Biallelic ELMO3 mutations and loss of function for DOCK-mediated RAC1 activation result in intellectual disability

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ABSTRACT

The engulfment and cell motility 3 (ELMO3) protein belongs to the ELMO-family of proteins. ELMO proteins form a tight complex with the DOCK1-5 guanine nucleotide exchange factors that regulate RAC1 spatiotemporal activation and signalling. DOCK proteins and RAC1 are known to have fundamental roles in central nervous system development. Here, we searched for homozygous or compound heterozygous mutations in the ELMO3 gene in 390 whole exomes sequenced in trio in individuals with neurodevelopmental disorders compatible with a genetic origin. We found a compound heterozygous mutation in ELMO3 (c.1153A>T, p.Ser385Cys and c.1009 G > A, p.Val337Ile) in a 5 year old male child with autism spectrum disorder (ASD) and developmental delay. These mutations did not interfere with the formation of an ELMO3/DOCK1 complex, but markedly impaired the ability of the complex to promote RAC1-GTP-loading. Consequently, cells expressing DOCK1 and either of the ELMO3 mutants displayed impaired migration and invasion. Collectively, our results suggest that biallelic loss-of-function mutations in ELMO3 may cause a developmental delay and provide new insight into the role of ELMO3 in neurodevelopmental as well as the pathological consequences of ELMO3 mutations.

KEYWORDS

ELMO3; dock1; rac1; cell migration; intellectual disability; developmental delay; autism spectrum disorder

Introduction

Neurodevelopmental disorders can lead to cognitive disabilities that affect both intellectual and adaptive functioning, and these can arise as a result of genetic mutations. As such, the discovery of novel mutations associated with such disorders might provide insight into the molecular and cellular defects that lead to cognitive disabilities.

DOCK-family proteins are RAC-specific guanine nucleotide exchange factors (GEFs) with established roles in neural differentiation, dendrite outgrowth, axon formation and axon-dendrite polarity. DOCK1, DOCK2, DOCK3, DOCK4 and DOCK5 can each form a complex with the Engulfment and Cell Motility (ELMO) family of scaffold proteins which includes ELMO1, ELMO2 and ELMO3 [1,2]. The ELMO1-DOCK1, ELMO1-DOCK3 and ELMO2-DOCK4 complexes participate in neurite and dendrite formation and the ELMO/DOCK pathway has been demonstrated to be involved in embryonic central nervous system (CNS) development [3–8]. While disturbance in these molecular functions likely explains the relation of DOCK and RAC1 proteins with a number of neurodevelopmental disorders, the specific roles of ELMO3 in CNS development or in neurodevelopmental disorders have yet to be investigated [9–11].

In the resting state, the ELMO/DOCK complex is auto-inhibited by intramolecular contacts (closed conformation) and exists in an open conformation when in complex with RAC1 and other binding partners [12]. The open conformation ELMO/DOCK complex promotes localized GTP-loading of the small GTPase RAC1 to promote actin cytoskeleton remodelling. The ELMO/DOCK complexes have been implicated in...
a number of cellular processes including migration/invasion, phagocytosis of apoptotic cells and myoblast fusion [1,2]. While ELMO1 and ELMO2 have been shown to function in cytoskeleton rearrangements during cell migration, phagocytosis and myoblast fusion, much less is known about the function of ELMO3 [11,13–15]. To date, ELMO3 has been linked to the migration and invasion of cancer cells and shown to contribute to stem cell renewal in the intestinal epithelium [16–19].

Here, we describe a patient with autism, developmental delay, and compound heterozygous mutations in ELMO3. Functionally, each of the ELMO3 mutations carried by this patient impairs DOCK1-mediated RAC1 activation and cell migration and invasion. These findings support the notion that biallelic loss-of-function ELMO3 mutations can cause a neurodevelopmental disorder characterized by developmental delay and intellectual disability.

