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

Abstract

RAC3 is a member of the Rho GTPases family, which has important regulatory functions in aspects of neuronal morphogenesis. Rho GTPases show a conformational change in two regions (switch I and II) through GTP binding, which provides a platform for selective interactions with functionally diverse proteins. Missense variants in the switch I and II regions of RAC3 were recently suggested to cause severe intellectual disability and brain malformations. Here, we report an individual with a novel *de novo RAC3* variant (c.101 C>G, p.(Pro34Arg)), which substitutes for an evolutionarily conserved amino acid within the switch I region. The patient showed severe global developmental delay, intellectual disability, epilepsy, and laryngeal dystonia. An imaging study revealed characteristic brain dysplasia, including coexistence of the middle interhemispheric variant of holoprosencephaly and brainstem dysmorphism. Our study supports that *RAC3* variants cause syndromic neurodevelopmental disorders and brain structural abnormality, and expands the phenotypic spectrum of *RAC3*-related disorders.

Introduction

RAC3 (Rac family small GTPase 3) encodes a small GTPase that belongs to the RAS superfamily of small GTP-binding proteins. RAC proteins (RAC1, RAC2, and RAC3) are members of the Rho GTPases family, which are highly evolutionarily conserved homologous proteins, sharing >88% identity in their amino acid sequences. They have important regulatory functions in aspects of neuronal morphogenesis, such as proliferation, migration, and synaptic plasticity [1]. *RAC3* has been reported to be expressed in the developing nervous system and is involved in interneuron development and maturation [2]. In a recent study, three missense variants in *RAC3* were identified in five individuals with severe global developmental delays (GDDs) and brain malformations [3].

Middle interhemispheric variant of holoprosencephaly (MIH) or syntelencephaly is a rare brain malformation characterized by an abnormal midline connection of the cerebral hemispheres in the posterior frontal and parietal regions [4]. MIH results from a failure of induction and patterning along the rostrocaudal axis of the neural tube, and leads to GDDs and seizures [5].

Here, we report an individual with a *de novo RAC3* missense variant with severe GDD, intellectual disability (ID), epilepsy, laryngeal dystonia, and MIH. We reviewed the literature and discussed the phenotypic features of *RAC3*-related disorders.

Case report

After 39 weeks and 3 days of gestation without asphyxia, a Japanese boy was born to nonconsanguineous healthy parents as their fourth child. There was no family history of neurodevelopmental disorders. His birth weight, body length, and head circumference were 2810 g (−0.97 standard deviation [SD]), 46.8 cm (−1.2 SD), and 32.1 cm

Supplementary information The online version of this article (https://doi.org/10.1038/s10038-019-0656-7) contains supplementary material, which is available to authorized users.

✉ Mitsuko Nakashima
mnakashi@hama-med.ac.jp

[1] Department of Biochemistry, Hamamatsu University School of Medicine, Hamamatsu, Japan

