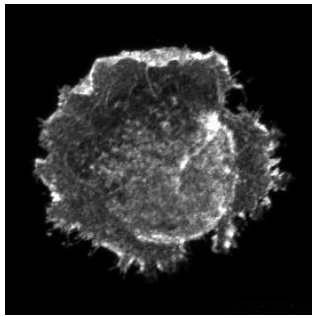
C18Y



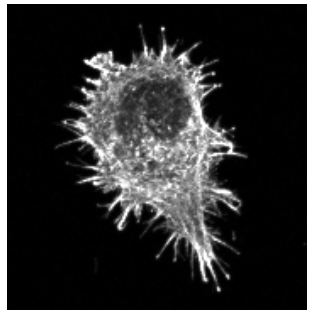
N39S



V51M



Y64D



P73L

RAC1-GTP

↓

WRC

↓

Arp2/3 complex

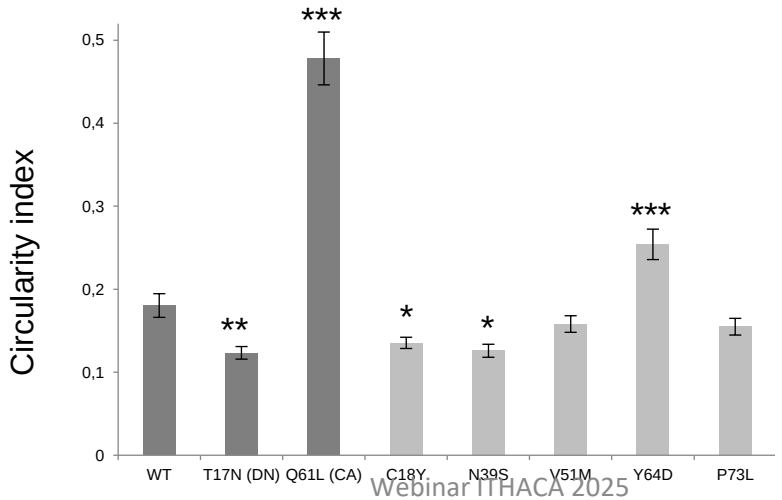
↓

Branched actin assembly

↓

Lamellipodia

Circularity index = 4π x Area / Perimeter^2



Nurhuda Ansor

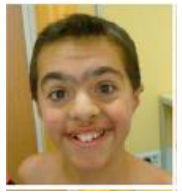
Reijnders *et al.* 2017 AJHG

De novo RAC1 missense variants cause a developmental disorder with extremely diverse phenotypes


