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Review

16p11.2 Copy Number Variations and Neurodevelopmental Disorders

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Copy number variations (CNVs) of the human 16p11.2 genetic locus are associated with a range of neurodevelopmental disorders, including autism spectrum disorder, intellectual disability, and epilepsy. In this review, we delineate genetic information and diverse phenotypes in individuals with 16p11.2 CNVs, and synthesize preclinical findings from transgenic mouse models of 16p11.2 CNVs. Mice with 16p11.2 deletions or duplications recapitulate many core behavioral phenotypes, including social and cognitive deficits, and exhibit altered synaptic function across various brain areas. Mechanisms of transcriptional dysregulation and cortical maldevelopment are reviewed, along with potential therapeutic intervention strategies.

The Link between 16p11.2 Copy Number Variations and Neurodevelopmental Disorders

Genetic factors comprise a large proportion of the risk for neurodevelopmental disorders (NDDs) such as autism spectrum disorder (ASD), schizophrenia (SZ), and intellectual disability (ID) [1]. Copy number variations (CNVs, i.e., deletion or duplication) of various susceptible genetic loci predispose individuals to these NDDs and other developmental abnormalities [2,3]. The human 16p11.2 gene locus (chromosome 16, position 11.2) is an approximately 500–600-kb region containing 27–29 genes [4–7] located on the proximal short arm of chromosome 16 [8]. Deletions and duplications of 16p11.2 have highly pleiotropic phenotypic effects, with strong links to ASD [8–19], ID [8,10,12,14–16,19–21], motor/developmental delay (DD) [8–10,12,13,15–19,22], dysmorphic features (DFs) [8,12,16,17,22], and epilepsy/seizures [10,12,17]. Deletions of 16p11.2 are associated with increased head circumference (macrocephaly) [10,12,17,23] and obesity [10,12,16,20], whereas duplications often result in below-average head size (microcephaly) [10,17,23,24] and low body weight/body mass index (BMI) [10,24]. SZ appears to be associated more strongly with 16p11.2 duplications [9,25–27].

The literature on 16p11.2 CNVs is large and growing. This review provides a synthesis of the most common neurodevelopmental phenotypes associated with 16p11.2 deletions/duplications (Figure 1). Studies performed in animal models of 16p11.2 CNVs have also begun highlighting core neurobiological mechanisms. Here we discuss key pathways and mechanisms of dysfunction identified through these studies (Figures 2, Key Figure, and 3), which may provide explanations for some of the phenomena observed in human 16p11.2 CNVs.

Analysis of Clinical Data from Humans with 16p11.2 CNVs
Prevalence of 16p11.2 CNVs
Genetics Home Reference estimates that 16p11.2 deletions and duplications each affect about three in every 10 000 individuals.[i,ii] A predictive algorithm estimated 16p11.2 deletions to affect one in every 3021 live births, and duplications one in every 4216 [28]. These estimates are supported by large genetic screenings [20,24,29]. 16p11.2 deletions have been reported at rates of 0.028–0.043% in the general population, while duplications have been reported between

Highlights
16p11.2 deletions or duplications predispose individuals to neurodevelopmental diseases, including autism spectrum disorder, intellectual disability, epilepsy/seizures, dysmorphic features, congenital anomalies, macrocephaly, and microcephaly.

16p11.2 CNV mouse models recapitulate many of the human behavioral phenotypes, with distinct representation of social and cognitive deficits.

Various forms of synaptic dysfunction are observed across distributed brain areas in 16p11.2 CNV mouse models.

Broad transcriptional dysregulation is found in 16p11.2 CNV mouse models and human carriers, extending far beyond genes within the 16p11.2 region.

Several 16p11.2 genes, including *Mapk3, Kctd13, Taok2*, are highly involved in abnormal cortical development in 16p11.2 CNVs.

Possible therapeutic intervention strategies include the restoration of excitation–inhibition balance and synaptic plasticity by targeting glutamate and GABA systems.

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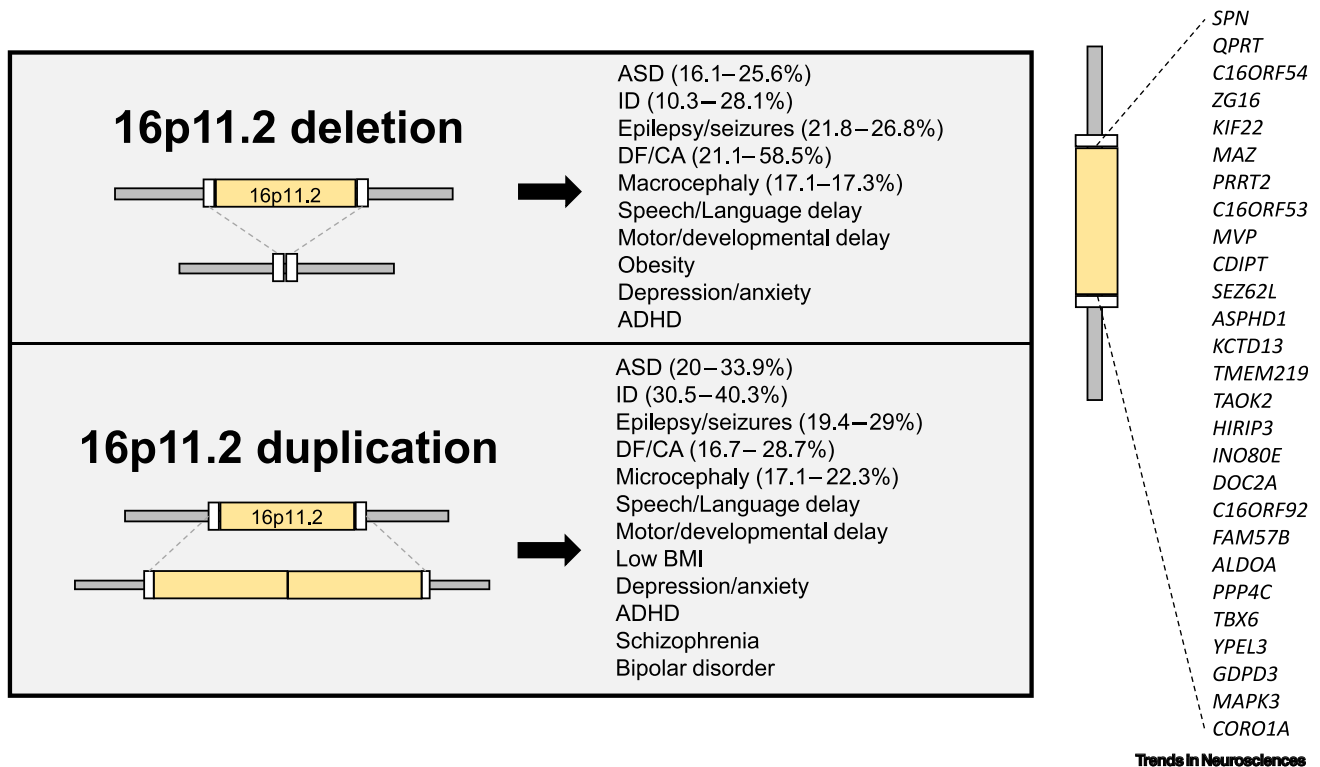brein@buffalo.edu (B. Rein) and
zhenyan@buffalo.edu (Z. Yan).