[2] Department of Neurology, Kanagawa Children's Medical Center, Tokyo, Japan

[3] Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan

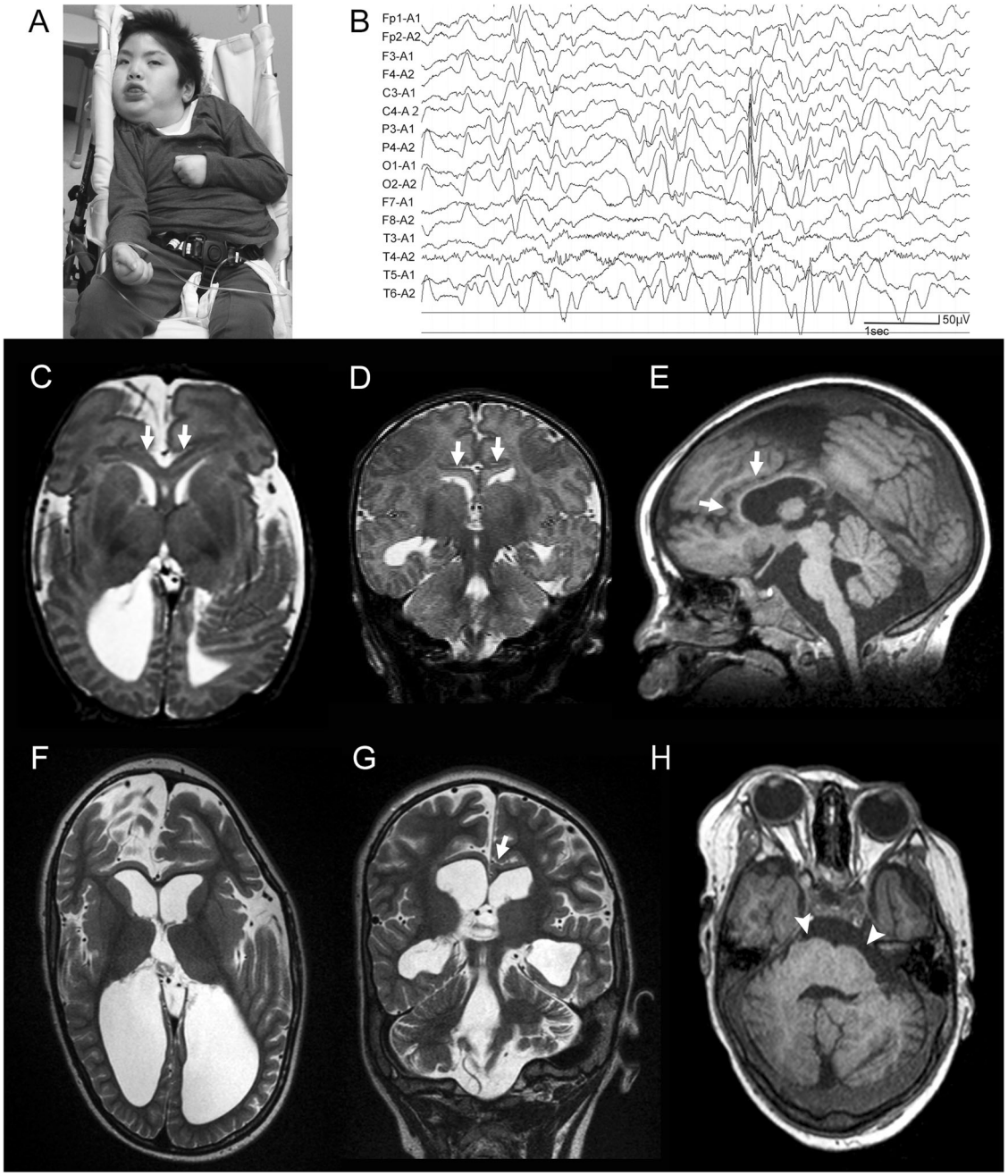

Fig. 1 Cranial and digital features in the patient. **a** A clinical photograph at 6 years and 5 months old. **b** Interictal electroencephalogram at 4 years and 3 months old shows the 2–3 Hz spike-and-waves in the bilateral central, parietal, and posterior areas with a slow wave-dominant background. **c**, **d** Axial **c** and coronal **d** T2-weighted images obtained at 1 day after birth. A thick supracallosal gray matter across the midline interhemispheric fusion (arrow) and irregularly enlarged bilateral posterior ventricles can be observed. **e**–**h** Brain MRI taken at 4 years and 3 months old. **e** Sagittal T1-weighted image shows a small cortex in the surface of a corpus callosum-like structure (arrows), which is not considered to be the corpus callosum. **f** Axial T2-weighted image shows acquired cerebral atrophy. The myelinization progressed and was completed. **g** A coronal T2-weighted image shows continuity of the cortical gray matter on the interhemispheric structure and a protrusion mimicking a gyrus over the left corpus callosum (arrow). **h** An axial T1-weighted image shows dysplasia of the pons, which shows oblong swelling and an irregular surface (arrowhead)

(−0.88 SD), respectively. He had no dysmorphic features (Fig. 1a). He developed seizures 5 h after birth and required respirator support. Daily laryngeal dystonia was observed early after birth. Irritability and rigidity of the shoulder, elbow, knee, ankle, and hip joints were noted when he was 1 month old. He underwent gastrostomy and fundoplication for dysphagia and gastroesophageal reflux disease when he was 2 months old. He experienced several types of seizures