Margot Reijnders

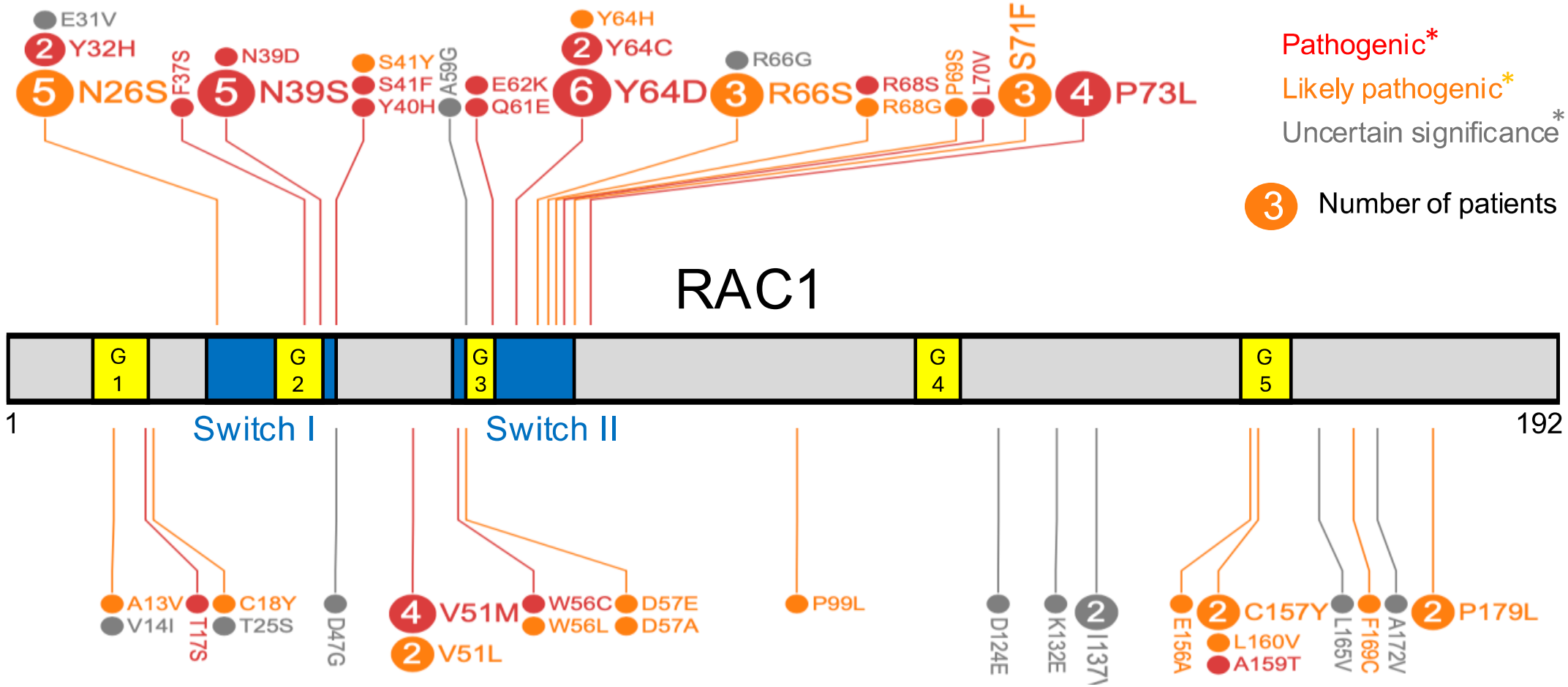

Han Brunner


Nicholas Katsanis

	Microcephaly				Macrocephaly	
	1	2	3	4	5	6
						
Variant	C18Y	N39S	P73L	Y64D	V51M	V51L
Head circumference	**-2.5 SD**	**-3 SD**	**-5 SD**	**+1 SD**	**+4.2 SD**	**+4.5 SD**
Intellectual disability	Yes	Yes	Yes	Yes	Yes	Yes
Neurological/ behavioural	Epilepsy Hypotonia	Hyperactivity	Not known	Hypotonia Stereotypies	Not known	Epilepsy, hypotonia Stereotypies, ASD
Brain MRI abnormalities	Yes	Yes	Not known	Yes	Yes	Yes
Cardiovascular	NS,LVC,IV	No	Not known	VSD	Not known	No
Effect on RAC1 activity	**DN**	**DN**	**Likely DN**	**Activating**	**?**	**?**

