



Principal Molecular Pathways Affected in Autism Spectrum Disorder

1

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Abstract

Autism spectrum disorder (ASD) development is a highly multifaceted process as evidenced by the complexity of the factors involved in the etiology of ASD, including genetic and nongenetic factors. Several forms of ASD result from genetic alterations in genes that regulate the process of protein synthesis. A growing body of evidence suggests that abnormal synaptic protein synthesis might contribute to ASD and ASD-like clinical features. Several reports of different mutated genes responsible for ASD cases and genetic models have emerged, revealing dysregulation of many crucial signaling pathways. In this chapter, the authors summarize the various factors described to contribute to ASD, both genetic and nongenetic, and their association with WNT, SHH, RA, FGF, and BMP/TGF- β signaling pathways. In addition, the authors discuss the scope for additional research for a better understanding of the pathophysiology of ASD in the context of disrupted signaling pathways, which could help open the doors to identify possible gene targets and novel therapeutic strategies.

Keywords

ASD · Autism · Signaling pathways · Genes · Therapeutic targets · Developmental mechanisms

1.1 Introduction

Autism spectrum disorder (ASD) encompasses a heterogenous set of multifactorial challenges in neurodevelopment, classified according to three fundamental features; compromised social correspondence skills, delayed dialect development, and raised stereotyped alternately tedium practices (Mohn et al. 2014; Golden et al. 2017; Abrahams and Geschwind 2008; Geschwind 2008; Sudhof 2008; Zoghbi 2003). ASD is believed to occur due to the complicated processes that involve numerous gene-environment interactions, as evidenced by the association of multiple elements (genetic and nongenetic). People with autism are recognized to have several traits, such as hampered social interactions, communication impairment, and increased tedium behaviors, implying that aberrant signaling pathways during brain development resulted in the disturbance of specific neural circuits in ASD (Styles et al. 2020; Al-Dewik et al. 2020). The disruption of multiple critical signaling pathways,

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including WNT (Bae and Hong 2018; Kalkman 2012; Mulligan and Cheyette 2017), BMP (Zhang et al. 2017; Kashima et al. 2016; Li et al. 2016; Sajan et al. 2011), SHH (Halepoto et al. 2015; Patel et al. 2017), and retinoic acid (RA) (Niculae and Pavál 2016), has been discovered in research using genetic models in ASD individuals. While direct evidence has not been found, indirect evidence of abnormal FGF or TGF- β signaling in ASD exists (Ansari et al. 2017; Chen et al. 2017; Iwata and Hevner 2009). The success of therapy treatments is severely constrained due to a lack of data on the etiology of ASD and the mechanisms involved. In this chapter the authors aim to summarize current knowledge on the various ASD-associated factors (genetic and nongenetic), and their interactions with signaling pathways that are frequently altered in ASD. This chapter also discusses what additional research could be conducted to gain better insights into altered pathways in ASD.

1.2 Principal Signaling Pathways

1.2.1 Altered WNT Signaling in ASD

WNT signaling involves the secretion of cysteine-rich glycolipoproteins that are WNT receptors themselves. The WNT signaling pathway controls important developmental and regulatory processes, including embryonic development and tissue homeostasis, through regulating receptors, such as frizzled (FZD), when the receptors interact with the WNT protein. WNT signaling is required for several developmental and post-developmental neuroscientific processes, including synaptogenesis and CNS regionalization (Tang 2014; Wada and Okamoto 2009; Wodarz and Nusse 1998; Rosso and Inestrosa 2013; Bielen and Houart 2014; Abu-Khalil et al. 2004; Bengoa-Vergniory and Kypta 2015; Burden 2000; Inestrosa and Varela-Nallar 2015; Onishi et al. 2014). Thus, any disturbance in WNT signaling has the potential to cause the development of CNS-related diseases (Mulligan and Cheyette 2017; Okerlund and Cheyette 2011).

Investigations of both genetically engineered animal models and Human Induced Pluripotent Stem Cell (hiPSC) models have shown the critical role of spatiotemporal WNT signaling throughout animal development (Mulligan and Cheyette 2017). It has also been shown that irregularities in WNT may cause several types of mental illnesses, such as autism, schizophrenia, bipolar disorder, and developmental problems (Kalkman 2012; Mulligan and Cheyette 2017; Okerlund and Cheyette 2011; Oron and Elliott 2017; Kwan et al. 2016a; Martin et al. 2013). Many of the genes and epigenetic factors involving ASD have been identified as affecting common biological processes such as epigenetic modification, WNT, and synaptic transmission (Oron and Elliott 2017; Hormozdiari et al. 2015; Krumm et al. 2014).

The WNT pathway is classified into two major pathways: (1) β -catenin-dependent (canonical pathways) and (2) β -catenin-independent (noncanonical pathways), both of which are key players in neural development and related neurodevelopmental disorders (Grainger and Willert 2018; Komiya and Habas 2008). Several genetic variations associated with and/or documented in ASD cases

include either fundamental elements of the β -catenin-dependent pathway, such as *CTNGB1* (β -catenin) (Kalkman 2012; Mulligan and Cheyette 2017; Krumm et al. 2014; O’Roak et al. 2012a) and adenomatous polyposis coli (*APC*) (Mohn et al. 2014), or β -catenin-independent pathway, such as *PRICKLE2* (Sowers et al. 2013), implying that these two pathways are key players in ASD etiology. A list of selected ASD-associated genes that the authors have found to influence ASD-related pathways is presented in Table 1.1. Possible interactions between the ASD-associated genes and WNT pathway are illustrated in Fig. 1.1.

1.3 Genetic Etiologies

Core elements of the canonical WNT signaling In humans, the components of the β -catenin-dependent route includes many WNT ligands, frizzled receptors, LDL receptor family co-receptors, and intracellular and extracellular modulators (Klaus and Birchmeier 2008). WNT ligands are modified cysteine-rich proteins, and to function, they need to be glycosylated and palmitoylated (Komiya and Habas 2008). WNT1, WNT2, WNT3, and WNT9B are known to be involved with ASD. ASD individuals usually harbor a unique missense genetic variant in *WNT1* (S88R, rs61758378) that has demonstrated higher activation of the WNT/ β -catenin pathway compared to WNT1 wild type i.e. without this variant (Martin et al. 2013). Beyond that, ASD Individuals have been found to have uncommon pathogenic variants in *WNT2*, *WNT3*, and *WNT9B* (Wassink et al. 2001; Marui et al. 2010; Lin et al. 2012; Levy et al. 2011). Therefore, it is surprising that in people with ASD, WNT3 expression in the prefrontal cortex is more pronounced (Chow et al. 2012). The above finding suggests that WNT signaling is overactive and that overactivation could play a key role in ASD etiology.

Several studies have revealed that WNT1 is crucial for cerebellar and midbrain development in animal models (Thomas and Capecchi 1990; McMahon and Bradley 1990; McMahon et al. 1992). Furthermore, it has been shown that WNT2 is necessary for the development of cortical dendrites as well as the production of dendritic spines. Moreover, it was shown that *WNT2* expression could be regulated by a protein referred to as brain-derived neurotrophic factor (BDNF), which is a key player in neuron survival and growth (Hiester et al. 2013). It has also been shown that altered dendritic spines were associated with neurodegenerative and neurodevelopmental problems (Hiester et al. 2013). In addition, WNT3 has been shown to be required for gastrulation and for the regulation of hippocampus neurogenesis (Lie et al. 2005; Liu et al. 1999). Increased inhibitory synaptic density and decreased excitatory synapse counts have been seen in cortical layer 6 neurons during late mouse gestation when the function of T-brain-1, a T-box transcription factor and one of the high-confidence ASD-associated genes, is lost (Fazel Darbandi et al. 2018). WNT9b is known to promote lip/palate formation and fusion (Jin et al. 2012; Juriloff et al. 2006), although it is not known what function it plays in neurodevelopment.

Table 1.1 ASD-related genes influencing WNT, SHH, RA, FGF, and BMP/TGF- β signaling pathways

ASD causal genes	Species	Chromosome	Genetic score ^a	Gene category ^a	Molecular function	Associated signaling pathway	Relevance to ASD	Ref
<i>ALDH1A3</i>	Human	15q26.3	3	Rare single-gene pathogenic variant, syndromic	This gene encodes an enzyme that uses retina as a substrate	RA	Loss of function leads to autistic traits	Moreno-Ramos et al. (2015)
<i>ALDH5A1</i>	–	6p22.3	1	Rare single-gene pathogenic variant, syndromic	Oxidoreductase	–	Gene has been linked to syndromic autism	De Rubeis et al. (2014); Iossifov et al. (2014)
<i>ANK3</i>	Mouse	10q21.2	1	Rare single-gene pathogenic variant, genetic association	Cadherin binding; cytoskeletal protein-binding source; ion channel-binding source; spectrin binding source	WNT (canonical)	Increases proliferation of neural progenitor cells and nuclear β -catenin	Durak et al. (2015)
<i>APC</i>	Mouse	5q22.2	–	–	Beta-catenin binding	WNT (canonical)	Increased repetitive behaviors, reduction in social interest, and other autistic like behaviors; memory and learning difficulties as well	Mohn et al. (2014)
<i>CD38</i>	Human and mouse	4p15.32	3	Rare single-gene pathogenic variant, genetic	Cell adhesion, signal transduction,	RA	Upregulation of CD38	Riebold et al. (2011); Kim et al. (2016)

(continued)

Table 1.1 (continued)

ASD causal genes	Species	Chromosome	Genetic score ^a	Gene category ^a	Molecular function	Associated signaling pathway	Relevance to ASD	Ref
<i>CHD1</i>	–	5q15-q21.1	3	Rare single-gene pathogenic variant	Chromatin regulator, DNA binding, helicase, hydrolase and calcium signaling	–	Missense and frameshift variants in the <i>CHD1</i> gene have been identified in ASD probands (Neale et al. 2012; Iossifov et al. 2014). Heterozygous <i>CHD1</i> missense variants were identified by the authors in patients with autism	Iossifov et al. (2014); Neale et al. (2012); Pilarowski et al. (2018)
<i>CHD2</i>	–	15q26.1	1	Rare single-gene pathogenic variant, syndromic	Chromatin regulator, DNA binding, helicase, hydrolase	–		
<i>CHD3</i>	–	17p13.1	1	Rare single-gene pathogenic variant, syndromic	Chromatin regulator, DNA binding, helicase, hydrolase	–	Two missense and one in-frame deletion variant were identified in the <i>CHD3</i> gene in ASD probands	Iossifov et al. (2014); Yuen et al. (2016, 2017)