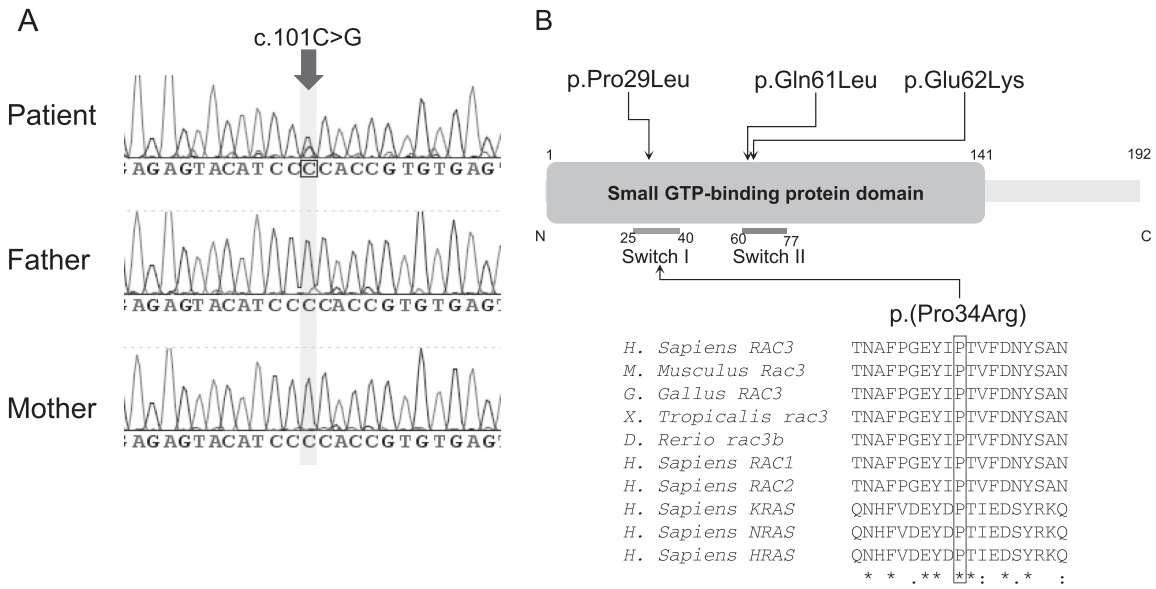

Fig. 2 *RAC3* variant in the patient. **a** Sanger sequencing confirmation of the *RAC3* variant (NM_005052.2:c.101 C>G). Electropherograms revealed a *de novo* heterozygous missense variant in the patient (red arrow). **b** Schematic presentation of the RAC3 protein and location of altered residues. Previously reported *RAC3* variants (p.Pro29Leu, p.Gln61Leu, and p.Glu62Lys) are depicted above [2]. The p.Pro34Arg variant identified in our case is shown below. The p.Pro29Leu and p.Pro34Arg are located in the switch I motif. The other variants are located in the switch II motif. Multiple amino acid sequences of RAC3 were aligned using the ClustalW tool (see http://www.genome.jp/tools/clustalw)

that were refractory to multiple antiepileptic drugs, including myoclonic seizures, tonic seizures, and epileptic spasms. His interictal electroencephalogram revealed spike-and-slow wave complexes in the bilateral central, parietal, and posterior areas (Fig. 1b).

Severe GDD was noticed at early infancy; i.e., he could not perform eye pursuit movements, social smiles, and head control. Brain magnetic resonance imaging (MRI) at 1 day after birth and a follow up study at 4 years revealed progressive cerebral atrophy, brainstem deformation, and deep midline fusion of frontoparietal lobes (Fig. 1c–h). A disorganized gyral formation with deep sulci was also observed mainly on the bilateral frontal and parietal lobes. At 3 years, he had left orchiectomy and right orchiopexy because of left testicular torsion. At 5 years, noninvasive positive pressure ventilation during the night was initiated because of his laryngeal dystonia and frequent respiratory infections.

Upon final examination at 5 years and 7 months old, his height, weight, and head circumference were 110 cm (−0.1 SD), 20 kg (+0.3 SD), and 51 cm (−0.2 SD), respectively. He was bedridden with severe muscle hypertonia and was unable to speak any meaningful words.

