

Review

# Rho GTPase Regulators and Effectors in Autism Spectrum Disorders: Animal Models and Insights for Therapeutics

Daji Guo , Xiaoman Yang and Lei Shi \*

JNU-HKUST Joint Laboratory for Neuroscience and Innovative Drug Research, College of Pharmacy, Jinan University, Guangzhou 510632, China; daji0321@stu2017.jnu.edu.cn (D.G.); yxm3644@stu2018.jnu.edu.cn (X.Y.)

\* Correspondence: t\_shilei@jnu.edu.cn or sophielshi80@gmail.com; Tel.: +86-020-85222120

Received: 26 February 2020; Accepted: 26 March 2020; Published: 31 March 2020



**Abstract:** The Rho family GTPases are small G proteins that act as molecular switches shuttling between active and inactive forms. Rho GTPases are regulated by two classes of regulatory proteins, guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs). Rho GTPases transduce the upstream signals to downstream effectors, thus regulating diverse cellular processes, such as growth, migration, adhesion, and differentiation. In particular, Rho GTPases play essential roles in regulating neuronal morphology and function. Recent evidence suggests that dysfunction of Rho GTPase signaling contributes substantially to the pathogenesis of autism spectrum disorder (ASD). It has been found that 20 genes encoding Rho GTPase regulators and effectors are listed as ASD risk genes by Simons foundation autism research initiative (SFARI). This review summarizes the clinical evidence, protein structure, and protein expression pattern of these 20 genes. Moreover, ASD-related behavioral phenotypes in animal models of these genes are reviewed, and the therapeutic approaches that show successful treatment effects in these animal models are discussed.

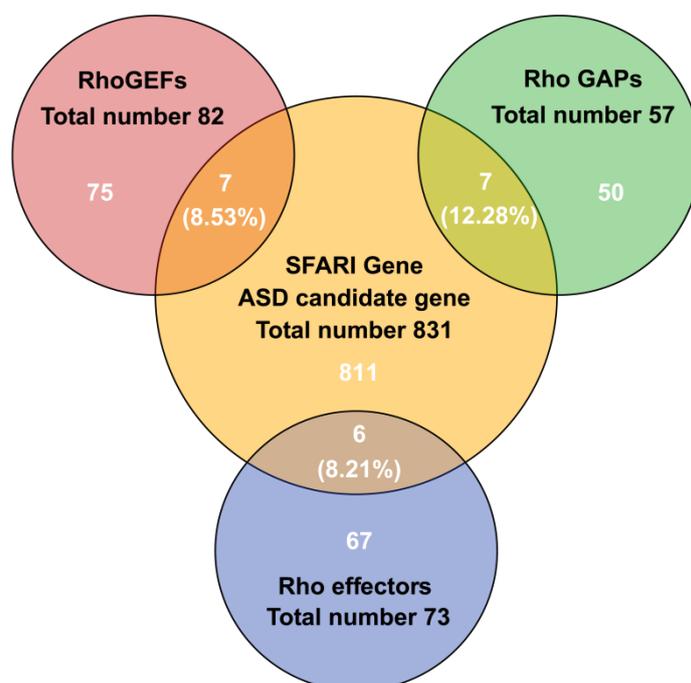
**Keywords:** Rho GTPase; autism spectrum disorder; guanine nuclear exchange factor; GTPase-activating protein; animal model; behavior

## 1. Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by two core symptoms: (1) impaired social interaction and communication, and (2) repetitive or restricted interest and behaviors. The average global prevalence of ASD is ~0.62% [1], and studies in Europe and Asia have identified individuals with ASD with an average prevalence between 1% and 2% [2–6]. Statistics from CDC (Centers for Disease Control and Prevention)'s Autism and Developmental Disabilities Monitoring (ADDM) Network revealed that one in 59 children were diagnosed with ASD in United States in 2018 [7]. In addition, there is a high rate of co-occurring mental health disorders in ASD patients [8]. Meta-analysis of twin studies show that monozygotic twins have significantly higher concordance rate of ASD than dizygotic twins [9,10], thus the aetiology of ASD is closely related to genetic component. However, the genetic causes of ASD are very complex as a huge number of genes contribute to the pathogenesis of ASD. Therefore, databases of ASD-related genes, such as SFARI (Simons foundation autism research initiative) Gene [11,12] and AutDB (the autism gene database) [13], have been established. With the development of genome sequencing, increasing genes related to ASD have been identified. As of November 2019, more than 800 genes have been included in SFARI Gene [14] and more than 1000 genes have been listed in AutDB [15]. Among these ASD susceptibility genes, many converge on synapse regulation such as the regulation of development and maturation of synaptic contacts and synaptic transmission [16–18].

In the nervous system, precise neuronal connectivity depends on synapses. It is well known that dendritic spines, which are enriched with filamentous actin, are dynamic structures important for synapse formation, function and plasticity [19]. Rho family GTPases are key regulators of the actin cytoskeleton that play critical roles in axonal outgrowth, dendritic spine morphogenesis, and synapse formation [20]. The Rho family GTPases, which belong to the Ras superfamily, are small G proteins sized ~20 KDa. Human Rho family GTPases include 20 members that can be classified into eight groups [21,22]. By cycling between GTP-bound active forms and GDP-bound inactive forms, Rho GTPases regulate a diverse array of cellular events, including the control of growth, migration, adhesion, and differentiation. Rho GTPase activity is regulated by two different kinds of regulatory protein: guanine nucleotide exchange factors (GEFs), which catalyze the replacement of GDP by GTP, enabling the GTPases to recognize downstream effectors, and GTP-activating proteins (GAPs), which negatively regulate GTPase activity by favoring the GDP-bound forms [19,23]. Rho GTPase activity regulation is a complex process as 82 GEFs [24,25] and 66 GAPs (of which 57 have a common GAP domain) [26] have been identified so far in humans. The complexity of Rho GTPase signaling is also contributed by their downstream effectors, as there are over 70 downstream effectors identified to be capable of transducing signals from Rho GTPases [27].

A number of extensive review articles have summarized the impact of Rho family GTPases in neural development and diseases [23,28–33]. Moreover, multiple lines of evidence have suggested that Rho GTPase signaling may contribute to the pathogenesis of ASD. By searching SFARI Gene, it is notable to find that 8.53% (seven in 82) of RhoGEFs, 12.28% (seven in 57) of RhoGAPs and 8.21% (six in 73) Rho effectors are categorized as ASD-risk genes (Figure 1); 2.40% (20 in 831) ASD-risk genes directly participate in Rho GTPase signaling. We also find that all these genes encode regulatory proteins or effectors of three most-well studied Rho GTPases, Ras homolog family member A (RhoA), cell division cycle 42 (Cdc42), and Ras-related C3 Botulinum Toxin Substrate 1 (Rac1) (Table 1). In this review, the clinical evidence and animal models of these 20 genes are summarized. Moreover, therapeutic approaches that are capable of correcting the abnormalities caused by dysfunctions of these Rho GTPase regulators and effectors are discussed.



**Figure 1.** Overlap of human gene sets of RhoGEFs, RhoGAPs, and Rho effectors with autism spectrum disorder (ASD) risk genes in Simons foundation autism research initiative (SFARI).

**Table 1.** Rho family GTPases involved in ASD.

ASD Candidate Gene	Gene Name	Chromosome Location	Genetic Category	SFARI Gene Score	Upstream/DOWNSTREAM Rho GTPase(s)
<b>Rho GTPase GEF</b>					
<i>ARHGEF9</i>	Cdc42 guanine nucleotide exchange factor (GEF) 9	Xq11.1-q11.2	Rare Single Gene Mutation, Syndromic	Category 1 (High Confidence)	CDC42
<i>TRIO</i>	Trio Rho guanine nucleotide exchange factor	5p15.2	Rare Single Gene Mutation, Syndromic	Category 1 (High Confidence)	RHOA, RAC1
<i>DOCK8</i>	Dedicator of cytokinesis 8	9p24.3	Rare Single Gene Mutation	Category 2 (Strong Candidate)	CDC42
<i>PREX1</i>	Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1	20q13.13	Genetic Association	Category 2 (Strong Candidate)	RAC1
<i>ARHGEF10</i>	Rho guanine nucleotide exchange factor 10	8p23.3	Rare Single Gene Mutation, Functional	Category 3 (Suggestive Evidence)	RHOA
<i>DOCK1</i>	Dedicator of cytokinesis 1	10q26.2	Rare Single Gene Mutation	Category 3 (Suggestive Evidence)	RAC1
<i>DOCK4</i>	Dedicator of cytokinesis 4	7q31.1	Rare Single Gene Mutation, Genetic Association, functional	Category 3 (Suggestive Evidence)	RAC1
<b>Rho GTPase GAP</b>					
<i>MYO9B</i>	Myosin IXB	19p13.11	Rare Single Gene Mutation	Category 2 (Strong Candidate)	RHOA
<i>OPHN1</i>	Oligophrenin 1	Xq12	Rare Single Gene Mutation, Syndromic	Category 2 (Strong Candidate)	RHOA, RAC1, CDC42
<i>ARHGAP5</i>	Rho GTPase activating protein 5	14q12	Rare Single Gene Mutation	Category 3 (Suggestive Evidence)	RHOA, RAC1, CDC42
<i>ARHGAP11B</i>	Rho GTPase activating protein 11B	15q13.2	Rare Single Gene Mutation	Category 3 (Suggestive Evidence)	Unknown
<i>ARHGAP32</i>	Rho GTPase activating protein 32	11q24.3	Rare Single Gene Mutation, Functional	Category 3 (Suggestive Evidence)	RHOA, RAC1, CDC42
<i>SRGAP3</i>	SLIT-ROBO Rho GTPase activating protein 3	3p25.3	Rare Single Gene Mutation	Category 3 (Suggestive Evidence)	CDC42, RAC1
<i>OCRL</i>	Oculocerebrorenal syndrome of Lowe	Xq26.1	Rare Single Gene Mutation, Syndromic	Syndromic	CDC42, RAC1

Table 1. Cont.

ASD Candidate Gene	Gene Name	Chromosome Location	Genetic Category	SFARI Gene Score	Upstream/DOWNSTREAM Rho GTPase(s)
<b>Rho GTPase Effector</b>					
<i>NCKAP1</i>	NCK-associated protein 1	2q32.1	Rare Single Gene Mutation	Category 1 (High Confidence)	RAC1
<i>CYFIP1</i>	Cytoplasmic FMR1 interacting protein 1	15q11.2	Rare Single Gene Mutation, Genetic Association, Functional	Category 2 (Strong Candidate)	RAC1
<i>PAK2</i>	p21 (RAC1) activated kinase 2	3q29	Rare Single Gene Mutation	Category 2 (Strong Candidate)	CDC42, RAC1
<i>ITPR1</i>	Inositol 1,4,5-trisphosphate receptor type 1	3p26.1	Rare Single Gene Mutation	Category 3 (Suggestive Evidence)	RHOA
<i>PRKCA</i>	Protein kinase C alpha	17q24.2	Rare Single Gene Mutation	Category 3 (Suggestive Evidence)	RHOA, RAC1, CDC42
<i>WASF1</i>	WAS protein family member 1	6q21	Syndromic	Syndromic	RAC1

## 2. Rho Family GTPases and ASD

Rho GTPases themselves have been rarely reported as risk genes of ASD. The only evidence so far is the linkage of *RAC1* with ASD. Rac1 is an important Rho GTPase family member which regulates actin polymerization and spine remodeling through multiple signaling pathways, including PAKs (p21-activated kinases)-LIMK (LIM-domain-containing protein kinase)-cofilin [34], IRSp5 (insulin receptor substrate p53)-WAVE (Wiskott–Aldrich syndrome protein (WASP) family verprolin-homologous protein)-Arp2/3 [35,36], and PKA (protein kinase A) [37]. *RAC1*, encoding RAC1 and RAC1B, is a candidate gene for ASD listed in AutDB. Seven individuals with de novo mutations of *RAC1* were identified in patients of developmental disorders with divergent phenotypes [38]. One of these individuals displayed hyperactive behavior, two presented stereotypic movements, and one was diagnosed with autism [38]. Rac1 is highly expressed in embryonic cortex [39], and is ubiquitously expressed in the hippocampus, neocortex, thalamus, and cerebellum [40,41]. Rac1 is essential for the formation of three germ layers during gastrulation [42], and lack of which leads to embryonic lethality in *Rac1* knockout (KO) mice. To understand the brain function of Rac1, several *Rac1* conditional KO (cKO) mouse models have been constructed and studied. For instance, Foxg1-Cre mediated deletion of *Rac1* in the ventricular zone (VZ) of telencephalon [43–45], Nkx2.1-Cre-mediated deletion in medial ganglionic eminence (MGE) [46], and Nestin-Cre-mediated deletion in precursors of neurons and glia during early embryonic stage [35] were used to study the important roles of Rac1 for brain development. Moreover, three studies investigated the behavioral changes in *Rac1* cKO mouse models. Haditsch and colleagues generated a mouse model in which *Rac1* is deleted in pyramidal neurons by Cre under CamKII $\alpha$  promoter to study the role of Rac1 in memory [47,48]. They demonstrated that loss of Rac1 in the hippocampus impairs long-term potentiation (LTP), and Rac1-deficient mice have impaired spatial memory and working or episodic-like memory [47]. They also found that the impaired working memory in these mice is due to prolonged memory retention or perseveration of the previously learned location [48]. Pennucci and colleagues generated a mouse model named Rac1N mice in which Rac1 is deleted in postmitotic neurons by Synapsin-I-Cre [49]. Rac1N mice show hyperactivity in several exploration tasks, impairment in spatial and working memory, and defects in retaining the context memory [49]. This study also reported failed synchronization of cortical networks in Rac1N mice by quantitative electroencephalogram (EEG). Moreover, spontaneous inhibitory synaptic currents (sIPSCs) are decreased in CA1 glutamatergic pyramidal cells in these mice [49]. However, these findings only focus on memory-related behaviors, but not the typical ASD-related ones such as social behaviors.

## 3. RhoGEF Family and ASD

There are 82 members of the human RhoGEF family, which are divided into two different subtypes: the classical Dbl family and the atypical Dock family [24]. So far, there are 71 members identified in Dbl RhoGEF family, which is characterized by a Dbl Homology (DH) domain, the catalytic GEF domain, and a Pleckstrin-Homology (PH) domain. The DH domain specifically catalyzes the exchange of GDP for GTP, whereas the role of PH domain varies considerably between different members, but is believed to facilitate the activation and localization of all Rho GTPases [25,50,51]. The Dock family, which contains 11 members, shows completely different structural features from the Dbl family. Dock family proteins have two main domains, the Dock homology region (DHR) 1 domain, which is responsible for phospholipid binding, and the DHR2 domain, which possesses the GEF activity [52]. Dock proteins are closely related to neurological disease [28,53]. By examining the overlap of RhoGEF genes and SFARI Gene, we find the following seven *RHOGEFs* as ASD-risk genes: *ARHGEF9*, *TRIO*, *DOCK8*, *PREX1*, *ARHGEF10*, *DOCK1*, and *DOCK4* (see Appendix A) (Table 1).

### 3.1. *ARHGEF9* (SFARI Gene Score: 1, High Confidence)

Rho guanine nucleotide exchange factor 9 (Arhgef9), also known as collybistin (CB), is a Dbl family GEF for Cdc42. *ARHGEF9* is located on chromosome Xq11.1-q11.2. The first report on the

linkage of *ARHGEF9* with ASD identified a de novo microdeletion of Xq11.1 including entire *ARHGEF9* in a male patient, who presented with severe intellectual disability (ID), epilepsy, and mild to moderate autism [54]. A second de novo mutation of *ARHGEF9* was identified in a female patient diagnosed with ASD, ID and speech delay [55]. Subsequently, more de novo deletions of *ARHGEF9* were found in patients with ASD co-occurring with developmental delay (DD) or other mental disorders [56–58], suggesting that *ARHGEF9* is a strong candidate for ASD. Alternative splicing of *Arhgef9* transcripts creates two CB variants, I and II [59]. CB I has typical domains of Dbl family that include an Src homology 3 domain (SH3), a PH and a DH domain followed by a predicted coiled-coil (CC) domain, whereas CB II lacks SH3 and CC domains [59,60] (Figure 2A). CB is expressed predominantly in the brain, with enrichment in the gray matter, cerebral cortex, hippocampus, and cerebellum [59,61,62]. It was found that the CB1 level is high during early brain developmental stage, whereas CB2 expression maintains high and constant levels throughout brain development [60]. CB KO mice exhibited elevated anxiety levels and impaired spatial memory (Table 2; Supplementary Table S1), and showed reduced GABAergic transmission, increased LTP, and decreased long-term depression (LTD) in hippocampal CA1 region [63]. Two studies using this mouse line investigated electrophysiological characteristics in hippocampal dentate gyrus (DG) region, demonstrating that CB plays an important role in maintaining normal granule cell excitability, GABAergic network inhibition, and synaptic plasticity [64,65].

**Table 2.** Summary of ASD-related behavior tests in Rho guanine nucleotide exchange factor (GEF), GTPase-activating protein (GAP), and effector mouse models.

Gene	Mouse Model	Core Symptoms				Comorbidities			Reference
		Social Related Behavior	Language Communication	Repetitive Behavior	Anxiety and Depression	Learning and Memory	Basic locomotion and Motor Coordination	Schizophrenia and Epilepsy	
<b>Summary of ASD-related behavior tests in Rho GEF mouse models</b>									
<i>ARHGEF9</i>	<i>Arhgef9</i> KO mice *	NT <sup>1</sup>	NT	NT	Anxiety ↑	Spatial learning and memory ↓	Activity –	NT	[63]
	<i>Emx1-Trio</i> <sup>-/-</sup> mice #	NT	NT	NT	NT	Spatial learning and memory ↓ Fear memory ↓	NT	NT	[66]
<i>TRIO</i>	<i>NEX-Trio</i> <sup>+/-</sup> mice &	Social preference ↓	NT	Nestlet shredding (M <sup>2</sup> , ↑; F <sup>3</sup> , –)	Anxiety ↑ Depression –	Object recognition memory –	Activity ↓ Motor coordination ↓	Prepulse inhibition –	[67]
	<i>NEX-Trio</i> <sup>-/-</sup> mice &	Social preference ↓	NT	Nestlet shredding (M, ↑; E, –)	Anxiety (M, ↑; F, –) Depression ↑	Object recognition memory –	Activity ↑ Motor coordination ↓	Prepulse inhibition (M, ↓; E, –)	
<i>PREX1</i>	<i>Prex1</i> <sup>-/-</sup> mice *	Social preference ↓ Social learning and memory ↓ Olfactory function –	Ultrasonic vocalizations (pup) ↓	Grooming ↑	Anxiety –	Reversal learning ↓ Fear memory ↓ Object recognition memory –	Activity – Motor coordination –	Prepulse inhibition –	[68]
<i>ARHGEF10</i>	<i>Arhgef10</i> KO mice *	Sociability and social novelty preference ↓	NT	NT	Anxiety ↓ Depression ↓	Spatial learning and memory –	Activity ↑	Prepulse inhibition –	[69]
<i>DOCK4</i>	<i>Dock4</i> KO mice &	Social novelty preference ↓	Ultrasonic vocalizations (pup) ↓	Stereotyped circling (~9% F; M, –) Marble burying (M, –; F, NT) Grooming (M, –; E, NT)	Anxiety ↑	Object recognition memory (F, ↓; M, –) Spatial memory (M, ↓; F, –) Working memory (M, ↓; E, –)	Activity (~9% F, ↑; M, –)	NT	[70]
	<i>Dock4</i> HET mice &	Social novelty preference (F, ↓; M, –)	Ultrasonic vocalizations (pup) –	Stereotyped circling (~1.7% F; M, –) Marble burying (M, –; F, NT) Grooming (M, –; E, NT)	Anxiety –	Object recognition memory – Spatial memory (F, ↓; M, –) Working memory –	Activity (~1.7% F, ↑; M, –)	NT	

Table 2. Cont.