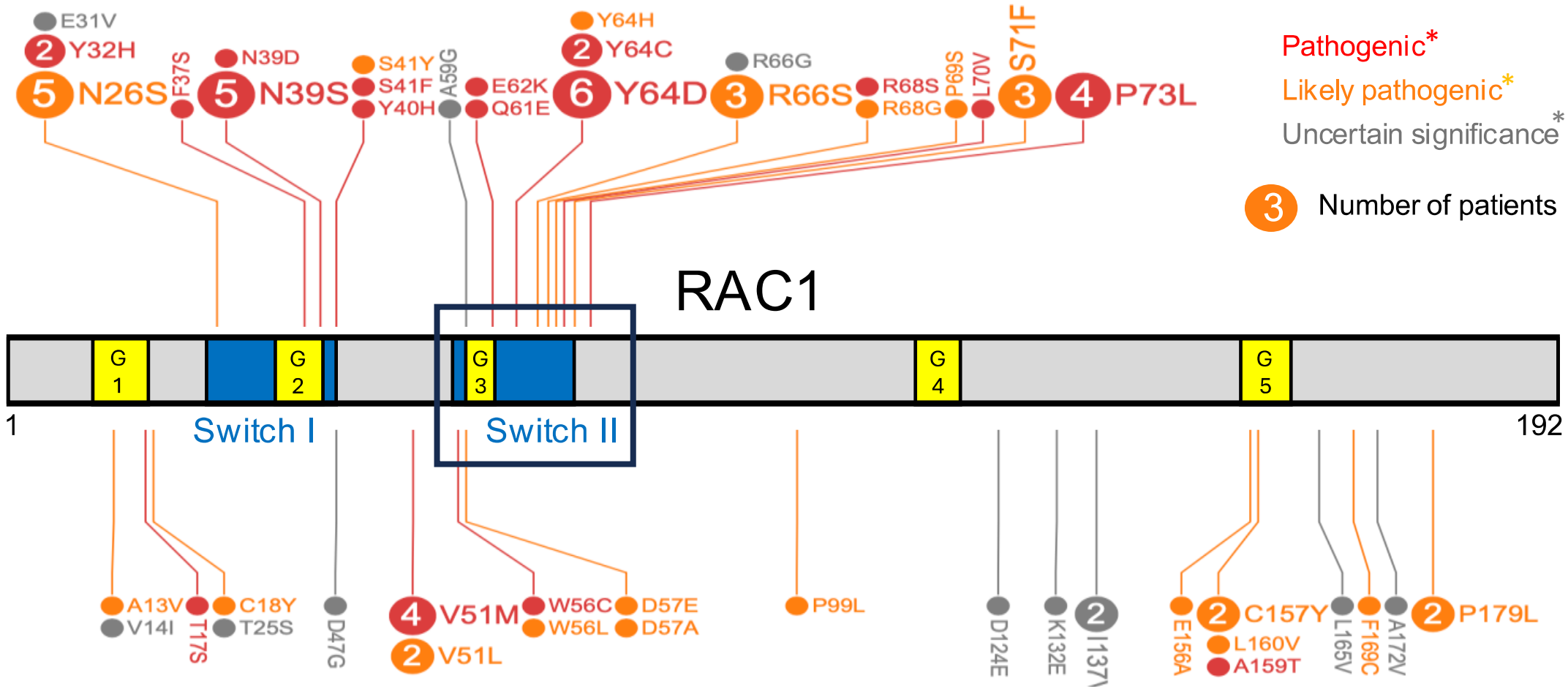
Reijnders *et al.* 2017 AJHG

2025: >80 individuals with >50 distinct variants identified



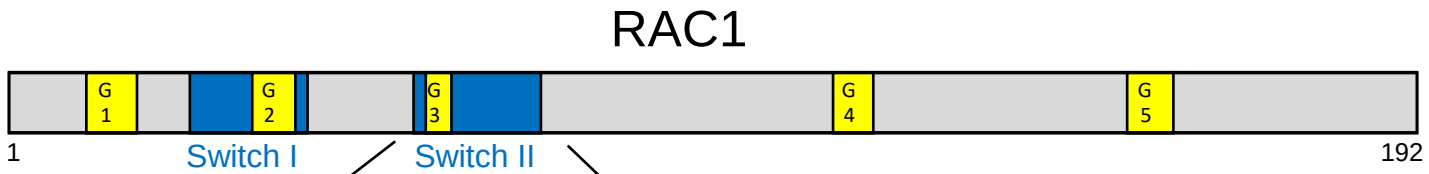
*Based on ACMG guidelines

2025: >80 individuals with >50 distinct variants identified



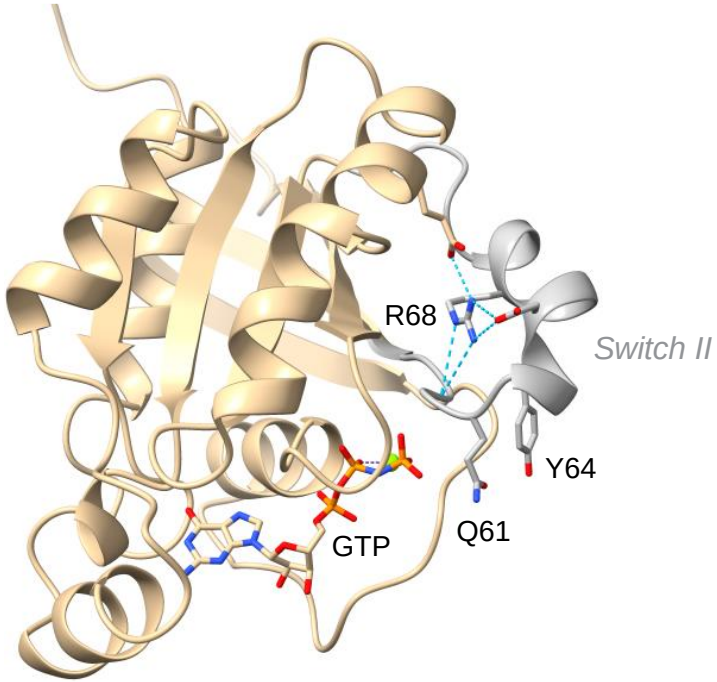
Highest concentration of pathogenic variants in switch II region of RAC1

Focus on variants affecting switch II



Human RAC1	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human RAC2	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human RAC3	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human CDC42	*54*	G	L	F	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human RHOA	*56*	A	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	D	T	D	V	I	80
Mouse RAC1	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Zebrafish RAC1	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Drosophila RAC1	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
C. elegans RAC1	*54*	G	L	W	D	T	A	G	**Q**	E	D	**Y**	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human KRAS	*54*	D	I	L	D	T	A	G	**Q**	E	E	**Y**	S	A	M	**R**	D	Q	Y	M	R	T	G	E	G	F	*78*

Switch II



Eight individuals identified with variants affecting Q61-R68 within switch II region

	1	2	3	4	5	6	7	8
Variant	Q61E	Y64D	Y64D	Y64D	Y64D	Y64C	R68S	R68G
Head circumference	-2 SD	+0.7 SD	-0.8 SD	+3.1 SD	+0.2 SD	-1.6 SD	-2.2 SD	-0.8 SD
Intellectual disability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neurological/ behavioural	Eating disorder	Stereotypies	Hypotonia	Dyspraxia ADHD	Sleep difficulties	None	Hypotonia	Hypotonia
Brain MRI Abnormalities	None	Yes	Not known	Yes	Not known	Yes	Yes	Yes
Cardiovascular	ASD, VSD	VSD	None	Not known	VSD	None	PDA,PFO,TI	None

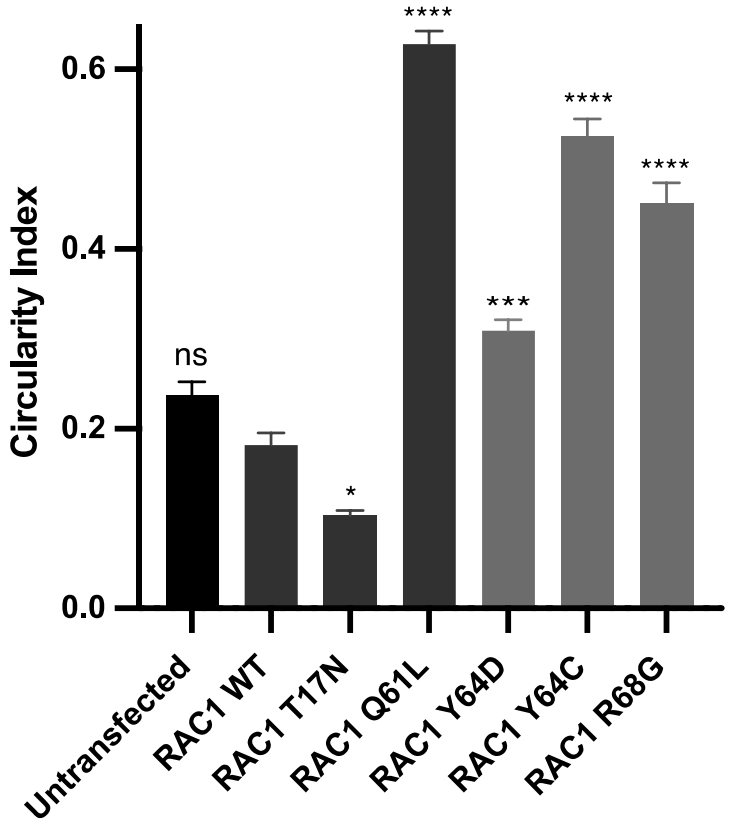
Variants affecting Q61-R68 do not cause extreme macro- or microcephaly

	1	2	3	4	5	6	7	8
Variant	Q61E	Y64D	Y64D	Y64D	Y64D	Y64C	R68S	R68G
Head circumference	**-2 SD**	**+0.7 SD**	**-0.8 SD**	**+3.1 SD**	**+0.2 SD**	**-1.6 SD**	**-2.2 SD**	**-0.8 SD**
Intellectual disability	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neurological/ behavioural	Eating disorder	Stereotypies	Hypotonia	Dyspraxia ADHD	Sleep difficulties	None	Hypotonia	Hypotonia
Brain MRI Abnormalities	None	Yes	Not known	Yes	Not known	Yes	Yes	Yes
Cardiovascular	ASD, VSD	VSD	None	Not known	VSD	None	PDA,PFO,TI	None

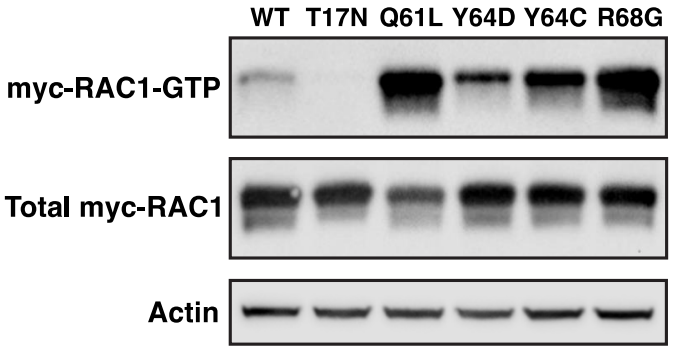
Do variants affecting Q61-R68 have similar effects on RAC1 activity?