Materials and methods

Identification of mutations in the ELMO3 gene

We analysed 390 patients suffering from neurodevelopmental disorders of probable genetic origin by whole exome sequencing for either homozygous or compound heterozygous mutations in the ELMO3 gene (Engulfment and Cell Motility Gene 3, NM_024712.3). We identified a novel compound heterozygous mutation in ELMO3 (c.1153A>T, p.Ser385Cys and c.1009 G>A, p.Val337Ile) in a young boy with autism. The study was carried out in accordance with the Declaration of Helsinki of the World Medical Association and approved by the Local Ethics Committees (Madrid, Spain; Ref. 30,062,019). Informed consent was obtained from parents after a full explanation of the procedures.

Genetic study

Exome sequencing was performed using genomic DNA isolated (MagnaPure, Roche) from whole blood from proband and parents. Libraries were prepared using the Ion AmpliSeq™ Exome Kit (Life Technologies) and quantified by qPCR. The enriched libraries were prepared using Ion Chef™ and sequenced on PI™ Chip in the Ion Proton™ System (Life Technologies) to provide >90% of amplicons with at least 20X coverage. Signal processing, base calling, alignment and variant calling were performed on a Proton™ Torrent Server using the Torrent Suite™ Software. Variants were annotated using Ion Reporter™ Software, and pedigree analysis was performed using the Genetic Disease Screen (GDS) trio workflow. Variant filtering and prioritization were performed with an in-house software and a local database. Candidate variants were visualized using Integrative Genomics Viewer (IGV) and were evaluated based on stringent assessments at both the gene and variant levels, taking into consideration both the patient’s phenotype and the inheritance pattern. Variants were classified following the guidelines of the American College of Medical Genetics and Genomics (ACMG). A board of molecular clinical geneticists evaluated each variant classified as pathogenic, likely pathogenic, or variant of uncertain significance, and decided which, if any, had to be reported. In every case, causal variants were discussed with the referring physician and/or clinical geneticist. Identified variants were confirmed by Sanger sequencing.

Plasmids, cell culture and transfection

pcDNA3.1+/C-(K)DYK-ELMO3 (obtained from Genscript) was used as a template, and the human ELMO3 constructs were added to the pEGFP-C2 plasmid by Gibson Assembly (HiFi, NEB), to generate the following constructs: GFP-ELMO3 WT, GFP-ELMO3 Val337Ile and GFP-ELMO3 Ser385Cys. The pCNX2-Flag-DOCK1 plasmid was a kind gift from M. Matsuda (Kyoto University, Japan). HeLa and HEK293 T cells were maintained in DMEM 10% FBS penicillin/streptomycin. HeLa cells were transfected using Lipofectamine 2000 (Thermo Fisher Scientific) and HEK293T cells by the calcium phosphate method as described in [13].

Co-Immunoprecipitation

Forty-eight hours following transfection with the indicated plasmids by calcium phosphate, HEK293T cells were lysed in 1% NP-40 lysis buffer (150 mM NaCl, 50 mM Tris pH7.5, 1% NP-40) supplemented with 1X Complete protease inhibitor cocktail (Roche). Following centrifugation to clarify cell lysates, 500 ug of total protein extracts were incubated with anti-Flag M2 affinity gel beads (Sigma) for 90 minutes at 4 °C with rotation. Beads containing the immune-complexes were washed three times with the lysis buffer and proteins were analysed by SDS-PAGE and immunoblotting, as previously described [13].

Purification of GST-PAK-PBD

BL21 bacterial cells harbouring the pGEX4T1-PAK-PBD plasmid were grown in 5 ml of LB+ampicillin
media. The next day, 45 ml of LB-ampicillin media was added to the cultured bacteria and induced at 37 °C for 3 hrs in the presence of IPTG. The cells were collected by centrifugation, resuspended in 1 ml of PBS/1% Triton and 1X Complete protease inhibitor cocktail (Roche), and sonicated. Clarified cell lysates were incubated with glutathione beads (Genscript) for 30 min at 4 °C with rotation. Beads were washed three times with PBS/0.1% Triton. The purified GST-PAK-PBD protein was separated by SDS-PAGE and quantified after Coomassie Blue staining of the gel and comparing against a range of concentrations of albumin (Bio-Rad) as a standard.