<i>CHD7</i>	-		8q12.2	1	Rare single-gene pathogenic variant, syndromic	Chromatin regulator, DNA binding, helicase, hydrolase	-	Gene has been associated with syndromic autism. A rare pathogenic variant in the <i>CHD7</i> gene has been identified in an individual with ASD	O'Roak et al. (2012a)
<i>CHD8</i>	Mouse		14q11.2	1	Rare single-gene pathogenic variant, syndromic, functional	Chromatin remodeling	WNT (canonical)	Lethal embryonically	Nishiyama et al. (2009)
<i>CHD8</i>	Mouse		14q11.2	1	Rare single-gene pathogenic variant, syndromic, functional	Chromatin remodeling	WNT (canonical)	Craniofacial abnormalities, macrocephaly, and behavioral issues; WNT signaling is upregulated in the NAC region of the brain	Platt et al. (2017)
<i>CTNNB1</i>	Mouse		3p22.1	1	Rare single-gene pathogenic variant, syndromic	Activator; cell-cell adhesion and gene transcription	WNT (canonical)	Object recognition and social interactions are impaired; repetitive behaviours are increased, and spatial memory is enhanced	Dong et al. (2016)
<i>CTNNB1</i>	Mouse		3p22.1	1	Rare single-gene pathogenic	Activator; cell-cell adhesion	WNT (canonical)	Caudal axis bending, spina bifida	Zhao et al. (2014)

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Table 1.1 (continued)

ASD causal genes	Species	Chromosome	Genetic score ^a	Gene category ^a	Molecular function	Associated signaling pathway	Relevance to ASD	Ref
<i>CTNNB1</i>	Human and mouse	3p22.1	1	variant, syndromic Rare single-gene pathogenic variant, syndromic	and gene transcription Activator; cell-cell adhesion and gene transcription	WNT (canonical)	aperta, and tail truncation Craniofacial abnormalities, neuronal loss and hair follicle defects	Dubruc et al. (2014)
<i>DHCR7</i>	Mouse	11q13.4	1	Rare single-gene pathogenic variant, syndromic	Oxidoreductase	SHH	Impairment of SMO and a decrease in SHH signaling	Blassberg et al. (2016)
<i>DIXDC1</i>	Human and mouse	11q23.1	3	Rare single-gene pathogenic variant, functional	This gene regulates positively WNT signaling pathway and is present in the centrosome, located at the base of the spindle, together with gamma tubulin	WNT (canonical)	Dendrite impairment and spine growth, positive modulator of WNT signaling	Kwan et al. (2016b)
<i>DLX2</i>	-	2q31.1	3	Genetic association	Encodes for a homeobox transcription factor, activator,	TGF- β /BMP signaling	The genetic association has been reported in ASD CARC cohorts	Liu et al. (2009)

<i>DLX3</i>	–		2	17q21.33		Rare single-gene pathogenic variant	developmental protein, DNA binding Developmental protein, DNA binding; likely to play a regulatory role in the development of the ventral forebrain and might play a role in craniofacial patterning and morphogenesis	TGF- β /BMP signaling	De novo missense variants in the <i>DLX3</i> gene have been identified in two ASD probands (Iossifov et al. 2014)	Iossifov et al. (2014)
<i>DLX5</i>	Mouse		–	7q21.3		–	Activator, developmental protein, DNA binding	TGF- β /BMP signaling	Bmp6 upregulation	Sajan et al. (2011)
<i>DLX6</i>	–		3	7q21.3		Rare single-gene pathogenic variant	Transcription factor, developmental protein, DNA binding	TGF- β /BMP signaling	Rare pathogenic variants in the <i>DLX6</i> gene have been identified with autism (Nakashima et al. 2010)	Nakashima et al. (2010)
<i>EN2</i>	Human		3	7q36.3		Rare single-gene pathogenic variant, genetic association, functional	Developmental protein, DNA binding	SHH	Gain of function pathogenic variant leads to an elevation in SHH expression	Choi et al. (2014)

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Table 1.1 (continued)

ASD causal genes	Species	Chromosome	Genetic score ^a	Gene category ^a	Molecular function	Associated signaling pathway	Relevance to ASD	Ref
<i>FGF22</i>	Mouse	19p13.3-11q14.2-	-	-	Growth factor	FGF	Synapse formation impairment	Terauchi et al. (2010)
<i>FGF7</i>	Mouse	15q21.2	-	-	Heparin binding, growth factor, mitogen	FGF	Synapse formation impairment	Terauchi et al. (2010)
<i>FGFR1</i>	-	8p11.23	2	Genetic association	Transferase, tyrosine-protein kinase, heparin binding, kinase, receptor	FGF	An intronic polymorphism in the <i>FGFR1</i> gene was the index SNP for a loci that reached genome-wide significance with ASD	Matoba et al. (2020)
<i>FMRP</i>	Monkey	Xq27.3	-	-	RNA binding	BMP	Increase in <i>BMPR2</i> and activation of <i>LIMK1</i> , stimulation of actin reorganization for promoting neurite outgrowth and synapse formation	Kashima et al. (2016)
<i>FOXP1</i>	Human	17q11.2	-	-	Developmental protein, DNA binding	RA		Moreno-Ramos et al. (2015)
<i>mGluR5</i>	Mouse	11q14.2-q14.3	3	Rare single-gene pathogenic variant, genetic	G-protein-coupled receptor,	FGF	Increased mRNA levels of both <i>FGF10</i> and <i>NGF</i>	Huang and Lu (2017)

<i>PRICKLE2</i>	Mouse				3p14.1		2	Rare single-gene pathogenic variant, functional	association, functional	Zinc ion binding receptor, transducer	WNT (noncanonical)	Altered social interactions, learning abnormalities, and behavioral inflexibility	Sowers et al. (2013)
<i>PTCH1</i>	Mouse				Xp22.11		1	Rare single-gene pathogenic variant, genetic association	Transmembrane protein	SHH (hypothetical)	SHH (hypothetical)	Disrupted synaptic transmission, SHH independent	Tora et al. (2017)
<i>PTGS2</i>	Humans				1q31.1		3	Rare single-gene pathogenic variant, genetic association, functional	Oxidoreductase, dioxygenase, peroxidase	WNT (canonical)	WNT (canonical)	There are significant associations between the genotypes and the specific symptom domain scores of ADOS and ADI-R	Yoo et al. (2008)
<i>RERE</i>	Human				1p36.23		1	Rare single-gene pathogenic variant, syndromic, genetic association	Transcriptional repressor during development	RA	RA		Fregeau et al. (2016)
<i>RORA</i>	Human				15q22.2		S	Rare single-gene pathogenic variant, syndromic, genetic association, functional	A member of the nuclear hormone-receptor superfamily	RA	RA	Reduction in the levels of <i>BCL-2</i> and <i>RORA</i> in the brain, as well as aberrant methylation	Nguyen et al. (2010)

(continued)

Table 1.1 (continued)

ASD causal genes	Species	Chromosome	Genetic score ^a	Gene category ^a	Molecular function	Associated signaling pathway	Relevance to ASD	Ref
<i>TCF7L2</i>	Human and mouse	10q25.2-q25.3	1	Rare single-gene pathogenic variant, syndromic		WNT (canonical)	Required for thalamocortical axonal projection formation	De Rubéis et al. (2014); Iossifov et al. (2014); Zhou et al. (2004); Lee et al. (2017)
<i>UBE3A</i>	Human	15q11.2	1	Rare single-gene pathogenic variant, syndromic, genetic association	Transferase	WNT (canonical)	Nuclear β -catenin is stabilized, and canonical WNT signaling is stimulated	Yi et al. (2017)
<i>UBE3A</i>	Mouse	15q11.2	1	Rare single-gene pathogenic variant, syndromic, genetic association	Transferase	RA	ALDH1A2 negative regulation and impaired synaptic plasticity	Xu et al. (2018)
<i>UBE3A</i>	Drosophila	15q11.2	1	Rare single-gene pathogenic variant, syndromic, genetic association	Transferase	BMP	Upregulation of BMP signaling in the nervous system and compromising endocytosis in the NMJs	Li et al. (2016)
<i>WNT1</i>	Human	12q13.12	3	Genetic association	Developmental protein	WNT (canonical)	Increased expression	Martin et al. (2013)

^aGenetic scores and categories that were retrieved from SFARI Gene database, S: syndromic. The genetic scores rank the strength of association of each gene with ASD from the strongest association to the weakest association as follows: (1) high confidence; (2) strong candidate; (3) suggestive evidence