Variants affecting Q61-R68 increase RAC1 activity

Cell spreading assay:



PAK pulldown assay:

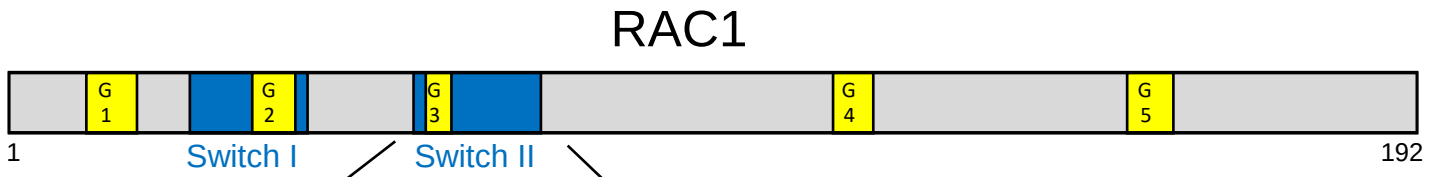


Abi Bennington, Martin Baker

Banka et al (2022) *Brain*

Downstream PAK and WRC pathways both activated by these variants

Variants affecting Q61-R68 are activating throughout the Rho GTPase family

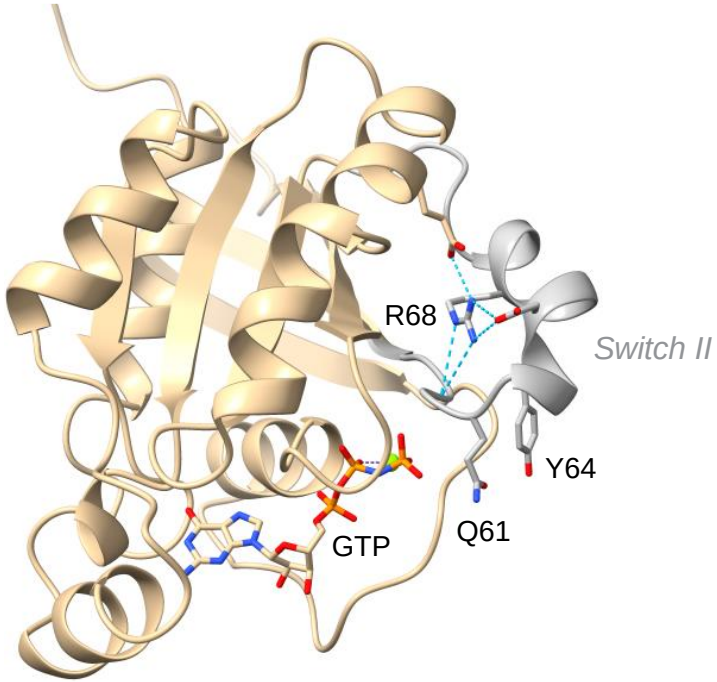


Switch II

Human RAC1	*54*	G	L	W	D	T	A	G	**Q**	**E**	D	**Y**	D	R	L	**R**	P	L	S	Y	**P**	Q	T	D	V	F	*78*
Human RAC2	*54*	G	L	W	**D**	T	**A**	G	**Q**	**E**	**D**	Y	D	R	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human RAC3	*54*	G	L	W	D	T	**A**	G	**Q**	**E**	**D**	**Y**	D	R	L	R	P	L	S	Y	P	Q	T	D	V	F	*78*
Human CDC42	*54*	G	L	F	D	T	A	G	Q	E	D	**Y**	D	**R**	L	**R**	P	L	S	Y	P	Q	T	D	V	F	*78*
Human RHOA	*56*	A	L	W	D	T	A	G	Q	E	D	Y	D	R	L	**R**	**P**	L	S	Y	P	D	T	D	V	I	80
Mouse RAC1	*54*	G	L	W	D	T	A	G	Q	E	D	Y	D	R	L	R	P	L	S	Y	P	Q	T	D	V	F	*78*
Zebrafish RAC1	*54*	G	L	W	D	T	A	G	Q	E	D	Y	D	R	L	R	P	L	S	Y	P	Q	T	D	V	F	*78*
Drosophila RAC1	*54*	G	L	W	D	T	A	G	Q	E	D	Y	D	R	L	R	P	L	S	Y	P	Q	T	D	V	F	*78*
C. elegans RAC1	*54*	G	L	W	D	T	A	G	Q	E	D	Y	D	R	L	R	P	L	S	Y	P	Q	T	D	V	F	*78*
Human KRAS	*54*	D	I	L	D	T	A	G	Q	E	E	Y	S	A	M	R	D	Q	Y	M	R	T	G	E	G	F	*78*