**GST-PAK-PBD pull down assay**

GST-PAK-PDB affinity precipitation was performed to analyse the GTP-loading status of RAC1 [20]. HEK293T cells were plated on a 10 cm tissue culture dish and transfected with calcium phosphate with pEGFP-C2 alone, Flag-DOCK1 and pEGFP-C2, or Flag-DOCK1 and GFP-ELMO3 WT or either of the ELMO3 mutants (with 1:2 ratio, with a total DNA of 12ug). Forty-eight hours after transfection, cells were lysed in lysis buffer (20 mM Tris pH7.2, 150 mM NaCl, 5 mM MgCl2, 0.5% NP40, 5 mM β-glycerophosphate, 1 mM DTT; final pH = 7.4) and 1X Complete protease inhibitor cocktail (Roche). Lysates were incubated with 10ug of the purified GST-PAK-PDB proteins for 30 minutes at 4 °C with rotation. The beads were washed 3 times with lysis buffer. GTP-loaded RAC1 levels and total cell lysate (for normalization) were analysed by SDS-PAGE and immunoblotting. Quantifications were performed as follow: Pull-Down (GTP-loaded RAC1)/Input (total Rac1). The following antibodies were used: anti-Flag M2 (1:10,000) (Sigma), anti-GFP (B-2) (1:2000) (Santa Cruz), anti-RC1 (1:3333) (Millipore) and anti-Tubulin (1:10,000) (Sigma).

**Wound-Healing assays**

HeLa cells were transfected in 6-well plates using Lipofectamine 2000 with 2ug of pEGFP-C2 alone, or Flag-DOCK1 together with constructs coding for either GFP-ELMO3 WT, GFP-ELMO3 Val337Ile or GFP-ELMO3 Ser385Cys for 48 h before performing the experiments. Boyden Chambers (24-well, 8 μm pore, Co-Star) coated with 100 ul of Matrigel (BD Biosciences) were used. The top chamber was seeded with 50 000 cells and their ability to migrate towards the bottom chamber containing 10% FBS media over a period of 16 h was measured. Cells in the upper chambers were physically removed using cotton swabs and cells on the bottom were fixed with 4% PFA. The membrane was then mounted on a glass slide using SlowFade Gold reagent (Invitrogen) and the average number of GFP positive migrating cells in five independent 10X microscope fields was evaluated.

**Statistics**

Data are presented as mean ± SEM from at least three independent experiments. Statistical analyses were performed with the GraphPad Prism Software using the Student’s t test and p-values <0.05 were considered as significant (*p < 0.05, **p < 0.001, ***p < 0.0001).

**Results**

**Clinical case**

Whole exome in trio analyses were performed from individuals suffering from a neurodevelopmental disorder and revealed two genetic variants in ELMO3 in a young patient with autism spectrum disorder (ASD) (Figure 1a). A missense variant chr16:67,235,720 A/T, c.1153A>T, p.Ser385Cys and a missense variant chr16:67,235,477 G/A, c.1009 G > A, p.Val337Ile were identified (Figure 1a, b). Segregation analysis confirmed a compound heterozygous state, as one of the mutations is present in one allele of the mother and the other mutation is carried by the father, consistent with an autosomal recessive inheritance (Figure 1a). These mutations were further confirmed by Sanger sequencing.
The young patient with the two genetic variants in ELMO3 is a young male child, who was referred to our clinic. At that time, he was 3 years old, the first child of non-consanguineous, apparently healthy parents of Spanish origin. He was the product of a 36-week pregnancy delivered via an uncomplicated C-section to his 40 years old mother. Apgar scores were 9–10 at 1 and 5 minutes, respectively. The birth weight was 2600 g (<5th centile), length 47 cm (5th centile), and the occipitofrontal circumference (OFC) was 34 cm (20th centile). The father of this child needed surgical treatment for an acoustic neuroma. Global developmental delays were noted in the first months of this child's life. He walked unsupported late at 17 months. At 3 years of age, he was speech impaired and showed no interest in interacting with other children, always played alone and avoided social eye contact. Clinical examinations disclosed a weight of 15 kg (50th centile), a height of 98 cm (70th centile), and an OFC of 51 cm (50th centile), without dysmorphic features. Observation of the patient revealed verbal and nonverbal communication deficits, echolalia, neologisms, stereotyped and idiosyncratic language. Routine laboratory screening including thyroid function and neurometabolic tests were within normal range. Sleep video-EEG test and auditory evoked potentials displayed normal results. Brain 3T MRI did not reveal any significant
structural malformations. Conventional genetic studies (karyotype and array comparative genomic hybridization analysis) also revealed no abnormalities.