Gene	Mouse Model	Core Symptoms				Comorbidities			Reference
		Social Related Behavior	Language Communication	Repetitive Behavior	Anxiety and Depression	Learning and Memory	Basic locomotion and Motor Coordination	Schizophrenia and Epilepsy	
Summary of ASD-related behavior tests in Rho GAP mouse models									
<i>OPHN1</i>	<i>Ophn1<sup>-ly</sup></i> mice *	Aggressivity ↓ Social memory – Olfactory function ↓	NT	NT	Anxiety –	Working, object recognition, and spatial learning and memory ↓ Fear memory extinction ↓ Vicarious trial and error (VTE) behavior ↓	Activity ↑ Motor coordination – Behavioral lateralization ↓	NT	[71–75]
<i>ARHGAP32</i>	<i>PX-RICS<sup>-/-</sup></i> mice (M were used in most behavior tests unless otherwise stated)	Social novelty preference ↓ social interaction ↓	Ultrasonic vocalizations (M and F, ↓)	Grooming ↑ Marble burying ↑	NT	Reversal learning ↓ Fear memory ↓	Motor coordination ↓	Epilepsy (Severe progressive seizures)	[76,77]
	<i>PX-RICS<sup>+/-</sup></i> mice (M were used in most behavior tests unless otherwise stated)	Social novelty preference ↓ social interaction ↓	Ultrasonic vocalizations (M and F, –)	Grooming – Marble burying ↑	NT	Reversal learning –	Motor coordination –	NT	[76]
<i>SRGAP3</i>	<i>WRP<sup>-/-</sup></i> mice &	NT	NT	NT	Anxiety –	Object recognition and long-term memory ↓ Spatial and reversal learning ↓ Working memory –	Activity – Motor coordination –	NT	[78]
	<i>WRP<sup>+/-</sup></i> mice &	NT	NT	NT	Anxiety –	Object recognition and long-term memory ↓ Spatial and reversal learning ↓ Working memory –	Activity – Motor coordination –	NT	
	<i>SrGAP3<sup>-/-</sup></i> mice &	Social interaction ↓	NT	Marble burying (M, –; F, NT)	Anxiety –	Working memory ↓ Spatial and object recognition memory – Fear memory ↑	Activity (M, ↓; F, –)	Prepulse inhibition (F, ↓; M, –)	[79,80]

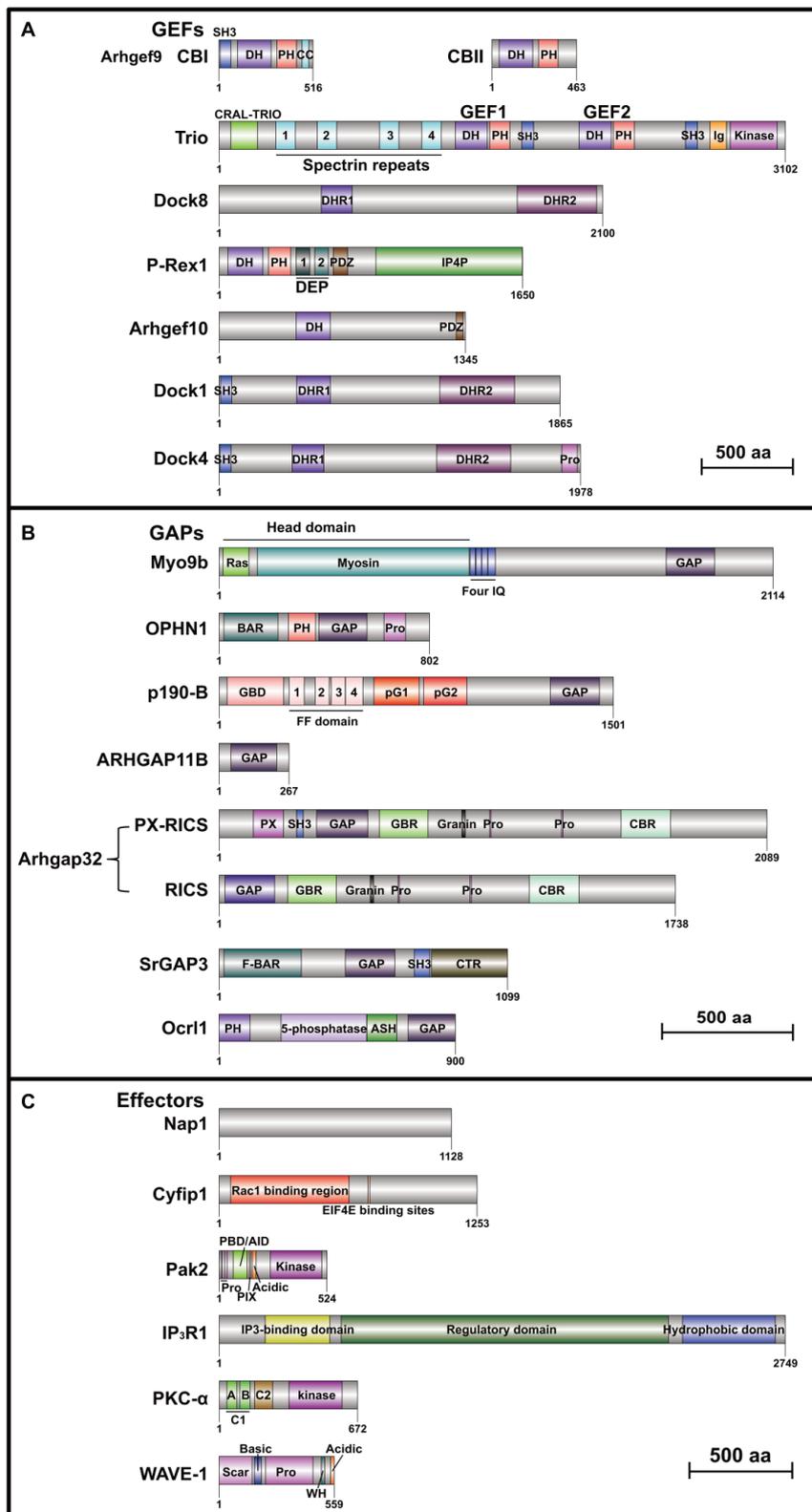
Table 2. Cont.

Gene	Mouse Model	Core Symptoms				Comorbidities			Reference
		Social Related Behavior	Language Communication	Repetitive Behavior	Anxiety and Depression	Learning and Memory	Basic locomotion and Motor Coordination	Schizophrenia and Epilepsy	
OCRL	<i>Ocr11<sup>-ly</sup></i> mice *	NT	NT	NT	NT	Passive avoidance preference –	Activity – Motor coordination ↓	NT	[81]
	<i>Ocr11<sup>-ly</sup></i> mice * ( <i>Inpp5b</i> deleted but human <i>INPP5B</i> overexpressed)	Social preference – Social novelty –	NT	NT	NT	Spatial learning and memory –	Activity ↓	NT	[82]
<b>Summary of ASD-related behavior tests in Rho effector mouse models</b>									
CYFIP1	<i>Cyfi1</i> HET mice *	Social interaction –	NT	NT	Anxiety –	Hippocampus-dependent memory ↓ Working, spatial, and fearing memory –	Activity –	Prepulse inhibition –	[83]
	<i>Cyfi1<sup>HET</sup></i> mice #	Social interest ↓	Ultrasonic vocalizations –	Marble burying –	NT	NT	Activity – Motor coordination ↓	NT	[84]
	<i>Cyfi1</i> m+/p– (Paternal origin) and <i>Cyfi1</i> m–/p+ (maternal origin) mice #	NT	NT	NT	Anxiety-like behavior	Fear memory (m+/p–, ↑; m–/p+, –)	Activity (m+/p–, –; m–/p+, ↓)	NT	[85]
	<i>Cyfi1<sup>+/-</sup></i> mice *	NT	NT	Self-grooming – Marble burying –	NT	Spatial memory and flexibility – Object recognition memory ↓ Working memory –	Activity – Motor coordination ↓	Prepulse inhibition ↓	[86]
	<i>Cyfi1<sup>+/-</sup></i> rat *	NT	NT	NT	NT	Behavioral flexibility ↓	NT	NT	[87]
	Human <i>CYFIP1</i> overexpressing mice (Tg line 1 and Tg line 2) &	Social preference –	Ultrasonic vocalizations –	Grooming – Digging –	Anxiety –	Fear memory (Line 1 and line 2, ↑) Spatial learning memory (Line 2, ↓; line 1, –) Working memory (M and F of both lines, –)	Activity –	Prepulse inhibition –	[88]

Table 2. Cont.

Gene	Mouse Model	Core Symptoms			Comorbidities				Reference
		Social Related Behavior	Language Communication	Repetitive Behavior	Anxiety and Depression	Learning and Memory	Basic locomotion and Motor Coordination	Schizophrenia and Epilepsy	
PAK2	PAK2 <sup>+/-</sup> mice *	Social preference ↓ Social memory ↓	Ultrasonic vocalizations –	Marble burying ↑ Grooming ↑	Anxiety –	Spatial learning and memory –	Activity –	Prepulse inhibition – Acoustic startle response –	[89]
ITPR1	IP3R1 <sup>+/-</sup> mice <sup>&amp;</sup>	NT	NT	NT	NT	NT	Activity – Motor coordination ↓	NT	[90]
	L7-Cre; Itpr1 <sup>flox/flox</sup> mice #	NT	NT	NT	NT	NT	Motor coordination ↓	NT	[91]
	Wnt1-Cre; Itpr1 <sup>flox/flox</sup> mice #	NT	NT	NT	NT	NT	Motor coordination ↓	NT	[92]
WASF1	WAVE-1 KO mice #	NT	NT	NT	Anxiety ↓	Spatial learning and memory ↓ Object recognition memory ↓ Passive avoidance –	Activity ↓ Motor coordination ↓	NT	[93]
	WAVE-1 HET mice #	NT	NT	NT	Anxiety –	Learning and memory –	Activity ↓ Motor coordination ↓	NT	

\*: Only male were used in behavior tests; &: Both male and female mice were used in behavior tests; #: Mice gender was not mentioned; <sup>1</sup> NT: not tested; <sup>2</sup> M: male mice; <sup>3</sup> F: female mice; ↑ is increased and ↓ is decreased; – is no change; More detailed information is shown in Supplementary Table S1.



**Figure 2.** Schematics of protein domain structures of 20 ASD-related RhoGEFs, RhoGAPs, and Rho effectors. (A) Seven ASD-related RhoGEF protein domain architectures. Arhgef9 (which has two variants,

CB I and CB II), Trio, P-Rex1, and Arhgef10 belong to Dbl family, which is characterized by a DH domain (dark violet) and a PH domain (light pink). Dock8, Dock1, and Dock4 belong to Dock family, which contains two main domains, DHR1 domain (dark orchid) and DHR2 domain (dark magenta). (B) Seven ASD-related RhoGAP protein domain architectures. In addition to a common catalytic GAP domain (purple), most RhoGAPs have multiple other functional domains. (C) Six ASD-related Rho effector protein domain architectures. All protein structures are generated using DOG 2.0 (Domain Graph, version 2.0) [94] based on corresponding mouse protein sequences except ARHGAP11B, for which human protein structure is shown as no homologs exist in rodents. Scales represent amino acid numbers of 500. AID, Autoinhibitory Domain; ASH, ASPM/SPD2/Hydin; BAR, Bin/Amphiphysin/Rvs; C1, binding site of diacylglycerol (DAG); C2, binding site of Ca<sup>2+</sup>; CBR,  $\beta$ -catenin-binding region; CC, coiled-coil; CRAL-TRIO, cellular retinaldehyde-binding protein and TRIO guanine exchange factor; CTR, C-terminal region with proline-rich; DEP, Disheveled, EGL-10, Pleckstrin; DH, Dbl Homology; DHR1, Dock homology region 1; DHR2, Dock homology region 2; F-BAR, Fes-Cip4 homology Bin/Amphiphysin/Rvs; FF, domain with two conserved phenylalanine residues; GAP, GTPase-activating proteins; GBD, guanosine triphosphate (GTP)-binding; GBR, GABARAP-binding region; Ig, immunoglobulin; IP4P, inositol polyphosphate 4-phosphatase; IQ, short calmodulin-binding motif containing conserved isoleucine and glutamine residues; PBD, p21-binding domain; PDZ, PSD95/SAP90, DlgA, ZO-1; pG1, pseudoGTPase domain 1; pG2, pseudoGTPase domain 2; PH, Pleckstrin-Homology; PIX, Pak-interacting exchange factor; Pro, proline-rich; PX, phox homology; Scar, Scar homology; SH3, Src homology 3; WH, WASF homology.

### 3.2. TRIO (SFARI Gene Score: 1, High Confidence)

Trio Rho guanine nucleotide exchange factor (Trio), a large protein of the mammalian Dbl family, activates both Rac1 and RhoA. *TRIO*, located on chromosome 5p15.2, is a strong candidate gene for ASD. By using whole-exome sequencing (WES) and transmission and de novo association (TADA) analysis of rare coding variations, a study identified *TRIO* as a gene strongly enriched for variants likely to affect autism risk from 3871 autism cases [95]. Subsequently, several whole-exome and genome sequencing studies identified several *TRIO* variations in subjects with autism [96,97] or ID co-occurring with autism [98,99]. Moreover, a large number of *TRIO* mutations leading to either reduced or excessive TIRO activity were found in ASD and neurodevelopmental disorder (NDD)/ASD/ID patients [100,101]. Trio protein contains two SH3 domains, a CRAL-TRIO (cellular retinaldehyde-binding protein and TRIO guanine exchange factor) domain, an immunoglobulin (Ig) domain, several spectrin-like repeats, two RhoGEF domains and a serine kinase domain [102] (Figure 2A). The GEF domain 1 activates Rac1 and RhoG, whereas the GEF domain 2 acts as an exchange factor for RhoA [103]. Because of the alternative splicing in *Trio*, Trio family is consisted of several isoforms, namely TrioA-E and Tgat [102,104,105]. Trio is enriched in the nervous system, and different isoforms are highly expressed in the cortex, hippocampus, striatum, and cerebellum, except for TrioC, which is highly expressed in the cerebellum [105]. Trio has important roles for embryonic development, as *Trio*<sup>-/-</sup> mice die between embryonic day (E) 15.5 and birth [106]. Trio is also essential for brain development, because *Trio*<sup>NKO</sup> mice, in which the *Trio* is deleted in neuronal and glia progenitors by Nestin-Cre, have high death rate after birth; hypoplasia was found in the residual survival individuals with disruptive development of the cerebellum [107]. Two recent studies on deletion of *Trio* in the forebrain showed that these cKO mice can survive to adulthood. In one study, *Emx1-Trio*<sup>-/-</sup> mice in which *Trio* deletion is restricted to the cerebral cortex and hippocampus show deficient spatial learning [66] (Table 2; Supplementary Table S1). In another study, *Trio* was deleted by NEX-Cre in neocortex and hippocampus starting from E11.5 [108]. Both *NEX-Trio*<sup>+/-</sup> and *NEX-Trio*<sup>-/-</sup> mice displayed impaired social preference and impaired motor coordination, and anxious behaviors were observed in all *Trio* mutant mice except female *NEX-Trio*<sup>-/-</sup> mice [67] (Table 2; Supplementary Table S1). Moreover, partial or full loss of Trio expression in motor cortex Layer 5 pyramidal neurons lead to disruption in presynaptic release probability, postsynaptic currents, and LTP [67]. However, the change in the ratio of NMDA ((N-methyl-D-aspartate)-type glutamate) receptor/AMPA

( $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor-mediated excitatory postsynaptic currents (EPSCs) was opposite in these two mouse lines [67]. These findings show comprehensive phenotypic changes on behaviors and neural function by *Trio* deficiency. It was also found in vitro that gain-of-function forms of *TRIO* variants lead to increased Rac1 activity and synaptic AMPA receptor function, whereas loss-of-function forms lead to decreased Rac1 activity and AMPA receptor function [100,101]. However, gain-of-function *Trio* mouse models have not been generated and investigated for ASD pathology thus far.

Katrantha and colleagues found decreased levels of PDE4A5, a negative regulator of PKA signaling, and corresponding increased PKA signaling in *NEX-Trio<sup>+/-</sup>* and *NEX-Trio<sup>-/-</sup>* motor cortex [67]. To examine approaches for correcting the abnormalities caused by *Trio* deficiency, they transfected *Trio<sup>+/-</sup>/lox* neurons with GFP-P2A-Cre to establish *Trio* haploinsufficiency neurons as an in vitro model. These neurons showed increased spine density and decreased dendritic branch number, mimicking the phenotypes observed in *NEX-Trio<sup>+/-</sup>* mouse brain [67]. Moreover, they treated the *Trio* deficient neurons with Rp-cAMPS, a competitive PKA antagonist, which reversed the increased dendritic spine density [67] (Table 3). For a non-pharmacological approach, PDE4A5 overexpression was capable of correcting the increased spine density but not branching deficits in *Trio<sup>+/-</sup>* neurons [67] (Table 3). However, whether these two therapeutic approaches could rescue abnormal behaviors in *NEX-Trio<sup>+/-</sup>* mice has not been investigated.

**Table 3.** Treatments for Rho GTPase mouse models.

Gene	Mouse/Cellular Model	Therapeutic Type	Therapeutic Strategy	Result	Reference
<i>TRIO</i>	<i>Trio</i> deficient neurons	Pharmacological	Rp-cAMPS treatment (100 $\mu$ M)	Increased dendritic spine density reversed	[67]
		Non-pharmacological	PDE4A5 overexpression		
<i>P-REX1</i>	<i>Prex1<sup>-/-</sup></i> mice	Pharmacological	D-serine (for electrophysiology: 20 $\mu$ M; for mouse: 0.8 g/kg i.p.(intraperitoneal))	NMDAR-LTD restored; disruptive social novelty corrected	[68]
		Non-pharmacological	WT P-Rex1 or WT Rac1 overexpression (in CA1 pyramidal neurons)	NMDAR-LTD restored; disruptive social novelty and reversal learning corrected	
<i>DOCK4</i>	<i>Dock4</i> KO mice	Pharmacological	D-cycloserine (DCS, 20 mg/kg i.p.)	Social novelty restored	
		Non-pharmacological	WT Rac1 overexpression (in CA1 region)	Social novelty and synaptic transmission (mEPSC and LTP) restored	[70]
	<i>Dock4</i> knockdown neurons	Non-pharmacological	WT Rac1 overexpression	Decreased dendritic spine density reversed	[109]
<i>OPHN1</i>	<i>Ophn1<sup>-/-</sup></i> mice	Pharmacological	Rp-cAMPS (bilaterally infused into PFC; 10 $\mu$ g/ $\mu$ L; 300–400 nl)	Cognitive dysfunction in Y-maze ameliorated	[75]
			Fasudil (dissolved in daily drinking water at 0.65 mg/mL for 3 weeks)	Spine morphology in olfactory bulbs, frequency and amplitude of mIPSC in olfactory neurons, and olfactory behaviors rescued	[73]
				Fear memory extinction restored	[74]
			Fasudil (orally a daily dose of 3 mg for 3 months)	Locomotor activity and object recognition memory restored; abnormal brain morphology ameliorated	[72]

Table 3. Cont.

Gene	Mouse/Cellular Model	Therapeutic Type	Therapeutic Strategy	Result	Reference
<i>ARHGAP32</i>	<i>PX-RICS</i> <sup>-/-</sup> mice	Pharmacological	Clonazepam (CZP, 0.03 mg/kg i.p.)	Deficits of social preference, reversal learning, and cued fear learning memory reversed	[76,77]
<i>CYFIP1</i>	<i>Cyfi1</i> HET mice hippocampal slices	Pharmacological	LY367385 (100 μM) and MPEP (2-Methyl-6-phenylethynyl pyridine), (10 μM) (Incubated in slices)	mGluR-LTD normalized to control levels	[83]
	<i>Cyfi1</i> <sup>HET</sup> mice	Non-pharmacological	Motor training (at postnatal days 40, 50, and 51)	Motor deficits alleviated	[84]
<i>PAK2</i>	<i>Pak2</i> <sup>+/-</sup> mice	Non-pharmacological	p-cofilin peptide (15 pmol/g i.v. (intravenous))	Social behaviors moderately improved	[89]
<i>ITPR1</i>	<i>Wnt1-Cre;Itpr1</i> <sup>fllox/fllox</sup> mice	Pharmacological	CNQX (5 mM; infused into the cerebellum; 0.5 μL/min for 20 min)	Dyskinesia ameliorated	[92]
		Non-pharmacological	Mating with <i>Lurcher</i> mice ( <i>GluD2</i> <sup>LC/+</sup> )	Dystonic movements eliminated	

### 3.3. *DOCK8* (SFARI Gene Score: 2, Strong Candidate)

Dedicator of cytokinesis 8 (*Dock8*) belongs to Dock-C subfamily, which lacks recognizable domains besides the DHR1-DHR2 module [28,110] (Figure 2A). *Dock8* displays Cdc42-specific GEF activity [111,112]. *DOCK8* is located on chromosome 9p24.3, which is identified as a linkage region in large autism extended pedigrees [113,114]. Moreover, multiple variants of *DOCK8* are found in several genome sequencing studies on ASD patients [95,115–119]. *Dock8* is primarily expressed in hematopoietic tissues, and *Dock8* deficiency causes a combined immunodeficiency syndrome [110,112,120]. Recently, a study examined the expression of *Dock8* in various cell types in the central nervous system (CNS), which reported that *Dock8* is specifically expressed in microglia, but not neurons, astroglia, and retinal Müller glia [121]. A *Dock8* KO mouse line has been generated, which showed abnormal microglial activity in retina [121]. However, the role of *Dock8* in regulating neural behaviors has not been explored yet.

### 3.4. *PREX1* (SFARI Gene Score: 2, Strong Candidate)

Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1 (P-Rex1) is a member of Dbl family. *PREX1* is located on chromosome 20q13.13. A study on the Chinese Han population revealed that common variations in *PREX1* are found in autistic individuals, and *PREX1* mRNA levels are lower in the peripheral blood cells of autism subjects [68]. The main domain structures of P-Rex1 include a DH domain which has GEF activity, a PH domain which binds to PIP3 (phosphatidylinositol (3,4,5) trisphosphate), two DEP (Disheveled, EGL-10, Pleckstrin) and two PDZ (PSD95/SAP90, DlgA, ZO-1) protein interaction domains, and a C-terminal domain which is similar to IP4P (inositol polyphosphate 4-phosphatase) [122] (Figure 2A). P-Rex1 activates several RhoGEF family members in vitro, but it only activates the Rac family and RhoG in vivo [123]. A study showed that P-Rex1 is expressed mainly in peripheral blood leukocytes and the brain in human [124]. In the developing mouse brain, the expression of P-Rex1 is present in the cerebral cortex, hippocampus, olfactory bulbs, and the cerebellum [125], and P-Rex1 is expressed in different types of cell, including neurons, neural precursor cells, and glial cells [125]. Thus, P-Rex1 may have multiple roles in the nervous system. A study reported several motor behavioral deficits in *Prex1*<sup>-/-</sup>, *Prex2*<sup>-/-</sup> double-knockout mice [126]. A more recent study using a *Prex1* KO mouse line investigated the behavioral, electrophysiological and biochemical changes after P-Rex1 is absent. These *Prex1*<sup>-/-</sup> mice display core ASD-like features, including impaired social novelty and social memory, decreased ultrasonic calls in pups, increased time

in grooming, and disrupted behavioral inflexibility [68] (Table 2; Supplementary Table S1). Moreover, mice with specific knockdown P-Rex1 in hippocampal CA1 region recapitulate the social defects and disrupted behavioral inflexibility in *Prex1*<sup>-/-</sup> mice [68]. The *Prex1*<sup>-/-</sup> mice exhibit impairment in NMDA receptors-dependent LTD in hippocampal neurons [68].

To examine therapeutic approaches to rescue abnormal social behaviors, D-serine, a selective full agonist of the glycine modulatory sites on the NMDA receptors, was examined [68]. *Prex1*<sup>-/-</sup> mice treated with D-serine restore normal behavior of social novelty in the Three-chamber test. Moreover, NMDA receptor-dependent LTD impairment in *Prex1*<sup>-/-</sup> mice is restored after D-serine treatment [68] (Table 3). As *Prex1*<sup>-/-</sup> mice show reduced Rac1 activity in the hippocampus [68], overexpression of Rac1 in the hippocampus of *Prex1*<sup>-/-</sup> mice is able to correct the failure of social preference and reversal learning, and the disruption of NMDA receptors function [68] (Table 3). Furthermore, re-expressing P-Rex1 in *Prex1*<sup>-/-</sup> mice also ameliorates impaired NMDA receptor-dependent LTD, deficient social behavior, and disruptive reversal learning [68] (Table 3).