Genetic analyses

This study was approved by the Institutional Review Board Committee at Hamamatsu University School of Medicine and Showa University School of Medicine. After receiving written informed consent, peripheral blood samples were obtained from the patient and his parents, and their genomic DNA was extracted from blood leukocytes. The patient's genomic DNA was analyzed by whole-exome sequencing (WES). Data processing, variant calling, annotation, and filtering were performed, as described previously [6]. At least 95.8% of the target RefSeq coding sequences were covered by 20 reads or more. Using the WES data, we found a candidate variant in *RAC3* (NM_005052.2:c.101 C>G, p.(Pro34Arg)). Sanger sequencing revealed that this variant occurred *de novo* (Fig. 2a) and biological parentage was confirmed by analyzing 10 microsatellite markers (data not shown). This variant was absent in our 218 in-house Japanese control exome data and public databases, including the Genome Aggregation Database (gnomAD, see http://gnomad.broadinstitute.org/; accessed on July 2019) and the Integrative Japanese Genome Variation Database (2KJPN, see https://ijgvd.megabank.tohoku.ac.jp/) [7]. The small GTPases have two highly conserved regions, termed switch I and II, whose conformational change accompanied by GTP binding regulates the recognition and binding to effector molecules after cell stimulation [1, 8]. All previously reported *RAC3* variants were positioned in the switch regions and the Pro34 residue was also located in the switch I motif (Fig. 2b). This variant was predicted to be deleterious by *in silico* pathogenicity prediction tools (Supplemental Table S1) and considered as "likely pathogenic" according to the American College of Medical

Genetics Standards and Guidelines (Supplemental Table S2, PS2, PM2, PP3). We also found three other candidate variants in this case, two compound heterozygous variants in *HSD17B4* and one hemizygous variant in *P2RY4* meeting the autosomal recessive or X-linked models (Supplemental Tables S1, S2). It is known that biallelic variants in *HSD17B4* cause D-bifunctional protein deficiency (OMIM# 261515), which is a disorder of peroxisomal fatty acid beta-oxidation leading to infantile death characterized by neonatal hypotonia, seizures and severely impaired psychomotor development, however, both variants found in this case were predicted to be tolerate. In contrast, the hemizygous variant in *P2RY4* predicted to be deleterious. *P2RY4* encodes a purinergic nucleotide receptor coupled to G-protein which highly expresses in small intestine and regulates various signal transduction pathways [9]. Previous study suggested P2RY4 may play a key role in head organizer formation in Xenopus development [10], however, there have been no obvious association with human diseases. Moreover, we examined possible pathogenic copy number variants (CNVs) using WES data with the eXome-Hidden Markov Model (XHMM) [11] and the methods developed by Nord et al. [12]; however, no possible pathogenic CNVs were found in this case. Therefore, we considered that the *RAC3* variant was the most likely to be causative in this case.

Table 1 Clinical findings of cases with the *RAC3* variant

Case	*RAC3* variants	
	This case	Previously reported cases [3]
Neurologic phenotype		
Global developmental delays	Yes	5/5
Abnormal muscle tone	Yes	5/5
Intellectual disability	Yes	5/5
Structural abnormality on brain MRI	Yes	5/5
MIH	Yes	0/5
Polymicrogyria	No	3/5
Heterotopia	No	2/5
Ventriculomegaly	Yes	4/5
Corpus callosum anomaly	Yes	5/5
Macro/microcephaly in childhood	No	0/5
Seizures	Yes	2/5
Extraneurologic phenotype		
Facial dysmorphism	No	5/5
Feeding difficulties	Yes	4/5
Respiratory failure	Yes	0/5
Laryngeal dystonia	Yes	NA

MRI magnetic resonance imaging, *MIH* middle interhemispheric variant of holoprosencephaly, NA not assessed or not available

Discussion

Five individuals with *RAC3* variants were recently described [3]. The clinical manifestations of six individuals with *RAC3* variants are summarized in Table 1, including our case and the other previously reported cases. All cases had GDD, ID, abnormal muscle tone, and brain structural abnormalities, such as corpus callosum (CC) anomaly (agenesis or dysgenesis of the CC, thinned CC and hypoplasia of CC) (6/6), polymicrogyria (3/6), ventriculomegaly (5/6), and heterotopia (2/6). Facial dysmorphisms (5/6) and seizures (3/6) were frequently observed. Hypoplasia of the hindbrain, Chiari type I malformations in two half-sibling cases [3], and dysplasia of pons with cerebellar atrophy in our case were recognized. Meanwhile, two characteristic findings, MIH and laryngeal dystonia causing respiratory failure, were only noted in our case. Laryngeal dystonia, which is one form of movement disorder, is a chronic voice disorder because of uncontrolled spasms in the vocal cords. A case of severe motor and intellectual disabilities with laryngeal dystonia had been reported previously [13], but it very rarely presents with laryngeal dystonia in early infancy. Rho GTPases regulate the actin dynamics important for axonal growth and axonal transport [1, 14]. Axonal defects including decreased axonal outgrowth and impaired axonal transport are characteristic pathological changes in some neurodegenerative diseases [15]. The axons of upper motor neurons convey signals to the lower motor neurons to control muscle movements; therefore, *RAC3* mutants may possibly cause impairment of muscle contractions from early infancy.