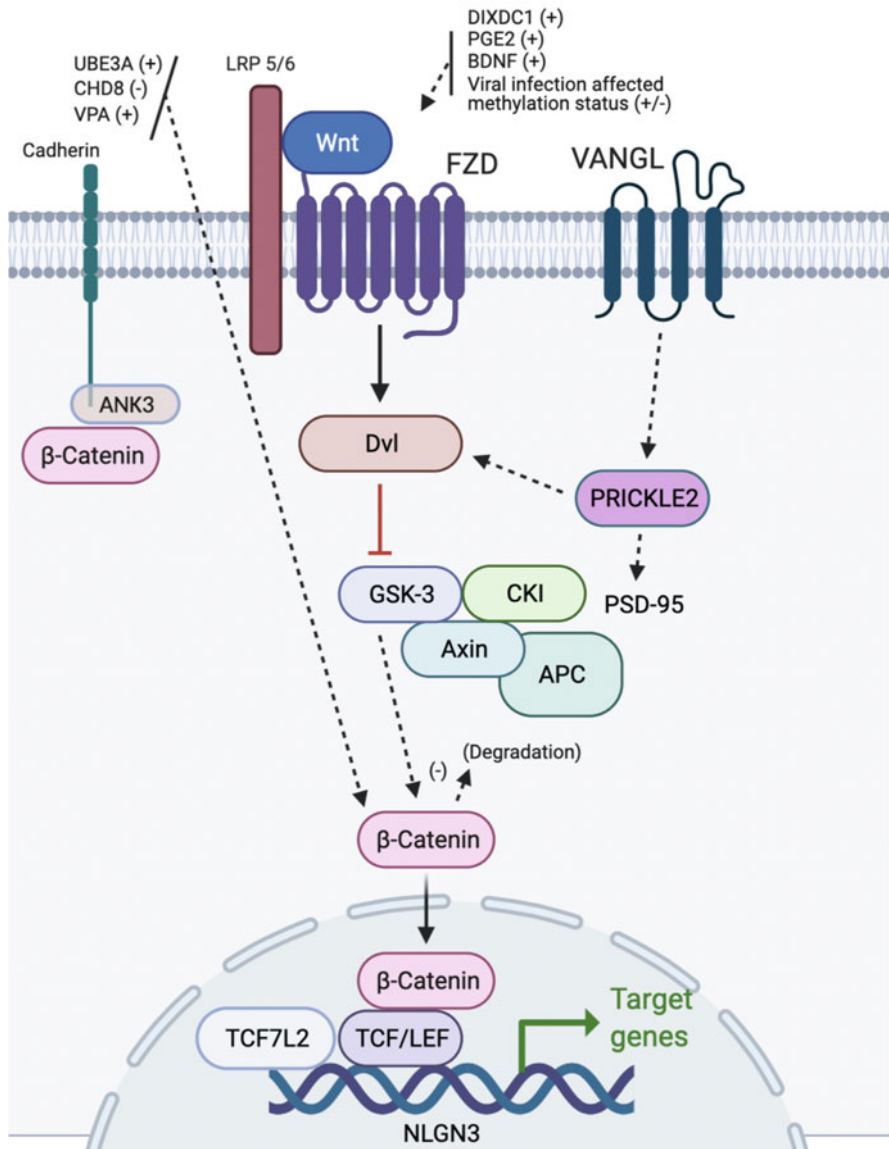


Fig. 1.1 Plausible interactions between the ASD-related genes and WNT signaling. The majority of molecules encoded by ASD-related genes either play a key role in WNT signaling pathways or are modulators. A plus sign signifies upregulation and a negative sign implies downregulation. The figure was created using BioRender (<https://biorender.com/>)

The functions of WNT receptors, in addition to the other WNT ligands (such as FZD1-FZD10 protein family), in ASD etiology remain poorly understood (Onishi et al. 2014; MacDonald and He 2012). Research shows that the duplication or deletion in FZD9 receptors might potentially impair brain development and thus cause ASD (Kalkman 2012). In addition, the administration of WNT2 has been found to stimulate the overexpansion of neurons in dopaminergic pathways in the midbrain of mice, resulting in the mice engaging in repetitive behavior (Sousa et al. 2010).

β -Catenin, an adherent junction component linked to E-cadherin, is an endogenous protein encoded by the gene *CTNNB1*, a key regulator of the WNT signaling pathway. β -Catenin is a major intracellular molecule in the classic WNT signaling pathway that plays essential roles in development and illness (Clevers and Nusse 2012; Grigoryan et al. 2008). High levels of the β -catenin signaling pathway have been reported to contribute to aberrant brain development in people with ASD. De novo *CTNNB1* mutations have been implicated, not only in ASD, but also in intellectual disabilities, microcephaly, speech impairment, and motor delay (Krumm et al. 2014; O’Roak et al. 2012a, b; Kuechler et al. 2015; Sanders et al. 2012). In the Simons Foundation Autism Research Initiative (SFARI) database, *CTNNB1* has a genetic score of “1,” defined as high confidence, indicating that there are at least three de novo likely gene-disrupting genetic variants being reported in association with ASD (Table 1.1). De novo pathogenic variants in this gene were discovered in two different studies utilizing ASD probands from the Simons Simplex Collection (Sanders et al. 2012). In both humans and animals, *CTNNB1* haploinsufficiency has been linked to neuronal loss, craniofacial deformities, and hair follicle abnormalities (Dubruc et al. 2014). Conditional β -catenin ablation in mouse embryo dorsal neural folds represses PAX3 and CDX2 expression at the dorsal posterior neuropore. Leading to reduced expression of the WNT/ β -catenin signaling target genes *T*, *TBX6*, and *FGF8* at the tailbud, resulting in spina bifida aperta, caudal axis bending, and tail truncation (Zhao et al. 2014). Conditional ablation of catenin in parvalbumin interneurons in mice resulted in poor object recognition and social interactions, and increased repetitive behaviors, both of which are essential features of ASD individuals, but they also showed improvement in spatial memory (Dong et al. 2016). The mice exhibited reduced c-Fos activity in the cortex but not in the dentate gyrus or the amygdala, suggesting that β -catenin has a cell type-specific role in the regulation of cognitive and autistic-like behaviors (Dong et al. 2016).

There are a number of ASD-linked genes, besides *CTNNB1*, that are believed to affect β -catenin functions; among them is adenomatous polyposis coli (*APC*) (Zhou et al. 2007). APC is a tumor suppressor that functions as a negative regulator of β -catenin and plays a significant role in the β -catenin-destruction complex (MacDonald et al. 2009). APC-inactivating gene variations in humans have been linked to ASD (Zhou et al. 2007; Barber et al. 1994). APC protein deletion mutation in mice was found to lead to autism-like disabilities, as well as learning and memory deficits in APC conditional knockout compared to wild-type littermates (Mohn et al. 2014). In addition, APC knockout forebrain neurons had higher levels of β -catenin

and increased transcript levels of the canonical WNT target genes *Dkk1*, *Sp5*, *Neurog1*, and *SYN2* (Mohn et al. 2014). Furthermore, APC knockout mouse lysates from the hippocampus, cortical, and striatal areas were shown to have greater β -catenin levels than control mice (Mohn et al. 2014), along with seizures, behavioral traits, and cognitive deficits (Pirone et al. 2017). These findings imply that WNT/ β -catenin signaling activity might be associated with ASD. Besides being an important regulator of the β -catenin level, APC has other roles that are critical for neurogenesis and function, including its role in regulating microtubule and actin cytoskeleton dynamics (Zumbrunn et al. 2001; Akiyama and Kawasaki 2006) and as an mRNA-binding protein with several of its targets involved in brain development (Preitner et al. 2014).

Another gene that plays an important role in the WNT pathway is the transcription factor 7-like 2 (*TCF7L2*), also known as *TCF4*. *TCF7L2* is one of the TCF/LEF1 transcription factors in the WNT/ β -catenin signaling pathway that aid in the initiation of gene transcriptional responses when WNT ligands engage their receptors on the membrane and the signal is transduced to the nucleus (Grant et al. 2006) (Fig. 1.1). *TCF7L2* has been implicated in developmental delays (DDs), intellectual disabilities (IDs), neurodevelopmental disorders (NDDs), and attention-deficit hyperactivity disorder (ADHD) (De Rubeis et al. 2014; Iossifov et al. 2014; Dias et al. 2021).

Recently, two de novo loss-of-function mutations in the *TCF7L2* gene have been identified in ASD cases (De Rubeis et al. 2014; Iossifov et al. 2014). In addition, 11 people with de novo *TCF7L2* mutations who had a syndromic neurodevelopmental condition were identified, four of which had ASD (Dias et al. 2021). It has been shown that *TCF7L2*, like the key WNT co-receptor *Lrp6*, is crucial for the development of thalamocortical axonal projections in mice (Zhou et al. 2004; Lee et al. 2017) implying that abnormal thalamocortical axonal inputs might be playing a vital role in the development of the disorder. Nevertheless, there is still a paucity of knowledge about the connection of other transcription factors from the TCF/Lef1 family with ASD. Furthermore, the significance of *TCF4* in brain development remains unknown and will need to be investigated further in future research.

Core elements of the noncanonical WNT signaling Whole-exome sequencing (WES) conducted on ASD-affected families has identified genetic variations of the WNT/PCP pathway genes *PRICKLE1* and *PRICKLE2*. *PRICKLE1* acts as a nuclear receptor that negatively regulates the WNT/ β -catenin signaling pathway. Moreover, it is involved in the planar cell polarity route, which has several roles, such as convergent extension during gastrulation and neural tube closure, whereas the function of *PRICKLE2* remains unclear. Pathogenic variants in *PRICKLE1* and *PRICKLE2* have previously been linked to epilepsy (EP) (Bosoi et al. 2011; Tao et al. 2011), a disease that is often associated with ASD (Buckley and Holmes 2016). According to an in vitro study, *PRICKLE1* and *PRICKLE2* promote neurite outgrowth through a dishevelled-dependent mechanism (Fujimura et al. 2009). *PRICKLE1* +/- mice were reported to have ASD-like characteristics, such as

abnormal social interactions and disturbed circadian rhythms. In addition, *PRICKLE1* is required for synapsin1 (Syn1) function in the pre-synapse (Paemka et al. 2013), while *PRICKLE2* interacts with postsynaptic density protein-95 (PSD95) and NMDA receptors in the postsynapse (Hida et al. 2011). Mice with disrupted *PRICKLE2* were reported exhibiting impaired social behavior, learning disabilities, and behavioral rigidity. Moreover, mouse models with ASD were shown to exhibit behavioral and physiological abnormalities that are similar to those of *PRICKLE2* mice (Sowers et al. 2013). Furthermore, rare on-synonymous *PRICKLE2* genetic variants (p.E8Q and p.V153I) have been identified in individuals with ASD (Sowers et al. 2013). Dendrite branching, synapse number, and PSD size were all reduced in *PRICKLE2*-deficient mice's hippocampal neurons (Sowers et al. 2013). The discovery of a *PRICKLE2*-containing 3p interstitial deletion in identical twins with ASD adds to the evidence that *PRICKLE2* plays a role in ASD (Okumura et al. 2014). *PRICKLE2*'s interaction with PSD-95 is improved by Vangl2, a key component in the noncanonical WNT/PCP pathway (Nagaoka et al. 2015). The role of other PCP genes in ASD etiology, and the signaling interaction between the PCP and WNT/ β -catenin pathways, should be investigated in the future.

1.4 Modulators and Effectors of WNT Signaling in ASD Etiology

Several genes have been implicated in ASD, such as chromodomain helicase DNA-binding protein 8 (*CHD8*) (Willsey et al. 2013; Cotney et al. 2015), ankyrin-G (*ANK3*) (Shi et al. 2013; Iqbal et al. 2013; Bi et al. 2012), DIX domain-containing 1 (*DIXDC1*) (Kwan et al. 2016b), prostaglandin E2 (*PGE2*) (Wong et al. 2016), and HECT domain E3 ubiquitin ligase (*UBE3A*) (Yi et al. 2017) (Fig. 1.1). In addition, recent research finds that neuroligin 3 (*NLGN3*), an ASD-associated gene, is a direct downstream target of WNT/ β -catenin signaling during synaptogenesis (Medina et al. 2018) (Fig. 1.1). Furthermore, due to the identification of multiple people with genetic variants in phosphatase and tensin homolog (*PTEN*), it has been described as a high-risk candidate ASD-associated gene that plays a role in WNT signaling (O'Roak et al. 2012b; Spinelli et al. 2015; Frazier et al. 2015; McBride et al. 2010).