### 3.5. ARHGEF10 (SFARI Gene Score: 3, Suggestive Evidence)

Rho guanine nucleotide exchange factor 10 (Arhgef10) belongs to the Dbl family, with a DH domain functioning as the GEF for RhoA and a putative PDZ-binding motif [127–129] (Figure 2A). *ARHGEF10* is located on chromosome 8p23.3. Several de novo and inherited missense variants in *ARHGEF10* have been identified in ASD patients [95,119,130]. Arhgef10 is ubiquitously expressed in the central and peripheral nervous system during embryonic development [131] and is widely expressed in the frontal cortex, striatum, hippocampus, and amygdala in adulthood [69]. An *Arhgef10* KO mouse line has been generated, and the mice display defective sociability and social novelty, hyperactivity, and reduced levels of anxiety and depression [69] (Table 2; Supplementary Table S1).

### 3.6. DOCK1 (SFARI Gene Score: 3, Suggestive Evidence)

Dedicator of cytokinesis 1 (Dock1), a member of the Dock-A family, activates Rac1 by its DHR2 domain [132]. *DOCK1* is located on chromosome 10q26.2. A genome-wide association study reported a loss-of-function variant of *DOCK1* in the affected proband as well as the ASD-affected mother, but not in the unaffected sibling [133]. A recent study reported that two autistic siblings have unbalanced translocation on chromosome 10 which leads to *DOCK1* deletion [134]. Dock1 consists of an SH3 domain, a DHR1 domain, and a DHR2 domain [132] (Figure 2A). The levels of Dock1 protein are downregulated during developmental stages in hippocampal neurons [135]. Dock1 has a critical role for embryonic development, and it has been shown that whole-body *Dock1* KO mice are perinatal or neonatal lethal [136]. There have been no *Dock1* cKO mouse models so far for the investigation of neuronal function and behavioral changes related to ASD.

### 3.7. DOCK4 (SFARI Gene Score: 3, Suggestive Evidence)

Dedicator of cytokinesis 4 (Dock4), a member of the Dock-B family, is an atypical Rac1 GEF. *DOCK4* is located on chromosome 7q31.1 which belongs to AUTS1 (designated as autism susceptibility locus 1), in which several ASD-associated genes reside. A comprehensive single nucleotide polymorphism (SNP) genotyping, association and copy number variation study in Caucasian autism families identified the linkage between *DOCK4* and ASD [137]. Several subsequent studies using SNP analysis reported multiple SNPs and chromosome microdeletions or duplications of *DOCK4* in autism and/or dyslexia patients [138,139]. A summary of *DOCK4* variations associated with ASD was provided in our previous study [70]. Dock4 contains an SH3 domain followed by a DHR1-DHR2 module, of which DHR2 is responsible for its GEF activity, and a proline-rich region [28] (Figure 2A). Dock4 is expressed at the highest level in the hippocampus, cortex, and cerebellum in adult rat brain [140], and the expression of Dock4 is upregulated along development in hippocampus in vivo and in hippocampal neurons cultured in vitro [140]. Our recent study used a *Dock4* whole-body KO mouse line to investigate the phenotypes in behaviors, synapse transmission, and molecular alterations. We found that *Dock4* KO

mice display impaired social novelty preference, increased vocalizations, elevated anxiety levels, and disrupted spatial and working memory [70] (Table 2; Supplementary Table S1). Heterozygous (*Dock4* HET) mice also show defective social novelty preference and disrupted spatial memory in Y-maze [70] (Table 2; Supplementary Table S1). Both male and female mice were studied in this study, and the *Dock4* deficient mice show sex-dependent differences in anxiety levels and learning and memory. Notably, a small population of female *Dock4* KO and HET mice exhibit repetitive circling behaviors in home cage and open field arena [70] (Table 2; Supplementary Table S1). Moreover, mice with specific KO of *Dock4* in hippocampal CA1 region also exhibit defective social preference [70]. The hippocampal CA1 neurons of *Dock4* KO mice show impaired excitatory synaptic transmission especially NMDA receptor-dependent transmission, and decreased LTP [70].

As NMDA receptor impairment appeared to be responsible for the synaptic dysfunction in *Dock4* KO hippocampus, a widely used NMDA receptor agonist D-cycloserine (DCS) was used as a pharmacological therapeutic strategy. Indeed, social novelty in the Three-chamber test was restored in *Dock4* KO mice treated with DCS [70] (Table 3). For non-pharmacological therapeutic approaches, overexpressing Rac1 in hippocampus of *Dock4* KO mouse corrects defective social preference and disruptive NMDA receptor function [70] (Table 3). Moreover, overexpressing Rac1 in cultured *Dock4*-knockdown hippocampal neurons also reverses the decreased spine density [109] (Table 3).

#### 4. RhoGAP Family and ASD

To date, 66 RhoGAPs have been identified, most of which contain a common RhoGAP domain that has the catalytic GAP activity [141]. In addition, almost all RhoGAPs have at least two to three additional domains, which may interact with different proteins and are thus engaging the RhoGAPs in different signaling pathways [30,141]. RhoGAPs play irreplaceable roles in axonal and dendritic development, and synaptic plasticity [19,30,31], disruption of which may contribute to the pathological mechanism of ASD. By overlapping 57 GAP domain-containing RhoGAP genes [26] with SFARI Gene, we find eight *RHO*GAPs as ASD-risk genes: *MYO9B*, *OPHN1*, *ARHGAP5*, *ARHGAP11B*, *ARHGAP32*, *SRGAP3*, and *OCRL* (Table 1).

##### 4.1. *MYO9B* (SFARI Gene Score: 2, Strong Candidate)

Myosin IXB (*Myo9b*), a unique member of myosin family, contains a RhoGAP domain in its C-terminal tail, which stimulates the GTP hydrolysis of RhoA but not Cdc42 or Rac1 in vitro [142,143]. *MYO9B* is located on chromosome 19p13.11. Using WES and TADA analysis of rare coding variations of autism patients, *MYO9B* was identified as a gene strongly enriched for variants likely to affect autism risk [95]. *Myo9b* has a three-part structure: a head domain, four calmodulin-binding motifs containing conserved isoleucine and glutamine residues (IQ motifs) in the neck, and a RhoGAP tail [142,144] (Figure 2B). Human *MYO9B* is highly expressed in the immune system, and has minor levels in the respiratory system, digestive system, reproductive system, and nervous system [145]. As *MYO9B* is related to inflammatory bowel diseases [146] and celiac disease [147], the use of *Myo9b* KO mice has been mostly limited in studies of immune cells [148,149]. The role of *Myo9b* in the nervous system has been so far investigated in one study, which reported that *Myo9b* expression in the cerebral cortex reaches peak at around E18, and is decreased during development [150]. Knockdown of *Myo9b* in cultured cortical neurons or in developing cortex results in decreased dendrite length and number [150]. Nonetheless, the function of *Myo9b* in regulating neural behaviors has not been explored.

##### 4.2. *OPHN1* (SFARI Gene Score: 2, Strong Candidate)

Oligophrenin 1 (*OPHN1*) is a RhoGAP family member that is capable of inhibiting RhoA, Rac1, and Cdc42 in vitro without any specificity [151]. *OPHN1* is located on chromosome Xq12 and is closely related to mental retardation (MR) and cerebellar hypoplasia [152]. Rare missense variants and rare hemizygous deletions in *OPHN1* have been identified in ASD patients in different studies [153,154]. In recent exome sequencing studies, several de novo and maternally inherited

variants of *OPHN1* have been found in ASD patients with other mental disorders [99,155]. *OPHN1* possesses a Bin/Amphiphysin/Rvs (BAR) dimerization domain and a PH domain at the N-terminus, followed by the GAP domain and the proline-rich region at the C-terminus [156,157] (Figure 2B). *OPHN1* is ubiquitously expressed with highest levels in various brain regions, including the olfactory bulb, frontal lobes, sensory cortex, hippocampus, thalamus, and cerebellum [151,157], and its brain expression remains high throughout development [150,151,157]. Due to the central role of *OPHN1* for maintaining dendritic spines, *Ophn1* KO mice (*Ophn1*<sup>-/-</sup>) have been generated for studying its in vivo function. *Ophn1*<sup>-/-</sup> mice show hyperactivity, decreased behavioral lateralization in paw preference test, altered spatial memory [71], and impaired object recognition memory [72] (Table 2; Supplementary Table S1). Furthermore, a study showed that *Ophn1*<sup>-/-</sup> mice are impaired in olfactory behavior [73], but another study reported that olfactory function is normal in the mice during social memory test [71] (Table 2; Supplementary Table S1). Lack of *Ophn1* leads to decreased paired-pulse facilitation (PPF) in hippocampal CA1 neurons [71] and increased miniature inhibitory postsynaptic current (mIPSC) amplitude and frequency in olfactory neurons [73]. A further study revealed that *Ophn1*<sup>-/-</sup> mice exhibit disruptive presynaptic plasticity at cortico-lateral amygdala and hippocampal synapses in a PKA dependent manner [74]. The abnormal presynaptic function leads to deficient fear memory extinction in *Ophn1*<sup>-/-</sup> mice [74] (Table 2; Supplementary Table S1). Another recent study demonstrated that *Ophn1*<sup>-/-</sup> mice display cognitive impairment in Y-maze spatial working memory test with high occurrence of perseverative behaviors [75] (Table 2; Supplementary Table S1), which suggests poor behavioral flexibility in *Ophn1*<sup>-/-</sup> mice. This study also found that *Ophn1*<sup>-/-</sup> mice show decreased vicarious trial and error (VTE) behavior [75] (Table 2; Supplementary Table S1), which is a pause and look back and forth behavior reflecting a deliberation process during decision making in rodents [158].

Interestingly, the abnormal behaviors observed in *Ophn1*<sup>-/-</sup> mice are found to be resulted from PKA-dependent dysfunction of medial prefrontal cortex (mPFC) neuronal networks [75]. Consistently, increasing PKA activity in mPFC of WT mice causes similar impairments in Y-maze as observed in *Ophn1*<sup>-/-</sup> mice [75]. Hence, local infusion of Rp-cAMPS, a competitive PKA antagonist, into mPFC of *Ophn1*<sup>-/-</sup> ameliorates the spatial working memory deficits [75] (Table 3). Moreover, RhoA and its effector Rho kinase (ROCK) are well-studied downstream mediators of *OPHN1* in vivo. As over-activation of the RhoA-ROCK pathway was observed in *Ophn1*<sup>-/-</sup> mice [73], fasudil, a clinically approved inhibitor of ROCK as well as PKA, has been examined. Chronic fasudil treatment in *Ophn1*<sup>-/-</sup> mice is able to reverse the alterations of spine morphology and mIPSC in olfactory neurons, and restore olfactory behaviors [73] (Table 3). Furthermore, chronic fasudil treatment restores fear memory extinction, locomotor activity, and object recognition memory in *Ophn1*<sup>-/-</sup> mice (Table 3), but it does not correct the abnormal working and spatial memory in these mice [72,74]. The abnormal brain morphology in *Ophn1*<sup>-/-</sup> mice, including the enlargement of brain lateral ventricles and the increase in hippocampal mushroom-shaped spines, are all ameliorated by fasudil treatment [72] (Table 3).

#### 4.3. *ARHGAP5* (SFARI Gene Score: 3, Suggestive Evidence)

Rho GTPase activating protein 5 (Arhgap5), also known as p190-B, is a member of the RhoGAP family that acts on inhibiting RhoA, Cdc42 and Rac1 [159,160]. *ARHGAP5* is located on chromosome 14q12. Several de novo mutations in *ARHGAP5*, which lead to loss-of-function or missense variants, are found in genome sequencing studies [95,96,161]. From N- to C-terminus, p190-B is composed of a guanosine triphosphate (GTP)-binding domain (GBD), four FF domains, which characterize two conserved phenylalanine residues in each domain, two pseudoGTPase domains (pG1 and pG2), and a GAP domain [162,163] (Figure 2B). p190-B is highly expressed in the brain, stomach, and thymus [164]. A study on the expression pattern of RhoA GAPs shows that the protein level of p190-B is increased in the cerebral cortex during postnatal development [150]. p190-B plays an important role during embryonic development in the CNS [165,166] and hematopoietic system [167,168], and homozygous deletion of p190-B results in perinatal lethality [164]. However, mouse models in which p190-B

is deficient in CNS are still missing for investigation of this protein on regulating neural function and behaviors.

#### 4.4. *ARHGAP11B* (SFARI Gene Score: 3, Suggestive Evidence)

Rho GTPase activating protein 11B (*ARHGAP11B*) is a truncated version of *ARHGAP11A*, which contains 267 amino acids (aa) mostly comprised of a truncated GAP-domain and a unique C-terminal sequence [169] (Figure 2B). *ARHGAP11B* is located on chromosome 15q13.2. Copy number variations of *ARHGAP11B* are found in patients with autism, ID [170], and schizophrenia (SCZ) [171]. Besides, a rare deletion overlapping *ARHGAP11B* is identified in monozygotic twins with SCZ [172]. Another study on 1257 autistic patients reported that loss of *ARHGAP11B* is detected in eight patients, and two of them carry de novo deletions of *SHANK2*, a high risk gene of ASD [173]. *ARHGAP11B* is a human-specific gene with an important role in human neocortex expansion [174,175], especially for the amplification of basal radial glial cells. However, *ARHGAP11B* does not exhibit RhoGAP activity in vivo [174,176], thus it may not truly belong to RhoGAP family. As there is no homolog gene of *ARHGAP11B* in rodents, it is not possible to investigate its function using rodent models.

#### 4.5. *ARHGAP32* (SFARI Gene Score: 3, Suggestive Evidence)

Rho GTPase activating protein 32 (*Arhgap32*), also designated as RICS, Grit, p200RhoGAP, p250RhoGAP, or GC-GAP, is a member of RhoGAP family. *ARHGAP32* is located on chromosome 11q24.3. In a study of 17 patients with Jacobsen syndrome, also called 11q terminal deletion disorder, eight patients with autistic behaviors have 8.7–14.6 Mb deletions of chromosome 11q, affecting four genes including *ARHGAP32* [177]. Another study on 1543 Chinese ASD probands discovered the first de novo LGD (likely gene-disrupting) mutation in *ARHGAP32* [115]. Recently, a study using single-molecule molecular inversion probes on ASD patients found an inherited mutation of *ARHGAP32* [118]. Some studies showed that RICS possesses GAP activity toward Cdc42, Rac1, and RhoA equally [178,179], while others reported that RICS prefers RhoA and Cdc42 [180,181] or even only RhoA [182] as its substrate in vitro. RICS contains five domains, a GAP domain, a GABARAP-binding region (GBR), a granin motif (Granin), a polyproline stretch (Pro-rich), and a  $\beta$ -catenin-binding region (CBR) [183] (Figure 2B). RICS is abundant in the nervous system [178–182], especially in the cerebral cortex, amygdala, thalamus, and hippocampus [181,184]. The expression of RICS in mouse brain reaches to peak at about postnatal day (P) 12 during development, and is downregulated afterwards [185]. Similar expression pattern of RICS is also observed in cultured hippocampal neurons [185]. Immunofluorescent staining of cultured hippocampal neurons and immunoblotting of subcellular fractionations reveal that RICS is concentrated in the postsynaptic density [179,181]. Another longer spliced isoform of RICS, namely PX-RICS, has been reported, which has an additional phospho-homology (PX) domain and an SH3 domain in its N-terminal region [186] (Figure 2B). PX-RICS protein is also predominantly expressed in the nervous system [186], and at relatively low levels in the lung, kidney and spleen [186]. A *RICS* KO (*PX-RICS*<sup>-/-</sup>) mouse line has been generated, in which both RICS and PX-RICS protein are absent [184]. *PX-RICS*<sup>-/-</sup> mice exhibit defective social novelty preference, reduced passive social interaction, decreased ultrasonic calls in pups, increased repetitive behaviors, poor behavioral flexibility, impaired motor coordination, a seizure-prone phenotype, and abnormal cued fear learning memory [76,77] (Table 2; Supplementary Table S1). Moreover, *PX-RICS*<sup>+/-</sup> mice also exhibit moderate defects in social and repetitive behavior [76] (Table 2; Supplementary Table S1). Notably, PX-RICS-deficient hippocampal CA1 neurons show decreased mIPSC amplitude, suggesting impaired GABA<sub>A</sub>R-mediated synaptic transmission in *PX-RICS*<sup>-/-</sup> mice [76]. Hence, clonazepam (CZP), a benzodiazepine agonist of GABA<sub>A</sub>R, was examined as a treatment for *PX-RICS*<sup>-/-</sup> mice. Indeed, CZP administration leads to restoration of normal social preference and improved reversal learning and cued fear learning memory [76,77] (Table 3).

#### 4.6. *SRGAP3* (SFARI Gene Score: 3, Suggestive Evidence)

SLIT-ROBO Rho GTPase activating protein 3 (SrGAP3), also called mental disorder-associated GAP protein (MEGAP) and WAVE-associated RacGAP protein (WRP), is a member of RhoGAP family for Rac1 and Cdc42 but not RhoA [187,188]. *SRGAP3* is located on chromosome 3p25.3. Two de novo missense variants in *SRGAP3* are identified in ASD probands from a genome sequencing study [161]. *SRGAP3* is also listed as a neurodevelopmental-disorder risk gene in ID patients co-occurring with autism [98]. SrGAP3 has four main domains including an N-terminal Fes-Cip4 homology Bin/Amphiphysin/Rvs (F-BAR) domain, a central GAP domain for its Rac1-GAP activity, a C-terminal SH3 domain, and a C-terminal region (CTR) with proline-rich motif [189,190] (Figure 2B). Both human and mouse SrGAP3 is widely expressed in the whole CNS during embryonic development [187,191,192], and highly expressed in the hippocampus, amygdala, thalamus, cortex, and cerebellum of the adult brain [187,193]. So far, two SrGAP3-deficient mouse models have been studied. First, a conditional *SrGAP3* KO mouse model named *WRP* KO mice was generated, in which *SrGAP3* is deleted by Nestin-Cre [78]. The *WRP* HET and KO mice both show impaired long-term memory and spatial memory in several behavior tests, and also display abnormal reversal learning in Morris water maze [78] (Table 2; Supplementary Table S1). Second, a SrGAP3-deficient mouse (*SrGAP3*<sup>-/-</sup> mice) was generated, in which an N-terminal 141 aa of SrGAP3 protein instead of the full length protein was expressed, mimicking the *SRGAP3* deletion of a patient with severe ID [79]. The *SrGAP3*<sup>-/-</sup> male mice display hypoactivity, abnormal social exploration, and impaired working memory, whereas female mice show normal locomotion but severely impaired social behaviors (Table 2; Supplementary Table S1) and decreased prepulse inhibition (PPI) [79]. However, another study using this mouse model reported normal locomotion but reduced marble burying in *SrGAP3*<sup>-/-</sup> male mice [80] (Table 2; Supplementary Table S1).

#### 4.7. *OCRL* (SFARI Gene Score: 5, Syndromic)

Inositol polyphosphate 5-phosphatase *OCRL* (*Ocr1l*), which encodes a type II phosphatidylinositol bisphosphate (PtdIns4,5P<sub>2</sub>) 5-phosphatase, is a RhoGAP family member for Rac1 and Cdc42 [194]. *OCRL* is located on chromosome Xq26.1. Mutations in *OCRL* are related to Lowe syndrome, a multisystem disorders affecting eyes, the nervous system, and kidney [195]. An assessment of 52 male patients with Lowe syndrome using the Autism Screening Questionnaire found that 71.2% of patients met the cut-off score for ASD [196]. Moreover, a study has identified a full gene duplication of *OCRL* in a male ASD patient [197]. *Ocr1l* has four main domains including a PH domain, a central 5-phosphatase domain, an ASPM/SPD2/Hydin (ASH) domain, and a C-terminal RhoGAP domain [198,199] (Figure 2B). Human *OCRL1* is widely expressed in different tissues, with the highest levels observed in the brain, liver, and kidney [81]. A study showed that mice deficient with *Ocr1l* alone fail to recapitulate the abnormalities observed in human [81] (Table 2; Supplementary Table S1). However, another mouse model with deletion of both *Ocr1l* and another type II PtdIns4,5P<sub>2</sub> 5-phosphatase *Inpp5b*, but overexpression of human *INPP5B* display disorders related to Lowe syndrome [200]. Moreover, these mice show dysfunctional locomotor activity caused by muscular defects but normal sociability and learning memory [82] (Table 2; Supplementary Table S1), suggesting that this mouse model may not be used as an ASD model.

### 5. Rho GTPase Effectors and ASD

It is well known that Rho GTPases act as molecular switches that transduce upstream signals to downstream effectors to engage specific signaling cascades. Once in the GTP-bound active forms, the conformations of effector-binding regions of Rho GTPases are changed to allow interaction with the effectors [201]. This interaction regulates the function of effectors, resulting in a series of cell responses to the initial stimuli. There are a large number of molecules involved in Rho GTPase signaling, and more than 70 proteins have been identified as potential effectors of RhoA, Rac1, and Cdc42 [27].

We examine the overlap between these effector genes and SFARI Gene and find the following six effectors as ASD-risk genes: *NCKAP1*, *CYFIP1*, *PAK2*, *ITPR1*, *PRKCA*, and *WASF1* (Table 1).

### 5.1. *NCKAP1* (SFARI Gene Score: 1, High Confidence)

Nck-associated protein 1, also known as Nap1, is a component of the WAVE1/2 complex that interacts with activated Rac1 [202]. *NCKAP1* is located on chromosome 2q32.1. A de novo LGD mutation of *NCKAP1* has been identified in ASD probands [203]. Subsequently, several de novo or maternally inherited mutations in *NCKAP1* are found in multiple WES studies on ASD probands [95,161,204]. Recently, two studies on Chinese and Caucasian ASD cohorts found more de novo LGD mutations in *NCKAP1*, which suggests that *NCKAP1* is a strong candidate gene for ASD [115,205]. Nap1 does not contain any known functional motif [206] (Figure 2C). Human Nap1 is extensively expressed in multiple tissues except peripheral blood leukocytes, with highest expression detected in the brain, heart, and skeletal muscle [207]. The expression of human Nap1 is ubiquitously observed in all brain regions, with relatively higher levels in the cerebellum, hippocampus, and amygdala [207]. A study reported that human Nap1 is preferentially expressed in neuronal cells and may participate in neuronal apoptotic pathway [207]. Immunoblots from different embryonic ages indicate a pattern of developmentally increased Nap1 expression in the mouse cerebral cortex [208]. Yokota and colleagues found that knockdown of endogenous Nap1 leads to defective neuronal differentiation in mouse cortical neurons [208]. They then generated a Nap1 mutant mouse line in which the N-terminal 898 aa of Nap1 fused with a 1291 aa  $\beta$ -geo reporter is expressed instead of the full-length protein [208]. However, these Nap1 mutant mice are embryonic lethal from E8.5–E10.5 due to neural tube and neuronal differentiation defects [208]. Therefore, mouse models of Nap1 deficiency are still absent for the investigation of Nap1 on regulating neural behaviors.