Figure 1. Common Phenotypes in Carriers of 16p11.2 CNVs. Both 16p11.2 deletions and duplications predispose individuals to ASD, ID, epilepsy/seizures, and DFs/CAs with high penetrance, in addition to several other common phenotypes. Numbers inside parentheses indicate ranges of reported penetrance within cohorts of 16p11.2 CNV patients. Inset: Genes in the human 16p11.2 region. Abbreviations: ADHD, attention-deficit/hyperactivity disorder; ASD, autism spectrum disorder; BMI, body mass index; CNV, copy number variation; DFs/CAs, dysmorphic features/congenital anomalies; ID, intellectual disability.

0.035% and 0.053% (Table 1). Triplications of 16p11.2 have also been reported [30], though the frequency of this presumably rarer CNV is unknown.

Genetic screenings of individuals with NDDs find starkly higher rates of 16p11.2 CNVs. Data compiled from eight studies [13,15–17,20,24,26,31] screening individuals with either ASD, ID, DD, DFs, multiple congenital anomalies (CAs), obesity, or seizures find 16p11.2 deletions at rates of 0.25–2.9%, and duplications at rates between 0.15% and 0.78% (Table 1).

Inheritance Patterns of 16p11.2 CNVs

We compiled data from 13 studies in which the rates of *de novo* versus inherited cases of 16p11.2 deletions and duplications were reported [10,12,13,15–17,19,20,26,31–34]. In studies with at least 50 subjects, between 60% and 76% of 16p11.2 deletions were reported as *de novo* events [10,12,32,34], while only 16–29% of 16p11.2 duplications occurred *de novo* [10,19,32,34]. Both the deletion and duplication appear to be preferentially maternally inherited.

Differing pathological severity of 16p11.2 deletions/duplications may underlie these dissimilar inheritance patterns. One study estimates that the penetrance of any pediatric phenotype in deletion carriers is 62.4%, relative to only 11.2% in duplication carriers [35]. The more severe outcomes associated with 16p11.2 deletions preclude these patients from having children, resulting in lower rates of inherited deletions [32]. However, *de novo* and inherited deletion carriers do not perform differently on several cognitive tasks [33]. *De novo* and inherited duplication carriers also show no differences in most clinical outcomes [19], and on cognitive tests

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Key Figure

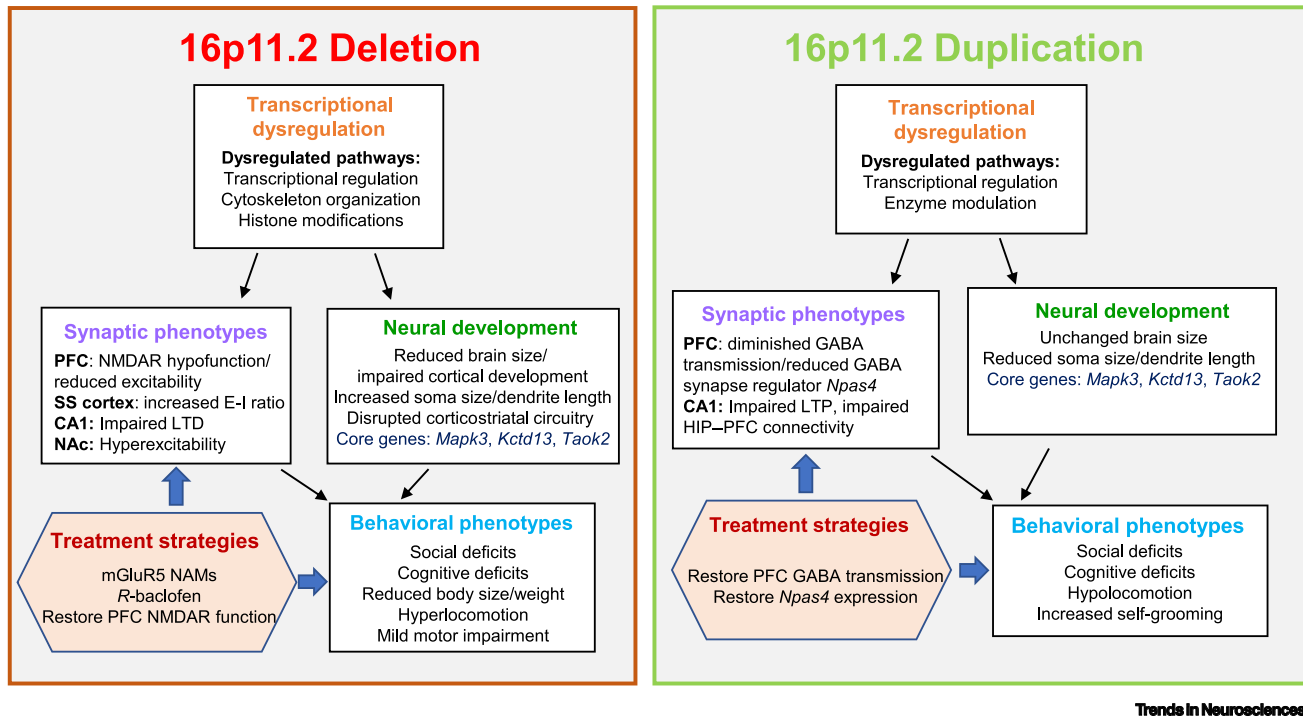Summary of Main Preclinical Findings from Mouse Models of 16p11.2 Deletion and Duplication

Figure 2. Studies in 16p11.2 CNV transgenic mice have identified several core behavioral abnormalities, along with associated neurobiological disruptions. The deletion or duplication of genes within the 16p11.2 region drives transcriptional dysregulation of various downstream pathways. These transcriptional changes in turn lead to the altered developmental trajectories and synaptic changes across distributed brain regions. A range of behavioral phenotypes are reported in both 16p11.2 deletion and duplication models, likely driven by structural/functional neurological changes. Preclinical studies have identified several therapeutic approaches targeting selected disrupted systems. Abbreviations: CNV, copy number variation; E/I, excitation–inhibition; HIP, hippocampus; LTD, long-term depression; NMDAR, NMDA receptor; PFC, prefrontal cortex; SS, somatosensory.

[10]. Altogether, these results suggest that 16p11.2 deletions are more likely to be *de novo* events than duplications.