At the age of 5 years, this child still displayed severe verbal and nonverbal communication deficits. At this age, because of additional concerns regarding social communication and repetitive behaviours, further evaluation was performed using the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Scale (ADOS). He met the criteria for autism on both instruments. Battelle Developmental Inventory revealed scaled scores for each of the five domains (adaptive, social, communication, motor, and cognitive domains) below the 5th centile (equivalent age of 2 years).

**Functional results**

ELMO3, similar to other ELMO proteins, is composed of an N-Terminal Ras-Binding Domain (RBD), an ELMO inhibitory domain (EID), an ELMO domain, an atypical PH domain, an ELMO auto-regulatory domain (EAD) and a C-Terminal proline-rich motif (Figure 1c). Here, we identified a compound heterozygous mutation in ELMO3: c.1153A>T, p.Ser385Cys and c.1009 G > A, p.Val337Ile in the aforementioned patient. Interestingly, one of these mutations (Val337Ile) is found immediately preceding the ELMO domain, while the other one (Ser385Cys) lies within this domain (Figure 1c). Val337 is conserved among the three human ELMO proteins while Ser385 is not. Notably, no function has yet been ascribed to the ELMO domain of ELMO1-3 proteins.

We carried out functional studies to evaluate the impact of each of these mutations on the formation of an ELMO3/DOCK1 complex. Since ELMO3 complexes with DOCK1, DOCK3 and DOCK4 in neurons, it was considered possible that the mutations that are located near or in the ELMO domain of ELMO3 might cause structural aberrations that would affect the interaction with DOCK proteins, which is mediated by the adjacent atypical PH domain [3–7]. However, we found that each of the GFP-ELMO3 mutants displayed a robust interaction with Flag-DOCK1, similar to the wild-type GFP-ELMO3 (Figure 1d). This finding ruled out the possibility that the phenotype was due to abnormal ELMO3/DOCK interactions.

To determine if the ELMO3 mutations affected DOCK1-mediated RAC1 activation, we co-expressed Flag-DOCK1 with either wild-type ELMO3 (GFP-ELMO3 WT) or with each of the mutants in HEK293T and assessed RAC1 activation by PAK-PBD pull-down assays. As expected, these experiments revealed that wild-type ELMO3/DOCK1 robustly promoted RAC1 GTP-loading in comparison to cells transfected with a control plasmid expressing GFP alone (Figure 1e, f). In contrast, co-expression of Flag-DOCK1 with either of the GFP-ELMO3 mutants resulted in greatly decreased activation of RAC1 (Figure 1e, f). Collectively, these experiments strongly suggest that both of the mutations in ELMO3 affect the ability of the ELMO3/DOCK1 complex to stimulate RAC1 GTP-loading.

We next explored whether the defects in RAC1 activation seen with the mutant ELMO3/DOCK1 complexes affected cellular migration and invasion. As expected, expression of wild-type GFP-ELMO3 with Flag-DOCK1 significantly increased cell migration in a wound healing assay in HeLa cells as compared to cells expressing GFP alone (Figure 2a, b). In contrast, when Flag-DOCK1 was co-expressed with either of the GFP-ELMO3 mutants, cell migration in the wound healing assay was significantly diminished (Figure 2a, b). Identical results were obtained when these cells were subjected to Boyden chamber Matrigel invasion assays (Figure 2c). Together, these results demonstrate that while neither of the ELMO3 mutations identified in this patient prevented the formation of a complex with DOCK1, they both profoundly inhibited the ability of the resulting ELMO3/DOCK1 complex to promote RAC1 activation as well as cell migration and invasion, thereby implicating the signalling activity of this axis in the development of the CNS.