### 5.2. *CYFIP1* (SFARI Gene Score: 2, Strong Candidate)

Cytoplasmic FMR1 interacting protein 1 (Cyfip1), also named Shyc and Sra1, is a Rac1-interacting protein and a partner of the WAVE complex that regulates actin filament. *CYFIP1* is located on chromosome 15q11.2. Several studies have reported that patients with 15q11.2 microdeletions and microduplications between breakpoints 1 and 2, which encompass several genes including *CYFIP1*, are diagnosed with neurodevelopmental disorders including ASD, ID, SCZ, attention-deficit/hyperactivity disorder (ADHD), and obsessive-compulsive disorder (OCD) [209–212]. A paternally inherited rare variant of *CYFIP1* is found in an autistic patient with a de novo *SHANK2* deletion [173]. Another paternally inherited rare variant of *CYFIP1* is found in a study on high functioning ASD patients [213]. Additionally, SNPs in *CYFIP1* were reported to correlate with ASD in two studies [214,215]. A study on *CYFIP1* mRNA expression in human dorsolateral prefrontal cortex revealed higher expression of *CYFIP1* mRNA in ASD and classical autism patients, and identified several common variants of *CYFIP1* in these patients [216]. A recent study also demonstrated significant increase in *CYFIP1* transcripts in the peripheral blood of ASD patients [217]. All these clinical findings reveal that altered *CYFIP1* dosage may contribute to the pathology of ASD. Cyfip1 has been shown to interact with Rac1, Fragile X mental retardation 1 (FMRP), and eukaryotic translation initiation factor 4E (EIF4E) [218,219] (Figure 2C). Cyfip1 is widely expressed in multiple tissues but not liver during development and is highly enriched in the hippocampus, cerebral cortex, cerebellum, olfactory bulb, and lateral septum in the adult brain [220]. Levels of Cyfip1 are high in the cortex and cerebellum at the stages of postnatal development, peaked at P23 and slightly decreased afterwards [221].

Four different strategies have been used for generation of *Cyfip1* KO mice. However, because Cyfip1 is very important for early embryonic development, none produce homozygous *Cyfip1* KO mice. Therefore, *Cyfip1* HET mice are used for studying behavioral and neural phenotypes in these studies. The first mouse model was generated by mutagenesis with a gene trap vector inserted into intron 1 of *Cyfip1*. There are no *Cyfip1* KO embryos in breeding [83]. *Cyfip1* HET mice display normal learning and memory abilities in several memory tests, but show more rapid extinction in inhibitory

avoidance test, a test for hippocampus-dependent memory [83] (Table 2; Supplementary Table S1). These results indicate that the accuracy of memory processing in HET mice is much poorer. *Cyfiip1* HET mice show increased mGluR-dependent LTD and abnormal presynaptic function in hippocampal slices [83,222]. The second one is generated by deleting exons 4–6 of *Cyfiip1*. Inbred from *Cyfiip1*<sup>HET</sup> mice, fertilized *Cyfiip1* KO oocytes are detectable in blastocyst stage [223]. The KO embryos can survive until E8.5, but become lethal due to complete developmental failure [223]. The *Cyfiip1*<sup>HET</sup> mice show absence of interest for social cues and deficits in motor learning [84] (Table 2; Supplementary Table S1). The third one is generated by deleting exon 5 of *Cyfiip1*, and the *Cyfiip1* homozygous KO embryos die before E9.5 [85]. Moreover, the difference between the maternal (m−/p+) and paternal (m+/p−) deficiency of *Cyfiip1* was investigated. Interestingly, *Cyfiip1* m−/p+ mice only display hypoactivity, whereas *Cyfiip1* m+/p− mice display increased freezing in cued fear conditioning and abnormal transitions in zero-maze test [85] (Table 2; Supplementary Table S1). Both *Cyfiip1* m−/p+ mice and *Cyfiip1* m+/p− mice show reduced field EPSC and increased PPF in hippocampal CA1 region, and enhanced mGluR-dependent LTD is observed in *Cyfiip1* m+/p− mice hippocampal CA1 neurons [85]. The fourth one is generated by inserting a gene trap cassette between exon 12 and 13 of *Cyfiip1*. *Cyfiip1*<sup>+/-</sup> mice display impaired motor coordination, deficient sensory processing/novelty seeking behavior, reduced PPI, and decreased sensory motor gating [86] (Table 2; Supplementary Table S1), recapitulating some ASD and SCZ-like behavioral phenotypes. *Cyfiip1*<sup>+/-</sup> mice show reduced spontaneous neuronal activity and presynaptic function in cortical slices [86]. Besides the whole-body mutant mice, one *Cyfiip1* cKO mouse model (*Cyfiip1*<sup>NEX</sup> cKO mice) has been generated, in which exon 4–6 of *Cyfiip1* was deleted in forebrain excitatory neurons by NEX-Cre. These mice are viable until adulthood with no obvious abnormalities [224]. *Cyfiip1*<sup>NEX</sup> cKO mice show increased mIPSC amplitude in hippocampal CA1 neurons and display similar deficits in dendrite morphology and spine maturation to those described in *Cyfiip1* haploinsufficient models [224]. There is also a *Cyfiip1* mutant rat model generated by CRISPR/Cas9 technology, in which a 4 bp heterozygous deletion is introduced in exon 7 of *Cyfiip1*, causing premature stop of the protein [87]. These *Cyfiip1* haploinsufficient rats exhibit normal learning ability during all behavioral tests, but show deficits in behavioral flexibility [87] (Table 2; Supplementary Table S1). As increased transcript levels of *CYFIP1* is found in some ASD patients, two transgenic (Tg) *Cyfiip1* mouse lines (Tg line 1 and Tg line 2) were generated by overexpressing human *CYFIP1*. mRNA of human *CYFIP1* was increased in the cortex and hippocampus of both Tg lines; *Cyfiip1* protein is also increased in the two brain regions of Tg line 1 and the hippocampus of Tg line 2, but not in the cortex of Tg line 2 [88]. Behaviorally, Tg line 2 mice display subtle defects of spatial learning memory and obvious increased fear response in contextual and cued fear conditioning test, whereas Tg line 1 mice show normal spatial learning memory and increased freezing only to the tone in the novel context during fear conditioning test [88] (Table 2; Supplementary Table S1). However, both Tg lines show no deficits in core ASD-related behaviors such as social and repetitive behaviors [88] (Table 2; Supplementary Table S1). Together, *Cyfiip1* deficient mice or rats recapitulate core features of ASD, whereas *Cyfiip1* Tg mice may not be appropriate ASD models.

As increased mGluR activation was caused by *Cyfiip1* deficiency, mGluR1 inhibitor LY367385 and mGluR5 antagonist MPEP (2-Methyl-6-(phenylethynyl) pyridine) were used together. Indeed, these mGluR blockers normalized the mGluR-LTD to control levels in hippocampal slices of *Cyfiip1* HET mice [83] (Table 3). However, the effect of these two inhibitors on behaviors has not been investigated. For non-pharmacological therapeutic approaches, motor training increased the number of newly formed dendritic spines in both WT and *Cyfiip1*<sup>HET</sup> mice, and the motor learning deficits in *Cyfiip1*<sup>HET</sup> mice can be alleviated by the behavioral training in early development but not in adult [84] (Table 3).

### 5.3. *PAK2* (SFARI Gene Score: 2, Strong Candidate)

P21 activated kinase 2 (Pak2), activated by Rac1 and Cdc42, is a member of the group I PAK family that belongs to a family of serine/threonine kinases. *PAK2* is located on chromosome 3q29, which is classified as one of six strong autism risk loci [225]. A study on six patients of 3q29 microdeletion

syndrome identified a ~1.5 Mb microdeletion, which includes entire *PAK2*. Two of these patients displayed autistic features [226]. Another study on two patients of 3q29 microdeletion syndrome with common ID, a history of autism, and other psychiatric symptoms also reported the same length deletion [227]. In a subsequent study, 11 of 44 patients with 3q29 microdeletion syndrome were found to display diverse neurodevelopmental disorders including autism [228]. Moreover, one de novo copy-number deletion containing *PAK2* was found in patients with ASD from the Simons Simplex Collection, and one de novo nonsense mutation and two inherited missense mutations were also found in *PAK2* in 914 Han Chinese patients with ASD [89]. All these four studies reveal that *PAK2* is a strong candidate for ASD. Pak2 has several recognized domains including two proline-rich regions and an AID (Autoinhibitory Domain) overlapping the PBD (p21-binding domain) in the N-terminal region, a kinase domain at the C-terminus, an acidic region, and a PIX (Pak-interacting exchange factor) binding site [229–231] (Figure 2C). Pak2 is ubiquitously expressed in multiple tissues [232]. Human *PAK2* shows high expression levels in the brain during the fetal period and low levels after birth. Mouse Pak2 is also down-regulated at the postnatal development in the cortex [89]. Loss of *Pak2* leads to embryonic lethality at ~E8 [232–234]. Therefore, a recent study used *Pak2*<sup>+/-</sup> mice to investigate the behavioral and neural functional changes caused by *Pak2* haploinsufficiency. This study revealed that *Pak2*<sup>+/-</sup> mice display repetitive and stereotyped behaviors, impaired social interaction, social avoidance, reduced social preference index, and disruptive social memory [89] (Table 2; Supplementary Table S1). *Pak2*<sup>+/-</sup> mice also exhibit decreased LTP in hippocampal CA1 region neurons [89]. Mechanistically, phosphorylation of both LIMK1, a major downstream target of group I PAKs, and its substrate cofilin were markedly decreased, suggesting abnormal LIMK1/cofilin-mediated actin polymerization in adult *Pak2*<sup>+/-</sup> mice cortex [89]. To normalize endogenous p-cofilin levels in the cortex and hippocampus, a p-cofilin peptide was intravenously injected into adult *Pak2*<sup>+/-</sup> mice as a substrate to compete with endogenous p-cofilin for phosphatases. As a result, this peptide moderately improved social behaviors but not repetitive behaviors in adult *Pak2*<sup>+/-</sup> mice [89] (Table 3).

#### 5.4. *ITPR1* (SFARI Gene Score: 3, Suggestive Evidence)

Inositol 1,4,5-trisphosphate receptor type 1, also known as IP<sub>3</sub>R1, is a RhoA effector. IP<sub>3</sub>R1 is a member of IP<sub>3</sub>Rs, which are Ca<sup>2+</sup> release channels on the endoplasmic reticulum. *ITPR1* is located on chromosome 3p26.1. Three de novo missense variants in *ITPR1* are found in ASD probands from WES studies [95,115,161]. In addition, a study on autism risk genes in probands from the Autism Clinical and Genetic Resources in China (ACGC) identified one maternally and one paternally inherited missense variant of *ITPR1* [118], and another variant is found in patients with NDDs [235]. IP<sub>3</sub>R1 has three main domains including a large N-terminal IP<sub>3</sub>-binding domain, a short C-terminal hydrophobic domain, and an intervening regulatory domain [236] (Figure 2C). The expression of IP<sub>3</sub>R1 is increased during embryogenesis [237]. Moreover, IP<sub>3</sub>R1 is predominately expressed in the nervous system [238,239] and is expressed in a wide range of brain regions including the cerebellum, cerebral cortex, hippocampus, olfactory bulb, globus pallidus, and striatum [238]. It was reported that homozygous *IP3R1*-deficient mice mostly die during the embryonic stage, and the born mice have severe ataxia and seizures and die at around P21 [240]. *IP3R1*<sup>+/-</sup> mice also show deficits in motor coordination [90] (Table 2; Supplementary Table S1). To study the neural function of IP<sub>3</sub>R1, several *IP3R1* brain cKO mouse lines have been generated. A study reported that *L7-Cre;Itpr1*<sup>flox/flox</sup> mice, in which *Itpr1* is deleted in Purkinje cells, exhibit cerebellar ataxia at around 6 weeks and severe ataxia at 8 weeks after birth [91] (Table 2; Supplementary Table S1). *L7-Cre;Itpr1*<sup>flox/flox</sup> mice could survive to adulthood, but display abnormal motor learning ability [91] (Table 2; Supplementary Table S1). In another study, three cKO lines were used, in which *Itpr1* deletion was restricted to the cerebral cortex and hippocampus (*Emx1-Cre;Itpr1*<sup>flox/flox</sup> mice), the cerebellum and brainstem (*Wnt1-Cre;Itpr1*<sup>flox/flox</sup> mice), and the caudate putamen and globus pallidus (*Gpr88-Cre;Itpr1*<sup>flox/flox</sup> mice) [92]. The *Emx1-Cre;Itpr1*<sup>flox/flox</sup> mice and *Gpr88-Cre;Itpr1*<sup>flox/flox</sup> mice were born normally and showed normal growth patterns until adulthood, without apparent dyskinesia like total *Itpr1*<sup>-/-</sup> mice.

However, *Wnt1-Cre;Itpr1<sup>flox/flox</sup>* mice began to show ataxia at around P9, and exhibited dyskinesia from 2 weeks after birth [92] (Table 2; Supplementary Table S1). *Wnt1-Cre;Itpr1<sup>flox/flox</sup>* mice show abnormal cerebellar Purkinje cell (PC) firing patterns, due to altered PC activity [92]. *Itpr1* deficiency induces a series of abnormal electrophysiological features, including failed LTD in the PCs [241], and excessive LTP induction, attenuated depotentiation and LTP suppression, and altered presynaptic activity in hippocampal CA1 neurons [242,243]. However, typical ASD-related behaviors such as social and repetitive behaviors have not been investigated using these mice.

As enhanced PC activity and dystonia were observed in *Wnt1-Cre;Itpr1<sup>flox/flox</sup>* mice, pharmacological inactivation of cerebellar activity by AMPA receptor antagonist (CNQX) infusion was examined for therapeutic effects. Indeed, CNQX ameliorates the dyskinesia in these mice [92] (Table 3). Furthermore, dystonic movements were completely absent in *Wnt1-Cre;Itpr1<sup>flox/flox</sup>* mice with genetic deletion of cerebellar PCs, achieved by mating *Wnt1-Cre;Itpr1<sup>flox/flox</sup>* mice with *Lurcher* mice (*GluD2<sup>LC/+</sup>*) to kill most of PCs by a mutation of the delta 2 glutamate receptor (*GluD2*) [92] (Table 3).

### 5.5. *PRKCA* (SFARI Gene Score: 3, Suggestive Evidence)

Protein kinase C alpha (PKC- $\alpha$ ) is a member of lipid-sensitive serine/threonine protein kinases that regulate various cellular functions including proliferation, migration, adhesion, differentiation, and apoptosis. PKC- $\alpha$  appears to be a common downstream effector of RhoA, Cdc42, Rac1. *PRKCA* is located on chromosome 17q24.2. In two WES studies, three de novo missense variants in *PRKCA* were reported in ASD probands [95,161]. PKC- $\alpha$  has a variable regulatory domain at the N-terminus consisting of a C1 domain and a C2 domain, which function as the binding sites of diacylglycerol (DAG) and Ca<sup>2+</sup>, respectively. PKC- $\alpha$  also has a highly conserved kinase domain at the C-terminus comprised by a smaller ATP-binding loop and a substrate-binding site [244,245] (Figure 2C). PKC- $\alpha$  is ubiquitously expressed in all tissues, including the heart, adrenal gland, testis, lung, kidney, spleen, and liver, and is also widespread in various regions of the brain [246]. PKC- $\alpha$  is enriched in both neuronal and glial cultured cells [246]. A PKC- $\alpha$  KO mouse line has been generated, which appears to be normal with regard to external characteristics, viability, and fertility [247]. However, ASD-related behavioral analysis of these mice has not been reported.

### 5.6. *WASF1* (SFARI Gene Score: S, Syndromic)

The Rac1 effector WAS protein family member 1 (*WASF1*), also known as WAVE-1/Scar1, is a member of the WASP-family. *WASF1* is located on chromosome 6q21. Using exome sequencing and whole-genome sequencing, three de novo truncated mutations of *WASF1* were reported in five unrelated individuals, all of whom presented ID with autistic features and seizures [248]. This is the only study so far that has reported the relationship between *WASF1* and ASD. WAVE-1 is composed by a Scar homology domain, a basic domain, a proline-rich region, a WASF homology (WH) domain, and an acidic domain [249] (Figure 2C). In human tissues, the expression of WAVE-1 is restricted to the brain [250]. Mouse study reveals that WAVE-1 shows high expression in the hippocampus, cortex, hypothalamus, amygdala, and cerebellum [93,251,252]. Two mouse lines of homozygous *Wave1* deletion are reported to be either postnatal lethal [251] or reduced in body size of offspring [93]. Behavioral analysis using *Wave1* KO mice (WAVE-1 null mice) generated by the second strategy showed hypoactivity, impaired motor coordination and balance, reduced anxiety levels, and defected spatial, nonspatial, and emotional learning and memory in the mice [93] (Table 2; Supplementary Table S1).

## 6. Conclusions

In this review, we summarize the findings of 20 ASD-risk genes of Rho GTPase regulators and effectors mainly from their mouse models. Most of these 20 genes are highly expressed in the hippocampus, cortex, amygdala and cerebellum in adult brains. These brain regions are important for behaviors such as social interaction and emotional regulation, and evidence from neuroimaging studies shows altered activation patterns in these regions in ASD individuals [253]. Therefore, mutations

of Rho GTPase regulators and effectors may cause impaired neural function in these brain regions and thus abnormal behaviors in ASD individuals. Regarding cellular function, 13 of these genes (*Arhgef9*, *Trio*, *Prex1*, *Dock1*, *Dock4*, *Myo9b*, *Ophn1*, *Arhgef32*, *SrGAP3*, *Cyfi1*, *Pak2*, *Itp1*, and *Wave1*) play important roles in regulating neuronal development and function, including axon guidance, dendrite and spine morphogenesis, and synaptic plasticity [60,67,68,71,80,89,92,135,140,150,224,251]. On the other hand, *Dock8* and *Myo9b* are highly expressed in immune cells. In the nervous system, *Dock8* regulates microglia migration [121] and *Myo9b* may have special function in microglia and astrocytes. It supports the view that microglia and astrocytes modulate synaptic function and contribute to the pathophysiology of ASD [254,255].

In particular, this review summarizes the recent advances of behavioral and biological phenotypes observed using genetic mouse models of these genes. Most of these 20 genes have mouse models, and multiple behavioral paradigms have been tested in 13 mouse model lines. Notably, seven of these 13 mouse model lines (*Trio* cKO, *Prex1* KO, *Dock4* KO, *Arhgef10* KO, *Ophn1* KO, *Argap32* KO, and *Pak2* HET mice) exhibit impaired social behaviors and/or repetitive behaviors (Table 2; Supplementary Table S1), which are the core ASD symptoms. Some mouse models, such as *Arhgef9* KO mice and *Wave1* KO mice, were generated before the genes were found to be related to ASD, therefore ASD-related behavior analyses have not been done in these mouse models and will need to be investigated. Moreover, for some genes, such as *DOCK1*, *ARHGEF5*, and *NCKAP1*, homozygous KO mice are lethal due to severe developmental failure, so their roles in neuronal function and behaviors have not been explored in vivo. For some genes, such as *DOCK8* and *MYO9b*, genetic models have not been studied. Besides core symptoms of ASD, other closely related behavioral defects, presented in ASD-comorbid psychiatric disorders such as ID, ADHD, SCZ, are commonly observed in 13 mouse model lines. These include altered anxiety levels and poor learning and memory ability (Table 2; Supplementary Table S1). Findings from these mouse models contribute substantially to the understanding of Rho family GTPase-involved molecular pathogenesis of ASD.

Eight of the abovementioned 13 mouse model lines show synaptic transmission dysfunctions in hippocampal neurons, but the changes vary in different models. Some of the changes may be attributable to the common alteration of a particular Rho GTPase function. For instance, LTP is decreased upon deficiency of the Rac1 GEF *Dock4* (*Dock4* KO mice) and the Rac1 effector *Pak2* (*Pak2* HET mice), but is increased when the RhoA effector *IP<sub>3</sub>R1* is deleted (*Itp1* KO mice). Moreover, *Arhgef9* KO and *Prex1* KO mice, leading to inhibited *Cdc42* and *Rac1*, respectively, fail to induce LTD. *Arhgef9* KO mice also show decreased GABAergic synaptic function, which is oppositely enhanced in the Rac1 effector *Cyfi1* cKO mice. These findings are consistent with the notion that RhoA and Rac1/*Cdc42* may play opposite roles in modulating synaptic function. However, many factors, such as *Ophn1* and *Cyfi1*, have additional functions besides participating Rho GTPase pathways. Altered activity of single Rho GTPase may not simply account for all the abnormalities in these mice. Nonetheless, these findings demonstrate that dysfunctions of Rho GTPase regulators and effectors lead to either too much or too little neurotransmission, supporting the hypothesis that impaired excitatory/inhibitory synaptic balance may be one of the pathogenic mechanisms generating ASD-like behaviors.