Canonical WNT signaling is one of the key pathways controlled by CHD8 (Thompson et al. 2008; Nishiyama et al. 2012). CHD8 acts as a transcription repressor by altering the structure of chromatin (Kwan et al. 2016a). It binds β -catenin and inhibits the WNT signaling pathway, which is vital in vertebrates' early development and morphogenesis. Alternatively, spliced transcript variants encoding various isoforms have been discovered in CHD8 (Thompson et al. 2008). Due to its presence at active transcription sites with H3K4me3 or H3K27ac histone modifications, it is believed that CHD8 directly activates genes by binding to the transcriptional start site and boosting transcription factor activity or recruitment, according to the theory. It might also have an indirect effect on transcription by interacting with altered histone sites and other co-regulators to make chromatin more

accessible to transcription factors (Barnard et al. 2015; Sugathan et al. 2014; Wilkinson et al. 2015; Cotney et al. 2015). CHD8 binding to p53 causes the development of a trimeric complex on chromatin with histone H1, which reduces p53-dependent transactivation and death during early embryogenesis (Nishiyama et al. 2009). CHD8 is also necessary for the expression of E2 adenovirus promoter-binding factor target genes during the cell cycle's G1/S transition (Subtil-Rodríguez et al. 2014). In mice, the *CHD8* gene deletion causes embryonic lethality (Nishiyama et al. 2009), whereas its heterozygous loss-of-function variants are associated with macrocephaly, craniofacial deformities, and behavioral impairments (Platt et al. 2017). Its knockdown in human neural progenitor cells changes the expression of neuronal development genes (Wilkinson et al. 2015). In the nucleus accumbens (NAc) region of the brain in *CHD8*+/- mice, WNT signaling is upregulated, suggesting the crucial function CHD8 plays in WNT signaling regulation in the NAc (Platt et al. 2017).

CHD8 is considered the most potential single candidate gene for non-syndromic ASDs (O'Roak et al. 2012a, b; Barnard et al. 2015; Krumm et al. 2014, 2015; Sanders 2015; Bernier et al. 2014). Multiple de novo, truncating, or missense mutations in *CHD8* have been found in people with ASDs (O'Roak et al. 2012a, b; Neale et al. 2012; Sugathan et al. 2014; Bernier et al. 2014; Talkowski et al. 2012; McCarthy et al. 2014). Novel risk loci as Balanced chromosomal abnormalities (BCAs) in the *CHD8* was found in people with ASD or other neurodevelopmental problems (Talkowski et al. 2012). An analysis of rare coding variation in 3871 ASD cases and 9937 ancestry-matched or paternal controls identified *CHD8* as a gene with high statistical significance with an FDR of 0.01, indicating that this gene had a 99% chance of being a true autism gene (De Rubeis et al. 2014). The gene *CHD8* has been identified as an antagonist to the signaling pathway that regulates canonical WNT signaling. This finding is consistent with the notion that increased canonical WNT signaling activity causes excessive proliferation of embryonic neural progenitor cells in the brain, which might help to explain in part the macrocephaly (or "big brain") phenotype observed in cases (Bernier et al. 2014). In Bernier and colleagues study (loss of function variants in *CHD8* was identified in children with developmental delay and ASD; a phenotypic comparison of patients with *CHD8* variants in this report revealed recurrent phenotypes and dysmorphic facial features suggestive of a syndromic form of ASD (Bernier et al. 2014). According to in vivo research, *CHD8* loss-of-function pathogenic variant might activate more canonical WNT signals, resulting in macrocephaly, and ASD-like symptoms (Platt et al. 2017). In addition, recent research found that multiple *CHD8*-controlled genes were implicated in abnormal head size (Sugathan et al. 2014; Wang et al. 2015; Merner et al. 2016). It has been revealed that CHD8 is a positive regulator of WNT/ β -catenin signaling neural progenitor cells while also negatively regulating the pathway in non-neuronal cells (Durak et al. 2016). This finding suggests that CHD8 modulates WNT signaling in a cell-specific manner and that some CHD8 mutations might not be as straightforward as WNT signaling loss-of-function mutations. Further studies are needed to understand how CHD8 modulates WNT signaling in diverse brain cell types, and how patients with

CHD8 pathogenic variants develop macrocephaly. It is also worth noting that WNT signaling is only one of several neurodevelopmental pathways regulated by *CHD8*, and recent research has revealed several other mechanisms, such as chromatin remodeling. Thus, more studies are required to explore the involvement of *CHD8* in WNT signaling at various stages in the development of brain. This is critical to gain deeper insights into how *CHD8* mutations might impair embryonic brain development.

Another gene that has been implicated in ASD is the *ANKK1* gene, which encodes a scaffolding protein referred to as ankyrin-G (Shi et al. 2013; Iqbal et al. 2013; Bi et al. 2012). The protein was first discovered in the axonal initial segment and nodes of Ranvier of neurons, where it plays a role in axonal initial segment assembly and neuronal polarity (Hedstrom et al. 2008; Kordeli et al. 1995). In general, the ankyrin family of proteins are thought to aid in anchoring integral membrane proteins to the cytoskeleton. Furthermore, they are involved in a wide range of activities, including cell motility, activation, proliferation, contact, and maintenance of specialized membrane domains, among other things. Ankyrin-G promotes cell-cell contact by binding to E-cadherin at a conserved location separate from that of β -catenin and transporting it to the cell adhesion site with 2-spectrin in early embryos and cultured epithelial cells (Kizhatil et al. 2007). Ankyrin-G is abundant in the embryonic brain's ventricular zone, where it governs neural progenitor cell growth (Durak et al. 2015). Because of alternative splicing and different beginning exons, there are multiple protein isoforms of ankyrin-G13,17. The isoforms have distinct roles and tissue distributions, with some being expressed exclusively in the brain. Rare polymorphisms identified in ASD patients are mostly found in the brain-specific exons 371, 2, 3, 4, 5, 6, 7, and 8 (Bi et al. 2012; Ferreira et al. 2008; Schulze et al. 2009; Psychiatric GWAS Consortium Bipolar Disorder Working Group 2011; Tesli et al. 2011; Baum et al. 2008; Iqbal et al. 2013; Kosmicki et al. 2017). In ASD cases, whole-genome and whole-exome sequencing investigations have documented several genetic variants in *ANKK1* (Shi et al. 2013; Iqbal et al. 2013; Bi et al. 2012). Loss of function in *ANKK1* promotes neural progenitor cell proliferation and nuclear β -catenin expression, most likely by disrupting the β -catenin/cadherin connection (Durak et al. 2015).

Several missense mutations in *DIXDC1* have been identified in individuals with ASD (Kwan et al. 2016b). These mutations hinder *DIXDC1* isoform 1 phosphorylation, resulting in dendritic and spine development defects (Kwan et al. 2016b). *DIXDC1* encodes for a protein that acts as a positive regulator of the WNT signaling pathway that modulates excitatory neuron dendrite formation and synapse function in the mouse cortex (Kwan et al. 2016b). *MARK1*, which has also been associated with ASD, phosphorylates *DIXDC1* to regulate dendritic and spine formation via isoform-specific cytoskeletal network regulation (Kwan et al. 2016b). *DIXDC1*-deficient animals were found to exhibit behavioral abnormalities, including decreased social interaction, which can be relieved by pharmacological inhibition of glycogen synthase kinase 3 (GSK3) to increase WNT/ β -catenin signaling (Martin et al. 2018; Kivimäe et al. 2011). These findings point to a possible approach to ASD treatment, including modification of WNT/ β -catenin signaling activity. Exome

sequencing of the *DIXDC1* gene has shown a higher burden of rare sequencing-disrupting SNVs among ASD cases in comparison to controls (Martin et al. 2018). In *DIXDC1* knockout neurons, it has been reported that rare *DIXDC1* missense variants in ASD cases failed to rescue deficits in glutamatergic synapse density and spine density, with a subset of *DIXDC1* missense variants displaying hyperactivity in WNT/ β -catenin signaling activity as opposed to dominant-negative effects on spine density and glutamatergic synapse density in wild-type neurons. The existence of functionally relevant ASD missense pathogenic variants in controls, on the other hand, complicates the genetic evidence connecting *DIXDC1* to ASD, with reports of high functionally relevant ASD missense variants in controls. Lack of information regarding the mode of inheritance and segregation of variants in ASD cases, along with the presence of sequence-disrupting *DIXDC1* variants in controls, all make it very challenging to understand the link between *DIXDC1* and ASD.

PGE₂, an endogenous lipid molecule, has been shown to alter the expression of downstream WNT pathway genes previously linked to neurodevelopmental problems (Wong et al. 2016). The primary regulator of PGE₂ synthesis is cyclooxygenase-2 (COX2). COX2/PGE₂ signaling abnormalities have been linked to ASD (Wong et al. 2016). In addition, a growing body of evidence shows that a variety of environmental risk factors, such as inadequate dietary supplementation, infections, misoprostol use during pregnancy, air pollutants, or chemicals, can have a negative effect on PGE₂ levels and can be indirectly linked to ASD (Tamiji and Crawford 2010; Wong et al. 2015; Bandim et al. 2003; Landrigan 2010). Furthermore, PGE₂ has been shown to downregulate *PTGS2* while upregulating *MMP9* and *CCND1* in undifferentiated stem cells. On the other hand, in differentiating neuronal cells, it upregulates *WNT3*, *TCF4*, and *CCND1* expression (Wong et al. 2016).

Another gene that has been implicated in ASD etiology is *UBE3A* which encodes an E3 ubiquitin-protein ligase, which is part of the ubiquitin protein degradation mechanism. This imprinted gene is expressed maternally in the brain and biallelically in other organs. Several studies have shown genetic associations and rare polymorphisms in the *UBE3A* gene that are linked to ASD. A link was discovered in the collaborative linkage study of autism families and rare variations were discovered in instances of European ancestry (Nurmi et al. 2001). Another polymorphism identified in *UBE3A* was T485A which is a de novo autism-linked *UBE3A* pathogenic variants that was reported to be involved in ubiquitinating numerous proteasome subunits, decreasing their number and activity, stabilizing nuclear β -catenin, and activating the canonical WNT pathway more efficiently in comparison to wild-type *UBE3A* (Yi et al. 2017). Dysfunction of *UBE3A* has been associated with cancer, Angelman syndrome, ADHD, DD/NDD, extrapyramidal symptoms (EPS), ID, and EP (Yi et al. 2017).