Q61-R68 activating variant region

Activating Dominant negative

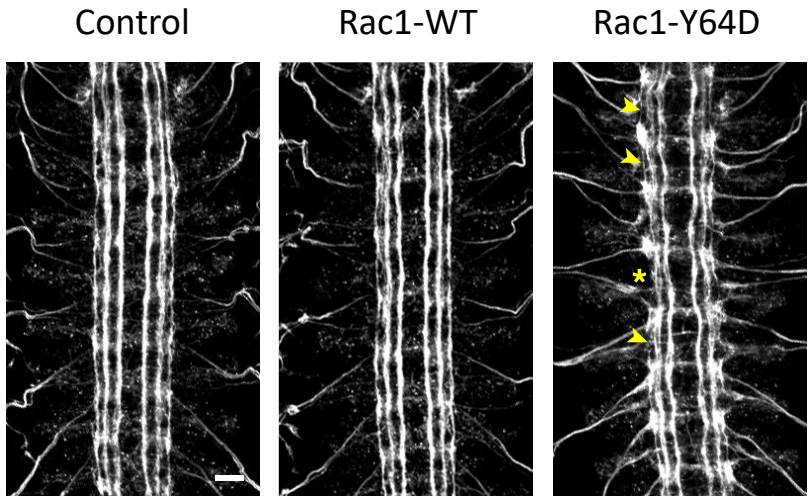


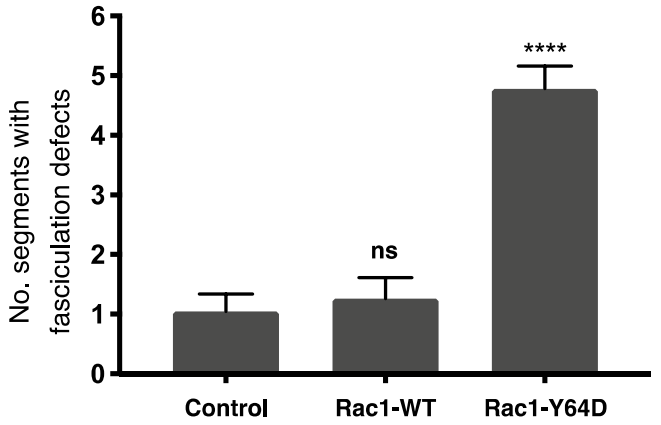
How do activating switch II variants affect neurodevelopment?

Drosophila melanogaster

RAC1 structure and function extremely well conserved

Neurons in developing *Drosophila* embryonic VNC (spinal cord):



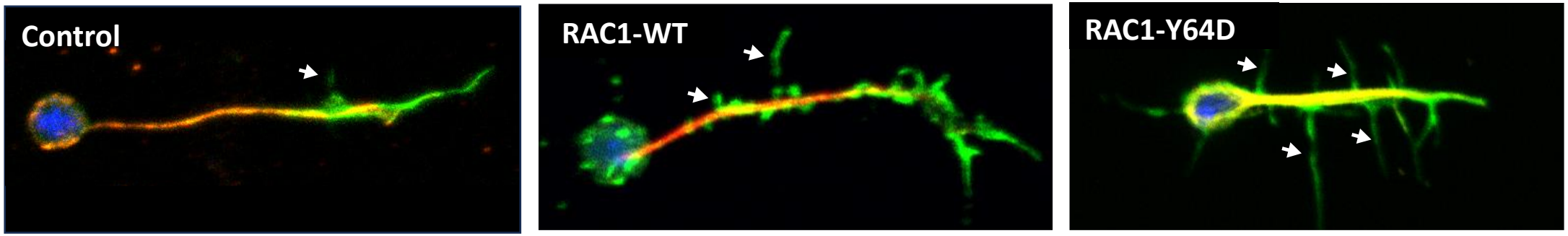


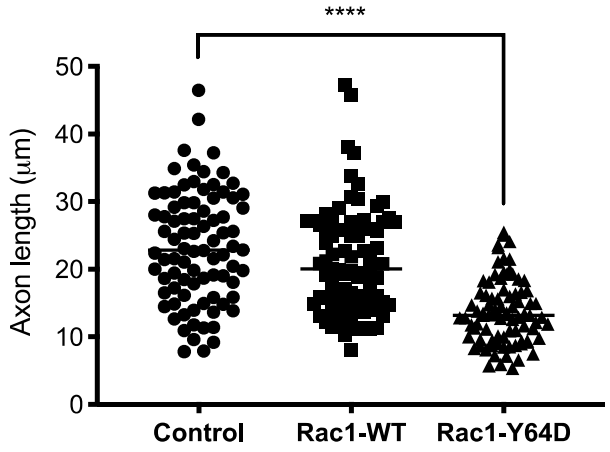
Banka et al (2022) *Brain*

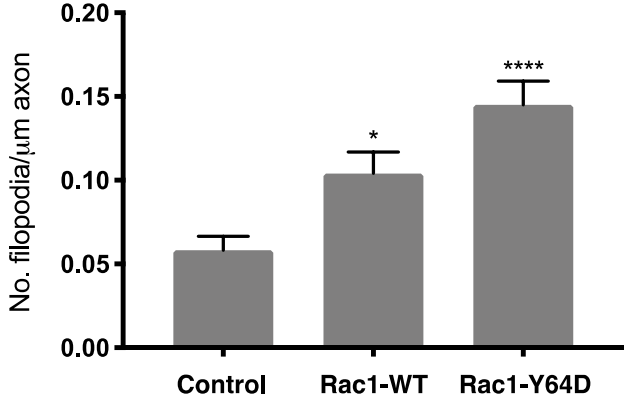
Expression of activating *RAC1* variant causes neuronal disorganization in embryonic CNS

How do activating *RAC1* variants affect the morphology of individual neurons?

