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CNV biology in neurodevelopmental disorders

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Copy number variants (CNVs), characterized in recent years by cutting-edge technology, add complexity to our knowledge of the human genome. CNVs contribute not only to human diversity but also to different kinds of diseases including neurodevelopmental delay, autism spectrum disorder and neuropsychiatric diseases. Interestingly, many pathogenic CNVs are shared among these diseases. Studies suggest that pathophysiology of disease may not be simply attributed to a single driver gene within a CNV but also that multifactorial effects may be important. Gene expression and the resulting phenotypes may also be affected by epigenetic alteration and chromosomal structural changes. Combined with human genetics and systems biology, integrative research by multi-dimensional approaches using animal and cell models of CNVs are expected to further understanding of pathophysiological mechanisms of neurodevelopmental disorders and neuropsychiatric disorders.

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Introduction

A copy number variant or copy number variation (CNV) is a genomic deletion, duplication or inversion [1,2]. Their size can range from Kb to Mb and recently even smaller CNVs have been detected. Before cutting-edge human genetics technologies, such as comparative genomic hybridization (CGH) and next generation sequencers were available, CNVs were called chromosomal aberrations or cytogenetic abnormalities that could be detected by microscopy. Not only do CNVs contribute to human diversity, similar to single nucleotide polymorphism or variation (SNP or SNV), but they have also been implicated in various diseases such as cancer, autoimmune diseases, lifestyle diseases and neuropsychiatric disorders, including autism spectrum disorder (ASD) and schizophrenia (SZ). A recent study that examined a large number of cases revealed that many critical target regions of CNVs seem to overlap among neuropsychiatric disorders such as ASD, SZ, and bipolar disorder [3••,4,5•,6,7]. Although the incidence of CNVs in diseases is very rare (*e.g.* less than 1% in ASD), the penetrance is high.

CNVs arise through non-allelic homologous recombination (NAHR) between low-copy repeat (LCR) sequences [1,8]. Single-cell sequencing analysis has shown that mosaic CNVs are abundant in the human brain [9] and large CNVs including a 2.9 Mb gain at chromosome 15q13.2-13.3 may arise in progenitor cells during brain development [10].

It was first reported among neuropsychiatric disorders that *de novo* CNV is strongly associated with ASD [11]. ASD is a childhood psychiatric disorder and a neurodevelopmental disorder. The major symptoms are impaired social interaction and communication, and restricted and repetitive behavior, interests and activity. The prevalence of ASD has surprisingly been increasing in many countries and a recent report from the United States claims that ASD affects 1 in every 68 children [12]. ASD is more often seen in males than females, with estimates of 1 in 42 boys versus 1 and 189 girls. Monozygotic twins have higher concordance rates for ASD, ranging from 60% to 90%, than dizygotic twins have [13] and the genetic contribution to the pathogenesis of ASD is higher compared to other psychiatric disorders. This fact has made ASD research a leading field of the neuropsychiatric disorder research. Many genetic and genomic CNVs have been revealed in patients with ASD [14–16], although most of the cases are still idiopathic. In this review, we focus on CNVs observed in patients with ASD. Recent studies using a large number of samples revealed several critical CNVs associated with ASD (Table 1). A study of 2591 families from the Simons Simplex Collection (SSC) combined with published CNV data from the Autism Genome Project (AGP) has identified 15q11.2-13.1, 16p11.2, 7q11.23, 1q21.1, 2p16.3 (only NRXN1 is included), 22q11.21, 15q13.2-13.3, 3q29, and 22q13.33 (SHANK3) [3••]. Another study of 1532 families from the autism genetic resource exchange (AGRE) has identified 15q11-13, 16p13.11, 22q11.2, 16p11.2, and 1q21.1 [4]. Pinto *et al.* analyze 2446 ASD-affected families from the AGP and show that the most frequent deletions are 16p11.2 and 2p16.3, accounting for 0.31% and 0.32% of affected subjects, respectively, and the most frequent duplication is 15q11-13 in 0.25% of affected siblings [5•]. More large-scale sequencing data of 5205 samples from families with ASD have recently been reported,

Table 1

ASD linked CNVs in SFARI. All CNVs were downloaded from SFARI (https://gene.sfari.org/database/cnv/). Two criteria including 'Individuals_case' >165 and 'Individuals_case/Individuals_control' >0.6 were set to extract major CNVs (40 CNVs). 'Autism Report' shows the number of autism-specific reports in which a CNV is implicated. 'Case/Control (%)' was calculated as Individuals_case/(Individuals_case + Individuals_control) × 100. NA, Associated human gene is not available in SFARI CNV database.

CNV-locus	CNV-type	Associated human gene	Autism report	Individuals_case	Individuals_control	Case/control (%)
15q11.2-q13.1	Duplication	NA	45	348	0	100.0
15q13.2-q13.3	Deletion–duplication	NA	33	278	11	96.2
7q11.23	Deletion–duplication	GTF2I, STX1A	50	341	33	91.2
22q11.21	Deletion–duplication	CLTCL1, GNB1L, LZTR1, PRODH, TBX1	64	802	103	88.6
1q21.1	Deletion–duplication	RBM8A	46	791	113	87.5
2p16.3	Deletion–duplication	NRXN1	54	350	74	82.5
17p13.3	Deletion–duplication	MNT, PAFAH1B1, SMG6, YWHAE	39	338	76	81.6
22q13.33	Deletion–duplication	CHKB, MAPK12, MAPK8IP2, SBF1, SHANK3	49	168	38	81.6
15q13.3	Deletion–duplication	CHRNA7, FAN1, TRPM1	58	317	77	80.5
17q12	Deletion–duplication	PPP1R1B	49	365	94	79.5
16p11.2	Deletion–duplication	BCKDK, KCTD13, MAPK3, SEZ6L2, SRCAP	91	1159	316	78.6
8p23.1	Deletion–duplication	MCPH1, PINX1	36	314	86	78.5
2q13	Deletion–duplication	NA	40	222	66	77.1
16p13.11	Deletion–duplication	NA	43	469	140	77.0
2q37.3	Deletion–duplication	HDAC4, PDCD1, PER2	39	177	53	77.0
4p16.3	Deletion–duplication	TNIP2	32	223	67	76.9
17p11.2	Deletion–duplication	KCNJ12, RAI1, RASD1	40	223	72	75.6
16p12.1	Deletion–duplication	NA	19	176	58	75.2
9q34.3	Deletion–duplication	CACNA1B, EHMT1, GRIN1, KCNT1	33	219	73	75.0
3q29	Deletion–duplication	DLG1, MUC4, TM4SF19	49	312	109	74.1
Xq28	Deletion–duplication	AFF2, CD99L2, GABRA3, GABRQ, HCFC1, MECP2, PDZD4, PLXNA3, RAB39B, RPL10, SLC6A8, TMLHE	48	282	102	73.4
16p13.3	Deletion–duplication	CACNA1H, CREBBP, RBFOX1, RNPS1, TSC2	38	314	126	71.4
10q26.3	Deletion–duplication	NA	33	207	91	69.5
14q11.2	Deletion–duplication	CHD8, SLC7A7	40	248	111	69.1
3p26.3	Deletion–duplication	CNTN6	41	252	116	68.5
16q23.1	Deletion–duplication	ADAMTS18, CHST5, CNTNAP4, TMEM231	31	170	80	68.0
6q26	Deletion–duplication	PARK2	32	183	89	67.3
Xq11.1	Deletion–duplication	NA	7	175	86	67.0
Xp11.1-q11.1	Duplication	NA	2	196	97	66.9
11p15.4	Deletion–duplication	OR52M1	31	178	89	66.7
12p13.31	Deletion–duplication	C12orf57, CLSTN3	25	175	90	66.0
6q27	Deletion–duplication	RPS6KA2	28	190	101	65.3
8p23.2	Deletion–duplication	CSMD1	26	183	100	64.7
20p12.1	Deletion–duplication	MACROD2	27	172	96	64.2
4q13.2	Deletion–duplication	UBA6	28	215	121	64.0
18q22.1	Deletion–duplication	NA	21	185	109	62.9
8p22	Deletion–duplication	MSR1, PSD3	34	357	211	62.9
1q44	Deletion–duplication	HNRNPU, OR1C1, OR2M4, OR2T10	42	348	214	61.9
15q11.2	Deletion–duplication	CYFIP1, MAGEL2, NIPA1, NIPA2, SNRPN, TUBGCP5, UBE3A	76	1287	823	61.0
13q12.11	Deletion–duplication	NA	18	218	145	60.1