Pathogenic variants in the neurexin and neuroligin families have also been implicated in the development of autism spectrum disorders (Südhof 2008). Neurexins and neuroligins interact cooperatively to regulate the synaptic activity, both excitatory and inhibitory, in the brain (Knight et al. 2011). *NRXN1*, *NLGNI*, *NLGNI3*, *CNTN4*, *CNTN6*, and *CNTNAP2* are examples of superfamily genes that have a function in autism spectrum disorder (Berg and Geschwind 2012). Knockout

ASD mouse models of genes such as *NRXN1*, *SHANK3*, *FMRI*, and *CNTNAP2* were found to have imbalances in excitation and inhibition in brain regions, and these knockout models exhibited social interaction deficits and reduced ultrasonic vocalizations, which overlapped with behavioral endophenotypes relevant to ASD (Rosti et al. 2014). Imbalances in excitation and inhibition in brain regions were discovered in knockout ASD mouse models of genes such as *NRXN1*, *CNTNAP2*, *FMRI*, and *SHANK3*. It has been shown that ASD-affected individuals have variants in the neuroligins *NLGN3* and *NLGN4* (Jamain et al. 2003). These type I transmembrane proteins serve as adhesion molecules for brain cells and are required to establish and develop synaptic connections in the brain (Zhang et al. 2017). According to chromatin immunoprecipitation and promoter luciferase experiments, WNT/ β -catenin signaling directly controls the production of *NLGN3*, a transcription factor (Medina et al. 2018). It would however be essential to establish whether or not WNT/ β -catenin signaling influences the expression of additional ASD-associated genes. It is noteworthy that neuronal activity is regulated by genes such as *GRIN2B*, *SCN1A*, and *SCN2A*, and these genes are thought to be involved in the mediation of synaptic plasticity. In addition, they encode for ion channels (O’Roak et al. 2012a). It is noteworthy to mention that *UBE3A*, *PCDH10*, *DIA1*, and *NHE9/SLC9A9* are similarly affected by neuronal activity that regulates transcription factors (Morrow et al. 2008; Flavell et al. 2008).

Given the finding of several individuals with phosphatase and tensin homolog (*PTEN*) mutations, *PTEN* is considered a high-risk autism candidate gene that plays a role in WNT signaling (O’Roak et al. 2012b; Spinelli et al. 2015; Frazier et al. 2015; McBride et al. 2010). Furthermore, individuals with heterozygous *PTEN* pathogenic variants are also at risk of developing macrocephaly, implying that *PTEN* regulates brain size, which is thought to affect specific ASD instances (Page et al. 2009; Kwon et al. 2006; Chen et al. 2015; Vogt et al. 2015; Clipperton-Allen and Page 2014, 2015; Takeuchi et al. 2013; Tilot et al. 2015; Zhou and Parada 2012).

1.5 Altered Sonic Hedgehog (SHH) Signaling in ASD

SHH signaling is one such pathway that regulates neurogenesis and neuronal processes during CNS development (Choy and Cheng 2012). SMO-SHH signaling is implicated in various neurological activities, including neuronal cell proliferation and survival (Álvarez-Buylla and Ihrie 2014). While the function of primary neural cilia in CNS patterning during embryonic development is well understood, their relevance in adult CNS plasticity has only recently been discovered (Kirschen and Xiong 2017). SHH signaling at the main cilium has been investigated and defined, as shown in Fig. 1.2 (Seppala et al. 2017). In the absence of SHH activity, *PTCH* suppresses SMO effectively. This causes Gli (glioma-associated oncogene/transcription factor) proteins to be phosphorylated, after which they are proteolytically truncated into repressor forms that inhibit transcriptional activity. SHH binding to *PTCH*, on the other hand, induces internalization followed by breakdown, resulting in SMO buildup and protein phosphorylation. As a consequence, Gli is carried to the

cytoplasm and enters the nucleus in its complete form, boosting target transcription even more. In children with ASD, pathological functions for SHH, Indian hedgehog (IHH), and BDNF have been proposed (Halepoto et al. 2015). SHH signaling influences both neurogenesis and neuronal patterning during the development of the central nervous system during pregnancy. Neurological diseases such as ASD are caused by an imbalance in SHH signaling in the brain (Patel et al. 2017). In the context of ASD, SHH has also been linked to oxidative stress (Ghanizadeh 2012). Autistic children exhibited substantially greater levels of oxygen free radicals (OFR) and serum SHH protein, suggesting that oxidative stress and SHH play a role in the development of ASD (Al-Ayadhi 2012). As shown in Fig. 1.2, the connection between ASD-associated genes and SHH signaling is illustrated.

The SHH pathway has also been implicated in ASD, and several pathogenic variants in patched domain-containing 1 (*PTCHD1*) have been identified. A pathogenic variant in this gene has been found to lead to problems with synaptic connections and irregularities in neuronal transmissions in male mice, resulting in hyperactivity and cognitive deficits (Tora et al. 2017; Ung et al. 2018) and behavioral abnormalities (Tora et al. 2017). However, while the *PTCHD1* protein does not seem to play a role in SHH-dependent signaling when tested using a loss-of-function approach, it does appear to affect synaptic transmission in the mouse dentate gyrus (Tora et al. 2017). In addition it has been shown that *PTCHD1* interacts with PSD95 and SAP102, two postsynaptic proteins (Ung et al. 2018). *PTCHD1* deficiency (*PTCHD1*^{-y}) in male mice causes widespread changes in synaptic gene expression, including changes in the expression of the immediate-early expression genes *EGR1* and *NPAS4*, and disruptions in excitatory synaptic structure and neuronal excitatory activity in the hippocampus, which results in cognitive dysfunction, motor impairments, and hyperactivity (Ung et al. 2018).

Pathogenic variants in the 7-dehydrocholesterol reductase (*DHCR7*) gene have also been linked to ASD. Increased cholesterol production was found to have a negative effect on the activation of the transmembrane protein smoothed (SMO), which is responsible for transmitting SHH signals, and its localization to the major cilium (Blassberg et al. 2016). The transcription factor engrailed 2 (*EN2*) has also been implicated in the development of ASD (Brune et al. 2008; Sen et al. 2010; Wang et al. 2008; Yang et al. 2008, 2010). The higher levels of *EN2* seen in individuals with the *EN2* ASD-associated haplotype (rs1861972-rs1861973 A-C) indicated that *EN2* is associated with ASD susceptibility (Choi et al. 2014; Bi et al. 2012; Gharani et al. 2004). According to the evidence from postmortem samples, rising *EN2* levels have been associated with increased *SHH* expression (Choi et al. 2014). During brain development, *SHH* is one of the genes that coexpress *EN2* and other genes (Wechsler-Reya and Scott 1999; Simon et al. 2005). Furthermore, both mice with *EN2* pathogenic variants and autistic humans had cerebellar structural abnormalities that were similar (Gharani et al. 2004).

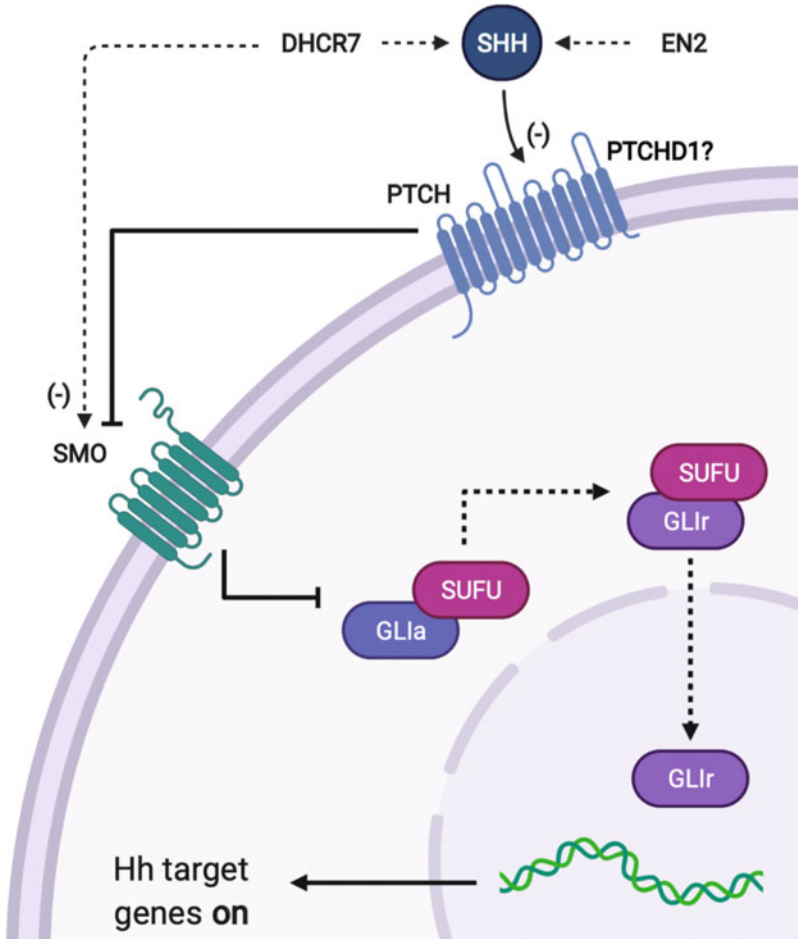


Fig. 1.2 Possible associations between the ASD-linked genes and SHH signaling. PTCHD1, EN2, and DHCR7 genes are all potential ASD-associated genes. A negative sign denotes downregulating activity. The figure was created using BioRender (<https://biorender.com/>)

1.6 Altered Retinoic Acid (RA) Signaling in ASD

RA, a functional metabolite of vitamin A, is a necessary morphogen during vertebrate development (Niederreither and Dollé 2008; Duester 2008). RA can influence several developmental genes that include RA response elements (RARE) and their regulatory areas. By binding to nuclear receptors known as retinoic acid receptors (RARs) and retinoid X receptors (RXRs), RA mediates both genomic transcriptional effects and non-genomic effects such as retinoylation (RA acylation), a posttranslational modification of proteins (Rhinn and Dollé 2012; Das et al. 2014). A number of

co-activators and co-repressors have been identified as modulators of RA signaling activity (Das et al. 2014). RA is necessary for neural patterning, differentiation, proliferation, and establishment of neurotransmitter systems in the developing CNS (Zieger and Schubert 2017). Cortical neuron generation is regulated by RA from the meninges (Siegenthaler et al. 2009). During embryonic development, RA aids in the regulation of a group of HOX (homeobox) genes that form the upper-body pattern, both anteriorly and posteriorly, and are implicated in brain patterning. Dopaminergic and GABAergic neurons are involved in brain cell development in RA. It is also a critical inducing factor for the development of motor neurons from pluripotent stem cells (Faravelli et al. 2014).