**Discussion**

Here we describe the first case of intellectual disability and developmental delay associated with compound heterozygous ELMO3 mutations. Each of the ELMO3 mutations described in this patient interfered with RAC1 activation and cell migration and invasion processes, but the mutant proteins ability to bind DOCK1 was not altered. According to these results, we hypothesize that a lower activity of the ELMO3/DOCK complex could alter the development of the CNS in these cases. Indeed, the involvement of the ELMO1 and ELMO2 proteins with the different DOCK proteins has an important role in neuronal development, dendritic and axonal formation [3–7]. In our study, we propose that the lower activity of RAC1 as well as the alteration of the cell migration process demonstrated in cells expressing ELMO3 mutants could result in neuronal dysfunction [9–11].

This study is the first report of a function of the ELMO domain in any ELMO-family proteins. Both mutations identified fall in or very close to this evolutionarily
conserved domain and result in decreased RAC1 GTP-loading. Exactly how the ELMO domain contributes to RAC1 activation and signalling remains an important area of investigation. Because each of the mutations represents a loss of biochemical function, in the biallelic heterozygosity state in the patient the two variant ELMO3/DOCK complexes are likely to be unable to carry out their main functions of activating RAC1. Since neither parent exhibited phenotypes, a single copy coding for the mutant ELMO3 protein is unlikely to be sufficient to cause developmental defects.

Although DOCK-family and RAC1 proteins have been related to different neurodevelopmental disorders, this is the first case that suggests the relation between ELMO proteins and developmental retardation. DOCK proteins, with known functions in neurite differentiation, dendrite development and axonal growth, have been implicated in different neurological disorders, including autism, psychosis, Alzheimer’s disease, intellectual disability or attention deficit hyperactivity disorder [9,21]. Furthermore, homozygous or compound heterozygous loss-of-functions of DOCK proteins have been proposed as a cause of neurodevelopmental delay [22–24].

Several neurological diseases associated with cognitive disability presenting aberrant spine formation associated with altered active cytoskeleton dynamics have been described. RAC1 is undoubtedly involved in this process and has critical roles in neuronal polarization, axonal development and cell differentiation. Indeed, RAC1 appears to be dysregulated in several neurodevelopmental disorders [10,25]. Additionally, heterozygous RAC1 missense mutations have been related to intellectual disability and abnormal cranial growth patterns [26].

The implication of DOCK-family and RAC1 proteins in other very different neurodevelopmental
disorders, such as attention deficit hyperactivity disorder (ADHD) or dyslexia, has also been described [9,27–29]. Dysfunction of DOCK proteins and their impact on neuronal migration or regulation of BDNF functions have been proposed as a possible cause of ADHD in some patients [30]. Indeed, a pericentric inversion breakpoint in the DOCK3 gene has been described in ADHD patients [31]. The dysregulation of cortical and hippocampal GABAergic interneuron maturation, its functional GAT1 association and glutamate signalling have been described as possible mechanisms to explain the relationship of RAC1 with ADHD [27,32,33].

Although ELMO1 has been previously associated with autism, our case is the first one that relates ELMO3 with this disorder [34,35]. However, no relationship has been previously described between ELMO proteins and other neurodevelopmental disorders. Because of the low haploinsufficiency score of ELMO3 gene, the dysfunction of ELMO3-DOCK1 and its secondary impact on brain development should only be observed in biallelic ELMO3 mutations. More studies are needed to understand the possible participation of the ELMO family in neurodevelopmental disorders that cause autism or intellectual disability. The implication of the DOCK-family and the RAC1 proteins in ADHD raises the possibility that the ELMO proteins could also be involved in other disorders. Indeed, heterozygous loss-of-function mutations of ELMO genes could combine with decreased function in other signalling genes to explain the polygenicity of certain neuronal disorders.

Neurodevelopmental disorders are aetiologically extremely heterogeneous. In recent years, the findings revealed by large-scale next-generation sequencing and heritability studies support the high causal role of genetics. Sequencing studies have identified several mutations that lead to the brain dysfunction resulting from these neurodevelopmental disorders. Although 60% of these gene mutations follow autosomal-recessive inheritance, de novo heterozygous mutations and copy number changes explain the majority of cases with intellectual disability or autism in non-consanguineous populations [36–38]. Rare disorders caused by non-recurrent mutations or infrequent biallelic mutations can provide evidence for the identity of biochemical pathways that can affect neuronal development and function and provide important insight into future avenues for clinical and functional studies.