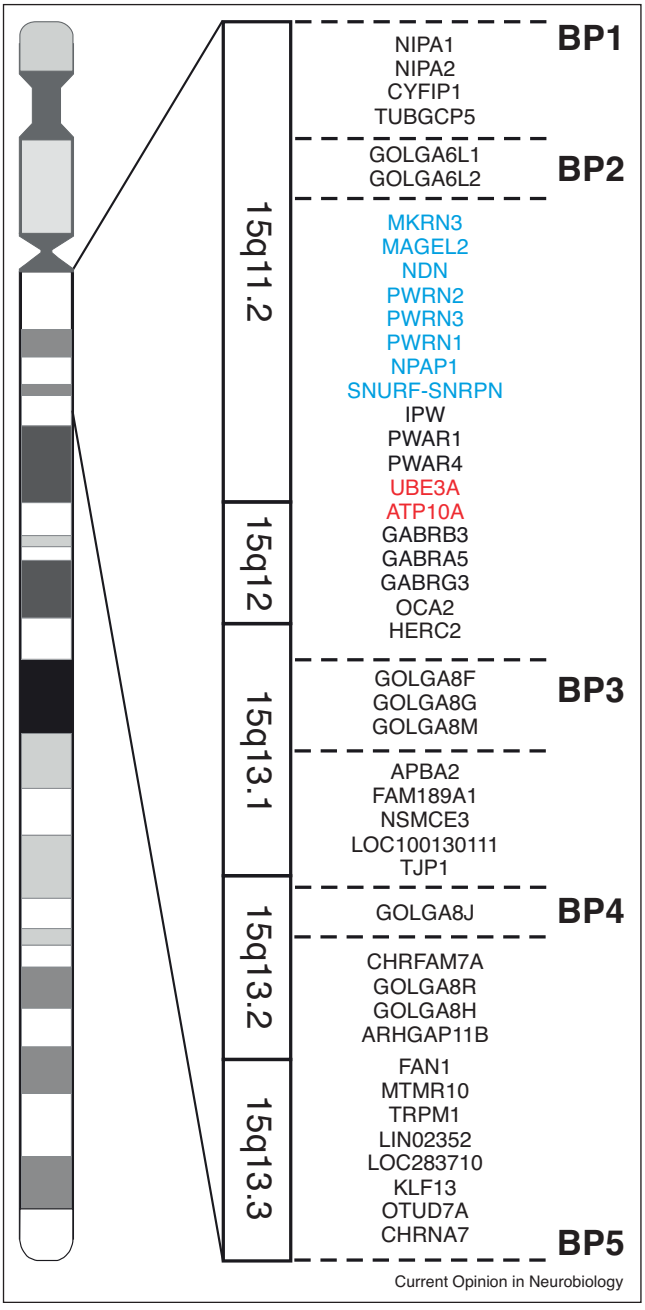
creating a database accessible on a cloud platform [17]. They find 189 of 2620 (7.2%) subjects carry pathogenic CNVs including 15q11-13, 16p13.1, and 1q21.1. Here we introduce recent findings on human genetics and animal models of CNVs associated with ASD mainly focusing on 15q11-13 and 16q11.2. Regarding 22q11.2, a comprehensive review has been published [18].

15q11.2-13.3

BP1–BP2

Chromosome 15 has a high rate of segmental duplications [19]. In chromosome region 15q11-13, there are 5 common break points (BPs) that can make different CNVs (Figure 1). The BP1–BP2 deletion affects brain structure [20]. Two brain regions, perigenual anterior cingulate cortex

Figure 1



Chromosome 15q11.2-q13.3. Gene names in blue and those in red are paternally-expressed and maternally-expressed genes, respectively. BP, break point.

and insula, show dosage effects in controls carrying the 15q11.2 (BP1–BP2) deletion and its reciprocal duplication. Cytoplasmic FMR1-interacting protein (CYFIP1), a binding partner of Fragile X mental retardation protein (FMRP), is a prime candidate within the interval between BP1 and BP2. CYFIP1/Sra-1 regulates the cytoskeleton through its competitive interactions between FMRP and the small GTPase RAC1 and may be involved in the development and maintenance of neuronal structures [21–23]. CYFIP1 also regulates translation at the synapse through binding to eukaryotic translation initiation factor 4E (eIF4E) together with FMRP [24]. *CYFIP1* is upregulated in transformed lymphoblastoid cell lines and postmortem brain of duplication of 15q11-13 (Dup15q) syndrome patients. *CYFIP1* bacterial artificial chromosome (BAC) transgenic mice show an increase in mature dendritic spines and dendritic spine density [25].

BP–BP3

Within the interval between BP2 and BP3, duplications (dup15q) are commonly observed. This is also the imprinting or Prader-Will and Angelman syndrome region [26]. The paternal deletion of this region causes Prader-Willi syndrome (PWS), while the maternal deletion of the same region causes Angelman syndrome (AS). The duplications of the region (interstitial and isodicentric 15q duplication) cause ASD and are among the most common and penetrant forms of ASD [27,28]. A recent post-mortem genome-wide transcriptome analysis of a large number of samples has demonstrated that dup15q exhibits similar changes to idiopathic ASD [29••]. Maternal duplications of the region are recognized risk factors for ASD with a high penetrance, while rare duplications of paternal origin have been regarded as benign [30]. However, there are case reports of paternal duplications and a recent analysis collecting a large number of samples has indicated that paternal duplications are pathogenic, increasing risk for ASD with a penetrance of 20% [31•].

The first CNV mouse model was generated by using a chromosome engineering technique [32]. Surprisingly, the *patDp*/+ (*15q dup*) mice in which the interstitial duplication is derived from paternal allele, but not the *matDp*/+ mice, display abnormal social behavior including impaired social interaction, abnormal ultrasonic vocalization (USV) and behavioral inflexibility. *In vivo* imaging of dendritic spines using two-photon microscopy shows an increased rate of spine formation and elimination in *15q dup* mice. This altered spine turnover rate might be a common endophenotype at a cellular level of ASD mouse models because other models such as *Neuroligin 3* knockin mice and BTBR mice also show a similar phenotype [33]. In terms of neurochemistry, Tamada *et al.* report that *15q dup* mice have lower serotonin (5-HT) levels in the brain during development [34]. Positron emission tomography (PET) imaging and electrophysiological analyses indicate that the serotonergic activity in the dorsal raphe nucleus (DRN) of *15q dup* mice is repressed [35••]. Additionally, an magnetic resonance imaging (MRI) study has revealed that *15q dup* mice have a smaller DRN [36]. *15q dup* mice show altered excitatory/inhibitory (E/I) balance in the somatosensory cortex due to the impairment of inhibitory synapses [35••]. Recovery of serotonin during development can rescue electrophysiological and behavioral phenotypes, suggesting that serotonin is important for neural