Cultured *Drosophila* embryonic neurons



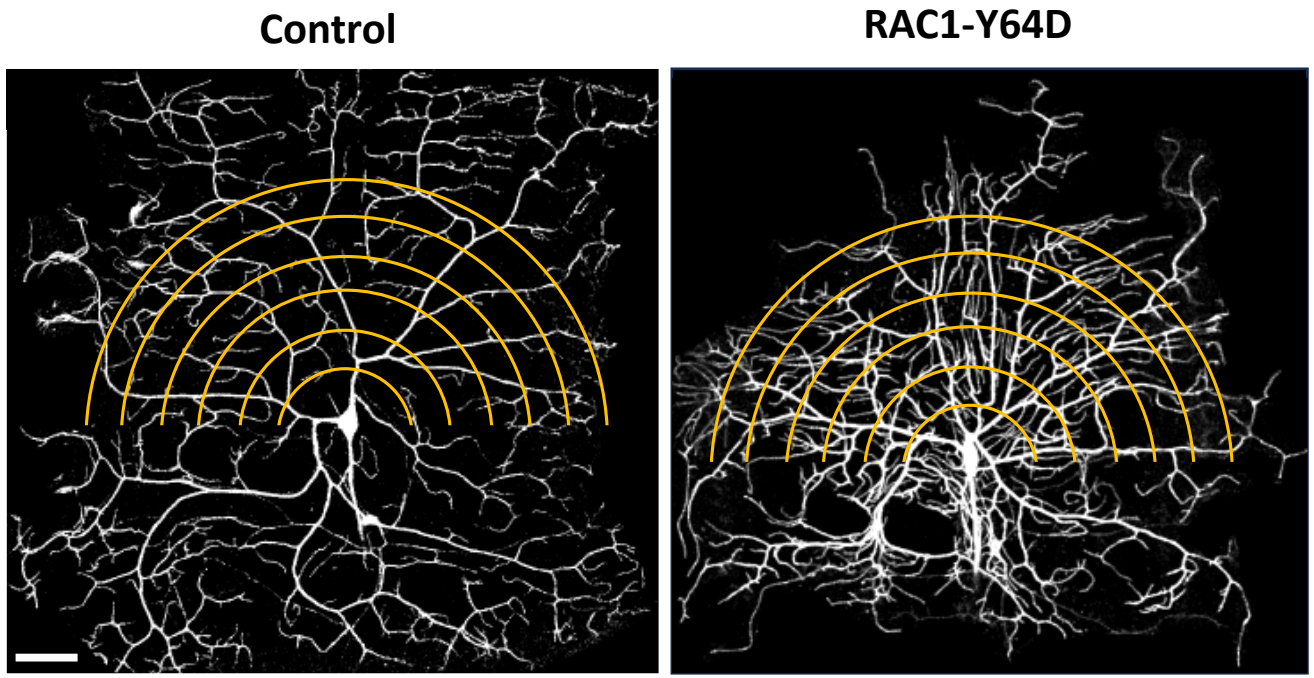




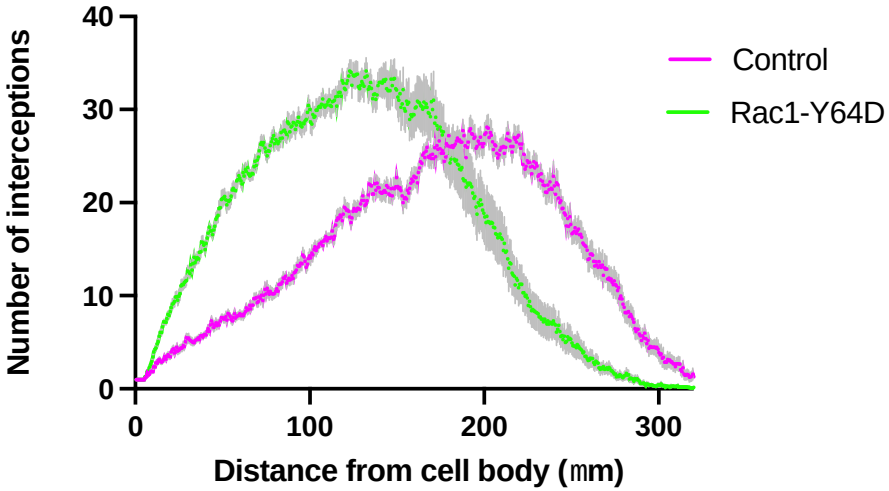
Banka et al (2022) *Brain*

Expression of activating *RAC1* variant reduces axonal length and increases lateral filopodia