Contributors

MM and SA provided molecular genetic data; AJDD, PT, BCP and AFJ provided clinical data and reviewed the manuscript; VT and MAG provided functional data and reviewed the manuscript; VT, SA, JFC and AFJ supervised the study and wrote the paper.

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Disclosure statement

The authors declare no conflict of interests.

Patient Consent

Obtained

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References

[1] Laurin M, Cote JF. Insights into the biological functions of dock family guanine nucleotide exchange factors. Genes Dev. 2014;28(6):533–547.
[2] Patel M, Pelletier A, Cote JF. Opening up on ELMO regulation: new insights into the control of Rac signalling by the DOCK180/ELMO complex. Small GTPases. 2011;2(5):268–275.
[3] Miyamoto Y, Yamauchi J. Cellular signaling of dock family proteins in neural function. Cell Signal. 2010;22(2):175–182.
[4] Namekata K, Watanabe H, Guo X, et al. Dock3 regulates BDNF-TrkB signaling for neurite outgrowth by forming a ternary complex with Elmo and RhoG. Genes Cells. 2012;17(8):688–697.
[5] Li W, Tam KM, Chan WWR, et al. Neuronal adaptor FE65 stimulates Rac1-mediated neurite outgrowth by recruiting and activating ELMO1. J Biol Chem. 2018;293(20):7674–7688.
[6] Ueda S, Negishi M, Rac KH. GEF dock4 interacts with cortactin to regulate dendritic spine formation. Mol Biol Cell. 2013;24(10):1602–1613.
[7] Makihara S, Morin S, Ferent J, et al. Polarized dock activity drives shh-mediated axon guidance. Dev Cell. 2018;46(4):417.
[8] Biersmith B, Liu ZC, Bauman K, et al. The DOCK protein sponge binds to ELMO and functions in drosophila embryonic CNS development. PLoS One. 2011;6(1):e16120.
[9] Shi L. Dock protein family in brain development and neurological disease. Commun Integr Biol. 2013;6(6):e26839.

[10] Zeidan-Chulia F, Salmina AB, Noda M, et al. Rho GTPase RAC1 at the molecular interface between genetic and environmental factors of autism spectrum disorders. Neuromolecular Med. 2015;17(4):333–334.

[11] Dong T, He J, Wang S, et al. Inability to activate Rac1-dependent forgetting contributes to behavioral inflexibility in mutants of multiple autism-risk genes. Proc Natl Acad Sci U S A. 2016;113(27):7644–7649.

[12] Chang L, Yang J, Jo CH, et al. Structure of the DOCK2-ELMO1 complex provides insights into regulation of the auto-inhibited state. Nat Commun. 2020;11(1):3464.

[13] Patel M, Margaron Y, Fradet N, et al. An evolutionarily conserved autoinhibitory molecular switch in ELMO proteins regulates Rac signaling. Curr Biol. 2010;20(22):2021–2027.

[14] Patel M, Chiang TC, Tran V, et al. The Arf family GTPase Arl4A complexes with ELMO proteins to promote actin cytoskeleton remodeling and reveals a versatile Rab-binding domain in the ELMO proteins family. J Biol Chem. 2011;286(45):38969–38979.

[15] Hamoud N, Tran V, Aimi T, et al. Spatiotemporal regulation of the GPCR activity of BA13 by C1qL4 and Stabilin-2 controls myoblast fusion. Nat Commun. 2018;9(1):4470.

[16] Peng HY, Yu Q-F, Shen W, et al. Knockdown of ELMO3 suppresses growth, invasion and metastasis of colorectal cancer. Int J Mol Sci. 2016;17(12):2119.

[17] Kristensen LS, Soes S, Hansen LL. ELMO3: a direct driver of cancer metastasis? Cell Cycle. 2014;13(16):2483–2484.