To investigate the possibility of treating the phenotypes in ASD mouse models, various strategies have been tried, which include genetic manipulation, cellular therapy, pharmacological intervention, and environmental stimulation [256]. We summarize the current successful treatments for mouse models of Rho GTPase regulators and effectors, including pharmacological and non-pharmacological approaches (Table 3). Interestingly, some strategies show similar therapeutic effects in different mouse models. For example, *Dock4* KO and *Prex1*<sup>-/-</sup> mice both have reduced hippocampal Rac1 activity and show disruptive NMDA receptor function and social defects. Therefore, similar therapeutic strategies, including drug treatment to restore NMDA receptor function or replenishing Rac1 activity, rescue the defects in neural function and behaviors in both mice. In another case, both *Trio* cKO and *Ophn1* KO mice show increased PKA activity in the cortex, and the abnormal phenotypes due to *Trio* or *Ophn1* deficiency can be restored by the PKA inhibitor Rp-cAMPS treatment. Moreover, a dual inhibitor of

PKA and ROCK, fasudil, also has therapeutic effects to treat the behavioral defects in *Ophn1<sup>-ly</sup>* mice. Other strategies, such as GABA receptor agonist, AMPA receptor antagonist, and mGluR inhibitors, show treatment effects in different individual mice models. These lines of evidence suggest that personalized therapy, through either single or multiple therapeutic strategies, could be possible for patients who share common or unique pathologies due to their genetic variations.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/2073-4409/9/4/835/s1>, Table S1: Summary of ASD-related behavior tests in Rho GEF, GAP, and effector mouse models.

**Author Contributions:** Conceptualization, L.S. and D.G.; writing—original draft preparation, D.G.; writing—review and editing, D.G., X.Y. and L.S. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by The Academy of Medical Sciences Newton Advanced Fellowship in partnership with The Royal Society and The National Natural Science Foundation of China [UK/China grant numbers: AOMS-NAF0051003/81761130084], Key Realm R&D Program of Guangdong Province (2019B030335001), Local Innovative and Research Teams Project of Guangdong Pearl River Talents Program (2017BT01Y036).

**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A

The order of genes discussed in the review is according to SFARI Gene scores which reflect the gene linkage strengths to ASD; Genes of same score are listed alphabetically.

## References

1. Elsabbagh, M.; Divan, G.; Koh, Y.-J.; Kim, Y.S.; Kauchali, S.; Marcín, C.; Montiel-Nava, C.; Patel, V.; Paula, C.S.; Wang, C.; et al. Global Prevalence of Autism and Other Pervasive Developmental Disorders. *Autism Res.* **2012**, *5*, 160–179. [[CrossRef](#)] [[PubMed](#)]
2. Baron-Cohen, S.; Gullon-Scott, F.; Allison, C.; Williams, J.; Bolton, P.; E Matthews, F.; Brayne, C. Prevalence of autism-spectrum conditions: UK school-based population study. *Br. J. Psychiatry* **2009**, *194*, 500–509. [[CrossRef](#)] [[PubMed](#)]
3. Kim, Y.S.; Leventhal, B.L.; Koh, Y.-J.; Fombonne, E.; Laska, E.; Lim, E.-C.; Cheon, K.-A.; Kim, S.-J.; Kim, Y.-K.; Lee, H.; et al. Prevalence of Autism Spectrum Disorders in a Total Population Sample. *Am. J. Psychiatry* **2011**, *168*, 904–912. [[CrossRef](#)] [[PubMed](#)]
4. Kočovská, E.; Biskupstø, R.; Gillberg, I.C.; Ellefsen, A.; Kampmann, H.; Stóra, T.; Billstedt, E.; Gillberg, C. The Rising Prevalence of Autism: A Prospective Longitudinal Study in the Faroe Islands. *J. Autism Dev. Disord.* **2012**, *42*, 1959–1966. [[CrossRef](#)]
5. Sun, X.; Allison, C.; E Matthews, F.; Sharp, S.; Auyeung, B.; Baron-Cohen, S.; Brayne, C. Prevalence of autism in mainland China, Hong Kong and Taiwan: a systematic review and meta-analysis. *Mol. Autism* **2013**, *4*, 7. [[CrossRef](#)]
6. Wan, Y.; Hu, Q.; Li, T.; Jiang, L.; Du, Y.; Feng, L.; Wong, J.C.-M.; Li, C.-B. Prevalence of autism spectrum disorders among children in China: a systematic review. *Shanghai Arch. Psychiatry* **2013**, *25*, 70–80. [[PubMed](#)]
7. Baio, J.; Wiggins, L.; Christence, D.L.; Maenner, M.J.; Daniels, J.; Warren, Z.; Kurzius-Spencer, M.; Zahorodny, W.; Rosenberg, C.R.; White, T.; et al. Prevalence of Autism Spectrum Disorder Among Children Aged 8 Years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2014. *MMWR Surveill. Summ.* **2018**, *67*, 1–23. [[CrossRef](#)]
8. Lai, M.C.; Kasse, C.; Besney, R.; Bonato, S.; Hull, L.; Mandy, W.; Szatmari, P.; Ameis, S.H. Prevalence of co-occurring mental health diagnoses in the autism population: A systematic review and meta-analysis. *Lancet Psychiatry* **2019**, *6*, 819–829. [[CrossRef](#)]
9. Colvert, E.; Tick, B.; McEwen, F.; Stewart, C.; Curran, S.R.; Woodhouse, E.L.; Gillan, N.; Hallett, V.; Lietz, S.; Garnett, T.; et al. Heritability of Autism Spectrum Disorder in a UK Population-Based Twin Sample. *JAMA Psychiatry* **2015**, *72*, 415–423. [[CrossRef](#)]
10. Tick, B.; Bolton, P.; Happé, F.; Rutter, M.L.; Rijdsdijk, F. Heritability of autism spectrum disorders: A meta-analysis of twin studies. *J. Child Psychol. Psychiatry* **2015**, *57*, 585–595. [[CrossRef](#)]

11. Banerjee-Basu, S.; Packer, A. SFARI Gene: An evolving database for the autism research community. *Dis. Model. Mech.* **2010**, *3*, 133–135. [[CrossRef](#)] [[PubMed](#)]
12. Abrahams, B.S.; Arking, D.E.; Campbell, D.; Mefford, H.C.; Morrow, E.M.; Weiss, L.A.; Menashe, I.; Wadkins, T.; Banerjee-Basu, S.; Packer, A. SFARI Gene 2.0: A community-driven knowledgebase for the autism spectrum disorders (ASDs). *Mol. Autism* **2013**, *4*, 36. [[CrossRef](#)] [[PubMed](#)]
13. Basu, S.N.; Kollu, R.; Banerjee-Basu, S. AutDB: A gene reference resource for autism research. *Nucleic Acids Res.* **2008**, *37*, D832–D836. [[CrossRef](#)] [[PubMed](#)]
14. SFARI Gene. Available online: [www.sfari.org/resource/sfari-gene/](http://www.sfari.org/resource/sfari-gene/) (accessed on 27 March 2020).
15. AutDB. Available online: <http://autism.mindspec.org/autdb/Welcome.do> (accessed on 27 March 2020).
16. Ebert, D.H.; Greenberg, M.E. Activity-dependent neuronal signalling and autism spectrum disorder. *Nature* **2013**, *493*, 327–337. [[CrossRef](#)] [[PubMed](#)]
17. Bourgeron, T. From the genetic architecture to synaptic plasticity in autism spectrum disorder. *Nat. Rev. Neurosci.* **2015**, *16*, 551–563. [[CrossRef](#)] [[PubMed](#)]
18. Joensuu, M.; LaNoue, V.; Hotulainen, P. Dendritic spine actin cytoskeleton in autism spectrum disorder. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* **2018**, *84 Pt B*, 362–381. [[CrossRef](#)]
19. Tolia, K.F.; Duman, J.; Um, K. Control of synapse development and plasticity by Rho GTPase regulatory proteins. *Prog. Neurobiol.* **2011**, *94*, 133–148. [[CrossRef](#)]
20. Govek, E.-E.; Newey, S.E.; Van Aelst, L. The role of the Rho GTPases in neuronal development. *Genome Res.* **2005**, *19*, 1–49. [[CrossRef](#)]
21. Heasman, S.J.; Ridley, A.J. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 690–701. [[CrossRef](#)]
22. Hodge, R.G.; Ridley, A.J. Regulating Rho GTPases and their regulators. *Nat. Rev. Mol. Cell Biol.* **2016**, *17*, 496–510. [[CrossRef](#)]
23. Bai, Y.; Xiang, X.; Liang, C.; Shi, L. Regulating Rac in the Nervous System: Molecular Function and Disease Implication of Rac GEFs and GAPs. *BioMed. Res. Int.* **2015**, *2015*, 632450. [[CrossRef](#)] [[PubMed](#)]
24. Fort, P.; Blangy, A. The Evolutionary Landscape of Dbl-Like RhoGEF Families: Adapting Eukaryotic Cells to Environmental Signals. *Genome Biol. Evol.* **2017**, *9*, 1471–1486. [[CrossRef](#)] [[PubMed](#)]
25. Rossman, K.L.; Der, C.J.; Sondek, J. GEF means go: Turning on RHO GTPases with guanine nucleotide-exchange factors. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 167–180. [[CrossRef](#)] [[PubMed](#)]
26. Amin, E.; Jaiswal, M.; Derewenda, U.; Reis, K.; Nouri, K.; Koessmeier, K.T.; Aspenström, P.; Somlyo, A.V.; Dvorsky, R.; Ahmadian, M.R. Deciphering the Molecular and Functional Basis of RHOGAP Family Proteins. *J. Biol. Chem.* **2016**, *291*, 20353–20371. [[CrossRef](#)] [[PubMed](#)]
27. Bustelo, X.R.; Sauzeau, V.; Berenjano, I.M. GTP-binding proteins of the Rho/Rac family: Regulation, effectors and functions in vivo. *BioEssays* **2007**, *29*, 356–370. [[CrossRef](#)] [[PubMed](#)]
28. Shi, L. Dock protein family in brain development and neurological disease. *Commun. Integr. Biol.* **2013**, *6*, e26839. [[CrossRef](#)] [[PubMed](#)]
29. Aguilar, B.J.; Zhu, Y.; Lu, Q. Rho GTPases as therapeutic targets in Alzheimer’s disease. *Alzheimer’s Res. Ther.* **2017**, *9*, 97. [[CrossRef](#)]
30. Huang, G.-H.; Sun, Z.-L.; Li, H.-J.; Feng, D.-F. Rho GTPase-activating proteins: Regulators of Rho GTPase activity in neuronal development and CNS diseases. *Mol. Cell. Neurosci.* **2017**, *80*, 18–31. [[CrossRef](#)]
31. Zamboni, V.; Jones, R.; Umbach, A.; Ammoni, A.; Passafaro, M.; Hirsch, E.; Merlo, G. Rho GTPases in Intellectual Disability: From Genetics to Therapeutic Opportunities. *Int. J. Mol. Sci.* **2018**, *19*, 1821. [[CrossRef](#)]
32. Niftullayev, S.; Lamarche-Vane, N. Regulators of Rho GTPases in the Nervous System: Molecular Implication in Axon Guidance and Neurological Disorders. *Int. J. Mol. Sci.* **2019**, *20*, 1497. [[CrossRef](#)]
33. Stankiewicz, T.R.; Linseman, D.A. Rho family GTPases: key players in neuronal development, neuronal survival, and neurodegeneration. *Front. Cell. Neurosci.* **2014**, *8*, 314. [[CrossRef](#)]
34. Edwards, D.C.; Sanders, L.C.; Bokoch, G.M.; Gill, G.N. Activation of LIM-kinase by Pak1 couples Rac/Cdc42 GTPase signalling to actin cytoskeletal dynamics. *Nat. Cell Biol.* **1999**, *1*, 253–259. [[CrossRef](#)] [[PubMed](#)]
35. Tahirovic, S.; Hellal, F.; Neukirchen, R.; Hindges, R.; Garvalov, B.K.; Flynn, K.C.; Stradal, T.; Chrostek-Grashoff, A.; Brakebusch, C.; Bradke, F. Rac1 Regulates Neuronal Polarization through the WAVE Complex. *J. Neurosci.* **2010**, *30*, 6930–6943. [[CrossRef](#)] [[PubMed](#)]
36. Kang, J.; Park, H.; Kim, E. IRSp53/BAIAP2 in dendritic spine development, NMDA receptor regulation, and psychiatric disorders. *Neuropharmacology* **2016**, *100*, 27–39. [[CrossRef](#)] [[PubMed](#)]

37. Mpey, S.; Davare, M.; Lasiek, A.; Fortin, D.; Ando, H.; Varlamova, O.; Obrietan, K.; Soderling, T.R.; Goodman, R.H.; Wayman, G.A. An activity-induced microRNA controls dendritic spine formation by regulating Rac1-PAK signaling. *Mol. Cell. Neurosci.* **2009**, *43*, 146–156.
38. Reijnders, M.R.; Ansor, N.M.; Kousi, M.; Yue, W.W.; Tan, P.L.; Clarkson, K.; Clayton-Smith, J.; Corning, K.; Jones, J.R.; Lam, W.W.; et al. RAC1 Missense Mutations in Developmental Disorders with Diverse Phenotypes. *Am. J. Hum. Genet.* **2017**, *101*, 466–477. [[CrossRef](#)]
39. Bolis, A.; Corbetta, S.; Cioce, A.; De Curtis, I. Differential distribution of Rac1 and Rac3 GTPases in the developing mouse brain: implications for a role of Rac3 in Purkinje cell differentiation. *Eur. J. Neurosci.* **2003**, *18*, 2417–2424. [[CrossRef](#)]
40. Tanabe, K.; Tachibana, T.; Yamashita, T.; Che, Y.H.; Yoneda, Y.; Ochi, T.; Tohyama, M.; Yoshikawa, H.; Kiyama, H. The Small GTP-Binding Protein TC10 Promotes Nerve Elongation in Neuronal Cells, and Its Expression Is induced during Nerve Regeneration in Rats. *J. Neurosci.* **2000**, *20*, 4138–4144. [[CrossRef](#)]
41. Corbetta, S.; Gualdoni, S.; Albertinazzi, C.; Paris, S.; Croci, L.; Consalez, G.G.; De Curtis, I. Generation and Characterization of Rac3 Knockout Mice. *Mol. Cell. Biol.* **2005**, *25*, 5763–5776. [[CrossRef](#)]
42. Sugihara, K.; Nakatsuji, N.; Nakamura, K.; Nakao, K.; Hashimoto, R.; Otani, H.; Sakagami, H.; Kondo, H.; Nozawa, S.; Aiba, A.; et al. Rac1 is required for the formation of three germ layers during gastrulation. *Oncogene* **1998**, *17*, 3427–3433. [[CrossRef](#)]
43. Chen, L.; Liao, G.; Waclaw, R.R.; Burns, K.A.; Linguist, D.; Campbell, K.; Zheng, Y.; Kuan, C.-Y. Rac1 Controls the Formation of Midline Commissures and the Competency of Tangential Migration in Ventral Telencephalic Neurons. *J. Neurosci.* **2007**, *27*, 3884–3893. [[CrossRef](#)] [[PubMed](#)]
44. Chen, L.; Melendez, J.; Campbell, K.; Kuan, C.-Y.; Zheng, Y. Rac1 deficiency in the forebrain results in neural progenitor reduction and microcephaly. *Dev. Biol.* **2009**, *325*, 162–170. [[CrossRef](#)] [[PubMed](#)]
45. Hua, Z.L.; Emiliani, F.E.; Nathans, J. Rac1 plays an essential role in axon growth and guidance and in neuronal survival in the central and peripheral nervous systems. *Neural Dev.* **2015**, *10*, 21. [[CrossRef](#)] [[PubMed](#)]
46. Vidaki, M.; Tivodar, S.; Doulgieraki, K.; Tybulewicz, V.L.; Kessaris, N.; Pachnis, V.; Karagogeos, D. Rac1-Dependent Cell Cycle Exit of MGE Precursors and GABAergic Interneuron Migration to the Cortex. *Cereb. Cortex* **2011**, *22*, 680–692. [[CrossRef](#)] [[PubMed](#)]
47. Haditsch, U.; Leone, D.P.; Farinelli, M.; Chrostek-Grashoff, A.; Brakebusch, C.; Mansuy, I.M.; McConnell, S.K.; Palmer, T.D. A central role for the small GTPase Rac1 in hippocampal plasticity and spatial learning and memory. *Mol. Cell. Neurosci.* **2009**, *41*, 409–419. [[CrossRef](#)] [[PubMed](#)]
48. Haditsch, U.; Anderson, M.P.; Freewoman, J.; Cord, B.; Babu, H.; Brakebusch, C.; Palmer, T.D. Neuronal Rac1 is required for learning-evoked neurogenesis. *J. Neurosci.* **2013**, *33*, 12229–12241. [[CrossRef](#)]
49. Pennucci, R.; Talpo, F.; Astro, V.; Montinaro, V.; Morè, L.; Cursi, M.; Castoldi, V.; Chiaretti, S.; Bianchi, V.; Marena, S.; et al. Loss of Either Rac1 or Rac3 GTPase Differentially Affects the Behavior of Mutant Mice and the Development of Functional GABAergic Networks. *Cereb. Cortex* **2016**, *26*, 873–890. [[CrossRef](#)]
50. Viaud, J.; Gaits-Iacovoni, F.; Payrastra, B. Regulation of the DH-PH tandem of guanine nucleotide exchange factor for Rho GTPases by phosphoinositides. *Adv. Biol. Regul.* **2012**, *52*, 303–3144. [[CrossRef](#)]
51. Lawson, C.D.; Ridley, A.J. Rho GTPase signaling complexes in cell migration and invasion. *J. Cell Biol.* **2017**, *217*, 447–457. [[CrossRef](#)]
52. Cote, J.F.; Vuori, K. In vitro guanine nucleotide exchange activity of DHR-2/DOCKER/CZH2 domains. *Methods Enzymol.* **2006**, *406*, 41–57.
53. Namekata, K.; Kimura, A.; Kawamura, K.; Harada, C.; Harada, T. Dock GEFs and their therapeutic potential: Neuroprotection and axon regeneration. *Prog. Retin. Eye Res.* **2014**, *43*, 1–16. [[CrossRef](#)] [[PubMed](#)]
54. Machado, C.O.F.; Griesi-Oliveira, K.; Rosenberg, C.; Kok, F.; Martins, S.; Passos-Bueno, M.R.; Sertie, A. Collybistin binds and inhibits mTORC1 signaling: A potential novel mechanism contributing to intellectual disability and autism. *Eur. J. Hum. Genet.* **2015**, *24*, 59–65. [[CrossRef](#)] [[PubMed](#)]
55. Bhat, G.; Lagrave, D.; Millson, A.; Herriges, J.; Lamb, A.N.; Matalon, R. Xq11.1-11.2 deletion involving ARHGEF9 in a girl with autism spectrum disorder. *Eur. J. Med. Genet.* **2016**, *59*, 470–473. [[CrossRef](#)] [[PubMed](#)]
56. Alber, M.; Kalscheuer, V.M.; Marco, E.; Sherr, E.; Lesca, G.; Till, M.; Gradek, G.; Wiesener, A.; Korenke, C.; Mercier, S.; et al. ARHGEF9 disease: Phenotype clarification and genotype-phenotype correlation. *Neurol. Genet.* **2017**, *3*, e148. [[CrossRef](#)]

57. Aarabi, M.; Infante, E.; Madan-Khetarpal, S.; Surti, U.; Bellissimo, D.; Rajkovic, A.; Yatsenko, S.A. Autism spectrum disorder in females with ARHGEF9 alterations and a random pattern of X chromosome inactivation. *Eur. J. Med. Genet.* **2018**, *62*, 239–242. [[CrossRef](#)]
58. Xiong, J.; Chen, S.; Pang, N.; Deng, X.; Yang, L.; He, F.; Wu, L.; Chen, C.; Yin, F.; Peng, J. Neurological Diseases with Autism Spectrum Disorder: Role of ASD Risk Genes. *Front. Mol. Neurosci.* **2019**, *13*, 349. [[CrossRef](#)]
59. Kins, S.; Betz, H.; Kirsch, J. Collybistin, a newly identified brain-specific GEF, induces submembrane clustering of gephyrin. *Nat. Neurosci.* **2000**, *3*, 22–29. [[CrossRef](#)]
60. De Groot, C.; Floriou-Servou, A.; Tsai, Y.-C.; Früh, S.; Kohler, M.; Parkin, G.; Schwerdel, C.; Bosshard, G.; Kaila, K.; Fritschy, J.-M.; et al. RhoGEF9 splice isoforms influence neuronal maturation and synapse formation downstream of  $\alpha 2$  GABAA receptors. *PLoS Genet.* **2017**, *13*, e1007073. [[CrossRef](#)]
61. Kneussel, M.; Engelkamp, D.; Betz, H. Distribution of transcripts for the brain-specific GDP/GTP exchange factor collybistin in the developing mouse brain. *Eur. J. Neurosci.* **2001**, *13*, 487–492. [[CrossRef](#)]
62. Ibaraki, K.; Mizuno, M.; Aoki, H.; Niwa, A.; Iwamoto, I.; Hara, A.; Tabata, H.; Ito, H.; Nagata, K.-I. Biochemical and Morphological Characterization of a Guanine Nucleotide Exchange Factor ARHGEF9 in Mouse Tissues. *Acta Histochem. ET Cytochem.* **2018**, *51*, 119–128. [[CrossRef](#)]
63. Papadopoulos, T.; Korte, M.; Eulenburg, V.; Kubota, H.; Retiounskaia, M.; Harvey, R.J.; Harvey, K.; O'Sullivan, G.A.; Laube, B.; Hülsmann, S.; et al. Impaired GABAergic transmission and altered hippocampal synaptic plasticity in collybistin-deficient mice. *EMBO J.* **2007**, *26*, 3888–3899. [[CrossRef](#)] [[PubMed](#)]
64. Jedlicka, P.; Papadopoulos, T.; Deller, T.; Betz, H.; Schwarzacher, S.W. Increased network excitability and impaired induction of long-term potentiation in the dentate gyrus of collybistin-deficient mice in vivo. *Mol. Cell. Neurosci.* **2009**, *41*, 94–100. [[CrossRef](#)] [[PubMed](#)]
65. Jedlicka, P.; Muellerleile, J.; Schwarzacher, S.W. Synaptic Plasticity and Excitation-Inhibition Balance in the Dentate Gyrus: Insights from In Vivo Recordings in Neuroligin-1, Neuroligin-2, and Collybistin Knockouts. *Neural Plast.* **2018**, *2018*, 6015753. [[CrossRef](#)] [[PubMed](#)]
66. Zong, W.; Liu, S.; Wang, X.; Zhang, J.; Zhang, T.; Liu, Z.; Wang, N.; Zhang, A.; Zhu, M.; Gao, J. Trio gene is required for mouse learning ability. *Brain Res.* **2015**, *1608*, 82–90. [[CrossRef](#)] [[PubMed](#)]
67. Katrancha, S.M.; Shaw, J.E.; Zhao, A.Y.; Myers, S.A.; Cocco, A.R.; Jeng, A.T.; Zhu, M.; Pittenger, C.; Greer, C.A.; Carr, S.A.; et al. Trio Haploinsufficiency Causes Neurodevelopmental Disease-Associated Deficits. *Cell Rep.* **2019**, *26*, 2805–2817.e9. [[CrossRef](#)]
68. Li, J.; Chai, A.; Wang, L.; Ma, Y.; Wu, Z.; Yu, H.; Mei, L.; Lu, L.; Zhang, C.; Yue, W.; et al. Synaptic P-Rex1 signaling regulates hippocampal long-term depression and autism-like social behavior. *Proc. Natl. Acad. Sci. USA* **2015**, *112*, E6964–E6972. [[CrossRef](#)]
69. Lu, D.-H.; Liao, H.-M.; Chen, C.-H.; Tu, H.-J.; Liou, H.-C.; Gau, S.S.-F.; Fu, W.-M. Impairment of social behaviors in Arhgef10 knockout mice. *Mol. Autism* **2018**, *9*, 11. [[CrossRef](#)]
70. Guo, D.; Peng, Y.; Wang, L.; Sun, X.; Wang, X.; Liang, C.; Yang, X.; Li, S.; Xu, J.; Ye, W.-C.; et al. Autism-like social deficit generated by Dock4 deficiency is rescued by restoration of Rac1 activity and NMDA receptor function. *Mol. Psychiatry* **2019**. [[CrossRef](#)]
71. Khelifaoui, M.; Denis, C.; van Galen, E.; de Bock, F.; Schmitt, A.; Houbron, C.; Morice, E.; Giros, B.; Ramakers, G.; Fagni, L.; et al. Loss of X-linked mental retardation gene oligophrenin 1 in mice impairs spatial memory and leads to ventricular enlargement and dendritic spine immaturity. *J. Neurosci.* **2007**, *27*, 9439–9450. [[CrossRef](#)]
72. Meziane, H.; Khelifaoui, M.; Morello, N.; Hiba, B.; Calcagno, E.; Reibel-Foisset, S.; Selloum, M.; Chelly, J.; Humeau, Y.; Riet, F.; et al. Fasudil treatment in adult reverses behavioural changes and brain ventricular enlargement in Oligophrenin-1 mouse model of intellectual disability. *Hum. Mol. Genet.* **2016**, *25*, 2314–2323. [[CrossRef](#)]
73. Redolfi, N.; Galla, L.; Maset, A.; Murru, L.; Savoia, E.; Zamparo, I.; Gritti, A.; Billuart, P.; Passafaro, M.; Lodovichi, C. Oligophrenin-1 regulates number, morphology and synaptic properties of adult-born inhibitory interneurons in the olfactory bulb. *Hum. Mol. Genet.* **2016**, *25*, 5198–5211. [[CrossRef](#)] [[PubMed](#)]
74. Khelifaoui, M.; Gambino, F.; Houbaert, X.; Ragazzon, B.; Müller, C.; Carta, M.; Lanore, F.; Srikumar, B.N.; Gastrein, P.; Lepleux, M.; et al. Lack of the presynaptic RhoGAP protein oligophrenin1 leads to cognitive disabilities through dysregulation of the cAMP/PKA signalling pathway. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2013**, *369*, 20130160. [[CrossRef](#)]