development. Among paternally expressed genes, small nuclear RNAs (*snoRNA*) have been considered as potential candidates; specifically, *snoRNA MBII-52* (*SNORD115*), which is involved in posttranscriptional modification of 5-HT2c receptor (5-HTR2C). The mRNA editing ratio of *5-htr2c* is altered in *15q dup* mice and the Ca^{2+} signal response against an agonist of 5-HTR2C is increased [32]. Interestingly, a Japanese macaque that spontaneously exhibits autistic traits harbors a nonsense mutation affecting HTR2C [37]. It remains unknown how 5-HT affects social behavior; however, the 5-HT system may impact social behavior via oxytocin release [38]. In *15q dup* mice, locomotion is characterized by greater stance width, longer stride length, reduced stride frequency and enhanced propulsion duration compared with wild-type controls, cerebellum-dependent motor learning and long term depression (LTD) at parallel fiber-Purkinje cell synapses are impaired, and activity-dependent synaptic pruning in the cerebellum is also impaired [39]. Considering that children with Dup15q syndrome demonstrate more impairment in motor and daily living skills compared to those in the non-syndromic ASD [40], the gait pattern might be a biomarker of Dup15q syndrome.

It has been reported that clinical cases of ASD with small duplications of paternally expressed genes, Makorin ring finger protein 3 (*MKRN3*), MAGE family member L2 (*MAGEL2*) and Necdin (*NDN*) [41]. *15q dup* mice with a 3 Mb duplication (including *snoRNA* and *miRNA*) were newly created and they show similar obesity like original *15q dup* mice with 6.3 Mb duplication [42], suggesting that these paternally expressed genes might be critical for late-onset obesity of *15q dup* mice. Similarly, it is possible that *15q dup* mice with a 3 Mb duplication exhibit abnormal social behavior but it remains unknown. Truncating mutations of *MAGEL2* in human cause Prader-Willi phenotypes and autism [43]. The screening of small molecules using a cell-based model with the *Snrpn*-EGFP fusion protein has identified histone methyltransferase (G9a, EHMT2) inhibitors as activators of paternally expressed genes [44••]. These inhibitors cause a selective reduction of the dimethylation of histone H3 lysine 9 (H3K9me2) at PWS-IC (imprinting center), suggesting a possibility for an epigenetic-based therapy for PWS.

Among maternally expressed genes, *UBE3A*, an E3 ubiquitin ligase, plays a causal role in Angelman syndrome (AS) and is a key candidate gene in this region. *Ube3a*-deficent neurons accumulate Ephexin-5, a UBE3A target, which limits the growth and maturation of dendritic spines by promoting RhoA activity [45,46]. Another UBE3A target, Arc, is increased in *Ube3a*-deficient neurons and enhances removal of the AMPA-type glutamate receptors, leading to a decrease in synaptic function [45,47]. *Ube3a* transcription is complex. Among three alternative *Ube3a* transcripts *Ube3a1* RNA, a transcript that encodes a truncated *Ube3a* protein lacking catalytic activity, regulates dendrite growth and spine maturation and its function is independent of its coding sequence [48]. Recently, Xu *et al.* identify ALDH1A2, the rate-limiting enzyme of retinoic acid (RA) synthesis, as a UBE3A target and show that reduced RA signaling may be an underlying mechanism in ASD phenotypes [49]. A core clock transcription factor, BMAL1, has also been identified as a UBE3A target and UBE3A works as an integral component of the molecular circadian clock [50]. Loss of *Ube3a* causes BMAL1 stabilization affecting circadian oscillation and resulting in the disturbance of sleep [51], which is often seen in AS and ASD. Drug screening has identified topoisomerase inhibitors that unsilence the paternal *Ube3a*, suggesting that topoisomerase inhibitors have the potential to rescue the molecular, cellular and behavioral deficits associated with loss of UBE3A [52]. Furthermore, topoisomerase facilitates expression of a large number of long genes linked to ASD [53]. Not glutamatergic but GABAergic *Ube3a* loss causes AS-like increases in neocortical EEG delta power, enhances seizure susceptibility, and leads to presynaptic accumulation of clathrin-coated vesicles without decreasing GABAergic inhibition onto L2/3 pyramidal neurons [54], consistent with the phenotype observed in mice with a maternal *Ube3a* null mutation [55]. An electrophysiological study using induced pluripotent stem cells (iPSCs) derived from AS shows disrupted neuronal maturation and the electrophysiological phenotypes could be rescued by a topoisomerase inhibitor [56].

Mice carrying a triple dose of *Ube3a* by BAC transgenesis show abnormal social behavior and communication, and impaired glutamatergic synaptic transmission [57]. UBE3A might repress CBLN1 expression to disrupt NRXN-CBLN1-GRIND1 complexes and compromise glutamatergic synaptic transmission, while *Ube3a* and seizure interactions in ventral tegmental area (VTA) glutamatergic neurons may impair sociability by down-regulating *Cbln1* [58•]. A new mouse model overexpressing *Ube3a* isoform 2 (human *UBE3A* isoform 3) in excitatory forebrain neurons shows anxiety-like behavior, cognitive impairments and reduced seizure thresholds, but displays normal social behavior [59•]. Dose-dependent *Ube3a* expression and its functional outcomes in neurons are essential for understanding the pathogenesis. Although altered *UBE3A* gene dosage is often proposed to be the mechanism responsible Dup15q syndrome, disruption of additional candidate genes may contribute significantly, as suggested by unexpected gene expression levels in postmortem brain and iPSC cell lines from Dup15q syndrome, and a neuronal cell culture model carrying 15q11.2-q13.3 maternal duplication [60–62].

Among the non-imprinting genes within the BP2–BP3 interval, there are three $GABA_A$ receptor subunits (*GABRB3*, *GABRA5*, and *GABARG3*). *GABRB3* is a key

susceptibility gene and *Gabrb3* deficient mice, a potential model of ASD, exhibit impaired social and exploratory behaviors [63]. Patients with Dup15q syndrome show abnormal EEG [41,64] and spontaneous beta oscillations can be a quantitative electrophysiological biomarker [65]. The distribution of beta power (with greater mean and variance) is similar to the distribution of *GABRB3* expression in the postmortem cortex of Dup15q syndrome [60]. *HERC2* within BP–BP3 encodes a ubiquitin ligase, which interacts with UBE3A and may act as a regulator of UBE3A [66].

Gene expression analyses using postmortem cortical samples from individuals with Dup15q syndrome and differentiated neurons from patient-derived iPSC lines reveals that copy number does not consistently predict expression levels [60,61], suggesting that epigenetic mechanisms influence expression. In fact, in the Dup15q postmortem brain, global DNA hypomethylation of synaptic genes is observed by using whole-genome bisulfite sequencing and acetylome alterations are shown by using H3K27ac chromatin immunoprecipitation sequencing (ChIP-seq) [67,68]. Considering all the evidence, we cannot simply conclude that dose-dependent gene expression can explain CNV pathogenesis. Epigenetic alteration and chromosomal structural changes may affect phenotypes. Epigenetic contribution to the ASD pathogenesis is reported in other reviews [69,70].

BP3–BP4

CNVs between BP3 and BP4 have also been reported [71,72], however, they are rare. Amyloid precursor protein-binding protein A2 (*APBA2*) within BP3-BP4 encodes a neuronal adaptor protein that interacts directly with *NRXN1* at the presynaptic membrane and may be a candidate gene for ASD [73].