[18] Goyette M-A, Cote J-F. NSCLC metastasis: going with ELMO3. Oncotarget. 2014;5(15):5850–5851.

[19] Coskun M, Boyd M, Olsen J, et al. Control of intestinal promoter activity of the cellular migratory regulator gene ELMO3 by CDX2 and SP1. J Cell Biochem. 2010;109(6):1118–1128.

[20] Cote JF, Vuori K. Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. J Cell Sci. 2002;115(24):4901–4913.

[21] Gadea G, Blangy A. Dock-family exchange factors in cell migration and disease. Eur J Cell Biol. 2014;93(10–12):466–477.

[22] Iwata-Otsubo A, Ritter AL, Weckelbatt B, et al. DOCK3 related neurodevelopmental condition: biallelic intragenic deletion of DOCK3 in a boy with developmental delay and hypotonia. Am J Med Genet A. 2018;176(1):241–245.

[23] Helbig KL, Mroske C, Moomy D, et al. Biallelic loss-of-function variants in DOCK3 cause muscle hypotonia, ataxia, and intellectual disability. Clin Genet. 2017;92(4):430–433.

[24] Riazuddin S, Hussain M, Razzaq A, et al. Exome sequencing of Pakistani consanguineous families identifies 30 novel candidate genes for recessive intellectual disability. Mol Psychiatry. 2017;22(11):1604–1614.

[25] Tejada-Simon MV. Modulation of actin dynamics by Rac1 to target cognitive function. J Neurochem. 2015;133(6):767–779.

[26] Reijnders MRF, Anson NM, Kousi M, et al. Rac1 missense mutations in developmental disorders with diverse phenotypes. Am J Hum Genet. 2017;101(3):466–477.

[27] Lesch KP, Merker S, Reif A, et al. Dances with black widow spiders: misderegulation of glutamate signalling enters centre stage in ADHD. Eur Neuropsychopharmacol. 2013;23(6):479–491.

[28] Bennett MR, Lagopoulos J. Neurodevelopmental sequelae associated with gray and white matter changes and their cellular basis: a comparison between autism spectrum disorder, ADHD and dyslexia. Int J Dev Neurosci. 2015;46(1):132–143.

[29] Chen Y-C, Sudre G, Sharp W, et al. Neuroanatomical, epigenetic and genetic differences in monozygotic twins discordant for attention deficit hyperactivity disorder. Mol Psychiatry. 2018;23(3):683–690.

[30] Namekata K, Harada C, Guo X, et al. Dock3 stimulates axonal outgrowth via GSK-3beta-mediated microtubule assembly. J Neurosci. 2012;32(1):264–274.

[31] de Silva MG, Elliot K, Dahl HH, et al. Disruption of a novel member of a sodium/hydrogen exchanger family and DOCK3 is associated with an attention deficit hyperactivity disorder-like phenotype. J Med Genet. 2003;40(10):733–740.

[32] de Curtis I. Roles of Rac1 and Rac3 GTPases during the development of cortical and hippocampal GABAergic interneurons. Front Cell Neurosci. 2014;8:307.

[33] Won H, Mah W, Kim E, et al. GIT1 is associated with ADHD in humans and ADHD-like behaviors in mice. Nat Med. 2011;17(5):566–572.

[34] Lin YC, Frei JA, Kilander MB, et al. Subset of autism-associated genes regulate the structural stability of neurons. Front Cell Neurosci. 2016;10:263.

[35] Mitra I, Lavillaureix A, Yeh E, et al. Reverse pathway genetic approach identifies epistasis in autism spectrum disorders. PLoS Genet. 2017;13(1):e1006516.

[36] Kochinke K, Zweier C, Nijhof B, et al. Systematic phenomics analysis deconvolutes genes mutated in intellectual disability into biologically coherent modules. Am J Hum Genet. 2016;98(1):149–164.

[37] Gilissen C, Hehir-Kwa JY, Thung DT, et al. Genome sequencing identifies major causes of severe intellectual disability. Nature. 2014;511(7509):344–347.

[38] Shi L, Zhang X, Golhar R, et al. Whole-genome sequencing in an autism multiplex family. Mol Autism. 2013;4(1):8.