75. Zhang, C.-L.; Aime, M.; Laheranne, E.; Houbaert, X.; El Oussini, H.; Martin, C.; Lepleux, M.; Normand, E.; Chelly, J.; Herzog, E.; et al. Protein Kinase A Deregulation in the Medial Prefrontal Cortex Impairs Working Memory in Murine Oligophrenin-1 Deficiency. *J. Neurosci.* **2017**, *37*, 11114–11126. [[CrossRef](#)] [[PubMed](#)]
76. Nakamura, T.; Arima-Yoshida, F.; Sakaue, F.; Nasu-Nishimura, Y.; Takeda, Y.; Matsuura, K.; Akshoomoff, N.; Mattson, S.N.; Grossfeld, P.D.; Manabe, T.; et al. PX-RICS-deficient mice mimic autism spectrum disorder in Jacobsen syndrome through impaired GABAA receptor trafficking. *Nat. Commun.* **2016**, *7*, 10861. [[CrossRef](#)] [[PubMed](#)]
77. Nakamura, T.; Sakaue, F.; Nasu-Nishimura, Y.; Takeda, Y.; Matsuura, K.; Akiyama, T. The Autism-Related Protein PX-RICS Mediates GABAergic Synaptic Plasticity in Hippocampal Neurons and Emotional Learning in Mice. *Ebiomedicine* **2018**, *34*, 189–200. [[CrossRef](#)] [[PubMed](#)]
78. Carlson, B.R.; Lloyd, K.E.; Kruszewski, A.; Kim, I.-H.; Rodriguiz, R.M.; Heindel, C.; Faytell, M.; Dudek, S.M.; Wetsel, W.C.; Soderling, S.H. WRP/srGAP3 Facilitates the Initiation of Spine Development by an Inverse F-BAR Domain, and Its Loss Impairs Long-Term Memory. *J. Neurosci.* **2011**, *31*, 2447–2460. [[CrossRef](#)] [[PubMed](#)]
79. Waltereit, R.; Leimer, U.; Halbach, O.v.B.U.; Panke, J.; Hoelter, S.M.; Garrett, L.; Wittig, K.; Schneider, M.; Schmitt, C.; Calzada-Wack, J.; et al. Srgap3(-/-) mice present a neurodevelopmental disorder with schizophrenia-related intermediate phenotypes. *FASEB J.* **2012**, *26*, 4418–4428. [[CrossRef](#)] [[PubMed](#)]
80. Bertram, J.; Koschuetzke, L.; Pfannmoeller, J.P.; Esche, J.; van Diepen, L.; Kuss, A.W.; Hartmann, B.; Bartsch, D.; Lotze, M.; van Halbach, O.B.U. Morphological and behavioral characterization of adult mice deficient for SrGAP3. *Cell Tissue Res.* **2016**, *366*, 1–11. [[CrossRef](#)] [[PubMed](#)]
81. Janne, P.A.; Suchy, S.F.; Bernard, D.; MacDonald, M.; Crawley, J.; Grinberg, A.; Wynshaw-Boris, A.; Westphal, H.; Nussbaum, R.L. Functional overlap between murine Inpp5b and Ocr1l may explain why deficiency of the murine ortholog for OCRL1 does not cause Lowe syndrome in mice. *J. Clin. Investig* **1998**, *101*, 2042–2053. [[CrossRef](#)]
82. Festa, B.P.; Berquez, M.; Gassama, A.; Amrein, I.; Ismail, H.M.; Samardzija, M.; Staiano, L.; Luciani, A.; Grimm, C.; Nussbaum, R.L.; et al. OCRL deficiency impairs endolysosomal function in a humanized mouse model for Lowe syndrome and Dent disease. *Hum. Mol. Genet.* **2019**, *28*, 1931–1946. [[CrossRef](#)]
83. Bozdagi, O.; Sakurai, T.; Dorr, N.; Pilorge, M.; Takahashi, N.; Buxbaum, J.D. Haploinsufficiency of Cyfip1 produces fragile X-like phenotypes in mice. *PLoS ONE* **2012**, *7*, e42422. [[CrossRef](#)] [[PubMed](#)]
84. Bachmann, S.O.; Sledziowska, M.; Cross, E.; Kalbassi, S.; Waldron, S.; Chen, F.; Ranson, A.; Baudouin, S.J. Behavioral training rescues motor deficits in Cyfip1 haploinsufficiency mouse model of autism spectrum disorders. *Transl. Psychiatry* **2019**, *9*, 29. [[CrossRef](#)] [[PubMed](#)]
85. Chung, L.; Wang, X.; Zhu, L.; Towers, A.J.; Cao, X.; Kim, I.H.; Jiang, Y.-H. Parental origin impairment of synaptic functions and behaviors in cytoplasmic FMRP interacting protein 1 (Cyfip1) deficient mice. *Brain Res.* **2015**, *1629*, 340–350. [[CrossRef](#)] [[PubMed](#)]
86. Domínguez-Iturza, N.; Lo, A.C.; Shah, D.; Armendáriz, M.; Vannelli, A.; Mercaldo, V.; Trusel, M.; Li, K.W.; Gastaldo, D.; Santos, A.R.; et al. The autism- and schizophrenia-associated protein CYFIP1 regulates bilateral brain connectivity and behaviour. *Nat. Commun.* **2019**, *10*, 3454. [[CrossRef](#)] [[PubMed](#)]
87. Silva, A.I.; Haddon, J.E.; Ahmed Syed, Y.; Trent, S.; Lin, T.-C.E.; Patel, Y.; Carter, J.; Haan, N.; Honey, R.C.; Humby, T.; et al. Cyfip1 haploinsufficient rats show white matter changes, myelin thinning, abnormal oligodendrocytes and behavioural inflexibility. *Nat. Commun.* **2019**, *10*, 3455. [[CrossRef](#)]
88. Fricano-Kugler, C.; Gordon, A.; Shin, G.; Gao, K.; Nguyen, J.; Berg, J.; Starks, M.; Geschwind, D.H. CYFIP1 overexpression increases fear response in mice but does not affect social or repetitive behavioral phenotypes. *Mol. Autism* **2019**, *10*, 25. [[CrossRef](#)]
89. Wang, Y.; Zeng, C.; Li, J.; Zhou, Z.; Ju, X.; Xia, S.; Li, Y.; Liu, A.; Teng, H.; Zhang, K.; et al. PAK2 Haploinsufficiency Results in Synaptic Cytoskeleton Impairment and Autism-Related Behavior. *Cell Rep.* **2018**, *24*, 2029–2041. [[CrossRef](#)]
90. Ogura, H.; Matsumoto, M.; Mikoshiba, K. Motor discoordination in mutant mice heterozygous for the type 1 inositol 1,4,5-trisphosphate receptor. *Behav. Brain Res.* **2001**, *122*, 215–219. [[CrossRef](#)]
91. Sugawara, T.; Hisatsune, C.; Le, T.D.; Hashikawa, T.; Hirono, M.; Hattori, M.; Nagao, S.; Mikoshiba, K. Type 1 Inositol Trisphosphate Receptor Regulates Cerebellar Circuits by Maintaining the Spine Morphology of Purkinje Cells in Adult Mice. *J. Neurosci.* **2013**, *33*, 12186. [[CrossRef](#)]

92. Hisatsune, C.; Miyamoto, H.; Hirono, M.; Yamaguchi, N.; Sugawara, T.; Ogawa, N.; Ebisui, E.; Ohshima, T.; Yamada, M.; Hensch, T.K.; et al. IP3R1 deficiency in the cerebellum/brainstem causes basal ganglia-independent dystonia by triggering tonic Purkinje cell firings in mice. *Front. Neural Circuits* **2013**, *7*, 156. [[CrossRef](#)]
93. Soderling, S.H.; Langeberg, L.K.; Soderling, J.A.; Davee, S.M.; Simerly, R.; Raber, J.; Scott, J.D. Loss of WAVE-1 causes sensorimotor retardation and reduced learning and memory in mice. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1723–1728. [[CrossRef](#)] [[PubMed](#)]
94. Ren, J.; Wen, L.; Gao, X.; Jin, C.; Xue, Y.; Yao, X. DOG 1.0: illustrator of protein domain structures. *Cell Res.* **2009**, *19*, 271–273. [[CrossRef](#)] [[PubMed](#)]
95. De Rubeis, S.; He, X.; Goldberg, A.P.; Poultney, C.S.; E Samocha, K.; Cicek, A.E.; Kou, Y.; Liu, L.; Fromer, M.; Singh, T.; et al. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* **2014**, *515*, 209–215. [[CrossRef](#)] [[PubMed](#)]
96. Turner, T.N.; Hormozdiari, F.; Duyzend, M.; McClymont, S.A.; Hook, P.W.; Iossifov, I.; Raja, A.; Baker, C.; Hoekzema, K.; Stessman, H.A.; et al. Genome Sequencing of Autism-Affected Families Reveals Disruption of Putative Noncoding Regulatory DNA. *Am. J. Hum. Genet.* **2016**, *98*, 58–74. [[CrossRef](#)]
97. Takata, A.; Miyake, N.; Tsurusaki, Y.; Fukai, R.; Miyatake, S.; Koshimizu, E.; Kushima, I.; Okada, T.; Morikawa, M.; Uno, Y.; et al. Integrative Analyses of De Novo Mutations Provide Deeper Biological Insights into Autism Spectrum Disorder. *Cell Rep.* **2018**, *22*, 734–747. [[CrossRef](#)]
98. Stessman, H.; Xiong, B.; Coe, B.P.; Wang, T.; Hoekzema, K.; Fenckova, M.; Kvarnung, M.; Gerds, J.; Trinh, S.; Cosemans, N.; et al. Targeted sequencing identifies 91 neurodevelopmental-disorder risk genes with autism and developmental-disability biases. *Nat. Genet.* **2017**, *49*, 515–526. [[CrossRef](#)]
99. Aspromonte, M.C.; Bellini, M.; Gasparini, A.; Carraro, M.; Bettella, E.; Polli, R.; Cesca, F.; Bigoni, S.; Carlet, O.; Negrin, S.; et al. Characterization of intellectual disability and autism comorbidity through gene panel sequencing. *Hum. Mutat.* **2019**, *40*, 1346–1363. [[CrossRef](#)]
100. Sadybekov, A.; Tian, C.; Arnesano, C.; Katritch, V.; Herring, B.E. An autism spectrum disorder-related de novo mutation hotspot discovered in the GEF1 domain of Trio. *Nat. Commun.* **2017**, *8*, 601. [[CrossRef](#)]
101. Barbosa, S.; Greville-Heygate, S.; Bonnet, M.; Godwin, A.; Fagotto-Kaufmann, C.; Kajava, A.V.; Laouteouet, D.; Mawby, R.; Wai, H.A.; Dingemans, A.J.; et al. Opposite Modulation of RAC1 by Mutations in TRIO Is Associated with Distinct, Domain-Specific Neurodevelopmental Disorders. *Am. J. Hum. Genet.* **2020**, *106*, 338–355. [[CrossRef](#)]
102. Schmidt, S.; Debant, A. Function and regulation of the Rho guanine nucleotide exchange factor Trio. *Small GTPases* **2014**, *5*, e29769. [[CrossRef](#)]
103. Medley, Q.G.; Serra-Pagès, C.; Iannotti, E.; Seipel, K.; Tang, M.; O'Brien, S.; Streuli, M. The Trio Guanine Nucleotide Exchange Factor Is a RhoA Target. *J. Biol. Chem.* **2000**, *275*, 36116–36123. [[CrossRef](#)] [[PubMed](#)]
104. McPherson, C.E.; Eipper, B.A.; Mains, R.E. Multiple novel isoforms of Trio are expressed in the developing rat brain. *Gene* **2005**, *347*, 125–135. [[CrossRef](#)]
105. Portales-Casamar, E.; Briançon-Marjollet, A.; Fromont, S.; Triboulet, R.; Debant, A. Identification of novel neuronal isoforms of the Rho-GEF Trio. *Biol. Cell* **2006**, *98*, 183–193. [[CrossRef](#)] [[PubMed](#)]
106. O'Brien, S.P.; Seipel, K.; Medley, Q.G.; Bronson, R.; Segal, R.; Streuli, M. Skeletal muscle deformity and neuronal disorder in Trio exchange factor-deficient mouse embryos. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 12074–12078. [[CrossRef](#)] [[PubMed](#)]
107. Peng, Y.-J.; He, W.-Q.; Tang, J.; Tao, T.; Chen, C.; Gao, Y.-Q.; Zhang, W.-C.; He, X.; Dai, Y.-Y.; Zhu, N.-C.; et al. Trio Is a Key Guanine Nucleotide Exchange Factor Coordinating Regulation of the Migration and Morphogenesis of Granule Cells in the Developing Cerebellum. *J. Biol. Chem.* **2010**, *285*, 24834–24844. [[CrossRef](#)]
108. Goebbels, S.; Bormuth, I.; Bode, U.; Hermanson, O.; Schwab, M.H.; Nave, K. Genetic targeting of principal neurons in neocortex and hippocampus of NEX-Cre mice. *Genesis* **2006**, *44*, 611–621. [[CrossRef](#)]
109. Huang, M.; Liang, C.; Li, S.; Zhang, J.; Guo, D.; Zhao, B.; Liu, Y.; Peng, Y.; Xu, J.; Liu, W.; et al. Two Autism/Dyslexia Linked Variations of DOCK4 Disrupt the Gene Function on Rac1/Rap1 Activation, Neurite Outgrowth, and Synapse Development. *Front. Cell. Neurosci.* **2020**, *13*, 577. [[CrossRef](#)]
110. Nishikimi, A.; Kukimoto-Niino, M.; Yokoyama, S.; Fukui, Y. Immune regulatory functions of DOCK family proteins in health and disease. *Exp. Cell Res.* **2013**, *319*, 2343–2349. [[CrossRef](#)]

111. Harada, Y.; Tanaka, Y.; Terasawa, M.; Pieczyk, M.; Habiro, K.; Katakai, T.; Hanawa-Suetsugu, K.; Kukimoto-Niino, M.; Nishizaki, T.; Shirouzu, M.; et al. DOCK8 is a Cdc42 activator critical for interstitial dendritic cell migration during immune responses. *Blood* **2012**, *119*, 4451–4461. [[CrossRef](#)]
112. Kunimura, K.; Uruno, T.; Fukui, Y. DOCK family proteins: Key players in immune surveillance mechanisms. *Int. Immunol.* **2020**, *32*, 5–15. [[CrossRef](#)]
113. Allen-Brady, K.; Miller, J.; Matsunami, N.; Stevens, J.; Block, H.; Farley, M.; Krasny, L.; Pingree, C.; Lainhart, J.; Leppert, M.; et al. A high-density SNP genome-wide linkage scan in a large autism extended pedigree. *Mol. Psychiatry* **2008**, *14*, 590–600. [[CrossRef](#)] [[PubMed](#)]
114. Coon, H.; E Villalobos, M.; Robison, R.J.; Camp, N.J.; Cannon, D.S.; Allen-Brady, K.; Miller, J.S.; McMahon, W.M. Genome-wide linkage using the Social Responsiveness Scale in Utah autism pedigrees. *Mol. Autism* **2010**, *1*, 8. [[CrossRef](#)] [[PubMed](#)]
115. Wang, T.; Guo, H.; Xiong, B.; Stessman, H.A.; Wu, H.; Coe, B.P.; Turner, T.; Liu, Y.; Zhao, W.; Hoekzema, K.; et al. De novo genic mutations among a Chinese autism spectrum disorder cohort. *Nat. Commun.* **2016**, *7*, 13316. [[CrossRef](#)] [[PubMed](#)]
116. Glessner, J.; Li, J.; Wang, D.; March, M.; Lima, L.; Desai, A.; Hadley, D.; Kao, C.; Gur, R.E.; Sleiman, P.M.; et al. Copy number variation meta-analysis reveals a novel duplication at 9p24 associated with multiple neurodevelopmental disorders. *Genome Med.* **2017**, *9*, 1–11. [[CrossRef](#)]
117. Krgovic, D.; Vokac, N.K.; Zagorac, A.; Kumperscak, H.G. Rare structural variants in the DOCK8 gene identified in a cohort of 439 patients with neurodevelopmental disorders. *Sci. Rep.* **2018**, *8*, 9449. [[CrossRef](#)]
118. Guo, H.; Wang, T.; Wu, H.; Long, M.; Coe, B.P.; Li, H.; Xun, G.; Ou, J.-J.; Chen, B.; Duan, G.; et al. Inherited and multiple de novo mutations in autism/developmental delay risk genes suggest a multifactorial model. *Mol. Autism* **2018**, *9*, 64. [[CrossRef](#)]
119. Ruzzo, E.K.; Pérez-Cano, L.; Jung, J.Y.; Wang, L.K.; Kashef-Haghighi, D.; Hartl, C.; Singh, C.; Xu, J.; Hoekstra, J.N.; Leventhal, O.; et al. Inherited and De Novo Genetic Risk for Autism Impacts Shared Networks. *Cell* **2019**, *178*, 850–866.e26. [[CrossRef](#)]
120. Biggs, C.; Keles, S.; Chatila, T.A. DOCK8 deficiency: Insights into pathophysiology, clinical features and management. *Clin. Immunol.* **2017**, *181*, 75–82. [[CrossRef](#)]
121. Namekata, K.; Guo, X.; Kimura, A.; Arai, N.; Harada, C.; Harada, T. DOCK8 is expressed in microglia, and it regulates microglial activity during neurodegeneration in murine disease models. *J. Biol. Chem.* **2019**, *294*, 13421–13433. [[CrossRef](#)]
122. Rynkiewicz, N.; Liu, T.; Balamatsias, D.; Mitchell, C.A. INPP4A/INPP4B and P-Rex proteins: Related but different? *Adv. Biol. Regul.* **2012**, *52*, 265–279. [[CrossRef](#)]
123. Welch, H. Regulation and function of P-Rex family Rac-GEFs. *Small GTPases* **2015**, *6*, 49–70. [[CrossRef](#)] [[PubMed](#)]
124. Welch, H.C.; Coadwell, W.J.; Ellson, C.D.; Ferguson, G.J.; Andrews, S.R.; Erdjument-Bromage, H.; Tempst, P.; Hawkins, P.T.; Stephens, L.R. P-Rex1, a PtdIns(3,4,5)P-3- and G beta gamma-regulated guanine-nucleotide exchange factor for Rac. *Cell* **2002**, *108*, 809–821. [[CrossRef](#)]
125. Yoshizawa, M.; Kawauchi, T.; Sone, M.; Nishimura, Y.V.; Terao, M.; Chihama, K.; Nabeshima, Y.-I.; Hoshino, M. Involvement of a Rac Activator, P-Rex1, in Neurotrophin-Derived Signaling and Neuronal Migration. *J. Neurosci.* **2005**, *25*, 4406–4419. [[CrossRef](#)] [[PubMed](#)]
126. Donald, S.; Humby, T.; Fyfe, I.; Segonds-Pichon, A.; Walker, S.; Andrews, S.R.; Coadwell, W.J.; Emson, P.; Wilkinson, L.S.; Welch, H. P-Rex2 regulates Purkinje cell dendrite morphology and motor coordination. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 4483–4488. [[CrossRef](#)] [[PubMed](#)]
127. Garcia-Mata, R.; Burrridge, K. Catching a GEF by its tail. *Trends Cell Biol.* **2007**, *17*, 36–43. [[CrossRef](#)] [[PubMed](#)]
128. Chaya, T.; Shibata, S.; Tokuhara, Y.; Yamaguchi, W.; Matsumoto, H.; Kawahara, I.; Kogo, M.; Ohoka, Y.; Inagaki, S. Identification of a Negative Regulatory Region for the Exchange Activity and Characterization of T332I Mutant of Rho Guanine Nucleotide Exchange Factor 10 (ARHGEF10). *J. Biol. Chem.* **2011**, *286*, 29511–29520. [[CrossRef](#)]
129. Shibata, S.; Teshima, Y.; Niimi, K.; Inagaki, S. Involvement of ARHGEF10, GEF for RhoA, in Rab6/Rab8-mediated membrane traffic. *Small GTPases* **2019**, *10*, 169–177. [[CrossRef](#)]
130. Li, J.; Wang, L.; Guo, H.; Shi, L.; Zhang, K.; Tang, M.; Hu, S.; Dong, S.; Liu, C.; Wang, T.; et al. Targeted sequencing and functional analysis reveal brain-size-related genes and their networks in autism spectrum disorders. *Mol. Psychiatry* **2017**, *22*, 1282–1290. [[CrossRef](#)]