Furthermore, RA is necessary for the appropriate functioning of motor neurons. RA has also been linked to neuronal migration and neurogenesis in the granular zone of the hippocampus, the sub-ventricular zone, and the olfactory bulb (Ghyselinck and Duester 2019). The above mentioned functions of RA indicate that neurodevelopmental diseases and RA signaling pathways are linked.

A sufficient quantity of retinol is required for the regular functioning of the RA signaling system, assuming that all of the enzymes involved in the RA pathway and nuclear factors operate properly. One of the key reasons for reduced intracellular RA signaling is retinol insufficiency. In China, some autistic people had lower levels of retinol than the normal control group, which might be a synergistic component in the development of ASD symptoms (Cheng et al. 2021). Retinol supplements have been shown to increase RAR expression and reduce ASD symptoms. Some investigations have shown that retinol deficiency in rats during pregnancy reduces RA receptor expression (RAR, beta isoform) in the hypothalamus, resulting in autistic-like symptoms in the neonates (Lai et al. 2018). In rats, a lack of vitamin A has been linked to ASD-like behavior (Lai et al. 2018). It has also been claimed that ASD is caused by an abnormality in the interaction of RA and sex hormones (Niculae and Pavál 2016).

The nuclear receptors for RA have also been implicated in the pathophysiology of ASD. RORs (retinoic acid-related orphan receptors) operate as transcriptional regulators upon RA binding, triggering transcription of numerous genes. Autistic people were shown to have reduced protein expression of the RA-related orphan receptor alpha (RORA) due to hypermethylation (Nguyen et al. 2010), and RORA mutations have been linked to ASD (Sayad et al. 2017). RORA has been shown to transcriptionally control numerous ASD-relevant genes, including *NLGNI* (Sarachana and Hu 2013). P89L missense variant of *NLGNI*, identified in ASD cases, has been linked to alterations in cellular localization, protein degradation, and spine formation impairment. Mice with heterozygous P89L in the *NLGNI* have also been found to exhibit aberrant social behavior, which is a crucial hallmark of ASD (Nakanishi et al. 2017).

Furthermore, an immunohistochemical examination of the postmortem brains of autistic people revealed a reduced quantity of ROR alpha protein (Nguyen et al. 2010). Disruption of the retinoic acid enzymatic production pathway was found to be associated with ASD phenotypes and retinoic acid nuclear receptors, which have also been implicated in the pathophysiology of ASD. Therefore, more research is

needed to establish a link between ASD pathogenesis and involvement of RAR and ROR agonists in autism treatment.

Another approach for detecting ASD signals is quantitative electroencephalography (EEG) analysis. Details of individual characterization of EEG fluctuations in ASD subjects could aid in examining brain issues, which would be helpful in observing automatic groupings and random draws of the patient population when analyzing sensory-processing issues of the brain and peripheral system (Ryu et al. 2021).

The conversion of retinol to RA with the aid of the enzyme (ALDH) retinaldehyde dehydrogenase, which assures the concentration of RA in the cell, is an essential metabolic step in the RA pathway. In vitro, over-ubiquitinylation by *UBE3A* has been shown to increase the breakdown rate of this enzyme's isoform aldehyde dehydrogenase 1 family member A2 (ALDH 1A2). Autistic characteristics were detected in mice with *UBE3A* overexpression (Xu et al. 2017). In addition, loss-of-function pathogenic variants in the *UBE3A* have been linked to Angelman syndrome, demonstrating the role of *UBE3A* in brain development (Khatri and Man 2019). Surprisingly, *UBE3A* overexpression suppresses ALDH1A2 and inhibits RA-mediated synaptic plasticity in ASD, which might be improved with RA supplementation (Xu et al. 2018). All-trans-RA can increase CD38 expression in ASD lymphoblastoid cell lines, but CD38-deficient animals display ASD-like behavior (Riebold et al. 2011; Kim et al. 2016). In BTBR mice, beta-carotene, a precursor of vitamin A, is a viable therapy for autistic-like behavior (Avraham et al. 2019). In a mouse model, a synthetic RORA/G agonist was investigated for its ability to ameliorate autistic symptoms (Wang et al. 2016). A South American cohort's WES has revealed a link between RA signaling genes, including a RA-synthesizing gene *ALDH1A3* and the RORA-regulated *FOXN1*, and ASD (Moreno-Ramos et al. 2015). A subpopulation of autistic people were shown to have low levels of *ALDH1A1* (Pavál et al. 2017). ASD and other abnormalities linked with proximal 1p36 deletions might be caused by de novo variants in arginine-glutamic acid dipeptide repeats (RERE) that encode a nuclear receptor coregulator for RA signaling (Fregeau et al. 2016). These findings point to potential pharmacological methods for ASD treatment that include targeting RA and associated signaling pathways. Figure 1.3 depicts the potential connections between ASD-associated genes and RA signaling.

1.7 Altered Fibroblast Growth Factor (FGF) Signaling in ASD

FGF signaling is critical in brain patterning, and its disruption might result in a variety of neurological disorders (Turner et al. 2016). In the mammalian FGF family, there are 18 secreted FGFs and 4 tyrosine kinase FGF receptors (FGFRs) whose interaction is controlled by cofactors and external binding proteins (Ornitz and Itoh 2015). When FGFRs are activated, tyrosine residues are phosphorylated, resulting in interactions between cytosolic adaptor proteins and RAS-MAPK, PI3K-AKT, PLC, and STAT intracellular signaling pathways (Ornitz and Itoh 2015). It has been

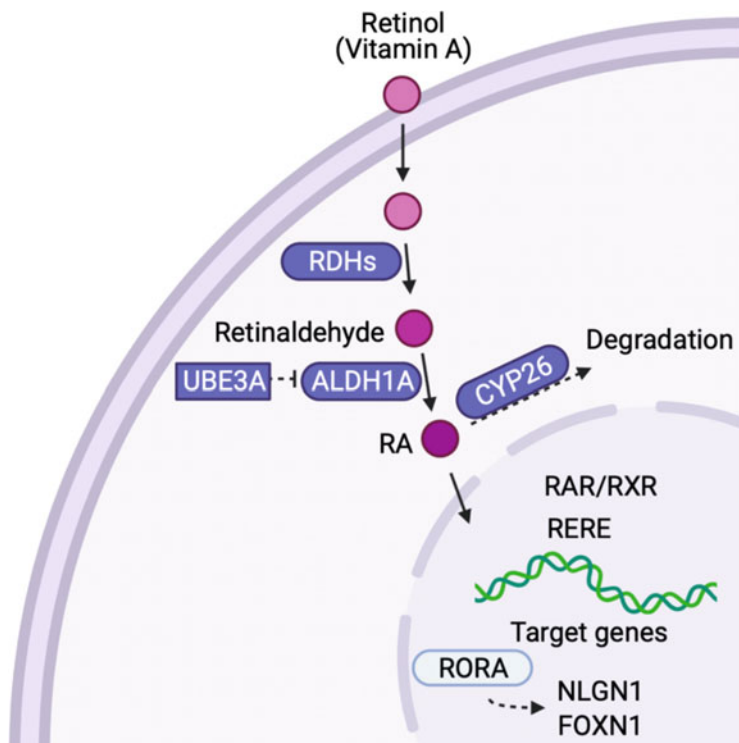


Fig. 1.3 Possible interactions between ASD-associated genes and RA signaling. UBE3A influences ALDH1A expression, which in turn affects the RA signaling pathway. RORA is linked to ASD, which impacts NLGN1. ASD is also linked to the RA signaling coregulator RERE. The figure was created using BioRender (<https://biorender.com/>)

proposed that dysregulation of FGF signaling plays a role in the etiology of ASD (Iwata and Hevner 2009). Cortical abnormalities in autistic brains, for example, have been linked to faulty FGF signaling (Turner et al. 2016; Amaral et al. 2008). ASD is thought to be caused by a disruption in the number of excitatory and inhibitory synapses (Terauchi et al. 2010). Mutant mice FGF22 or FGF7 knockout (KO) showed defective synapse formation in hippocampal CA3 pyramidal neurons (Terauchi et al. 2010), indicating the pathogenic significance of dysregulated FGF signaling in ASD. The metabotropic glutamate receptor 5 (mGluR5) loss-of-function pathogenic variant causes abnormal dendritogenesis in cortical neurons, which is one of the traits identified in autistic brains, by raising nerve growth factor (NGF) and *FGF10* mRNA levels (Huang and Lu 2017). Although several members of FGF gene subfamilies have been linked to CNS development and/or function, many of their roles remain largely unknown, and no direct evidence of altered FGF signaling in ASD has been reported. Nevertheless, it has been well documented that epigenetic mechanisms (Zhu et al. 2007), and posttranslational modifications of FGFs and FGFRs (Sarabipour and Hristova 2016; Triantis et al. 2010; Wheeler and

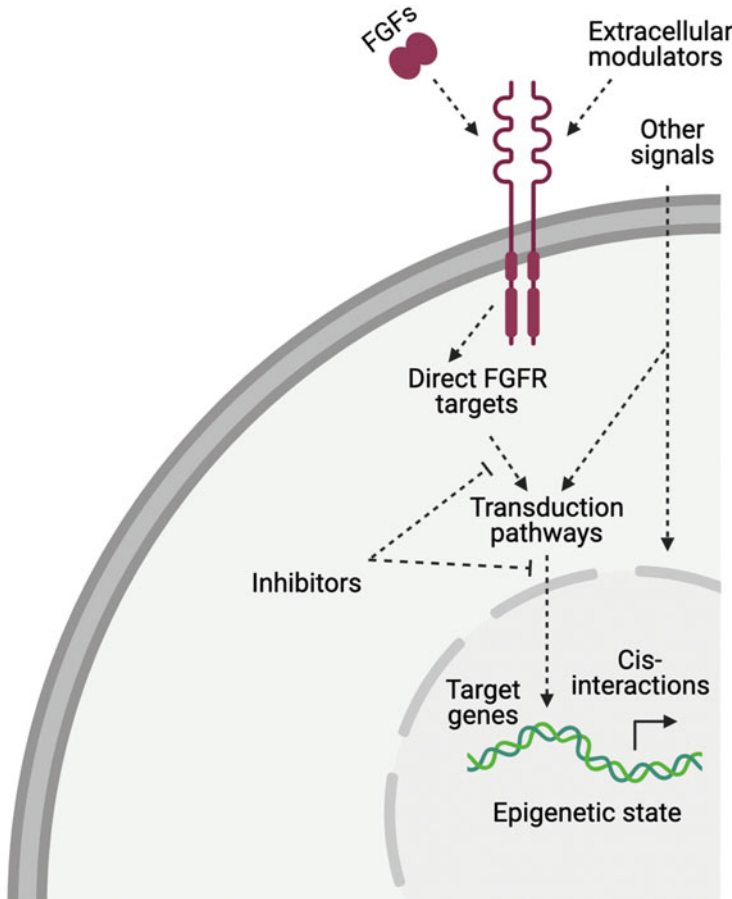


Fig. 1.4 Possible mechanisms of FGF signaling. A simplified depiction of the possible mechanisms through which an FGF signal might be modulated to influence cell fate. The figure was created using BioRender (<https://biorender.com/>)

Clinkenbeard 2019; Kucinska et al. 2019; Porebska et al. 2018), are involved in the regulation of the expressions of FGF/FGFR signaling (Xie et al. 2020). Figure 1.4 summarizes the plausible mechanisms by which FGF signaling modulates cell fate.