Expression of an activating *RAC1* variant increases dendritic arbor complexity

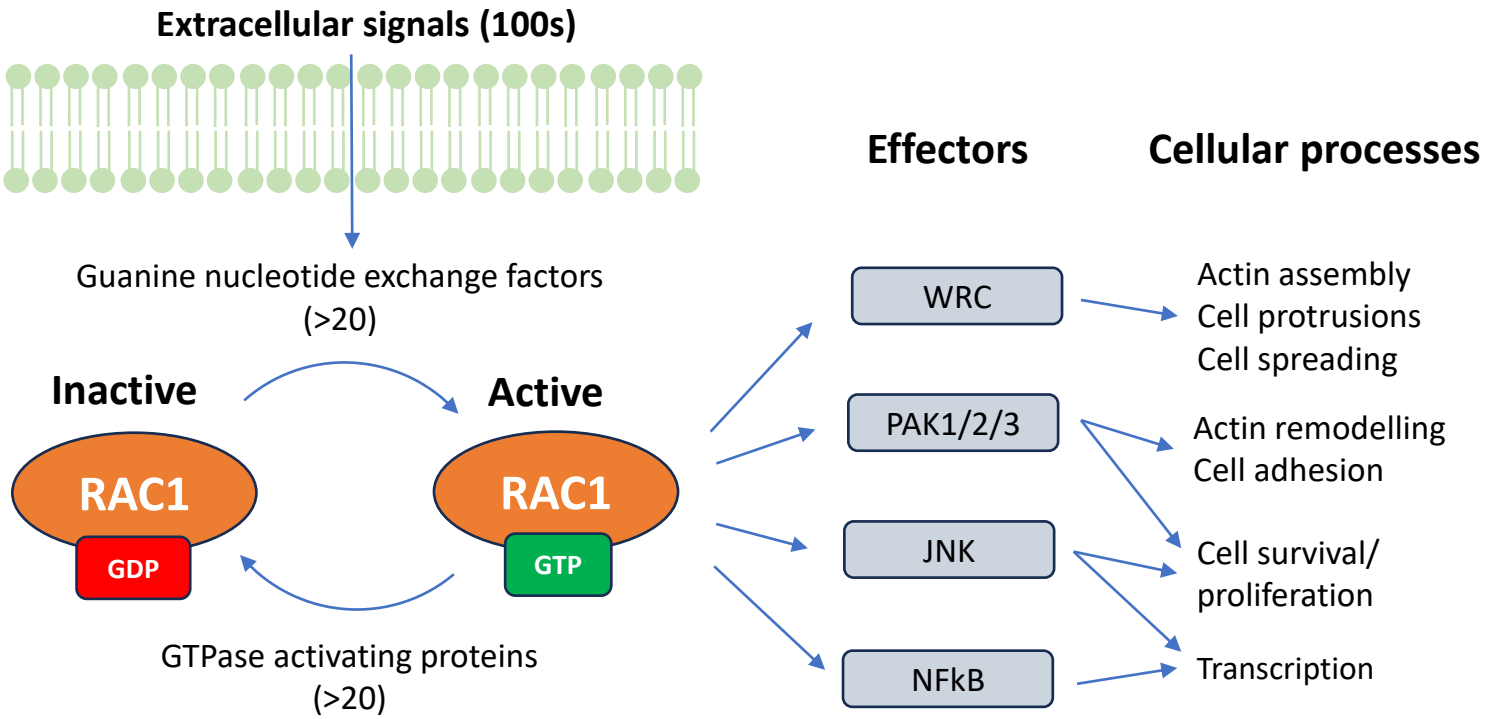


Type IV larval sensory neurons

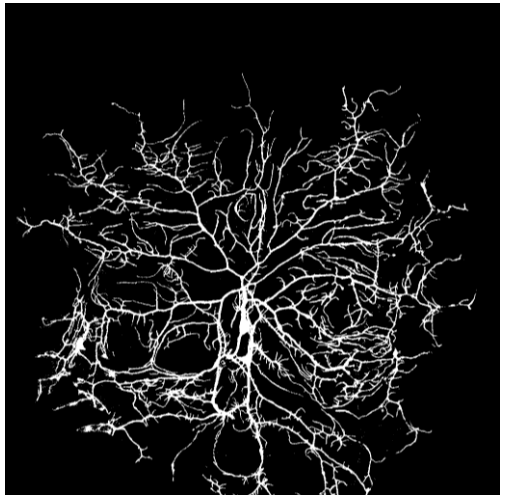


Sholl analysis

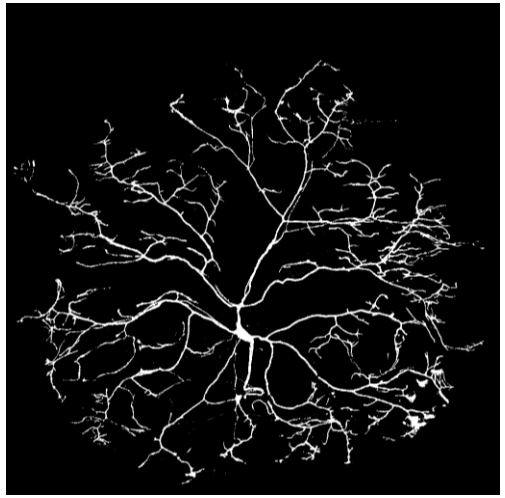
Which effector drives neuronal morphology changes caused by activating variants?



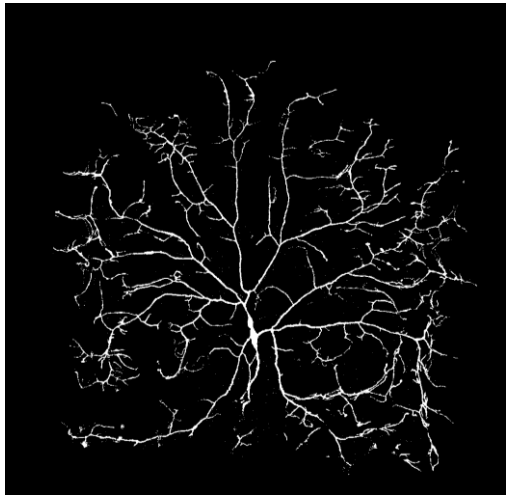
Knocking down components of the WAVE regulatory complex (WRC) rescues neuronal morphology