131. Verhoeven, K.; De Jonghe, P.; Van De Putte, T.; Nelis, E.; Zwijsen, A.; Verpoorten, N.; De Vriendt, E.; Jacobs, A.; Van Gerwen, V.; Francis, A.; et al. Slowed conduction and thin myelination of peripheral nerves associated with mutant Rho guanine-nucleotide exchange factor 10. *Am. J. Hum. Genet.* **2003**, *73*, 926–932. [[CrossRef](#)]
132. Cote, J.F.; Vuori, K. Identification of an evolutionarily conserved superfamily of DOCK180-related proteins with guanine nucleotide exchange activity. *J. Cell Sci.* **2002**, *115 Pt 24*, 4901–4913. [[CrossRef](#)]
133. Griswold, A.J.; Dueker, N.D.; Van Booven, D.J.; Rantus, J.A.; Jaworski, J.M.; Slifer, S.H.; Schmidt, M.A.; Hulme, W.; Konidari, I.; Whitehead, P.G.; et al. Targeted massively parallel sequencing of autism spectrum disorder-associated genes in a case control cohort reveals rare loss-of-function risk variants. *Mol. Autism* **2015**, *6*, 43. [[CrossRef](#)] [[PubMed](#)]
134. Coci, E.G.; Auhuber, A.; Langenbach, A.; Mrasek, K.; Riedel, J.; Leenen, A.; Liehr, T.; Lücke, T. Novel Unbalanced Translocations Affecting the Long Arms of Chromosomes 10 and 22 Cause Complex Syndromes with Very Severe Neurodevelopmental Delay, Speech Impairment, Autistic Behavior, and Epilepsy. *Cytogenet. Genome Res.* **2017**, *151*, 171–178. [[CrossRef](#)] [[PubMed](#)]
135. Kim, J.Y.; Oh, M.H.; Bernard, L.P.; Macara, I.G.; Zhang, H. The RhoG/ELMO1/Dock180 signaling module is required for spine morphogenesis in hippocampal neurons. *J. Biol. Chem.* **2011**, *286*, 37615–37624. [[CrossRef](#)] [[PubMed](#)]
136. Laurin, M.; Fradet, N.; Blangy, A.; Hall, A.; Vuori, K.; Coté, J.-F. The atypical Rac activator Dock180 (Dock1) regulates myoblast fusion in vivo. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 15446–15451. [[CrossRef](#)]
137. Maestrini, E.; Pagnamenta, A.T.; Lamb, J.; Bacchelli, E.; Sykes, N.H.; De Sousa, I.G.M.; Toma, C.; Barnby, G.; Butler, H.; Winchester, L.; et al. High-density SNP association study and copy number variation analysis of the AUTS1 and AUTS5 loci implicate the IMMP2L–DOCK4 gene region in autism susceptibility. *Mol. Psychiatry* **2009**, *15*, 954–968. [[CrossRef](#)]
138. Pagnamenta, A.T.; Bacchelli, E.; De Jonge, M.V.; Mirza, G.; Scerri, T.S.; Minopoli, F.; Chiocchetti, A.; Ludwig, K.U.; Hoffmann, P.; Paracchini, S.; et al. Characterization of a Family with Rare Deletions in CNTNAP5 and DOCK4 Suggests Novel Risk Loci for Autism and Dyslexia. *Biol. Psychiatry* **2010**, *68*, 320–328. [[CrossRef](#)]
139. Liang, S.; Wang, X.-L.; Zou, M.-Y.; Wang, H.; Zhou, X.; Sun, C.-H.; Xia, W.; Wu, L.-J.; Fujisawa, T.X.; Tomoda, A. Family-based association study of ZNF533, DOCK4 and IMMP2L gene polymorphisms linked to autism in a northeastern Chinese Han population. *J. Zhejiang Univ. Sci. B* **2014**, *15*, 264–271. [[CrossRef](#)]
140. Xiao, Y.; Peng, Y.; Wan, J.; Tang, G.; Chen, Y.; Tang, J.; Ye, W.-C.; Ip, N.Y.; Shi, L. The Atypical Guanine Nucleotide Exchange Factor Dock4 Regulates Neurite Differentiation through Modulation of Rac1 GTPase and Actin Dynamics. *J. Biol. Chem.* **2013**, *288*, 20034–20045. [[CrossRef](#)]
141. Tcherkezian, J.; Lamarche-Vane, N. Current knowledge of the large RhoGAP family of proteins. *Biol. Cell* **2007**, *99*, 67–86. [[CrossRef](#)]
142. Reinhard, J.; Scheel, A.; Diekmann, D.; Hall, A.; Ruppert, C.; Bähler, M. A novel type of myosin implicated in signalling by rho family GTPases. *EMBO J.* **1995**, *14*, 697–704. [[CrossRef](#)]
143. Müller, R.T.; Honnert, U.; Reinhard, J.; Bähler, M. The Rat Myosin myr 5 Is a GTPase-activating Protein for Rho In Vivo: Essential Role of Arginine 1695. *Mol. Biol. Cell* **1997**, *8*, 2039–2053. [[CrossRef](#)] [[PubMed](#)]
144. Liu, K.C.; E Cheney, R. Myosins in cell junctions. *BioArchitecture* **2012**, *2*, 158–170. [[CrossRef](#)] [[PubMed](#)]
145. Wirth, J.A.; Jensen, K.A.; Post, P.L.; Bement, W.M.; Mooseker, M.S. Human myosin-IXb, an unconventional myosin with a chimerin-like rho/rac GTPase-activating protein domain in its tail. *J. Cell Sci.* **1996**, *109 Pt 3*, 653–661.
146. Wang, M.-J.; Xu, X.-L.; Yao, G.-L.; Yu, Q.; Zhu, C.-F.; Kong, Z.-J.; Zhao, H.; Tang, L.-M.; Qin, X.-H. MYO9B gene polymorphisms are associated with the risk of inflammatory bowel diseases. *Oncotarget* **2016**, *7*, 58862–58875. [[CrossRef](#)] [[PubMed](#)]
147. Hunt, K.A.; Monsuur, A.J.; McArdle, W.; Kumar, P.J.; Travis, S.P.L.; Walters, J.R.F.; Jewell, D.P.; Strachan, D.P.; Playford, R.J.; Wijmenga, C.; et al. Lack of association of MYO9B genetic variants with coeliac disease in a British cohort. *Gut* **2006**, *55*, 969–972. [[CrossRef](#)] [[PubMed](#)]
148. Hanley, P.J.; Xu, Y.; Kronlage, M.; Grobe, K.; Schoen, P.; Song, J.; Sorokin, L.; Schwab, A.; Baehler, M. Motorized RhoGAP myosin IXb (Myo9b) controls cell shape and motility. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 12145–12150. [[CrossRef](#)] [[PubMed](#)]

149. Xu, Y.; Pektor, S.; Balkow, S.; Hemkemeyer, S.A.; Liu, Z.; Grobe, K.; Hanley, P.J.; Shen, L.; Bros, M.; Schmidt, T.; et al. Dendritic Cell Motility and T Cell Activation Requires Regulation of Rho-Cofilin Signaling by the Rho-GTPase Activating Protein Myosin IXb. *J. Immunol.* **2014**, *192*, 3559–3568. [[CrossRef](#)]
150. Long, H.; Zhu, X.; Yang, P.; Gao, Q.; Chen, Y.; Ma, L. Myo9b and RICS Modulate Dendritic Morphology of Cortical Neurons. *Cereb. Cortex* **2013**, *23*, 71–79. [[CrossRef](#)]
151. Govek, E.E.; Newey, S.E.; Akerman, C.J.; Cross, J.R.; Van der Veken, L.; Van Aelst, L. The X-linked mental retardation protein oligophrenin-1 is required for dendritic spine morphogenesis. *Nat. Neurosci.* **2004**, *7*, 364–372. [[CrossRef](#)]
152. Zanni, G.; Saillour, Y.; Nagara, M.; Billuart, P.; Castelnaud, L.; Moraine, C.; Faivre, L.; Bertini, E.; Durr, A.; Guichet, A.; et al. Oligophrenin 1 mutations frequently cause X-linked mental retardation with cerebellar hypoplasia. *Neurology* **2005**, *65*, 1364–1369. [[CrossRef](#)]
153. Celestino-Soper, P.B.S.; Shaw, C.A.; Sanders, S.J.; Li, J.; Murtha, M.T.; Ercan-Sencicek, A.G.; Davis, L.; Thomson, S.; Gambin, T.; Chinault, A.C.; et al. Use of array CGH to detect exonic copy number variants throughout the genome in autism families detects a novel deletion in TMLHE. *Hum. Mol. Genet.* **2011**, *20*, 4360–4370. [[CrossRef](#)] [[PubMed](#)]
154. Piton, A.; Gauthier, J.; Hamdan, F.F.; Lafreniere, R.G.; Yang, Y.; Henrion, E.; Laurent, S.; Noreau, A.; Thibodeau, P.; Karemera, L.; et al. Systematic resequencing of X-chromosome synaptic genes in autism spectrum disorder and schizophrenia. *Mol. Psychiatry* **2011**, *16*, 867–880. [[CrossRef](#)] [[PubMed](#)]
155. Guo, H.; Duyzend, M.H.; Coe, B.P.; Baker, C.; Hoekzema, K.; Gerds, J.; Turner, T.N.; Zody, M.C.; Beighley, J.S.; Murali, S.C.; et al. Genome sequencing identifies multiple deleterious variants in autism patients with more severe phenotypes. *Genet. Med.* **2019**, *21*, 1611–1620. [[CrossRef](#)] [[PubMed](#)]
156. Barresi, S.; Tomaselli, S.; Athanasiadis, A.; Galeano, F.; Locatelli, F.; Bertini, E.; Zanni, G.; Gallo, A. Oligophrenin-1 (OPHN1), a gene involved in X-linked intellectual disability, undergoes RNA editing and alternative splicing during human brain development. *PLoS ONE* **2014**, *9*, e91351. [[CrossRef](#)]
157. Fauchereau, F.; Herbrand, U.; Chafey, P.; Eberth, A.; Koulakoff, A.; Vinet, M.C.; Ahmadian, M.R.; Chelly, J.; Billuart, P. The RhoGAP activity of OPHN1, a new F-actin-binding protein, is negatively controlled by its amino-terminal domain. *Mol. Cell. Neurosci.* **2003**, *23*, 574–586. [[CrossRef](#)]
158. Redish, A.D. Vicarious trial and error. *Nat. Rev. Neurosci.* **2016**, *17*, 147–159. [[CrossRef](#)]
159. Burbelo, P.D.; Miyamoto, S.; Utani, A.; Brill, S.; Yamada, K.M.; Hall, A.; Yamada, Y. p190-B, a new member of the Rho GAP family, and Rho are induced to cluster after integrin cross-linking. *J. Biol. Chem.* **1995**, *270*, 30919–30926. [[CrossRef](#)]
160. Bustos, R.I.; Forget, M.-A.; Settleman, J.E.; Hansen, S.H. Coordination of Rho and Rac GTPase Function via p190B RhoGAP. *Curr. Biol.* **2008**, *18*, 1606–1611. [[CrossRef](#)]
161. Iossifov, I.; O’Roak, B.J.; Sanders, S.J.; Ronemus, M.; Krumm, N.; Levy, D.; Stessman, H.A.; Witherspoon, K.T.; Vives, L.; Patterson, K.E.; et al. The contribution of de novo coding mutations to autism spectrum disorder. *Nature* **2014**, *515*, 216–221. [[CrossRef](#)]
162. Stiegler, A.L.; Boggan, T.J. p190RhoGAP proteins contain pseudoGTPase domains. *Nat. Commun.* **2017**, *8*, 506. [[CrossRef](#)]
163. Héraud, C.; Pinault, M.; Lagrée, V.; Moreau, V. p190RhoGAPs, the ARHGAP35- and ARHGAP5-Encoded Proteins, in Health and Disease. *Cells* **2019**, *8*, 351. [[CrossRef](#)]
164. Sordella, R.; Classon, M.; Hu, K.Q.; Matheson, S.F.; Brouns, M.R.; Fine, B.; Zhang, L.; Takami, H.; Yamada, Y.; Settleman, J. Modulation of CREB activity by the Rho GTPase regulates cell and organism size during mouse embryonic development. *Dev. Cell* **2002**, *2*, 553–565. [[CrossRef](#)]
165. Matheson, S.F.; Hu, K.-Q.; Brouns, M.R.; Sordella, R.; VanderHeide, J.D.; Settleman, J. Distinct but overlapping functions for the closely related p190 RhoGAPs in neural development. *Dev. Neurosci.* **2006**, *28*, 538–550. [[CrossRef](#)] [[PubMed](#)]
166. Heckman, B.M.; Chakravarty, G.; Vargo-Gogola, T.; Gonzales-Rimbau, M.; Hadsell, D.L.; Lee, A.V.; Settleman, J.; Rosen, J.M. Crosstalk between the p190-B RhoGAP and IGF signaling pathways is required for embryonic mammary bud development. *Dev. Biol.* **2007**, *309*, 137–149. [[CrossRef](#)] [[PubMed](#)]
167. Raman, R.; Kumar, R.S.; Hinge, A.; Kumar, S.; Nayak, R.; Xu, J.; Szczur, K.; Cancelas, J.A.; Filippi, M.D. p190-B RhoGAP regulates the functional composition of the mesenchymal microenvironment. *Leukemia* **2013**, *27*, 2209–2219. [[CrossRef](#)] [[PubMed](#)]

168. Hinge, A.; Xu, J.; Javier, J.; Mose, E.; Kumar, S.; Kapur, R.; Srour, E.F.; Malik, P.; Aronow, B.J.; Filippi, M.-D. p190-B RhoGAP and intracellular cytokine signals balance hematopoietic stem and progenitor cell self-renewal and differentiation. *Nat. Commun.* **2017**, *8*, 14382. [[CrossRef](#)] [[PubMed](#)]
169. Heide, M.; Long, K.R.; Huttner, W.B. Novel gene function and regulation in neocortex expansion. *Curr. Opin. Cell Biol.* **2017**, *49*, 22–30. [[CrossRef](#)]
170. Chilian, B.; Abdollahpour, H.; Bierhals, T.; Haltrich, I.; Fekete, G.; Nagel, I.; Rosenberger, G.; Kutsche, K. Dysfunction of SHANK2 and CHRNA7 in a patient with intellectual disability and language impairment supports genetic epistasis of the two loci. *Clin. Genet.* **2013**, *84*, 560–565. [[CrossRef](#)]
171. Chen, J.; Calhoun, V.D.; Perrone-Bizzozero, N.I.; Pearlson, G.D.; Sui, J.; Du, Y.; Liu, J. A pilot study on commonality and specificity of copy number variants in schizophrenia and bipolar disorder. *Transl. Psychiatry* **2016**, *6*, e824. [[CrossRef](#)]
172. Vadgama, N.; Pittman, A.; Simpson, M.; Nirmalanathan, N.; Murray, R.; Yoshikawa, T.; De Rijk, P.; Rees, E.; Kirov, G.; Hughes, D.; et al. De novo single-nucleotide and copy number variation in discordant monozygotic twins reveals disease-related genes. *Eur. J. Hum. Genet.* **2019**, *27*, 1121–1133. [[CrossRef](#)]
173. Leblond, C.S.; Heinrich, J.; Delorme, R.; Proepper, C.; Betancur, C.; Huguet, G.; Konyukh, M.; Chaste, P.; Ey, E.; Rastam, M.; et al. Genetic and functional analyses of SHANK2 mutations suggest a multiple hit model of autism spectrum disorders. *PLoS Genet.* **2012**, *8*, e1002521. [[CrossRef](#)]
174. Florio, M.; Albert, M.; Taverna, E.; Namba, T.; Brandl, H.; Lewitus, E.; Haffner, C.; Sykes, A.; Wong, F.K.; Peters, J.; et al. Human-specific gene ARHGAP11B promotes basal progenitor amplification and neocortex expansion. *Science* **2015**, *347*, 1465–1470. [[CrossRef](#)] [[PubMed](#)]
175. Wilsch-Braeuning, M.; Florio, M.; Huttner, W.B. Neocortex expansion in development and evolution - from cell biology to single genes. *Curr. Opin. Neurobiol.* **2016**, *39*, 122–132. [[CrossRef](#)] [[PubMed](#)]
176. Florio, M.; Namba, T.; Pääbo, S.; Hiller, M.; Huttner, W.B. A single splice site mutation in human-specific ARHGAP11B causes basal progenitor amplification. *Sci. Adv.* **2016**, *2*, e1601941. [[CrossRef](#)] [[PubMed](#)]
177. Akshoomoff, N.; Mattson, S.N.; Grossfeld, P.D. Evidence for autism spectrum disorder in Jacobsen syndrome: identification of a candidate gene in distal 11q. *Genet. Med.* **2015**, *17*, 143–148. [[CrossRef](#)] [[PubMed](#)]
178. Zhao, C.M.; Ma, H.; Bossy-Wetzel, E.; Lipton, S.A.; Zhang, Z.H.; Feng, G.S. GC-GAP, a Rho family GTPase-activating protein that interacts with signaling adapters Gab1 and Gab2. *J. Biol. Chem.* **2003**, *278*, 34641–34653. [[CrossRef](#)]
179. Okabe, T.; Nakamura, T.; Nishimura, Y.N.; Kohu, K.; Ohwada, S.; Morishita, Y.; Akiyama, T. RICS, a novel GTPase-activating protein for Cdc42 and Rac1, is involved in the beta-catenin-N-cadherin and N-methyl-D-aspartate receptor signaling. *J. Biol. Chem.* **2003**, *278*, 9920–9927. [[CrossRef](#)]
180. Nakamura, T.; Komiyama, M.; Sone, K.; Hirose, E.; Gotoh, N.; Morii, H.; Ohta, Y.; Mori, N. Grit, a GTPase-activating protein for the Rho family, regulates neurite extension through association with the TrkA receptor and N-Shc and CrkL/Crk adapter molecules. *Mol. Cell. Biol.* **2002**, *22*, 8721–8734. [[CrossRef](#)]
181. Nakazawa, T.; Watabe, A.M.; Tezuka, T.; Yoshida, Y.; Yokoyama, K.; Umemori, H.; Inoue, A.; Okabe, S.; Manabe, T.; Yamamoto, T. p250GAP, a novel brain-enriched GTPase-activating protein for Rho family GTPases, is involved in the N-methyl-D-aspartate receptor signaling. *Mol. Biol. Cell* **2003**, *14*, 2921–2934. [[CrossRef](#)]
182. Moon, S.Y.; Zang, H.S.; Zheng, Y. Characterization of a brain-specific Rho GTPase-activating protein, p200RhoGAP. *J. Biol. Chem.* **2003**, *278*, 4151–4159. [[CrossRef](#)]
183. Nakamura, T.; Hayashi, T.; Nasu-Nishimura, Y.; Sakaue, F.; Morishita, Y.; Okabe, T.; Ohwada, S.; Matsuura, K.; Akiyama, T. PX-RICS mediates ER-to-Golgi transport of the N-cadherin/beta-catenin complex. *Genes Dev.* **2008**, *22*, 1244–1256. [[CrossRef](#)] [[PubMed](#)]
184. Nasu-Nishimura, Y.; Hayashi, T.; Ohishi, T.; Okabe, T.; Ohwada, S.; Hasegawa, Y.; Senda, T.; Toyoshima, C.; Nakamura, T.; Akiyama, T. Role of the Rho GTPase-activating protein RICS in neurite outgrowth. *Genes Cells* **2006**, *11*, 607–614. [[CrossRef](#)] [[PubMed](#)]
185. Yuan, J.; Huang, H.; Zhou, X.; Liu, X.; Ou, S.; Xu, T.; Li, R.; Ma, L.; Chen, Y. MicroRNA-132 Interact with p250GAP/Cdc42 Pathway in the Hippocampal Neuronal Culture Model of Acquired Epilepsy and Associated with Epileptogenesis Process. *Neural Plast.* **2016**, *2016*, 5108489. [[CrossRef](#)] [[PubMed](#)]
186. Hayashi, T.; Okabe, T.; Nasu-Nishimura, Y.; Sakaue, F.; Ohwada, S.; Matsuura, K.; Akiyama, T.; Nakamura, T. PX-RICS, a novel splicing variant of RICS, is a main isoform expressed during neural development. *Genes Cells* **2007**, *12*, 929–939. [[CrossRef](#)]