BP4–BP5

Within the interval between BP4 and BP5, the 15q13.3 deletion (1.5 Mb) is a CNV known to link with ASD, developmental delay and epilepsy [71], and recently the link with SZ has gained additional attention. Palindoromic *GOLGA8* core duplicons located in the boundary of the BP promote microdeletion [74]. Mouse models with the 15q13.3 deletion are also available and they demonstrate features related to ASD [75], and SZ, as well as epilepsy-related alterations [76]. A smaller recurrent deletion within 15q13.3 (680 Kb) in human [77] suggests that haploinsufficiency of *CHRNA7* causes neurodevelopmental phenotypes although scan statistic-based analysis of exome sequencing data identifies Fanconi-associated nuclease 1 (*FAN1*), a DNA repair enzyme, at 15q13.3 as a susceptibility gene for SZ and ASD [78].

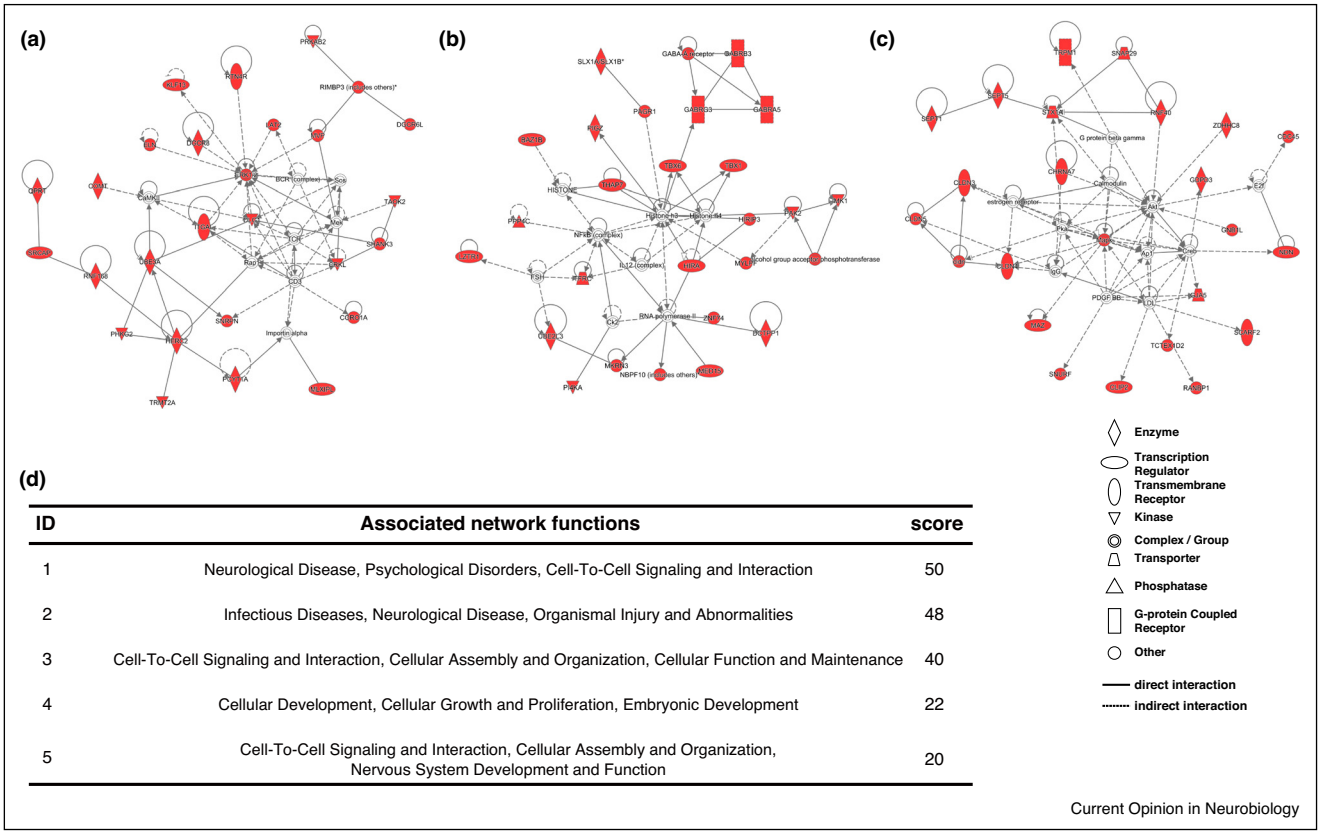
16p11.2

16p11.2 is a recently identified [79,80] but potentially frequently occurring CNV. Patients containing this CNV have been increasingly identified by the Simons foundation [81]. 16p11.2 is linked to not only ASD and SZ but also body mass index [82]. The first mouse model (the *Slx1b-Sept1* region) was developed using chromosome engineering and the deletion mice exhibit behaviors resembling sensorimotor deficits [83]. The second (*Coro1a-Spn*) [84] and third (*Sult1a1-Spn*) [85•] models were created independently. The second deletion models show hyperactivity, circling, and deficits in movement control and the third models show hyperactivity, repetitive behaviors, and recognition memory deficits, respectively. A zebrafish model has revealed that potassium channel tetramerization domain containing 13 (*KCTD13*) is a major driver gene of neuroanatomical phenotypes [86]. In contrast to this knockdown study, a recent report using *Kctd13* knockout mice exhibits no increase brain size or neurogenesis [87]. They also show that *Kctd13* deletion reduces synaptic transmission via increased RhoA. Spatiotemporal 16p11.2 protein network analysis implicates that KCTD13-Cul3-RhoA pathway may be dysregulated [88]. Increased dendritic arborization is found in primary cultured neurons from a mouse model of 16p11.2 duplication and network analysis identifies mitogen-activated protein kinase 3 (MAPK3), ERK MAP kinase, as the most topologically important hub in protein–protein interaction networks in 16p11.2 [89]. Thousand-and-one-amino acid kinase 2 (*TAOK2*) within the 16p11.12 region encodes a serine/threonine kinase that activates MAPK pathways. TAOK2 interacts with Neuropilin (Nrp1), a Semaphorin 3A receptor, and TAOK2 downregulation impairs basal dendrite formation [90]. Phosphorylation of TAOK2 by the hippo kinase homolog MST3 is required for dendritic spine formation in hippocampal neurons [91]. TAOK2 mediates PSD95 stability and dendritic spine maturation through Septin7 phosphorylation [92].

Conclusions and perspectives

Human genetics revealed many genetic bases, including CNVs, of the pathogenesis of neurodevelopmental disorders. Interestingly, critical CNVs are commonly found in patients with neurodevelopmental diseases and neuropsychiatric disorders. Systems biology and bioinformatics combined with human genetics enables us to understand the pathophysiological pathways affected by CNVs. To see functional convergence among the critical CNVs, we collected 271 genes from 9 different CNVs (25 from 1q21.1, 1 from 2p16.3, 31 from 3q29, 45 from 7q11.23, 25 from 15q11.2-q13.1, 19 from 15q13.3, 61 from 16p11.2, 63 from 22q11.21, and 1 from 22q13.33) and performed Ingenuity® Pathway Analysis (IPA®). Top canonical pathways were Signaling by Rho Family GTPase (p-value, 1.04E−03) and RhoA Signaling (p-value, 3.58E−03), which were also found in 16p11.2 protein