Neurodevelopmental Phenotypes Associated with 16p11.2 CNVs
Autism Spectrum Disorder
Genetic screenings of ASD patients repeatedly identify 16p11.2 deletions and duplications, placing them among the strongest genetic risk factors for ASD [11,13,16,36]. Data accumulated from 14 studies on the penetrance of ASD in 16p11.2 deletion and duplication carriers are shown in Table 2 [9,10,12,14–17,19–21,23,31–33]. In multiple studies of subjects with 16p11.2 deletion or duplication, autistic features or a formal ASD diagnosis were reported in 16.1–25.6% of deletion carriers, and 20–33.9% of duplication carriers. Overall, ASD appears to be a highly penetrant phenotype in both 16p11.2 duplications and deletions.

Intellectual Disability/Cognitive Impairment
Table 2 summarizes findings from six studies on the penetrance of ID in 16p11.2 deletion and duplication carriers [10,14,19,21,23,32]. In studies of at least 50 subjects, ID was reported in 10.3–28.1% of 16p11.2 deletion carriers, and 30.5–40.3% of duplication carriers. Note that
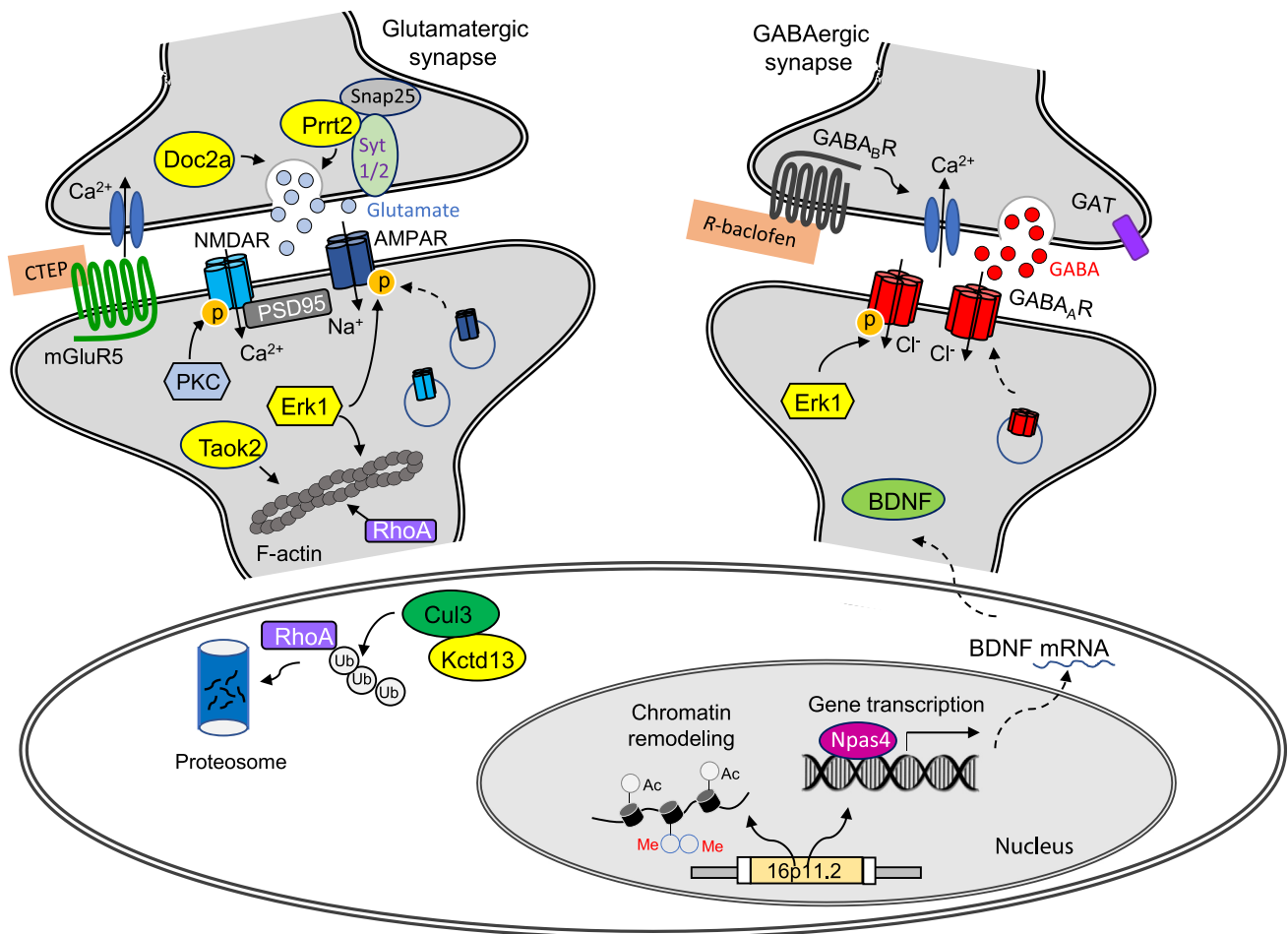
Figure 3. Schematic Showing Molecular and Cellular Mechanisms of 16p11.2 Genes and Interacting Partners in Nucleus, Soma, and Synapses.
Proteins encoded by genes in 16p11.2 region are labeled with yellow. At glutamatergic synapses, Prrt2 and Doc2a are involved in regulating presynaptic transmitter release. TaoK2 and Erk1 act on postsynaptic actin cytoskeleton via RhoA signaling, which contributes to the deficits of spine structure and synaptic excitation associated with 16p11.2 deletions. Kctd13 is an adaptor of the E3 ligase Cul3, controlling the degradation of RhoA and other protein substrates. The therapeutic agent CTEP acts on mGluR5 to modify glutamatergic responses. At GABAergic synapses, the therapeutic agent R-baclofen acts on presynaptic $GABA_B$ receptors to regulate GABA release. Erk1 regulates the phosphorylation of $GABA_A$ receptor channels. In the nucleus, 16p11.2 CNVs could induce the alterations of chromatin remodeling and gene transcription. The expression of Npas4, a key regulator of GABA synapses via BDNF, is downregulated in 16p11.2 duplication mice, which contributes to the deficits of synaptic inhibition. Abbreviations: AMPAR, AMPA receptor; CNV, copy number variation; BDNF, brain-derived neurotrophic factor; NMDAR, NMDA receptor; PKC, protein kinase C.

several of these studies were conducted in clinically ascertained populations of 16p11.2 CNV carriers, so the estimate of ID penetrance may be exaggerated.