187. Endris, V.; Wogatzky, B.; Leimer, U.; Bartsch, D.; Zatyka, M.; Latif, F.; Maher, E.R.; Tariverdian, G.; Kirsch, S.; Karch, D.; et al. The novel Rho-GTPase activating gene MEGAP/srGAP3 has a putative role in severe mental retardation. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 11754–11759. [[CrossRef](#)]
188. Ma, Y.; Mi, Y.-J.; Dai, Y.-K.; Fu, H.-L.; Cui, D.-X.; Jin, W.-L. The inverse F-BAR domain protein srGAP2 acts through srGAP3 to modulate neuronal differentiation and neurite outgrowth of mouse neuroblastoma cells. *PLoS ONE* **2013**, *8*, e57865. [[CrossRef](#)]
189. Bacon, C.; Endris, V.; Rappold, G.A. The cellular function of srGAP3 and its role in neuronal morphogenesis. *Mech. Dev.* **2013**, *130*, 391–395. [[CrossRef](#)]
190. Wuertenberger, S.; Groemping, Y. A single PXXP motif in the C-terminal region of srGAP3 mediates binding to multiple SH3 domains. *FEBS Lett.* **2015**, *589*, 1156–1163. [[CrossRef](#)]
191. Waltereit, R.; Kautt, S.; Bartsch, D. Expression of MEGAP mRNA during embryonic development. *Gene Expr. Patterns* **2008**, *8*, 307–310. [[CrossRef](#)]
192. Bacon, C.; Endris, V.; Rappold, G. Dynamic Expression of the Slit-Robo GTPase Activating Protein Genes during Development of the Murine Nervous System. *J. Comp. Neurol.* **2009**, *513*, 224–236. [[CrossRef](#)]
193. Yao, Q.; Jin, W.-L.; Wang, Y.; Ju, G. Regulated shuttling of Slit-Robo-GTPase activating proteins between nucleus and cytoplasm during brain development. *Cell. Mol. Neurobiol.* **2008**, *28*, 205–221. [[CrossRef](#)] [[PubMed](#)]
194. Faucherre, A.; Desbois, P.; Satre, V.; Lunardi, J.; Dorseuil, O.; Gacon, G. Lowe syndrome protein OCRL1 interacts with Rac GTPase in the trans-Golgi network. *Hum. Mol. Genet.* **2003**, *12*, 2449–2456. [[CrossRef](#)] [[PubMed](#)]
195. Kenworthy, L.; Park, T.; Charnas, L.R. Cognitive and behavioral profile of the oculocerebrorenal syndrome of Lowe. *Am. J. Med. Genet.* **1993**, *46*, 297–303. [[CrossRef](#)] [[PubMed](#)]
196. Oliver, C.; Berg, K.; Moss, J.; Arron, K.; Burbidge, C. Delineation of Behavioral Phenotypes in Genetic Syndromes: Characteristics of Autism Spectrum Disorder, Affect and Hyperactivity. *J. Autism Dev. Disord.* **2011**, *41*, 1019–1032. [[CrossRef](#)] [[PubMed](#)]
197. Schroer, R.J.; Beaudet, A.L.; Shinawi, M.; Sahoo, T.; Patel, A.; Sun, Q.; Skinner, C.; Stevenson, R.E. Duplication of OCRL and Adjacent Genes Associated with Autism but not Lowe Syndrome. *Am. J. Med. Genet. Part A* **2012**, *158A*, 2602–2605. [[CrossRef](#)] [[PubMed](#)]
198. Erdmann, K.S.; Mao, Y.; McCrea, H.J.; Zoncu, R.; Lee, S.; Paradise, S.; Modregger, J.; Biemesderfer, D.; Toomre, D.; De Camillil, P. A role of the Lowe syndrome protein OCRL in early steps of the endocytic pathway. *Dev. Cell* **2007**, *13*, 377–390. [[CrossRef](#)]
199. Mao, Y.; Balkin, D.M.; Zoncu, R.; Erdmann, K.S.; Tomasini, L.; Hu, F.; Jin, M.M.; Hodsdon, M.E.; De Camilli, P. A PH domain within OCRL bridges clathrin-mediated membrane trafficking to phosphoinositide metabolism. *EMBO J.* **2009**, *28*, 1831–1842. [[CrossRef](#)]
200. Bothwell, S.P.; Chan, E.; Bernardini, I.M.; Kuo, Y.-M.; Gahl, W.A.; Nussbaum, R.L. Mouse Model for Lowe Syndrome/Dent Disease 2 Renal Tubulopathy. *J. Am. Soc. Nephrol.* **2011**, *22*, 443–448. [[CrossRef](#)]
201. Takai, Y.; Sasaki, T.; Matozaki, T. Small GTP-binding proteins. *Physiol. Rev.* **2001**, *81*, 153–208. [[CrossRef](#)]
202. Kitamura, Y.; Kitamura, T.; Sakaue, H.; Maeda, T.; Ueno, H.; Nishio, S.; Ohno, S.; Osada, S.i.; Sakaue, M.; Ogawa, W.; et al. Interaction of Nck-associated protein 1 with activated GTP-binding protein Rac. *Biochem. J.* **1997**, *322 Pt 3*, 873–878. [[CrossRef](#)]
203. Iossifov, I.; Ronemus, M.; Levy, D.; Wang, Z.; Hakker, I.; Rosenbaum, J.; Yamrom, B.; Lee, Y.-h.; Narzisi, G.; Leotta, A.; et al. De Novo Gene Disruptions in Children on the Autistic Spectrum. *Neuron* **2012**, *74*, 285–299. [[CrossRef](#)] [[PubMed](#)]
204. Iossifov, I.; Levy, D.; Allen, J.; Ye, K.; Ronemus, M.; Lee, Y.-h.; Yamrom, B.; Wigler, M. Low load for disruptive mutations in autism genes and their biased transmission. *Proc. Natl. Acad. Sci. United States Am.* **2015**, *112*, E5600–E5607. [[CrossRef](#)] [[PubMed](#)]
205. Freed, D.; Pevsner, J. The Contribution of Mosaic Variants to Autism Spectrum Disorder. *PLoS Genet.* **2016**, *12*, e1006245. [[CrossRef](#)] [[PubMed](#)]
206. Kitamura, T.; Kitamura, Y.; Yonezawa, K.; Totty, N.F.; Gout, I.; Hara, K.; Waterfield, M.D.; Sakaue, M.; Ogawa, W.; Kasuga, M. Molecular cloning of p125Nap1, a protein that associates with an SH3 domain of Nck. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 509–514. [[CrossRef](#)]

207. Suzuki, T.; Nishiyama, K.; Yamamoto, A.; Inazawa, J.; Iwaki, T.; Yamada, T.; Kanazawa, I.; Sakaki, Y. Molecular cloning of a novel apoptosis-related gene, human Nap1 (NCKAP1), and its possible relation to Alzheimer disease. *Genomics* **2000**, *63*, 246–254. [[CrossRef](#)]
208. Yokota, Y.; Ring, C.; Cheung, R.; Pevny, L.; Anton, E.S. Nap1-regulated neuronal cytoskeletal dynamics is essential for the final differentiation of neurons in cerebral cortex. *Neuron* **2007**, *54*, 429–445. [[CrossRef](#)]
209. Doornbos, M.; Sikkema-Raddatz, B.; Ruijvenkamp, C.A.L.; Dijkhuizen, T.; Bijlsma, E.K.; Gijsbers, A.C.J.; Hilhorst-Hofstee, Y.; Hordijk, R.; Verbruggen, K.T.; Kerstjens-Frederikse, W.S.; et al. Nine patients with a microdeletion 15q11.2 between breakpoints 1 and 2 of the Prader-Willi critical region, possibly associated with behavioural disturbances. *Eur. J. Med. Genet.* **2009**, *52*, 108–115. [[CrossRef](#)]
210. Van der Zwaag, B.; Staal, W.G.; Hochstenbach, R.; Poot, M.; Spierenburg, H.A.; de Jonge, M.V.; Verbeek, N.E.; van't Slot, R.; van Es, M.A.; Staal, F.J.; et al. A Co-segregating Microduplication of Chromosome 15q11.2 Pinpoints Two Risk Genes for Autism Spectrum Disorder. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **2010**, *153B*, 960–966. [[CrossRef](#)]
211. Picinelli, C.; Lintas, C.; Piras, I.S.; Gabriele, S.; Sacco, R.; Brogna, C.; Persico, A.M. Recurrent 15q11.2 BP1-BP2 Microdeletions and Microduplications in the Etiology of Neurodevelopmental Disorders. *Am. J. Med. Genet. Part B Neuropsychiatr. Genet.* **2016**, *171*, 1088–1098. [[CrossRef](#)]
212. Cox, D.M.; Butler, M.G. The 15q11.2 BP1-BP2 Microdeletion Syndrome: A Review. *Int. J. Mol. Sci.* **2015**, *16*, 4068–4082. [[CrossRef](#)]
213. Isabel Alvarez-Mora, M.; Calvo Escalona, R.; Puig Navarro, O.; Madrigal, I.; Quintela, I.; Amigo, J.; Martinez-Elurbe, D.; Linder-Lucht, M.; Aznar Lain, G.; Carracedo, A.; et al. Comprehensive molecular testing in patients with high functioning autism spectrum disorder. *Mutat. Res. Fundam. Mol. Mech. Mutagenesis* **2016**, *784*, 46–52. [[CrossRef](#)]
214. Waltes, R.; Duketis, E.; Knapp, M.; Anney, R.J.L.; Huguette, G.; Schlitt, S.; Jarczok, T.A.; Sachse, M.; Kaempfer, L.M.; Kleinboeck, T.; et al. Common variants in genes of the postsynaptic FMRP signalling pathway are risk factors for autism spectrum disorders. *Hum. Genet.* **2014**, *133*, 781–792. [[CrossRef](#)]
215. Toma, C.; Torricco, B.; Hervas, A.; Valdes-Mas, R.; Tristan-Noguero, A.; Padillo, V.; Maristany, M.; Salgado, M.; Arenas, C.; Puente, X.S.; et al. Exome sequencing in multiplex autism families suggests a major role for heterozygous truncating mutations. *Mol. Psychiatry* **2014**, *19*, 784–790. [[CrossRef](#)]
216. Wang, J.; Tao, Y.; Song, F.; Sun, Y.; Ott, J.; Saffen, D. Common Regulatory Variants of CYFIP1 Contribute to Susceptibility for Autism Spectrum Disorder (ASD) and Classical Autism. *Ann. Hum. Genet.* **2015**, *79*, 329–340. [[CrossRef](#)]
217. Noroozi, R.; Omrani, M.D.; Sayad, A.; Taheri, M.; Ghafouri-Fard, S. Cytoplasmic FMRP interacting protein 1/2 (CYFIP1/2) expression analysis in autism. *Metab. Brain Dis.* **2018**, *33*, 1353–1358. [[CrossRef](#)]
218. Kobayashi, K.; Kuroda, S.; Fukata, M.; Nakamura, T.; Nagase, T.; Nomura, N.; Matsuura, Y.; Yoshida-Kubomura, N.; Iwamatsu, A.; Kaibuchi, K. p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase. *J. Biol. Chem.* **1998**, *273*, 291–295. [[CrossRef](#)]
219. De Rubeis, S.; Pasciuto, E.; Li, K.W.; Fernandez, E.; Di Marino, D.; Buzzi, A.; Ostroff, L.E.; Klann, E.; Zwartkruis, F.J.T.; Komiyama, N.H.; et al. CYFIP1 Coordinates mRNA Translation and Cytoskeleton Remodeling to Ensure Proper Dendritic Spine Formation. *Neuron* **2013**, *79*, 1169–1182. [[CrossRef](#)]
220. Koster, F.; Schinke, B.; Niemann, S.; Hermans-Borgmeyer, I. Identification of shyc, a novel gene expressed in the murine developing and adult nervous system. *Neurosci. Lett.* **1998**, *252*, 69–71. [[CrossRef](#)]
221. Bonaccorso, C.M.; Spatuzza, M.; Di Marco, B.; Gloria, A.; Barrancotto, G.; Cupo, A.; Musumeci, S.A.; D'Antoni, S.; Bardoni, B.; Catania, M.V. Fragile X mental retardation protein (FMRP) interacting proteins exhibit different expression patterns during development. *Int. J. Dev. Neurosci.* **2015**, *42*, 15–23. [[CrossRef](#)]
222. Hsiao, K.; Harony-Nicolas, H.; Buxbaum, J.D.; Bozdagi-Gunal, O.; Benson, D.L. Cyfip1 Regulates Presynaptic Activity during Development. *J. Neurosci.* **2016**, *36*, 1564–1576. [[CrossRef](#)]
223. Pathania, M.; Davenport, E.C.; Muir, J.; Sheehan, D.F.; López-Doménech, G.; Kittler, J.T. The autism and schizophrenia associated gene CYFIP1 is critical for the maintenance of dendritic complexity and the stabilization of mature spines. *Transl. Psychiatry* **2014**, *4*, e374. [[CrossRef](#)]
224. Davenport, E.C.; Szulc, B.R.; Drew, J.; Taylor, J.; Morgan, T.; Higgs, N.F.; Lopez-Domenech, G.; Kittler, J.T. Autism and Schizophrenia-Associated CYFIP1 Regulates the Balance of Synaptic Excitation and Inhibition. *Cell Rep.* **2019**, *26*, 2037. [[CrossRef](#)]

225. Yuen, R.K.C.; Merico, D.; Bookman, M.; Howe, J.L.; Thiruvahindrapuram, B.; Patel, R.V.; Whitney, J.; Deflaux, N.; Bingham, J.; Wang, Z.; et al. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. *Nat. Neurosci.* **2017**, *20*, 602. [[CrossRef](#)]
226. Willatt, L.; Cox, J.; Barber, J.; Cabanas, E.D.; Collins, A.; Donnai, D.; FitzPatrick, D.R.; Maher, E.; Martin, H.; Parnau, J.; et al. 3q29 Microdeletion syndrome: Clinical and molecular characterization of a new syndrome. *Am. J. Hum. Genet.* **2005**, *77*, 154–160. [[CrossRef](#)]
227. Quintero-Rivera, F.; Sharifi-Hannauer, P.; Martinez-Agosto, J.A. Autistic and Psychiatric Findings Associated With the 3q29 Microdeletion Syndrome: Case Report and Review. *Am. J. Med Genet. Part A* **2010**, *152A*, 2459–2467. [[CrossRef](#)]
228. Glassford, M.R.; Rosenfeld, J.A.; Freedman, A.A.; Zwick, M.E.; Mulle, J.G. Novel features of 3q29 deletion syndrome: Results from the 3q29 registry. *Am. J. Med Genet. Part A* **2016**, *170*, 999–1006. [[CrossRef](#)]
229. Jaffer, Z.M.; Chernoff, J. p21-activated kinases: three more join the Pak. *Int. J. Biochem. Cell Biol.* **2002**, *34*, 713–717. [[CrossRef](#)]
230. Rane, C.K.; Minden, A. P21 activated kinases: structure, regulation, and functions. *Small Gtpases* **2014**, *5*, e28003. [[CrossRef](#)]
231. Kreis, P.; Barnier, J.-V. PAK signalling in neuronal physiology. *Cell. Signal.* **2009**, *21*, 384–393. [[CrossRef](#)]
232. Arias-Romero, L.E.; Chernoff, J. A tale of two Paks. *Biol. Cell* **2008**, *100*, 97–108. [[CrossRef](#)]
233. Hofmann, C.; Shepelev, M.; Chernoff, J. The genetics of Pak. *J. Cell Sci.* **2004**, *117*, 4343–4354. [[CrossRef](#)]
234. Marlin, J.W.; Chang, Y.-W.E.; Ober, M.; Handy, A.; Xu, W.; Jakobi, R. Functional PAK-2 knockout and replacement with a caspase cleavage-deficient mutant in mice reveals differential requirements of full-length PAK-2 and caspase-activated PAK-2p34. *Mamm. Genome* **2011**, *22*, 306–317. [[CrossRef](#)]
235. Geisheker, M.R.; Heymann, G.; Wang, T.; Coe, B.P.; Turner, T.N.; Stessman, H.A.F.; Hoekzema, K.; Kvarnung, M.; Shaw, M.; Friend, K.; et al. Hotspots of missense mutation identify neurodevelopmental disorder genes and functional domains. *Nat. Neurosci.* **2017**, *20*, 1043. [[CrossRef](#)]
236. Patel, S.; Joseph, S.K.; Thomas, A.P. Molecular properties of inositol 1,4,5-trisphosphate receptors. *Cell Calcium* **1999**, *25*, 247–264. [[CrossRef](#)]
237. Roseblit, N.; Moschella, M.C.; Ondriasova, E.; Gutstein, D.E.; Ondrias, K.; Marks, A.R. Intracellular calcium release channel expression during embryogenesis. *Dev. Biol.* **1999**, *206*, 163–177. [[CrossRef](#)]
238. Nakanishi, S.; Maeda, N.; Mikoshiba, K. Immunohistochemical localization of an inositol 1,4,5-trisphosphate receptor, P400, in neural tissue: studies in developing and adult mouse brain. *J. Neurosci.* **1991**, *11*, 2075–2086. [[CrossRef](#)]
239. Taylor, C.W.; Genazzani, A.A.; Morris, S.A. Expression of inositol trisphosphate receptors. *Cell Calcium* **1999**, *26*, 237–251. [[CrossRef](#)]
240. Matsumoto, M.; Nakagawa, T.; Inoue, T.; Nagata, E.; Tanaka, K.; Takano, H.; Minowa, O.; Kuno, J.; Sakakibara, S.; Yamada, M.; et al. Ataxia and epileptic seizures in mice lacking type 1 inositol 1,4,5-trisphosphate receptor. *Nature* **1996**, *379*, 168–171. [[CrossRef](#)]
241. Inoue, T.; Kato, K.; Kohda, K.; Mikoshiba, K. Type 1 inositol 1,4,5-trisphosphate receptor is required for induction of long-term depression in cerebellar Purkinje neurons. *J. Neurosci.* **1998**, *18*, 5366–5373. [[CrossRef](#)]
242. Fujii, S.; Matsumoto, M.; Igarashi, K.; Kato, H.; Mikoshiba, K. Synaptic plasticity in hippocampal CA1 neurons of mice lacking type 1 inositol-1,4,5-trisphosphate receptors. *Learn. Mem.* **2000**, *7*, 312–320. [[CrossRef](#)]
243. Nagase, T.; Ito, K.I.; Kato, K.; Kaneko, K.; Kohda, K.; Matsumoto, M.; Hoshino, A.; Inoue, T.; Fujii, S.; Kato, H.; et al. Long-term potentiation and long-term depression in hippocampal CA1 neurons of mice lacking the IP3 type 1 receptor. *Neuroscience* **2003**, *117*, 821–830. [[CrossRef](#)]
244. Steinberg, S.F. Structural basis of protein kinase C isoform function. *Physiol. Rev.* **2008**, *88*, 1341–1378. [[CrossRef](#)]
245. Singh, R.K.; Kumar, S.; Gautam, P.K.; Tomar, M.S.; Verma, P.K.; Singh, S.P.; Kumar, S.; Acharya, A. Protein kinase C- $\alpha$  and the regulation of diverse cell responses. *Biomol. Concepts* **2017**, *8*, 143–153. [[CrossRef](#)]
246. Shuntoh, H.; Sakamoto, N.; Matsuyama, S.; Saitoh, M.; Tanaka, C. Molecular structure of the C beta catalytic subunit of rat cAMP-dependent protein kinase and differential expression of C alpha and C beta isoforms in rat tissues and cultured cells. *Biochim. Et Biophys. Acta* **1992**, *1131*, 175–180. [[CrossRef](#)]
247. Letiges, M.; Plomann, M.; Standaert, M.L.; Bandyopadhyay, G.; Sajan, M.P.; Kanoh, Y.; Farese, R.V. Knockout of PKC alpha enhances insulin signaling through PI3K. *Mol. Endocrinol.* **2002**, *16*, 847–858.

248. Ito, Y.; Carss, K.J.; Duarte, S.T.; Hartley, T.; Keren, B.; Kurian, M.A.; Marey, I.; Charles, P.; Mendonca, C.; Nava, C.; et al. De Novo Truncating Mutations in WASF1 Cause Intellectual Disability with Seizures. *Am. J. Hum. Genet.* **2018**, *103*, 144–153. [[CrossRef](#)]
249. Benachenhou, N.; Massy, I.; Vacher, J. Characterization and expression analyses of the mouse Wiskott-Aldrich syndrome protein (WASP) family member Wave1/Scar. *Gene* **2002**, *290*, 131–140. [[CrossRef](#)]
250. Suetsugu, S.; Miki, H.; Takenawa, T. Identification of two human WAVE/SCAR homologues as general actin regulatory molecules which associate with the Arp2/3 complex. *Biochem. Biophys. Res. Commun.* **1999**, *260*, 296–302. [[CrossRef](#)]
251. Dahl, J.P.; Wang-Dunlop, J.; Gonzalez, C.; Goad, M.E.P.; Mark, R.J.; Kwak, S.P. Characterization of the WAVE1 knock-out mouse: Implications for CNS development. *J. Neurosci.* **2003**, *23*, 3343–3352. [[CrossRef](#)]
252. Ceglia, I.; Lee, K.-W.; Cahill, M.E.; Graves, S.M.; Dietz, D.; Surmeier, D.J.; Nestler, E.J.; Nairn, A.C.; Greengard, P.; Kim, Y. WAVE1 in neurons expressing the D1 dopamine receptor regulates cellular and behavioral actions of cocaine. *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 1395–1400. [[CrossRef](#)]
253. Varghese, M.; Keshav, N.; Jacot-Descombes, S.; Warda, T.; Wicinski, B.; Dickstein, D.L.; Harony-Nicolas, H.; De Rubeis, S.; Drapeau, E.; Buxbaum, J.D.; et al. Autism spectrum disorder: neuropathology and animal models. *Acta Neuropathol.* **2017**, *134*, 537–566. [[CrossRef](#)] [[PubMed](#)]
254. Petrelli, F.; Pucci, L.; Bezzi, P. Astrocytes and Microglia and Their Potential Link with Autism Spectrum Disorders. *Front. Cell. Neurosci.* **2016**, *10*, 21. [[CrossRef](#)] [[PubMed](#)]
255. Matta, S.M.; Hill-Yardin, E.L.; Crack, P.J. The influence of neuroinflammation in Autism Spectrum Disorder. *Brain Behav. Immun.* **2019**, *79*, 75–90. [[CrossRef](#)]
256. Delorme, R.; Ey, E.; Toro, R.; Leboyer, M.; Gillberg, C.; Bourgeron, T. Progress toward treatments for synaptic defects in autism. *Nat. Med.* **2013**, *19*, 685–694. [[CrossRef](#)]



© 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).