Figure 2



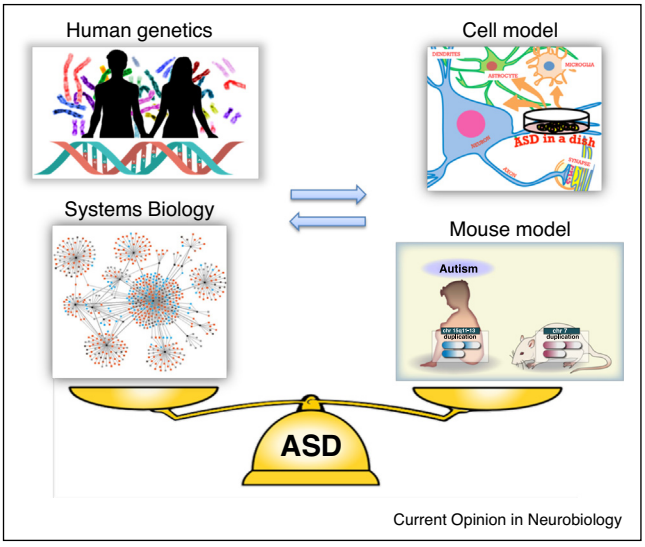
ID	Associated network functions	score
1	Neurological Disease, Psychological Disorders, Cell-To-Cell Signaling and Interaction	50
2	Infectious Diseases, Neurological Disease, Organismal Injury and Abnormalities	48
3	Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Cellular Function and Maintenance	40
4	Cellular Development, Cellular Growth and Proliferation, Embryonic Development	22
5	Cell-To-Cell Signaling and Interaction, Cellular Assembly and Organization, Nervous System Development and Function	20

Network analysis of genes in ASD-linked CNVs. **(a–c)** Top ranked network 1 **(a:** neurological disease, psychological disorders, cell-to-cell signaling and interaction), 2 **(b:** infectious diseases, neurological disease, organismal injury and abnormalities), and 3 **(c:** cell-to-cell signaling and interaction, cellular assembly and organization, cellular function and maintenance) analyzed by IPA are shown. Molecules are represented as nodes and the biological relationship between two nodes is shown as an edge (line). Red colored nodes indicate the genes located in ASD-linked CNVs. The shape of node and type of line are defined according to its functional class and type of relationship (http://ingenuity.force.com/ipa/). **(d)** Top 5 ranked network associated with ASD-linked CNVs. The core networks are ranked by score algorithmically based on their right-tailed Fisher's exact test *p*-value, which reflects the likelihood that the genes are grouped in a network by chance.

network analysis described above [88]. Top networks (network 1, 2 and 3) revealed neurological disease, psychological disorders, infectious diseases, cell-to-cell signaling and interaction (Figure 2). Network 1 included the ERK pathway, which is consistent with network analysis of 16p11.2 [89].

It is essential to make animal models to study CNVs. The original technique to develop CNVs in the genome was a chromosome engineering technique based on the Cre-loxP system using ES cells [93]. More than 20 years has passed since it is first developed; however, only few models have become available, probably because it is technically more difficult than conventional gene knockout technology and it takes longer time to make a model. A new genome editing technique, the clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated proteins (Cas) system, can be used to engineer chromosomes with more ease and speed. Using CRISPR/Cas9, mouse ESCs with structural variants of deletions, inversions, and duplications have already been generated and used to create mice [94]. Behavioral analyses, especially tests for social behavior, using model mice include confounding factors such as background species, environmental factors and, as a result, reproducibility is a challenge. For example, the phenotypes of social behavior of the C57BL/6N inbred background and C57BL/6NxC3B hybrid backgrounds are different, suggesting that the genetic background contributes to the manifestation of social behavior deficits induced by 16p11.2 CNV [85•]. It is known that in general, the genetic background of a mouse model strongly modulates phenotypes [95] and more caution on genetic background should be taken when considering genotype–phenotype relationships. More reproducible and quantitative biomarkers, such as EEG, motor or sensory phenotypes, should be characterized in patients. Another direction is cell models. CNVs of 16p11.2 and

Figure 3



CNV biology. From 'human genetics' and 'systems biology' to 'cell and animal models' and vice versa, these combinatorial approaches will enable us to understand the pathophysiology of ASD.

15q13.3 have recently been modeled in human iPSCs by using the CRISPR/Cas9 system [96••]. Combining examinations of postmortem brains cell models and mouse models will bring further understanding of the CNV pathophysiology. It is crucial for both basic and clinical scientists to work together, exchanging findings from clinical and basic research (Figure 3).

Further goals of CNV research are to develop of a new therapeutic strategy. One simple idea is dosage compensation of the target genes; however, strict control of gene expression ranging among 1 fold plus–minus 1 *in vivo* seems to be technically difficult. Since gene expression levels are not simply correlated to copy number, an epigenetic approach might be useful. Fusion of Tet1 or Dnmt3 with a catalytically inactive Cas9 (dCas9), dCas9-Tet1 or dCas9-Dnmt3a, enables precise editing of CpG methylation [97]. This specific controlling system of DNA methylation could become a potential therapeutic approach to CNV diseases in addition to G9a inhibitor and topoisomerase as described above.

Conflict of interest statement

Nothing declared.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Malhotra D, Sebat J: **CNVs: harbingers of a rare variant revolution in psychiatric genetics**. *Cell* 2012, **148**:1223-1241.

2. Zarrei M, MacDonald JR, Merico D, Scherer SW: **A copy number variation map of the human genome**. *Nat Rev Genet* 2015, **16**:172-183.

3. •• Sanders SJ, He X, Willsey AJ, Ercan-Sencicek AG, Samocha KE, Cicek AE, Murtha MT, Bal VH, Bishop SL, Dong S *et al.*: **Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci**. *Neuron* 2015, **87**:1215-1233.
This is a systematic analysis of CNVs from the full Simons Simplex Collection (SSC) in addition to published CNV data from the Autism Genome Project (AGP) and exome sequencing data from the SSC and the Autism Sequencing Consortium (ASC). A total of 71 ASD risk loci, including 6 CNV regions and 65 risk genes, were revealed.

4. Leppa VM, Kravitz SN, Martin CL, Andrieux J, Le Caignec C, Martin-Coignard D, DyBuncio C, Sanders SJ, Lowe JK, Cantor RM *et al.*: **Rare inherited and de novo CNVs reveal complex contributions to ASD risk in multiplex families**. *Am J Hum Genet* 2016, **99**:540-554.

5. • Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, Thiruvahindrapuram B, Xu X, Ziman R, Wang Z *et al.*: **Convergence of genes and cellular pathways dysregulated in autism spectrum disorders**. *Am J Hum Genet* 2014, **94**:677-694.
This is a systematic analysis of CNVs from the Autism Genome Project (AGP) to assess the impact of *de novo* and inherited rare CNV. The authors characterize candidate ASD-associated genes disrupted by CNVs and identify the biological relationships and common pathways they share.

6. Marshall CR, Howrigan DP, Merico D, Thiruvahindrapuram B, Wu W, Greer DS, Antaki D, Shetty A, Holmans PA, Pinto D *et al.*: **Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects**. *Nat Genet* 2017, **49**:27-35.

7. Green EK, Rees E, Walters JT, Smith KG, Forty L, Grozeva D, Moran JL, Sklar P, Ripke S, Chambert KD *et al.*: **Copy number variation in bipolar disorder**. *Mol Psychiatry* 2016, **21**:89-93.

8. Carvalho CM, Lupski JR: **Mechanisms underlying structural variant formation in genomic disorders**. *Nat Rev Genet* 2016, **17**:224-238.

9. McConnell MJ, Lindberg MR, Brennand KJ, Piper JC, Voet T, Cowing-Zitron C, Shumilina S, Lasken RS, Vermeesch JR, Hall IM *et al.*: **Mosaic copy number variation in human neurons**. *Science* 2013, **342**:632-637.

10. Cai X, Evrony GD, Lehmann HS, Elhosary PC, Mehta BK, Poduri A, Walsh CA: **Single-cell, genome-wide sequencing identifies clonal somatic copy-number variation in the human brain**. *Cell Rep* 2014, **8**:1280-1289.