A study of 270 duplication carriers and 442 deletion carriers found that full-scale IQ (FSIQ) was significantly lower than intrafamilial controls in both 16p11.2 duplication carriers (18.0 points) and deletion carriers (22.1 points) [10]. Several supporting studies also reported lower FSIQ scores in 16p11.2 deletion [12,33] and duplication [19,33] patients than intrafamilial controls. Higher variability in FSIQ scores is observed among duplication carriers than deletion carriers [10,19], suggesting a broader range of cognitive impairments in duplication patients. In one group of 16p11.2 duplication carriers, FSIQ scores range from 33 to 129, with 17% displaying

Table 1. Prevalence of 16p11.2 CNVs in the General Population and Within Groups of Clinical Cohorts with Various Neurodevelopmental Disorders

16p11.2 deletion prevalence (%)	16p11.2 duplication prevalence (%)	Sample group description	Refs
General population data			
110/396 725 (0.028)	138/396 725 (0.035)	General population (UK Biobank)	[29]
25/58 635 (0.043)	31/58 635 (0.053)	General European population	[24]
4/11 856 (0.034)	–	General Swiss, Finnish, and Estonian population	[20]
Clinical cohorts			
27/7400 (0.36)	18/7400 (0.24)	DD/MR[a], DF, seizures, CA, ASD, attention-deficit/hyperactivity disorder, or failure to thrive	[17]
98/38 779 (0.25)	59/38 779 (0.15)	Unexplained physical and/or intellectual disabilities, with or without DF	[26]
9/312 (2.9)	–	CA and/or DD with obesity	[20]
22/3947 (0.56)	–	CA and/or DD without obesity	[20]
11/2772 (0.40)	–	Childhood/adult obesity (combined data sets)	[20]
119/31 424 (0.38)	73/31 424 (0.23)	DD/ID	[24]
20/3450 (0.58)	–	DD, ID, DF or multiple CAs	[15]
14/4284 (0.33)	–	MR or multiple CAs	[31]
45/9773 (0.46)	32/9773 (0.33)	ASD, DD, DF, CA, or seizures	[16]
5/512 (0.98%)	4/512 (0.78%)	DD, MR, or suspected ASD	[13]

[a]Abbreviation: MR, mental retardation.

Table 2. Penetrance of Neurodevelopmental Disorders and Physical Abnormalities among 16p11.2 Deletion and 16p11.2 Duplication Carriers[a]

Number of 16p11.2 deletion carriers					Number of 16p11.2 duplication carriers					Refs
ASD	ID	E/S	Mac.	DF/CA	ASD	ID	E/S	Mic.	DF/CA	
41/217	61/217	–	–	–	26/114	36/114	–	–	–	[32]
3/62	–	–	–	–	5/44	–	–	–	–	[33]
4/25	5/25	–	6/25	–	1/17	1/17	–	4/17	–	[23]
3/11	–	5/16	11/16	5/16	0/10	–	3/10	6/10	5/10	[17]
0/14	4/14	–	–	–	2/17	3/17	–	–	–	[14]
51/317	–	69/317	–	67/317	36/180	47/154	35/180	48/215	30/180	[10]
6/21	–	–	–	–	–	–	–	–	–	[15]
20/78	8/78	–	–	–	–	–	–	–	–	[21]
0/14	–	3/14	–	9/14	–	–	–	–	–	[31]
9/16	–	2/18	–	–	–	–	1/10	–	–	[16]
4/24	–	–	–	–	–	–	–	–	–	[20]
8/55	–	47/195	29/170	76/130	–	–	–	–	–	[12]
–	–	–	–	–	4/21	–	–	–	–	[9]
–	–	–	–	–	21/62	25/62	–	–	–	[19]
–	–	22/82	14/81	–	–	–	22/76	13/76	–	[34]
–	–	–	–	–	–	–	–	–	29/101	[24]

[a]Abbreviations: E/S, epilepsy/seizures; Mac, macrocephaly; Mic, microcephaly.

severe impairment (<55), while 33% fall within the average range or better [19]. Interestingly, 16p11.2 duplication patients with ASD display more severe cognitive impairments than those without ASD [10], indicating that the severity of cognitive phenotypes may correlate with the pathogenesis of other NDDs, particularly ASD.

Epilepsy

Epilepsy/seizure ranks among the most common phenotypes observed in both 16p11.2 deletions and duplications [10,12,16,17,31,34] (Table 2). In studies of at least 50 subjects, epilepsy/seizure has been reported in 21.8–26.8% of deletion carriers, and 19.4–29% of duplication carriers. Epilepsy is thus a highly penetrant phenotype in 16p11.2 deletions and duplications.

Developmental Delay/Language Impairment

Motor delays are reported at rates of 57.1% [15] and 50% [17] in deletion carriers, and in 60% of duplication carriers [17]. A very delayed (>24 months) age of first walking is observed in 6.1% of deletion carriers and 15.9% of duplication carriers [10]. Balance impairment and gait abnormalities are reported in both 16p11.2 CNVs [37]. Developmental coordination disorder is reported at rates of 32% [23] and 57.7% [21] in deletion carriers, and 46.8% in duplication carriers [19]. Speech articulation abnormalities [34], phonological processing disorder [21,23], language or communication disorder [14,21,23], speech or language deficits [15,16], and speech delay [17,31] are present at high rates in deletion carriers. Duplications are similarly associated with speech articulation abnormalities [34], speech delay [17,19], and language deficits [16]. One study performed deep phenotyping of speech and language skills in 16p11.2 deletion carriers, providing further insight into the specific impairments present [38]. Interestingly, motor control of speech is impaired in 16p11.2 deletion carriers [39], indicating that the motor and language deficits may be fundamentally linked.

Schizophrenia/Psychosis

In a 2009 study, 21 duplications and one deletion were detected among 5877 SZ patients, indicating a 14.5-fold increased SZ risk in 16p11.2 duplication patients [9]. One follow-up study reported six duplications in 659 SZ patients [40]. An analysis of 22 screenings in various populations found 98 duplications in 36 676 SZ patients (0.26%), with only 12 duplications in 48 331 controls (0.025%), indicating a 10.79-fold increase in risk for developing SZ [41]. Psychotic symptoms have also been reported in duplication [7/114 (6.1%)] and deletion [5/217 (2.3%)] carriers [32]. In one study, both 16p11.2 deletion and duplication carriers exhibited psychotic symptoms, though only the duplication was a significant predictor of psychotic symptoms [42]. Thus, 16p11.2 duplications predispose robustly to SZ and associated psychotic symptoms, with greater penetrance than 16p11.2 deletions. The link between 16p11.2 duplications and SZ is particularly interesting, considering the growing perspective of SZ as a NDD [43]. The overlapping genetic predisposition by 16p11.2 CNVs to both SZ and more typical NDDs like ASD and ID appears to support a neurodevelopmental origin of SZ [44].

Other Psychiatric Phenotypes in 16p11.2 CNVs

16p11.2 deletions and duplications are linked to several other psychiatric conditions, including depression [14,45] and anxiety [12,14,18,19,23,32]. Attention-deficit/hyperactivity disorder is also observed in 16p11.2 CNV carriers [12,15–17,19,23,46], at rates as high as 29% (63/217) and 42% (48/114) in deletion and duplication carriers, respectively [32]. Bipolar disorder is also reported in 16p11.2 duplication carriers [9,14,24].

Dysmorphic Features/Congenital Anomalies
Summarized data from published reports on DFs/CAs in 16p11.2 CNVs are shown in Table 2 [10,12,17,24,31]. In studies of at least 50 subjects, DFs/CAs were reported in 21.1–58.5% of deletion carriers, and 16.7–28.7% of duplication carriers, indicating high penetrance in both CNVs.