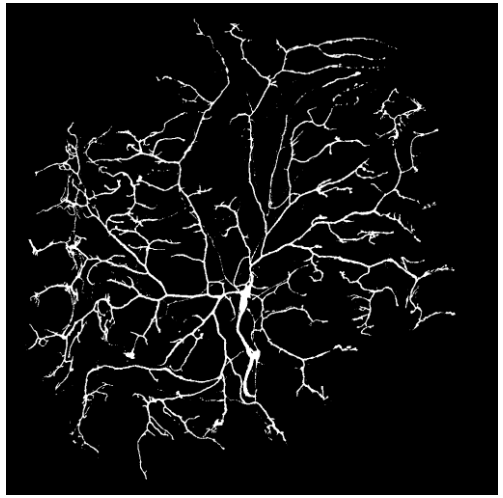
RAC1-Y64D



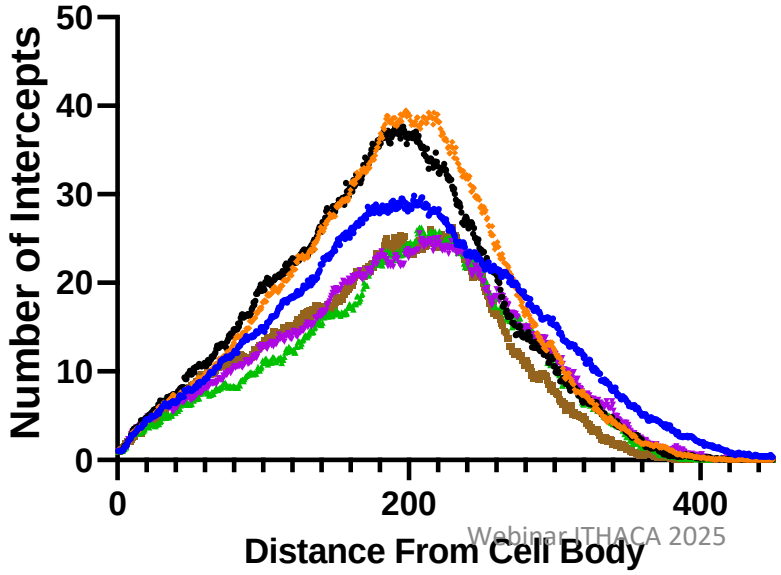
RAC1-Y64D + Abi RNAi



RAC1-Y64D + Scar RNAi



RAC1-Y64D + Cyfip RNAi



Y64D

Y64D + Luciferase RNAi

GFP

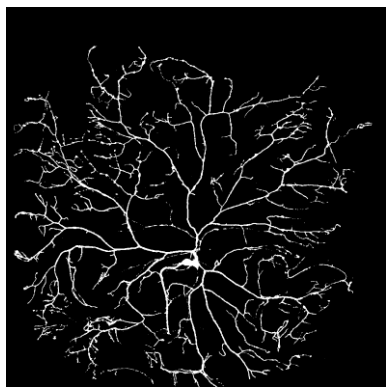
Y64D + Abi RNAi

Y64D + Scar RNAi

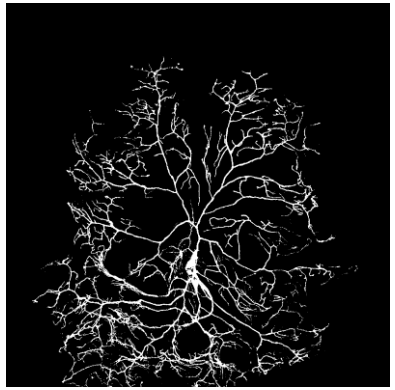
Y64D + Cyfip RNAi

Madeleine Smith

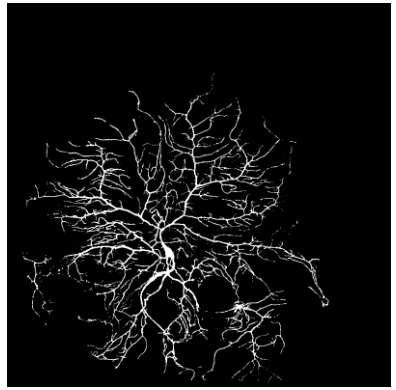
Dominant negative *RAC1* variants reduce dendritic complexity



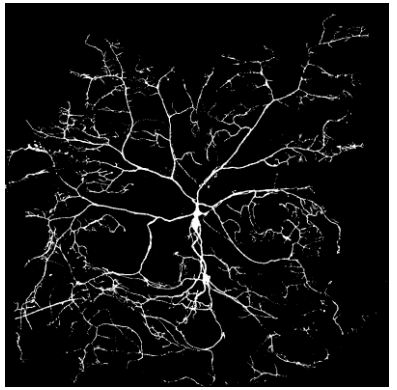
GFP



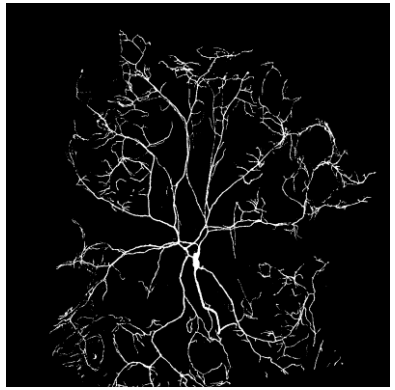
RAC1-WT



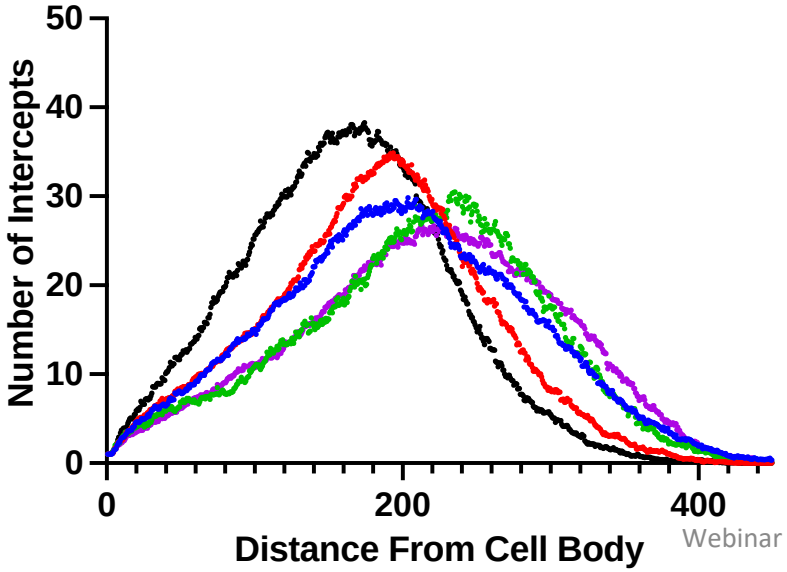
RAC1-R68G
(Activating variant)



RAC1-P73L
(DN variant)



RAC1-T17N



GFP

RAC1-WT

RAC1-T17N

RAC1-R68G (activating patient variant)

RAC1-P73L (dominant negative patient variant)

Conclusions

Variants in *RAC1* cause complex developmental disorders with a wide phenotypic spectrum

RAC1 variants result in at least 3 mechanistically distinct disorders:

Dominant negative	Microcephaly	Reduced dendritic complexity
Activating	Normocephaly	Increased dendritic complexity
?	Macrocephaly	No effect

Likely to be other mechanistically distinct variant groups/sub-groups

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Carlo Marcelis
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Maian Roifman
Ronald Cohn

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Kwame Anyane-Yeboa
Lauren Badalato
Boyan Dimitrov
David Fitzpatrick
Anna Hurst
Anna Jansen
Melissa Kelly
Ian Krantz
Claudine Rieubland
Meredith Ross
Natasha Rudy
Javier Sanz
Katrien Stouffs
Zhuo Luan

+ Patients and their families

tom.millard@Manchester.ac.uk

Discussion & Conclusion

- Time for questions

- Satisfaction Survey :
  - https://forms.office.com/e/kznh4KWQG9
- Website :
  - https://ern-ithaca.eu

Thank you for your participation