11. Sebat J, Lakshmi B, Malhotra D, Troge J, Lese-Martin C, Walsh T, Yamrom B, Yoon S, Krasnitz A, Kendall J *et al.*: **Strong association of de novo copy number mutations with autism**. *Science* 2007, **316**:445-449.

12. Developmental Disabilities Monitoring Network Surveillance Year Principal Investigators, Centers for Disease Control and Prevention (CDC): **Prevalence of autism spectrum disorder among children aged 8 years — autism and developmental disabilities monitoring network, 11 sites, United States, 2010**. *MMWR Surveill Summ* 2014, **63**:1-21.

13. Ronald A, Hoekstra RA: **Autism spectrum disorders and autistic traits: a decade of new twin studies**. *Am J Med Genet B Neuropsychiatr Genet* 2011, **156B**:255-274.

14. Liu X, Takumi T: **Genomic and genetic aspects of autism spectrum disorder**. *Biochem. Biophys. Res. Commun.* 2014, **452**:244-253.

15. de la Torre-Ubieta L, Won H, Stein JL, Geschwind DH: **Advancing the understanding of autism disease mechanisms through genetics**. *Nat Med* 2016, **22**:345-361.

16. Vorstman JAS, Parr JR, Moreno-De-Luca D, Anney RJL, Nurnberger JI Jr, Hallmayer JF: **Autism genetics: opportunities and challenges for clinical translation**. *Nat Rev Genet* 2017, **18**:362-376.

17. Yuen RKC, Merico D, Bookman M, Howe JL, Thiruvahindrapuram B, Patel RV, Whitney J, Deflaux N, Bingham J, Wang Z *et al.*: **Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder**. *Nat Neurosci* 2017, **20**:602-611.

18. Hiroi N, Takahashi T, Hishimoto A, Izumi T, Boku S, Hiramoto T: **Copy number variation at 22q11.2: from rare variants to common mechanisms of developmental neuropsychiatric disorders**. *Mol Psychiatry* 2013, **18**:1153-1165.

19. Bailey JA, Gu Z, Clark RA, Reinert K, Samonte RV, Schwartz S, Adams MD, Myers EW, Li PW, Eichler EE: **Recent segmental duplications in the human genome**. *Science* 2002, **297**:1003-1007.

20. Stefansson H, Meyer-Lindenberg A, Steinberg S, Magnusdottir B, Morgen K, Arnarsdottir S, Bjornsdottir G, Walters GB, Jonsdottir GA, Doyle OM *et al.*: **CNVs conferring risk of autism or schizophrenia affect cognition in controls**. *Nature* 2014, **505**:361-366.

21. Schenck A, Bardoni B, Langmann C, Harden N, Mandel JL, Giangrande A: **CYFIP/Sra-1 controls neuronal connectivity in Drosophila and links the Rac1 GTPase pathway to the fragile X protein**. *Neuron* 2003, **38**:887-898.

22. Kobayashi K, Kuroda S, Fukata M, Nakamura T, Nagase T, Nomura N, Matsuura Y, Yoshida-Kubomura N, Iwamatsu A, Kaibuchi K: **p140Sra-1 (specifically Rac1-associated protein) is a novel specific target for Rac1 small GTPase**. *J Biol Chem* 1998, **273**:291-295.

23. De Rubeis S, Pasciuto E, Li KW, Fernandez E, Di Marino D, Buzzi A, Ostroff LE, Klann E, Zwartkruis FJ, Komiyama NH *et al.*: **CYFIP1 coordinates mRNA translation and cytoskeleton remodeling to ensure proper dendritic spine formation**. *Neuron* 2013, **79**:1169-1182.

24. Napoli I, Mercaldo V, Boyl PP, Eleuteri B, Zalfa F, De Rubeis S, Di Marino D, Mohr E, Massimi M, Falconi M *et al.*: **The fragile X syndrome protein represses activity-dependent translation through CYFIP1, a new 4E-BP**. *Cell* 2008, **134**:1042-1054.

25. Oguro-Ando A, Rosensweig C, Herman E, Nishimura Y, Werling D, Bill BR, Berg JM, Gao F, Coppola G, Abrahams BS *et al.*: **Increased CYFIP1 dosage alters cellular and dendritic morphology and dysregulates mTOR**. *Mol Psychiatry* 2015, **20**:1069-1078.

26. Takumi T: **The neurobiology of mouse models syntenic to human chromosome 15q**. *J Neurodev Disord* 2011, **3**:2281-2710.

27. Vorstman JA, Staal WG, van Daalen E, van Engeland H, Hochstenbach PF, Franke L: **Identification of novel autism candidate regions through analysis of reported cytogenetic abnormalities associated with autism**. *Mol Psychiatry* 2006, **11**:18-28.

28. Depienne C, Moreno-De-Luca D, Heron D, Bouteiller D, Gennetier A, Delorme R, Chaste P, Siffroi JP, Chantot-Bastaraud S, Benyahia B *et al.*: **Screening for genomic rearrangements and methylation abnormalities of the 15q11-q13 region in autism spectrum disorders**. *Biol Psychiatry* 2009, **66**:349-359.

29. •• Parikshak NN, Swarup V, Belgard TG, Irimia M, Ramaswami G, Gandal MJ, Hartl C, Leppa V, Ubieta LT, Huang J *et al.*: **Genome-wide changes in lncRNA, splicing, and regional gene expression patterns in autism**. *Nature* 2016, **540**:423-427.

This is a post-mortem genome-wide transcript analysis of a large cohort including a genetically defined subtype of ASD, dup15q, demonstrating ASD-associated dysregulation of primate-specific long noncoding RNAs, downregulation of the alternative splicing of activity-dependent neuron-specific exons, and attenuation of normal differences in gene expression in the frontal and temporal lobes.

30. Cook EH Jr, Lindgren V, Leventhal BL, Courchesne R, Lincoln A, Shulman C, Lord C, Courchesne E: **Autism or atypical autism in maternally but not paternally derived proximal 15q duplication**. *Am J Hum Genet* 1997, **60**:928-934.

31. • Isles AR, Ingason A, Lowther C, Walters J, Gawlick M, Stober G, Rees E, Martin J, Little RB, Potter H *et al.*: **Parental origin of interstitial duplications at 15q11.2-q13.3 in schizophrenia and neurodevelopmental disorders**. *PLoS Genet* 2016, **12**: e1005993.

This is the largest and most detailed study of parental origin of 15q11.2-q13.3 interstitial duplications, showing that paternal duplications lead to an increased risk of developing DD/ASD/multiple congenital anomalies.

32. Nakatani J, Tamada K, Hatanaka F, Ise S, Ohta H, Inoue K, Tomonaga S, Watanabe Y, Chung YJ, Banerjee R *et al.*: **Abnormal behavior in a chromosome-engineered mouse model for human 15q11-13 duplication seen in autism**. *Cell* 2009, **137**:1235-1246.

33. Isshiki M, Tanaka S, Kuriu T, Tabuchi K, Takumi T, Okabe S: **Enhanced synapse remodelling as a common phenotype in mouse models of autism**. *Nat Commun* 2014, **5**:4742.

34. Tamada K, Tomonaga S, Hatanaka F, Nakai N, Takao K, Miyakawa T, Nakatani J, Takumi T: **Decreased exploratory activity in a mouse model of 15q duplication syndrome; implications for disturbance of serotonin signaling**. *PLoS ONE* 2010, **5**:e15126.