Obesity/Body Mass Index
Several reports indicate higher BMI in 16p11.2 deletion patients [10,12,23]. 16p11.2 deletions are present at higher-than-expected rates in obese individuals [29], and are more prevalent in DD patients with obesity than those without obesity [20]. Conversely, BMI of 16p11.2 duplication patients is significantly lower than controls [10,24]. Birth parameters for 16p11.2 duplication carriers are generally normal [24], indicating a postnatal effect.

Cephalic Phenotypes and Neurostructural Changes
Certain physical phenotypes, such as head size, display dosage-dependent effects in 16p11.2 CNVs. Deletions of 16p11.2 are associated with macrocephaly [12,17,23,34], while duplications are associated with microcephaly [10,17,23]. Summarized data on penetrance of microcephaly/macrocephaly in 16p11.2 CNVs are shown in Table 2 [10,12,17,23,34]. In studies of at least 50 subjects, macrocephaly was reported in 17.1–17.3% of deletion carriers, while microcephaly was reported in 17.1–22.3% of duplication carriers. Several studies also report increased or decreased head circumference in deletion [10,12,24,34] or duplication carriers [10,24], respectively.

Magnetic resonance imaging studies show reciprocal brain volume changes, with increased gray/white matter in 16p11.2 deletions, and decreased gray/white matter in 16p11.2 duplications [14,23,47,48]. The increased or decreased axial diffusivity of white matter and the thickening or thinning of the corpus callosum have also been found in deletion or duplication patients, respectively [49–51]. Regional volumetric differences are also found in 16p11.2 CNV humans. Several brain areas exhibit the increased volume in deletion carriers and reduced volume in duplications, including insula, calcarine cortex [47,48], accumbens, pallidum [52], transverse temporal gyrus [47,48], caudate, putamen [47,52], and thalamus [23]. Increased or decreased cortical surface area has been reported in 16p11.2 deletions or duplications, respectively [14,23,47]. 16p11.2 duplications also have the reduced cortical thickness [14,23], reduced hippocampal volume [47], and enlarged ventricles [48,51,53].

Sex Differences in 16p11.2 CNVs
Several NDDs display a sex bias where males are at a higher risk [54]. In agreement with this, the ratio of males to females with either ASD or ID shows a male predominance in both 16p11.2 CNVs [55], suggesting that being female is a protective factor when predicting overall ASD severity in either 16p11.2 CNV [56]. However, another study found that sex was not a significant predictor of psychosis in 16p11.2 CNV carriers [42]. Thus, the association between 16p11.2 CNVs and ASD/ID, but not psychosis, may involve a sex bias.

Insights from Preclinical Studies in 16p11.2 CNV Mouse Models
Here we describe behavioral phenotypes in 16p11.2 CNV mouse models, and synthesize the major biological takeaways and implications (Figures 2 and 3). Three lines of 16p11.2 CNV mice have been generated: 16p11.2 mice (Mills) carrying deletion ($16p11.2^{+/-}$) or duplication ($16p11.2^{dp/+}$) of the 7F4 region (*Slx1b-Sept1*) syntenic to human 16p11.2 [5]; 16p11.2 mice (Dolmetsch) with deletion of the *Coro1a-Spn* interval [4]; 16p11.2 mice (Herault) with deletion/duplication of the *Sult1a1-Spn* genetic interval [57]. Behavioral phenotypes in 16p11.2 deletion or duplication mice are summarized in Table 3.

Table 3. Behavioral Phenotypes Present in Various Mouse Models Carrying 16p11.2 Deletion (16p11.2$^{+/-}$) or Duplication (16p11.2$^{dp/+}$)

Mouse origin/ genetic interval	Behavioral phenotypes	Refs
16p11.2$^{+/-}$ mice		
Mills lab *Slx1b-Sept1*	• Reduced body size/body weight • Hyperlocomotion	[5]
Mills lab *Slx1b-Sept1*	• Barnes maze deficits • Reduced social approach	[60]
Mills lab *Slx1b-Sept1*	• Reduced body weight • More complex locomotor trajectory/shorter latency to approach stimulus • Deficits in righting from upside–down position • Normal three-chamber social preference/social novelty recognition • Normal startle/PPI	[59]
Mills lab *Slx1b-Sept1*	• Delayed learning in FR1 continuous reinforcement nose-poke task (males only) • Earlier response termination in a progressive ratio nose-poke task (males only) • Deficits in the Five-Choice Serial Reaction Time Test • No difference in sucrose preference test	[65]
Mills lab *Slx1b-Sept1*	• Hyperlocomotion over 24-h period (both sexes) • Reduced sleep time (males only) and less time in nonrapid eye movement sleep (rapid eye movement normal)	[68]
Mills lab *Slx1b-Sept1*	• Reduced open arm time in the elevated plus maze • Novel object recognition deficits	[64]
Mills lab *Slx1b-Sept1*	• Deficits in contextual fear conditioning (reduced freezing) • Impaired memory acquisition and extinction in inhibitory avoidance task	[63]
Mills lab *Slx1b-Sept1*	• Impaired object recognition memory • Reduced freezing in context-dependent aversive learning task • Open field hyperlocomotion	[62]
Dolmetsch lab *Coro1a-Spn*	• Deficient object location memory • Reduced male-to-female nose-to-nose/nose-to-anogenital sniffing, following time, following bouts, and ultrasonic vocalizations • Normal open field locomotion	[62]
Dolmetsch lab *Coro1a-Spn*	• Reduced body length/body weight • Severely impaired startle response • Lack of gait fluidity; frequent tremor • Hyperlocomotion in home cage and in an activity chamber • Increased hanging; reduced grooming; reduced resting; increased circling behavior • Impaired novel object recognition	[4]
Dolmetsch lab *Coro1a-Spn*	• Reduced body weight • Reduced male-to-female anogenital sniff, following time, and ultrasonic vocalizations (mixed-genotype housed only) • Increased open arm time in elevated plus maze (mixed-genotype housed only) • Reduced immobility time in tail suspension test (mixed-genotype housed only) • Impaired novel object recognition (mixed-genotype housed only) • Impaired object location memory (mixed-genotype housed only) • Increased arena exploration • Normal three-chamber social preference	[61]
Dolmetsch lab *Coro1a-Spn*	• Reduced body weight • Reduced male-to-female ultrasonic vocalizations, anogenital sniff, and follow • Severe deficits in startle response and PPI • Deafness, confirmed via auditory brainstem response test • Delayed hot plate response (reduced pain sensitivity) • Normal olfaction	[58]
Dolmetsch lab *Coro1a-Spn*	• Impaired novel object recognition • Impaired object location memory • Mild social novelty recognition deficits • Delayed acquisition and reversal in a touch screen discrimination task • Inability to swim • Normal contextual and cued fear conditioning	[66]

Table 3. (continued)