1.8 Altered TGF- β /BMP Signaling in ASD

TGF- β /activin and bone morphogenetic protein (BMP)/growth and differentiation factor (GDF) are the two subcategories of the TGF- β superfamily (Zi et al. 2012). BMPs are the most abundant in the TGF- β superfamily (Lory and Rosen 2018), and they play an essential role in nervous system development (Bond et al. 2012). Their

signaling has been demonstrated to be dysregulated in ASD. BMPs influence gene expression via both canonical (Smad-dependent) and noncanonical pathways (such as the MAPK cascade) (Wang et al. 2014). The binding of BMPs to type I or type II serine/threonine kinase receptors results in a heterotetrameric complex in the canonical pathway. The type II receptor then transphosphorylates the type I receptor, following which the type I receptor phosphorylates the R-Smads (Smad1/5/8). Phosphorylated Smad1/5/8 and the co-Smad (Smad4) translocate to the nucleus and regulate gene expression. BMP signaling is modulated by various factors, including plasma membrane co-receptors and external and intracellular factors (Wang et al. 2014). The BTBR T + Itpr3tf/J (BTBR) mice are commonly utilized in ASD studies (Ansari et al. 2017). TGF- β levels have been found to be lower in BTBR mice than in B6 mice (Ansari et al. 2017). TGF- β expression levels in the spleen and brain tissues of BTBR mice were found to be significantly higher than in adenosine A2A receptor (A2AR) agonist CGS 21680 (CGS)-treated mice (Ansari et al. 2017). Components of the TGF- β pathway were identified as novel hyperserotonemia-related ASD genes in a network-based gene set enrichment analysis (NGSEA) based on loss-of-function and missense de novo variants (Chen et al. 2017). Figure 1.5 summarizes the interactions between ASD-associated genes and TGF- β /BMP signaling.

Fragile X syndrome (FXS) is the most frequent heritable form of ASD and intellectual impairment caused by *FMR1* silencing (Kashima et al. 2016). Depletion of the *FMR1* protein (FMRP) leads to increased bone morphogenetic protein type II receptor (BMPR2) and activation of a noncanonical BMP signaling component, LIM domain kinase 1 (LIMK1), which stimulates actin rearrangement to promote neurite outgrowth and synapse formation (Kashima et al. 2016). Increased BMPR2 and LIMK1 activity has been observed in the prefrontal cortex of FXS patients compared to healthy participants (Kashima et al. 2016).

UBE3A has been associated with the regulation of synapse growth and endocytosis by inhibiting BMP signaling (Li et al. 2016). The ubiquitin-proteasome pathway degrades the BMP receptor Tkv, a direct substrate of *UBE3A* (Li et al. 2016). Through the BMP signaling pathway, *Drosophila UBE3A* is known to modulate neuromuscular junction (NMJ) development in presynaptic neurons (Li et al. 2016). *Drosophila UBE3A* mutants have been found to be viable and productive. Nevertheless, they have been demonstrated to have impaired endocytosis in the NMJs and upregulated BMP signaling in the nervous system, owing to an increase in Tkv (Li et al. 2016).

The *DLX* genes, which encode homeodomain transcription factors, have also been linked to ASD (Liu et al. 2009; Hamilton et al. 2005; Rubenstein and Merzenich 2003). These genes regulate craniofacial patterning, differentiation, and survival of forebrain inhibitory neurons (Hamilton et al. 2005). The BMP-binding endothelial regulator (Bmper) is upregulated in a cell line overexpressing *DLX5* (Sajan et al. 2011), indicating that dysregulated *DLX* activity in individuals with ASD might lead to aberrant BMP signaling.

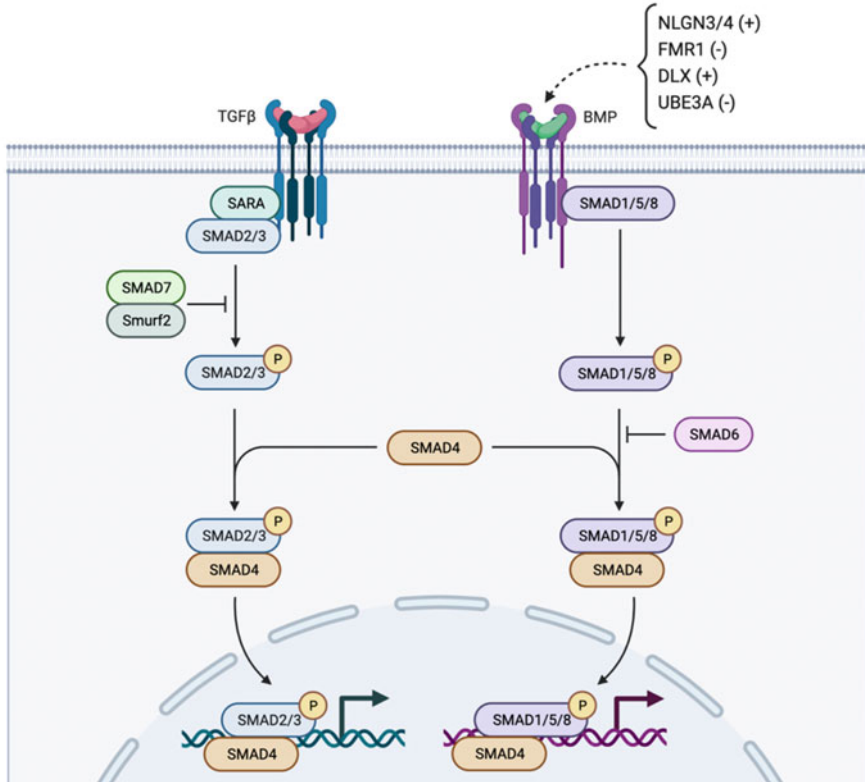


Fig. 1.5 Possible interactions between ASD-associated genes and TGF- β /BMP signaling. ASD-associated gene-encoded proteins, such as NLGN3/4, FMR1, DLX, and UBE3A, interact with BMP signaling. Plus sign indicates upregulation; minus sign indicates downregulation. The figure was created using BioRender (<https://biorender.com/>)

1.9 Signaling Crosstalk in ASD

While it is known that crosstalk occurs across signaling pathways in many developmental stages and illnesses, availability of evidence on autistic models is limited. Research has shown that the WNT signaling proteins might affect both developmental and inflammatory processes. It was shown that WNT signaling and SHH signaling are connected and influence each other at the transcriptional level (Chatterjee and Sil 2019). Cell-line studies have shown that Gli stimulates β -catenin nuclear translocation by E-cadherin and Snail (Li et al. 2007). Gli regulates WNT5a and WNT2b to elevate WNT signaling (Katoh and Katoh 2009). Casein kinase 1 (CK1), p53, PTEN, and GSK3 are protein modulators in these signaling pathways, and each serves a different purpose. They collaborate to boost β -catenin and Gli signaling (Song et al. 2014). Due to evidence of cross talk between WNT and SHH/Gli

signaling in colon cancer, this signaling pathway is now a possible candidate for colon cancer therapy (Song et al. 2015). CK1 and GSK3 are known to have antagonistic functions in regulating β -catenin and Gli1 (Wang and Li 2006; Tempé et al. 2006; Hart et al. 1999), and a detrimental impact on TCF and downstream genes of metastatic colon cancer. Also, they were shown to have opposing roles (Varnat et al. 2010). Another problem is that the gene for a kinase inhibitor (Sufu), which represses Gli1 activity, has been shown to change the distribution of β -catenin between the nucleus and cytoplasm (Meng et al. 2001; Dunaeva et al. 2003; Tukachinsky et al. 2010). Loss of p53 or PTEN causes Gli1 and β -catenin to activate in colon cancer (Varnat et al. 2010; Rychahou et al. 2008). The authors have seen that the use of SMO inhibitors keeps Gli1, an upstream effector, from becoming active, which prevents the amount of active β -catenin from rising and increases the nuclear exclusion of β -catenin (Arimura et al. 2009). Furthermore, Gli1 prevents Gli3R, whereas Gli3R stops Gli1 (Varnat et al. 2010). Additionally, β -catenin activity has been shown to reduce with Gli3R (Ulloa et al. 2007). There is evidence that Gli1 boosts the expression of WNT4, WNT2b, and WNT7b (Li et al. 2007).

Research on the embryonic development of WNT signaling, TGF- β , RA signaling, and BMP revealed that interactions exist among them (Hayward et al. 2008; Boyle et al. 2011; Pelullo et al. 2019). There are a number of ways that WNT signaling might also interact with cytokine signaling and NF- κ B to support inflammatory responses (Koopmans et al. 2017).