35. •• Nakai N, Nagano M, Saitow F, Watanabe Y, Kawamura Y, Kawamoto A, Tamada K, Mizuma H, Onoe H, Watanabe Y *et al.*: **Serotonin rebalances cortical tuning and behavior linked to autism symptoms in 15q11-13 CNV mice**. *Sci Adv* 2017, **3**: e1603001.

This paper includes multi-dimensional analyses of patDp/+ (15q dup) mice, showing that serotonergic activity in the DRN is decreased, and E/I balance is disrupted due to the impaired inhibitory synaptic network. These phenotypes as well as social behavior and anxiety are rescued by treatment of SSRI during developmental stages.

36. Ellegood J, Nakai N, Nakatani J, Henkelman M, Takumi T, Lerch J: **Neuroanatomical phenotypes are consistent with autism-like behavioral phenotypes in the 15q11-13 duplication mouse model**. *Autism Res* 2015, **8**:545-555.

37. Yoshida K, Go Y, Kushima I, Toyoda A, Fujiyama A, Imai H, Saito N, Iriki A, Ozaki N, Isoda M: **Single-neuron and genetic correlates of autistic behavior in macaque**. *Sci Adv* 2016, **2**:e1600558.

38. Muller CL, Anacker AM, Veenstra-VanderWeele J: **The serotonin system in autism spectrum disorder: from biomarker to animal models**. *Neuroscience* 2016, **321**:24-41.

39. Piochon C, Kloth AD, Grasselli G, Titley HK, Nakayama H, Hashimoto K, Wan V, Simmons DH, Eissa T, Nakatani J *et al.*: **Cerebellar plasticity and motor learning deficits in a copy-number variation mouse model of autism**. *Nat Commun* 2014, **5**:5586.

40. DiStefano C, Gulsrud A, Huberty S, Kasari C, Cook E, Reiter LT, Thibert R, Jeste SS: **Identification of a distinct developmental and behavioral profile in children with Dup15q syndrome**. *J Neurodev Disord* 2016, **8**:19.

41. Al Ageeli E, Drunat S, Delanoe C, Perrin L, Baumann C, Capri Y, Fabre-Teste J, Aboura A, Dupont C, Auvin S *et al.*: **Duplication of the 15q11-q13 region: clinical and genetic study of 30 new cases**. *Eur J Med Genet* 2014, **57**:5-14.

42. Kishimoto R, Tamada K, Liu X, Okubo H, Ise S, Ohta H, Ruf S, Nakatani J, Kohno N, Spitz F *et al.*: **Model mice for 15q11-13 duplication syndrome exhibit late-onset obesity and altered lipid metabolism**. *Hum Mol Genet* 2015, **24**:4559-4572.

43. Schaaf CP, Gonzalez-Garay ML, Xia F, Potocki L, Gripp KW, Zhang B, Peters BA, McElwain MA, Drmanac R, Beaudet AL *et al.*: **Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism**. *Nat Genet* 2013, **45**:1405-1408.

44. •• Kim Y, Lee HM, Xiong Y, Sciaky N, Hulbert SW, Cao X, Everitt JI, Jin J, Roth BL, Jiang YH: **Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of Prader-Willi syndrome**. *Nat Med* 2017, **23**:213-222.

Using a high-content screening of small molecules, the authors discovered that inhibitors of G9a (histone lysine N-methyltransferease-2) activate the maternal copy of genes by maternal derepression.

45. Scheiffele P, Beg AA: **Neuroscience: Angelman syndrome connections**. *Nature* 2010, **468**:907-908.

46. Margolis SS, Salogiannis J, Lipton DM, Mandel-Brehm C, Wills ZP, Mardinly AR, Hu L, Greer PL, Bikoff JB, Ho HY *et al.*: **EphB-mediated degradation of the RhoA GEF Ephexin5 relieves a developmental brake on excitatory synapse formation**. *Cell* 2010, **143**:442-455.

47. Greer PL, Hanayama R, Bloodgood BL, Mardinly AR, Lipton DM, Flavell SW, Kim TK, Griffith EC, Waldon Z, Maehr R *et al.*: **The Angelman Syndrome protein Ube3A regulates synapse development by ubiquitinating arc**. *Cell* 2010, **140**:704-716.

48. Valluy J, Bicker S, Aksoy-Aksel A, Lackinger M, Sumer S, Fiore R, Wust T, Seffer D, Metge F, Dieterich C *et al.*: **A coding-independent function of an alternative Ube3a transcript during neuronal development**. *Nat Neurosci* 2015, **18**:666-673.

49. Xu X, Li C, Gao X, Xia K, Guo H, Li Y, Hao Z, Zhang L, Gao D, Xu C *et al.*: **Excessive UBE3A dosage impairs retinoic acid signaling and synaptic plasticity in autism spectrum disorders**. *Cell Res* 2017.

50. Gossan NC, Zhang F, Guo B, Jin D, Yoshitane H, Yao A, Glossop N, Zhang YQ, Fukada Y, Meng QJ: **The E3 ubiquitin ligase UBE3A is an integral component of the molecular circadian clock through regulating the BMAL1 transcription factor**. *Nucleic Acids Res* 2014, **42**:5765-5775.

51. Shi SQ, Bichell TJ, Ihrie RA, Johnson CH: **Ube3a imprinting impairs circadian robustness in Angelman syndrome models**. *Curr Biol* 2015, **25**:537-545.

52. Huang HS, Allen JA, Mabb AM, King IF, Miriyala J, Taylor-Blake B, Sciaky N, Dutton JW Jr, Lee HM, Chen X *et al.*: **Topoisomerase inhibitors unsilence the dormant allele of Ube3a in neurons**. *Nature* 2012, **481**:185-189.

53. King IF, Yandava CN, Mabb AM, Hsiao JS, Huang HS, Pearson BL, Calabrese JM, Starmer J, Parker JS, Magnuson T *et al.*: **Topoisomerases facilitate transcription of long genes linked to autism**. *Nature* 2013, **501**:58-62.

54. Judson MC, Wallace ML, Sidorov MS, Burette AC, Gu B, van Woerden GM, King IF, Han JE, Zylka MJ, Elgersma Y *et al.*: **GABAergic neuron-specific loss of Ube3a causes Angelman syndrome-like EEG abnormalities and enhances seizure susceptibility**. *Neuron* 2016, **90**:56-69.

55. Wallace ML, Burette AC, Weinberg RJ, Philpot BD: **Maternal loss of Ube3a produces an excitatory/inhibitory imbalance through neuron type-specific synaptic defects**. *Neuron* 2012, **74**:793-800.

56. Fink JJ, Robinson TM, Germain ND, Sirois CL, Bolduc KA, Ward AJ, Rigo F, Chamberlain SJ, Levine ES: **Disrupted neuronal maturation in Angelman syndrome-derived induced pluripotent stem cells**. *Nat Commun* 2017, **8**:15038.

57. Smith SE, Zhou YD, Zhang G, Jin Z, Stoppel DC, Anderson MP: **Increased gene dosage of Ube3a results in autism traits and decreased glutamate synaptic transmission in mice**. *Sci Transl Med* 2011, **3** 103ra197.

58. • Krishnan V, Stoppel DC, Nong Y, Johnson MA, Nadler MJ, Ozkaynak E, Teng BL, Nagakura I, Mohammad F, Silva MA *et al.*: **Autism gene Ube3a and seizures impair sociability by repressing VTA Cbln1**. *Nature* 2017, **543**:507-512.
Using *in vivo* mouse genetics, the authors showed that increasing UBE3A in the nucleus downregulates Cbln1, and Ube3a and seizure interactions in VTA glutamatergic neurons impair sociability by downregulating Cbln1.