Mouse origin/ genetic interval	Behavioral phenotypes	Refs
Herault lab *Sult1a1-Spn*	C57BL/6N inbred background • Increased dark cycle vertical activity • Increased time in center of open field • Increased jumping and rearing • Impaired object recognition memory • Increased hindlimb errors in notched bar test • Normal social interaction C57BL/6N-C3B hybrid background • Increased dark cycle ambulatory/vertical activity and light cycle vertical activity • Increased climbing behavior • Reduced social interaction • Impaired object recognition memory • Normal rotarod motor coordination • Normal open field locomotion and time in center	[57]
16p11.2$^{dp/+}$ mice		
Mills lab *Slx1b-Sept1*	• Hypolocomotion • Increased grooming	[5]
Mills lab *Slx1b-Sept1*	• Three-chamber social preference deficits (normal social novelty recognition) • Social approach deficits • Temporal order recognition memory deficits • Increased self-grooming • Hypolocomotion • Normal novel object recognition • Normal startle response/PPI • Lack of MK-801-induced hyperlocomotion • Normal elevated plus maze and rotarod	[70]
Mills lab *Slx1b-Sept1*	• Hypolocomotion (males only) • Reduced time in center of open field (males only) • Reduced ratio of open/closed arm time in elevated plus maze (males only) • Normal startle response • Reduced PPI (females only) • Increased distance between cagemates • Reduced time spent in close proximity to cagemates • Impaired performance in 'N-back' working memory task • Impaired performance in continuous performance task	[71]
Herault lab *Sult1a1-Spn*	C57BL/6N inbred background • Reduced light cycle ambulatory/vertical activity and dark cycle vertical activity • Open field hypolocomotion and reduced time in center • Enhanced object recognition memory • Normal social interaction • Normal motor coordination in notched bar test C57BL/6N-C3B hybrid background • Reduced dark cycle vertical activity • Reduced social interaction • Decreased climbing behavior • Enhanced object recognition memory • Normal open field locomotion/time in center • Normal rotarod motor coordination	[57]

Behavioral Phenotypes in 16p11.2 Deletion or Duplication Mice Recapitulate Neurodevelopmental Deficits of Human Carriers

All three lines of 16p11.2$^{+/−}$ mice display social deficits in various testing paradigms [57–62], and an array of cognitive deficits [4,57,60–66]. Sleep abnormalities [67,68] and anxiety [64] have been reported in 16p11.2$^{+/−}$ mice (Mills). Startle response [4,58] and prepulse inhibition (PPI) [58] are severely impaired in the 16p11.2$^{+/−}$ mice (Dolmetsch). However, these mice are deaf [58], which likely underlies startle/PPI deficits and may be related to altered ultrasonic vocalizations.

Mild motor deficits are also present in these models [4,59,66], consistent with human phenotypes. However, 16p11.2$^{+/-}$ models show reduced body size/weight [4,5,58,59,61], in contrast to the human obesity phenotype. Hyperlocomotion is also broadly reported in 16p11.2$^{+/-}$ mice [4,5,57,59,61,62,68], another behavioral feature that does not coincide directly with human symptomology.

16p11.2$^{+/-}$ mice display impairments in cognition, sociability, and motor function, coinciding with phenotypes present in human 16p11.2 deletion carriers. However, several phenotypes, such as body weight, are discordant between mice and humans. Another limitation is that many deficits present in deletion carriers, such as apraxia of speech, cannot be tested or modeled in animals [69]. Thus, 16p11.2$^{+/-}$ mice may represent a suitable preclinical model system for evaluating mechanisms of certain social and cognitive dysfunctions, but several of the deficits associated with 16p11.2 deletions cannot be modeled in these animals.

The 16p11.2$^{dp/+}$ mice (Mills) exhibit social and cognitive deficits [70,71] along with increased repetitive self-grooming [5,70], with the absence of motor coordination deficits [70]. Two behavioral phenotypes associated with SZ, PPI of startle responses and MK-801-induced hyperlocomotion, were not observed in 16p11.2$^{dp/+}$ mice [70], despite a report on female-specific PPI deficits [71]. Based on these results, the SZ-linked changes should be further investigated in 16p11.2$^{dp/+}$ mice. 16p11.2$^{dp/+}$ mice also exhibit hypolocomotion [5,70,71], in direct contrast to 16p11.2$^{+/-}$ animals. Hypolocomotion and enhanced object-recognition memory are reported in 16p11.2$^{dp/+}$ mice (Herault) on a C57BL/6N inbred genetic background, whereas on an F$_1$ C57BL/6N-C3B hybrid background, mice additionally exhibit social approach deficits [57]. These studies indicate that 16p11.2$^{dp/+}$ mice recapitulate several, but not all, human 16p11.2 duplication phenotypes, including social deficits, repetitive behaviors, and cognitive impairment.

Sex-Specific Behavioral Phenotypes in Mouse Models of 16p11.2 CNVs

Male, but not female, 16p11.2$^{+/-}$ mice (Mills) display reduced and altered pup isolation calls, indicating sex-specific communication impairments in perinatal development [72]. In addition, male 16p11.2$^{+/-}$ mice (Mills) exhibit sleep deficits [68] and impairments in a reward-directed learning task [65], while females do not. In 16p11.2$^{dp/+}$ mice (Mills), male-specific reductions in locomotion, time in the center of an open field, and ratio of open arm/closed arm time in the elevated plus maze test have also been reported [71].

16p11.2 CNV Mouse Models Exhibit Synaptic Dysfunction in Distributed Brain Regions

The 16p11.2$^{+/-}$ mice (Mills) display deficient NMDA-receptor-mediated glutamatergic transmission and reduced frequency of action potential firing in medial prefrontal cortex (mPFC) pyramidal neurons [60], along with increased excitation–inhibition (E/I) ratio in somatosensory cortex [73]. Additionally, GABAergic neurons in the ventral medulla display hyperpolarized resting membrane potential and increased membrane resistance [67]. Long-term potentiation (LTP) and long-term depression appear to be intact in hippocampal CA1 of 16p11.2$^{+/-}$ mice (Mills), though protein-synthesis-dependent mGluR5 long-term depression is impaired [63]. Recordings from CA1 of 16p11.2$^{+/-}$ mice (Herault) reveal intact synaptic transmission, along with modest but not statistically significant reductions in LTP [57]. Nucleus accumbens medium spiny neurons (MSNs) in 16p11.2$^{+/-}$ mice (Dolmetsch) display increased AMPA receptor/NMDA receptor (NMDAR) ratio and decreased paired pulse ratios, along with increased miniature excitatory postsynaptic current frequency [4]. The authors hypothesize that these results are due to increased release probability of excitatory synapses on nucleus accumbens MSNs [4]. Synaptic phenotypes in 16p11.2$^{+/-}$ mice evidently vary across brain areas, suggesting that 16p11.2 deletion may cause region- or cell-type-specific impairments.