Our case had another interestingly finding, i.e., MIH, which is a variant of holoprosencephaly resulting from the failure of dorsal and ventral patterning in the prosencephalon by gestation day 35. Individuals with variants in *RAC1* and *RAC3* showed CC abnormalities and various types of neuronal migration disorders [3, 16]. The knockout mice models indicated that Rac1 deficiency caused midline commissures defects [17]. These findings supported that RAC proteins played important role in the axonal migration. However, no one showed MIH in individuals with variants in both RAC and RAS proteins. Holoprosencephaly is likely to be caused by chromosomal aberrations and oligogenic inheritance [18] or affected by some environmental factors, but its etiology remains unclear. Our case suggests that RAC3 also may have a role in the cleavage process of prosencephalon in embryonic development, whether another genetic or environmental factors will be involved in its pathology. However, further investigations will be necessary to fully elucidate its pathogenesis.

RAC proteins act as a binary switch of cell signaling pathway by cycling between an inactive GDP-bound form and an active GTP-bound state. This cycling process is tightly regulated by multiple guanine nucleotide exchange factors (GEFs) [19], which promote the exchange of GDP for GTP resulting in conformational change to activated form [2, 19]. Previous studies revealed that amino acid alteration in the switch I regions caused binding disability with GEFs or loss of GEF catalysis leading to impairment of signal transduction [20]. The altered Pro34 residue is highly evolutionarily conserved and common in RAC proteins and RAS oncoproteins (KRAS, NRAS and HRAS), which are family members of small GTPase and highly homologous to RAC proteins. (Fig. 2b) [1]. No alteration at Pro34 residue in these proteins had been observed in gnomAD, but plural somatic variants at Pro34 residue in RAC and RAS proteins are registered in the Catalogue Of Somatic Mutations In Cancer (COSMIC, see http://cancer.sanger.ac.uk/cosmic) leading to cause various types of carcinomas. In addition, the pathogenic germline variants at Pro34 in RAC2, KRAS and NRAS was identified in individuals with combined immunodeficiency [21], Cardio-facio-cutaneous syndrome (OMIM# 615278) and Noonan syndrome (OMIM# 609942) [22], respectively. These findings suggest that the amino acid alteration in switch region may have influence on GTPase activation and the alteration at Pro34 residue in RAC3 is likely to cause human diseases.

In conclusion, we identified a novel *de novo RAC3* variant in a case of GDD, epilepsy, and MIH. This case expands the phenotypic spectrum of *RAC3*-related disorders and may be a clue in the explication of the genetic background of holoprosencephaly.