The development of the zebrafish tailbud (Stulberg et al. 2012) and the mouse craniofacial area are thought to be influenced by the crosstalk between the FGF and WNT pathways (Wang et al. 2011). Researchers have shown that increased FGF and WNT signaling is positive (Stulberg et al. 2012). The gene *Fgf8* was shown to play a key role in the induction of neural tissues in the face. It is particularly active in the anterior neural ridge and face ectoderm, with WNT/ β -catenin signaling enhancing its role and β -catenin mutation leading to aberrant levels of *Fgf8* expression in the face ectoderm (Glinka et al. 2011). WNT boosts FGF signaling by boosting Erk phosphorylation in the MAPK branch (Stulberg et al. 2012). FGF blocks WNT antagonists *dkk1* and *notum1a*, which results in WNT signaling being raised (Stulberg et al. 2012). A mutated *UBE3A* gene, linked to ASD, affects both the WNT and BMP signaling pathways, indicating their possible connection (Li et al. 2016; Yi et al. 2017). Xu and her colleagues observed *UBE3A* as negatively regulating *ALDH1A2*, which means that this gene makes RA formation more difficult (Xu et al. 2018). The ASD-associated gene might additionally influence BMP signaling, even though it has been suggested that it was likely to be a direct target of WNT/ β -catenin signaling (Medina et al. 2018). The above results indicate that ASD-connected genes enhance the signaling of cross talk via morphogenetic pathways, emphasizing the necessity for further study into the interconnections between signals in normal physiological and pathological circumstances. WNT and BMP signaling cross talk seems to be the result of a tissue-specific process (Itasaki and Hoppler 2010). Signaling interactions occur contextually in the eye, because WNT signaling positively controls RA signaling in the dorsal optic cup but suppresses RA signaling during orofacial development (Itasaki and Hoppler 2010).

Cyclosporine-enhanced cell proliferation has also been seen in human gingival fibroblasts (Chung and Fu 2013). In addition, interactions between TGF- β and SHH pathways have been found in cancer (Javelaud et al. 2012). TGF- β , a growth factor, binds to receptors found on cancer cell membranes, including the neuropilin-1 (NRP1). NRP1 has been demonstrated to promote the canonical SMAD2/3 signaling in response to TGF- β (Glinka et al. 2011). Also, NRP1 transduction is strengthened by HH signaling, and NRP1 has been shown to increase HH target gene activation by assisting the SMO/SUFU interconnection (Hillman et al. 2011; Hochman et al. 2006). While it is essential in the growth of SMO-associated cancers (Fan et al. 2010), it has been shown that TGF- β might increase GLI2 and GLI1 expression by blocking PKA activity (Pierrat et al. 2012). According to the hierarchical structure of cross talk, TGF- β upregulates SHH, resulting in increased SHH synthesis and cell proliferation in gingival fibroblasts. This occurs through cyclosporine, which enhances SHH production (Chung and Fu 2013).

1.10 Nongenetic ASD Etiologies

With ASD susceptibility estimated to be 40–80% hereditary, the risk is probably driven by environmental variables, which are most likely involved in ASD etiology via epigenetic regulation as the primary mechanism.

Hundreds of plausible environmental risk variables have been identified, including increased parental age, viral infections, and prenatal exposure to anticonvulsants, such as valproic acid (VPA). All the proposed risk factors can cause various neurodevelopmental diseases with disrupted WNT signaling (O’Roak et al. 2012a; Rasalam et al. 2005; Kong et al. 2012; Ohkawara et al. 2015).

1.11 Advancing Paternal Age

Since the 1970s, there has been speculation about a connection between paternal age and ASD (Allen et al. 1971; Treffert 1970). A study has reported that increasing paternal age was significantly associated with the susceptibility to ASD, even after adjusting for confounding factors, such as maternal age (Reichenberg et al. 2006). De novo germline pathogenic variants or changes in genomic imprinting might have a role, or at least in part, in the observed association. Nevertheless, further research is needed to validate these findings.

1.12 Viral Infection

New research suggests a link between viral infections and neurodegenerative and neurobehavioral disorders such as autism. Infection with a virus at key stages of early in utero neurodevelopment might increase the chance of autism in the child. Clinical and epidemiological research has revealed associations between congenital

CMV infection and autistic symptoms (Stubbs 1978; Stubbs et al. 1984; Markowitz 1983). An antibody response to the virus, positive viral culture from the urine, decreased hearing, and retinal inflammation revealed evidence of congenital CMV infection. During pregnancy or at birth, CMV infection is a significant cause of sensorineural hearing loss (Grosse et al. 2008; Fowler et al. 1999) and other neurological impairments (Dollard et al. 2007; Townsend et al. 2013). Earlier case studies found that children with congenital CMV infection had typical autistic traits such as inability to build strong interpersonal relationships, poor eye contact, delayed use of language, and nonthematic usage of items.

In 1990, there were numerous reports of severely impaired children with autism who also had congenital CMV infection (Ivarsson et al. 1990). Yamashita and colleagues (Sarabipour and Hristova 2016) demonstrated positive serum CMV-specific IgM antibodies and CMV-DNA in the urine of children with typical autistic disorders. The brain magnetic resonance imaging (MRI) findings indicated an unusually intense region in the periventricular white matter, indicating disrupted myelination. Seten et al. (Seten et al. 2004) discussed the potential function of congenital and perinatal CMV infection in inducing an altered immune response or autoimmune process. The infection or the resulting immune response might impair the development of brain regions or structures, leading to autism. Engman et al. (Engman et al. 2015) also examined the frequency of congenital CMV infection in a representative sample of children with ASD. Congenital CMV infection was found in 1 of the 33 infants with autistic disorder and intellectual deficits, corresponding to a 0.2% prevalence in the general Swedish newborn population. Sakamoto et al. (Sakamoto et al. 2015) hypothesized that congenital CMV infection was involved in a subset of children with ASD since the rate of CMV infection was greater than the incidence of congenital CMV infection in Nagasaki, Japan.

There has been growing evidence describing a connection between rubella infection in early pregnancy and ASD (Chess 1971). Recent studies have demonstrated that in utero viral immune activation results in persistent hyper- and hypomethylated CpGs at WNT signaling genomic regions (WNT3, WNT7B, WNT8A) (Richetto et al. 2017), leading to the disruption of the transcription of downstream target genes, which is thought to have a role in the development of ASD. Other viral infections that have been implicated in ASD include Zika (Nielsen-Saines et al. 2019; Abtibol-Bernardino et al. 2020), cytomegalovirus, and seasonal and pandemic influenza infections (Atladóttir et al. 2012; Deykin and Macmahon 1979).

1.13 Valproic Acid (VPA)

VPA is a medication used to treat EP and bipolar disorder. VPA usage during early pregnancy has been associated with ASD and autistic features in children (Moore et al. 2000). Prenatal VPA exposure in rats has been reported to be related to autism-like phenotypes (Zhang et al. 2012). Prenatally VPA treatment in rats has been shown to cause an imbalance in oxidative homeostasis, which increases vulnerability

to autism (Zhang et al. 2012; Qin and Dai 2015). In addition, VPA-administered rats demonstrated reduced social contact, among other autism-like behavioral features. Sulindac, a small-molecule inhibitor of the WNT/ β -catenin signaling pathway (Zhang et al. 2012; Qin and Dai 2015), has been reported to be capable of reversal of the VPA-induced autistic-like behaviors. It also caused a decrease in p-GSK3 β expression and an increase in β -catenin expression in the hippocampus, prefrontal lobe, and cerebellum (Qin and Dai 2015).

In addition, GATA-3 is a transcription factor that is required for brain development (Tsarovina et al. 2010). It is implicated in the WNT (Notani et al. 2010) and TGF- β /BMP (Kim et al. 2018; Forsman et al. 2013) signaling pathways. It has been shown to be associated with ASD, after exposure to valproate, thalidomide, and alcohol, which might contribute to the development of ASD (Rout and Clausen 2009).

1.14 Precision Medicine Approaches in ASD

Various pathophysiological processes and etiologies characterize ASD and therefore precision medicine seems to be the most promising way of finding treatment options for ASD (Uddin et al. 2019; Al-Dewik and Alsharshani 2020; Styles et al. 2020). The field of precision medicine attempts to mix groundbreaking pathophysiologically based treatments (biomarker stratification) with testing to determine which therapeutic course of action might assist a particular person (Loth et al. 2016). Understanding the molecular and cellular pathways associated with ASD might enable the development of more effective treatment options. The current approach has more significant information about the molecular and cellular processes involved in ASD (Loth et al. 2016). However, following recent therapeutic research failures involving monogenic ASD variants, new challenges have emerged which include conceptual and methodological problems (i.e., the difficulty of translating from animal models to humans and then conducting suitable clinical trials). Other issues are the impact of placebo effects, clinical trial design, and the need to identify mechanistically plausible, measurable outcomes (Loth et al. 2016). Additionally, as many as 70% of individuals with ASD are likely to have a psychiatric or physical co-condition (Simonoff et al. 2008), which might complicate treatment, which could cause an impairment that will last far into adulthood (Shaltout et al. 2020). A transdisciplinary, multidisciplinary, and collaborative approach would be required to deal with the many issues that future research projects must cover.

1.15 Future Perspectives and Conclusions

Research and knowledge of ASD are growing, thanks to many efforts that have been rigorous and comprehensive. Nonetheless, a multi-domain expert collaboration is required to have a cohesive picture of ASD and adequately evaluate its increasing genetic, epidemiological, and environmental components. During embryogenesis,

dysregulation of various signaling neurodevelopmental pathways such as WNT, BMP/TGF, SHH, FGF, or RA seems to induce ASD and alter neurogenesis. The signaling pathways might function upstream or downstream of ASD causative genes. Several studies have revealed that the pathways mentioned above play important roles in developing targeted therapies for ASD. However, comprehensive developmental investigations are needed to determine the time window during which disruption of these signals has the most significant impact on brain structure and function, and the subsequent impairment in behavior.

Furthermore, such research might aid in the understanding of the processes involved in ASD etiology, and the upstream and downstream signaling pathways involved. The interaction between different signaling pathways in neuronal and glial cells for ASD should be explored, as this might aid in devising therapy and addressing the disturbed signaling in a cell-specific manner. Only a few ASD risk genes have been investigated in the context of disrupted signaling pathways so far. The exploration of functions of additional ASD genes in neurodevelopment and the control of other signaling pathways might improve the existing knowledge of the processes underlying ASD etiology. Early identification is necessary for a successful therapy that treats the symptoms while reversing neurons to normal circumstances. In addition to the clinical value of a genetic diagnosis, early diagnosis has also been proven to enhance the understanding, give a feeling of empowerment, and improve the quality of life of the affected child and the parents. As a result, additional investigations are needed to comprehend the mechanistic picture of signaling pathways, communication in ASD, and causative gene interaction with these pathways.

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