The 16p11.2$^{dp/+}$ mice (Herault) show impaired LTP in CA1 [57]. However, these findings have not yet been confirmed in other 16p11.2$^{dp/+}$ models, and may benefit from behavioral validation with corresponding memory tasks such as contextual fear conditioning [63]. The 16p11.2$^{dp/+}$ mice (Mills) display GABAergic synaptic deficits in mPFC pyramidal neurons and increased action potential firing rates [70]. Restoring mPFC GABAergic synaptic activity is sufficient to reverse the social and cognitive deficits [70], implicating prefrontal cortical synaptic dysfunction in 16p11.2 duplication pathology. In addition, disrupted connectivity between hippocampal–orbitofrontal and hippocampal–amygdala circuits has been found in 16p11.2$^{dp/+}$ mice (Mills), as well as reduced expression of several GABAergic markers, including parvalbumin, calbindin, and somatostatin in frontal cortex [71].

16p11.2 CNVs Induce Broad Transcriptional Disruptions in Mice and Human Cells

Sequencing studies have revealed broad transcriptional disruptions in 16p11.2 CNVs. RNA-sequencing identifies 2344 and 1504 significant differentially expressed genes (DEGs) in the cortex of 16p11.2$^{+/-}$ and 16p11.2$^{dp/+}$ mice (Mills), respectively, as well as 908 and 1290 nominally significant DEGs in lymphoblastoid cell lines from human 16p11.2 deletion or duplication carriers, respectively [74]. Gene ontology analysis of DEGs in mouse cortex indicates the strongest disruption of genes related to 'regulation of transcription'. The top pathways implicated in human lymphoblastoid cell lines are 'microtubule cytoskeleton organization' and 'cell surface receptor-linked signal transduction'.

A separate RNA-seq experiment in mPFC of 16p11.2$^{dp/+}$ mice (Mills) identifies 388 DEGs (277 downregulated, 111 upregulated), confirming broad transcriptional disruption extending far beyond the genes located within the 16p11.2 region [70]. Gene ontology analysis indicates that the largest portion of downregulated genes is classified as 'transcription factors' (17.7%), consistent with prior RNA-seq data [74]. The largest portion of upregulated DEGs (21.6%) is identified as 'enzyme modulators'. These findings highlight the wide transcriptional impacts of 16p11.2 CNVs, but further studies should clarify the specific contributions of 16p11.2 genes to these far-reaching downstream transcriptional disruptions and their links to the heterogeneous associated behavioral phenotypes.

Abnormal Cortical Development Driven by 16p11.2 CNVs Involves Several Genes in the 16p11.2 Region

Abnormal cortical development has been proposed as a core mechanism in 16p11.2 CNVs. As reviewed earlier, 16p11.2 deletions are associated with macrocephaly, whereas duplications are linked to microcephaly. Human-induced pluripotent stem cell-derived neurons from 16p11.2 deletion/duplication carriers display corresponding features, with increased soma size/dendrite length in 16p11.2 deletion neurons, and reduced size/dendrite length in duplication neurons [75]. By contrast, 16p11.2$^{+/-}$ mice display reduced brain size and reductions in upper layer cortical projection neurons, driven by increased progenitor proliferation and premature cell cycle exit, resulting in depleted progenitor pools and maldeveloped cortical structures [64]. The precise reasons for the reduced brain size remain unclear, although the reduced brain size is associated with reduced body weight phenotype in 16p11.2$^{+/-}$ mice, suggesting that at least in part, a broader developmental physiological impairment might be involved.

Corticostriatal circuits have been implicated in the pathophysiology of 16p11.2 deletion. Various structural abnormalities are reported in the striatum and cortex of 16p11.2$^{+/-}$ mice (Dolmetsch), along with an increased population of Drd2$^+$ MSNs in the striatum, and reduced Drd1$^+$ neurons in the cortex [4]. An elevated number of MSNs and the increased expression of Drd2 in the striatum are found in male 16p11.2$^{+/-}$ mice (Mills) [4,65]. Disrupted synaptic function in striatal MSNs is thought to underlie locomotion-related behavioral phenotypes in 16p11.2$^{+/-}$ mice [4].

The *MAPK3* gene located within the 16p11.2 region encodes the signaling molecule ERK1. Human genetic screenings link MAPK3 signaling to ASD [76], and ERK dysregulation is associated with ASD-related behavioral phenotypes in mice [77]. This pathway is also linked to ID [78]. Cultured pyramidal neurons from 16p11.2$^{dp/+}$ mice exhibit increased ERK1 phosphorylation, and greater dendritic arborization, which can be reversed by ERK inhibition [79]. These findings support a role for *Mapk3* in cortical development abnormalities associated with 16p11.2 CNVs.

The 16p11.2 gene *KCTD13* is also associated with cranial size phenotypes. In zebrafish, out of all 16p11.2 gene transcripts, only *KCTD13* overexpression drives a microcephalic phenotype with reduced brain cell counts, whereas its suppression produces a macrocephalic phenotype with increased brain cell counts [6]. However, these findings do not generalize to mice: two follow-up studies reported normal brain size in *Kctd13*-deficient mice [80,81]. *Kctd13*-deficient mice display reduced dendritic length/complexity and spine density, which is linked to downstream RhoA overexpression [80]. *Kctd13*-deficient mice also display cognitive deficits in several memory tasks [81], raising the possibility that similar mechanisms may contribute to ID pathogenesis in 16p11.2 CNVs.

Kctd13 is one of the adaptors of Cullin 3 (Cul3), a core component of the E3 ubiquitin ligase complex mediating protein ubiquitination and degradation [82]. The *Cul3* gene is one of the top-ranking high-risk factors for autism and related NDDs [83,84]. In mice, Cul3 deficiency in the forebrain or PFC induces social interaction impairment and sensory-gating deficiency, as well as NMDA receptor hypofunction, whereas Cul3 loss in the striatum causes stereotypic behaviors, as well as cell type-specific changes in neuronal excitability [85]. Abnormality in gene transcription or protein translation may underlie the involvement of Cul3 in NDDs [85,86]. It has also been suggested that dysregulation of the KCTD13–Cul3–RhoA pathway during the critical period for establishing the connectivity of 16p11.2 proteins with their coexpressed partners determines the abnormal brain sizes associated with 16p11.2 CNVs [87].

Knockout of the 16p11.2 gene *Taok2* produces cognitive and social deficits in mice, and also results in increased brain volume [88]. *Taok2*-deficient mice display reduced dendritic growth and deficient excitatory synaptic transmission in PFC through a mechanism involving the reduced RhoA expression [88], suggesting that the 16p11.2 genes *Kctd13* and *Taok2* may act upon convergent downstream molecules. *Taok2* has also been implicated in synaptic stabilization and spine maturation [89].

In humans, mutations in *MAPK3* [90] and *TAOK2* [91,92] but not in *KCTD13* [83,93] have been found in ASD cases. Missense variants in *MAPK3* are identified in ASD probands [83,90], but no loss-of-function *MAPK3* mutations have been reported in ASD, leaving the direct link to ASD uncertain. Loss-of-function variants in *TAOK2* are identified in ASD probands [91,92]; however, it is not a top-ranking ASD risk factor by large genetic screening [83]. Additionally, results from rodent studies of individual genes need to be considered with caution, as the phenotypes of 16p11.2 CNVs may depend on the collective effects of multiple genes in 16p11.2 locus.