Acknowledgements We would like to thank the patient's family for participating in this work. This work was supported by the Japan Society for the Promotion of Science, a Grant-in-Aid for Scientific Research (B) (grant number: JP16H05160) and (C) (grant number: 16K09975), the Japan Agency for Medical Research and Development, Initiative on Rare and Undiagnosed Diseases (IRUD) Beyond (grant number: JP18ek0109297), a Grant-in-Aid from Hamamatsu University School of Medicine, Health and Labour Sciences Research Grants on rare and intractable diseases from the Ministry of Health, Labour and Welfare, Japan (H29- nanchitou-ippan-025), and Intramural Research Grant (30–6) for Neurological and Psychiatric Disorders of NCNP.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Gonzalez-Billault C, Muñoz-Llancao P, Henriquez DR, Wojnacki J, Conde C, Caceres A. The role of small GTPases in neuronal morphogenesis and polarity. Cytoskeleton 2012;69:464–85.
2. de Curtis I. Roles of Rac1 and Rac3 GTPases during the development of cortical and hippocampal GABAergic interneurons. Front Cell Neurosci. 2014;8:307.
3. Costain G, Callewaert B, Gabriel H, Tan TY, Walker S, Christodoulou J, et al. De novo missense variants in RAC3 cause a novel neurodevelopmental syndrome. Genet Med. 2019;21:1021–6.
4. Barkovich AJ, Quint DJ. Middle interhemispheric fusion: an unusual variant of holoprosencephaly. AJNR Am J Neuroradiol. 1993;14:431–40.
5. Lewis AJ, Simon EM, Barkovich AJ, Clegg NJ, Delgado MR, Levey E, et al. Middle interhemispheric variant of holoprosencephaly: a distinct cliniconeuroradiologic subtype. Neurology 2002;59:1860–5.
6. Nakashima M, Negishi Y, Hori I, Hattori A, Saitoh S, Saitsu H. A case of early-onset epileptic encephalopathy with a homozygous TBC1D24 variant caused by uniparental isodisomy. Am J Med Genet A. 2019;179:645–9.
7. Nagasaki M, Yasuda J, Katsuoka F, Nariai N, Kojima K, Kawai Y, et al. Rare variant discovery by deep whole-genome sequencing of 1,070 Japanese individuals. Nat Commun. 2015;6:8018.
8. Dvorsky R, Ahmadian MR. Always look on the bright site of Rho: structural implications for a conserved intermolecular interface. EMBO Rep. 2004;5:1130–6.
9. Abbracchio MP, Boeynaems JM, Barnard EA, Boyer JL, Kennedy C, Miras-Portugal MT, et al. Characterization of the UDP-glucose receptor (re-named here the P2Y14 receptor) adds diversity to the P2Y receptor family. Trends Pharm Sci. 2003;24:52–55.
10. Harata A, Hirakawa M, Sakuma T, Yamamoto T, Hashimoto C, et al. Nucleotide receptor P2RY4 is required for head formation via induction and maintenance of head organizer in Xenopus laevis. Dev Growth Differ. 2019;61:186–97.
11. Fromer M, Moran JL, Chambert K, Banks E, Bergen SE, Ruderfer DM, et al. Discovery and statistical genotyping of copy-number variation from whole-exome sequencing depth. Am J Hum Genet. 2012;91:597–607.
12. Nord AS, Lee M, King MC, Walsh T. Accurate and exact CNV identification from targeted high-throughput sequence data. BMC Genom. 2011;12:184.
13. Hamano K, Kumada S, Hayashi M, Naito R, Hayashida T, Uchiyama A, et al. Laryngeal dystonia in a case of severe motor and intellectual disabilities due to Japanese encephalitis sequelae. Brain Dev. 2004;26:335–8.
14. Harrington AW, Ginty DD. Long-distance retrograde neurotrophic factor signalling in neurons. Nat Rev Neurosci. 2013;14:177–87.
15. Denton KR, Xu C, Shah H, Li XJ. Modeling axonal defects in hereditary spastic paraplegia with human pluripotent stem cells. Front Biol. 2016;11:339–54.
16. Reijnders MRF, Ansor NM, Kousi M, Yue WW, Tan PL, Clarkson K, et al. RAC1 missense mutations in developmental disorders with diverse phenotypes. Am J Hum Genet. 2017;101:466–77.
17. Chen L, Liao G, Waclaw RR, Burns KA, Linquist D, Campbell K, et al. Rac1 controls the formation of midline commissures and the competency of tangential migration in ventral telencephalic neurons. J Neurosci. 2007;27:3884–93.
18. Kim A, Savary C, Dubourg C, Carré W, Mouden C, Hamdi-Rozé H, et al. Integrated clinical and omics approach to rare diseases:

novel genes and oligogenic inheritance in holoprosencephaly. Brain 2019;142:35–49.
19. Zheng Y. Dbl family guanine nucleotide exchange factors. Trends Biochem Sci. 2001;26:724–32.
20. Gao Y, Xing J, Streuli M, Leto TL, Zheng Y. Trp(56) of rac1 specifies interaction with a subset of guanine nucleotide exchange factors. J Biol Chem. 2001;276:47530–41.
21. Lougaris V, Chou J, Beano A, Wallace JG, Baronio M, Gazzurelli L, et al. A monoallelic activating mutation in RAC2 resulting in a combined immunodeficiency. J Allergy Clin Immunol. 2019;143:1649–.e3.
22. Schubbert S, Bollag G, Lyubynska N, Nguyen H, Kratz CP, Zenker M, et al. Biochemical and functional characterization of germ line KRAS mutations. Mol Cell Biol. 2007;27:7765–70.