Neurostructural Changes in Mouse Models of 16p11.2 CNVs
In contrast to the human data [14,23,47,48], 16p11.2$^{+/−}$ mice (Mills) do not exhibit changes in gray matter [7], but they show male-specific increases in medial and peristriatal fiber tracts [7], coinciding with the increased medial fiber tracts in human deletion carriers [14]. Increased volumes of several brain areas, including the hypothalamus, midbrain, cerebellar cortex, striatum, nucleus accumbens, and globus pallidus, are observed in 16p11.2$^{+/−}$ mice (Mills, Dolmetsch)

[4,5,94], concordant with the increased volume of these structures in human deletion patients [52]. No significant differences were found between 16p11.2$^{dp/+}$ (Mills) and wild-type mice, though trends toward reduced volume were observed in several regions [5].

Therapeutic Interventions: Targeting Glutamatergic and GABAergic Systems

Many valuable findings have been drawn from attempted intervention strategies in 16p11.2 mouse models. Inhibitory avoidance (IA) deficits are observed in 16p11.2$^{+/-}$ mice (Mills) [63]. In a mouse model of fragile X syndrome, IA deficits are reversed via chronic postadolescent administration of the mGluR5-negative allosteric modulator CTEP [95]. This approach was thus tested in 16p11.2$^{+/-}$ mice and proved effective in ameliorating IA deficits [63].

The GABA$_B$-receptor selective agonist R-baclofen was tested in 16p11.2$^{+/-}$ mice (Mills and Dolmetsch), as R-baclofen displayed therapeutic efficacy in $Fmr1$-deficient mice with a similar synaptic plasticity phenotype [62]. R-baclofen improves performance of 16p11.2$^{+/-}$ mice in several cognitive tasks, while hyperlocomotion and USVs are unaffected [62]. However, R-baclofen and several mGluR5-negative allosteric modulators have produced negative results in clinical trials with fragile X patients [96]. Thus, findings from preclinical studies in animal models should be interpreted with caution. It has also been demonstrated that GABA$_A$ receptor availability is unaffected in human 16p11.2 deletion carriers [97], though this does not preclude the possibility of presynaptic GABAergic alterations or effects on other GABA receptor subunits.

As reviewed earlier, 16p11.2$^{+/-}$ mice exhibit NMDAR deficits and hypoactivity in PFC pyramidal neurons [60]. Both humans and mice carrying 16p11.2 deletions display reduced prefrontal cortical connectivity [98], further suggesting a role for PFC disruption in 16p11.2 CNVs. Chemogenetic activation of PFC leads to increased NMDAR phosphorylation and function, resulting in amelioration of social and cognitive deficits [60]. This study suggests NMDAR hypofunction in PFC as a core mechanism in 16p11.2 deletion-linked phenotypes, consistent with the significant involvement of NMDARs in ASD [99–101].

As to the rescue of phenotypes in 16p11.2$^{dp/+}$ mice (Mills), a recent study from our group [70] revealed that restoring expression of Npas4, a key regulator of GABA synapses [102,103], reverses GABAergic deficits and ameliorates social and cognitive deficits in these mice, but not repetitive grooming behavior. The therapeutic potential of pharmaceutical interventions to boost GABAergic transmission by targeting $Npas4$ or related molecules for 16p11.2 duplication syndrome awaits to be further explored.

Concluding Remarks and Future Perspectives

Clinical and preclinical investigations illustrate the diverse neurobiological impact of 16p11.2 CNVs. A number of highly penetrant developmental phenotypes are linked to both 16p11.2 deletions and duplications (Figure 1). Given the effects of 16p11.2 CNVs on 27–29 genes, these mutations have the capacity to cause broad and severe downstream biochemical insults across various brain areas. The array of cellular changes in 16p11.2 models, including transcriptional and synaptic dysregulation, aberrant cell proliferation, and cortical development, provides a window into the molecular pathologies underlying behavioral syndromes associated with 16p11.2 CNVs. Restoring E/I balance and synaptic plasticity by targeting glutamate and GABA systems is suggested as a core intervention strategy (Figure 2).

Characterizations of 16p11.2 CNV mouse models have illuminated several important pathophysiological clues, though more work must be done before one could come up with a clear disease mechanism (see Outstanding Questions). Future studies could expand electrophysiological,

Outstanding Questions

What are the mechanistic links between the molecular, electrophysiological, and behavioral abnormalities driven by 16p11.2 CNVs? How do synaptic disruptions across different brain regions integrate to produce diverse behavioral phenotypes in 16p11.2 CNVs?

Are the structural and functional changes found in 16p11.2 deletion or duplication mice relevant to the pathophysiological mechanisms of human 16p11.2 syndromes? Do human stem cell-derived neurons from 16p11.2 CNV carriers display analogous electrophysiological, biochemical, and transcriptional phenotypes to those observed in corresponding mouse models?

Which genes in the chromosome 16p11.2 region are core drivers of the molecular, cellular and behavioral aberrations associated with 16p11.2 CNVs?

What is the role of transcriptomic change in the pathogenesis of 16p11.2 syndromes? Given the prominent role of chromatin remodelers and histone modifiers in neurodevelopmental disorders, including ASD and ID, is epigenetic dysregulation of gene expression also critically involved in 16p11.2 CNVs?

How might preclinical findings from 16p11.2 CNV mouse models translate to clinical treatment of humans with 16p11.2 CNVs or ASD in general? What is the therapeutic value of targeting the NMDAR or mGluR system in 16p11.2 deletions and the GABA system in 16p11.2 duplications?

biochemical, and genomic investigations into stem cell-derived neuronal models from human 16p11.2 CNV carriers, to test the existence of similar molecular and cellular aberrations. Additionally, optogenetic and chemogenetic approaches could be used to investigate long-range and local circuits in 16p11.2 CNV mice, which will help explain how the distributed physiological changes integrate to produce broad behavioral phenotypes. To identify the genes in the 16p11.2 region that drive morphological and functional alterations, future studies could use CRISPR-Cas9 technology to manipulate individual 16p11.2 genes or combinations of them in human induced pluripotent stem cell-derived neurons or transgenic mice. Combined transcriptomic analyses of 16p11.2 CNV mice and human RNA-seq data sets have identified overrepresentation of pathways related to histone methylation [74], thus future studies are encouraged to explore the role of epigenetic modifications of gene expression in 16p11.2 CNVs. The ultimate goal is to find mechanism-based treatment strategy for NDDs related to 16p11.2 CNVs and beyond. Key molecular targets or biological pathways will guide translational research for therapeutic intervention.

Resources

ⁱhttps://ghr.nlm.nih.gov/condition/16p112-deletion-syndrome#statistics

ⁱⁱhttps://ghr.nlm.nih.gov/condition/16p112-duplication#statistics

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