59. • Copping NA, Christian SGB, Ritter DJ, Islam MS, Buscher N, Zolkowska D, Pride MC, Berg EL, LaSalle JM, Ellegood J *et al.*: **Neuronal overexpression of Ube3a isoform 2 causes behavioral impairments and neuroanatomical pathology relevant to 15q11.2-q13.3 duplication syndrome**. *Hum Mol Genet* 2017, **26**:3995-4010.
The authors developed and characterized a new mouse overexpressing Ube3a isoform 2 in excitatory neurons. The model mice exhibited increased anxiety-like behaviors, learning impairments, and reduced seizure threshold.

60. Scoles HA, Urraca N, Chadwick SW, Reiter LT, Lasalle JM: **Increased copy number for methylated maternal 15q duplications leads to changes in gene and protein expression in human cortical samples**. *Mol Autism* 2011, **2**:19.

61. Germain ND, Chen PF, Plocik AM, Glatt-Deeley H, Brown J, Fink JJ, Bolduc KA, Robinson TM, Levine ES, Reiter LT *et al.*: **Gene expression analysis of human induced pluripotent stem cell-derived neurons carrying copy number variants of chromosome 15q11-q13.1**. *Mol Autism* 2014, **5**:44.

62. Meguro-Horike M, Yasui DH, Powell W, Schroeder DI, Oshimura M, Lasalle JM, Horike S: **Neuron-specific impairment of inter-chromosomal pairing and transcription in a novel model of human 15q-duplication syndrome**. *Hum Mol Genet* 2011, **20**:3798-3810.

63. DeLorey TM, Sahbaie P, Hashemi E, Homanics GE, Clark JD: **Gabrb3 gene deficient mice exhibit impaired social and exploratory behaviors, deficits in non-selective attention and hypoplasia of cerebellar vermal lobules: a potential model of autism spectrum disorder**. *Behav Brain Res* 2008, **187**:207-220.

64. Urraca N, Cleary J, Brewer V, Pivnick EK, McVicar K, Thibert RL, Schanen NC, Esmer C, Lamport D, Reiter LT: **The interstitial duplication 15q11.2-q13 syndrome includes autism, mild facial anomalies and a characteristic EEG signature**. *Autism Res* 2013, **6**:268-279.

65. Frohlich J, Senturk D, Saravanapandian V, Golshani P, Reiter LT, Sankar R, Thibert RL, DiStefano C, Huberty S, Cook EH *et al.*: **A quantitative electrophysiological biomarker of duplication 15q11.2-q13.1 syndrome**. *PLoS ONE* 2016, **11**:e0167179.

66. Kuhnle S, Kogel U, Glockzin S, Marquardt A, Ciechanover A, Matentzoglu K, Scheffner M: **Physical and functional interaction of the HECT ubiquitin–protein ligases E6AP and HERC2**. *J Biol Chem* 2011, **286**:19410-19416.

67. Dunaway KW, Islam MS, Coulson RL, Lopez SJ, Vogel Ciernia A, Chu RG, Yasui DH, Pessah IN, Lott P, Mordaunt C *et al.*: **Cumulative impact of polychlorinated biphenyl and large chromosomal duplications on DNA methylation, chromatin, and expression of autism candidate genes**. *Cell Rep* 2016, **17**:3035-3048.

68. Sun W, Poschmann J, Cruz-Herrera Del Rosario R, Parikshak NN, Hajan HS, Kumar V, Ramasamy R, Belgard TG, Elanggovan B, Wong CC *et al.*: **Histone acetylome-wide association study of autism spectrum disorder**. *Cell* 2016, **167** 1385-1397 e1311.

69. Nakai N, Otsuka S, Myung J, Takumi T: **Autism spectrum disorder model mice: focus on copy number variation and epigenetics**. *Sci China Life Sci* 2015, **58**:976-984.

70. Vogel Ciernia A, LaSalle J: **The landscape of DNA methylation amid a perfect storm of autism aetiologies**. *Nat Rev Neurosci* 2016, **17**:411-423.

71. Sharp AJ, Mefford HC, Li K, Baker C, Skinner C, Stevenson RE, Schroer RJ, Novara F, De Gregori M, Ciccone R *et al.*: **A recurrent 15q13.3 microdeletion syndrome associated with mental retardation and seizures**. *Nat Genet* 2008, **40**:322-328.

72. Rosenfeld JA, Stephens LE, Coppinger J, Ballif BC, Hoo JJ, French BN, Banks VC, Smith WE, Manchester D, Tsai AC *et al.*: **Deletions flanked by breakpoints 3 and 4 on 15q13 may contribute to abnormal phenotypes**. *Eur J Hum Genet* 2011, **19**:547-554.

73. Babatz TD, Kumar RA, Sudi J, Dobyns WB, Christian SL: **Copy number and sequence variants implicate APBA2 as an autism candidate gene**. *Autism Res* 2009, **2**:359-364.

74. Antonacci F, Dennis MY, Huddleston J, Sudmant PH, Steinberg KM, Rosenfeld JA, Miroballo M, Graves TA, Vives L, Malig M *et al.*: **Palindromic GOLGA8 core duplicons promote chromosome 15q13.3 microdeletion and evolutionary instability**. *Nat Genet* 2014, **46**:1293-1302.

75. Kogan JH, Gross AK, Featherstone RE, Shin R, Chen Q, Heusner CL, Adachi M, Lin A, Walton NM, Miyoshi S *et al.*: **Mouse model of chromosome 15q13.3 microdeletion syndrome**

demonstrates features related to autism spectrum disorder. *J Neurosci* 2015, **35**:16282-16294.

76. Fejgin K, Nielsen J, Birknow MR, Bastlund JF, Nielsen V, Lauridsen JB, Stefansson H, Steinberg S, Sorensen HB, Mortensen TE *et al.*: **A mouse model that recapitulates cardinal features of the 15q13.3 microdeletion syndrome including schizophrenia- and epilepsy-related alterations**. *Biol Psychiatry* 2014, **76**:128-137.

77. Shinawi M, Schaaf CP, Bhatt SS, Xia Z, Patel A, Cheung SW, Lanpher B, Nagl S, Herding HS, Nevinny-Stickel C *et al.*: **A small recurrent deletion within 15q13.3 is associated with a range of neurodevelopmental phenotypes**. *Nat Genet* 2009, **41**:1269-1271.

78. Ionita-Laza I, Xu B, Makarov V, Buxbaum JD, Roos JL, Gogos JA, Karayiorgou M: **Scan statistic-based analysis of exome sequencing data identifies FAN1 at 15q13.3 as a susceptibility gene for schizophrenia and autism**. *Proc Natl Acad Sci U S A* 2014, **111**:343-348.

79. Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T *et al.*: **Association between microdeletion and microduplication at 16p11.2 and autism**. *N Engl J Med* 2008, **358**:667-675.

80. Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH Jr, Dobyns WB *et al.*: **Recurrent 16p11.2 microdeletions in autism**. *Hum Mol Genet* 2008, **17**:628-638.

81. Simons Vip C: **Simons variation in individuals project (Simons VIP): a genetics-first approach to studying autism spectrum and related neurodevelopmental disorders**. *Neuron* 2012, **73**:1063-1067.

82. Jacquemont S, Reymond A, Zufferey F, Harewood L, Walters RG, Kutalik Z, Martinet D, Shen Y, Valsesia A, Beckmann ND *et al.*: **Mirror extreme BMI phenotypes associated with gene dosage at the chromosome 16p11.2 locus**. *Nature* 2011, **478**:97-102.

83. Horev G, Ellegood J, Lerch JP, Son YE, Muthuswamy L, Vogel H, Krieger AM, Buja A, Henkelman RM, Wigler M *et al.*: **Dosage-dependent phenotypes in models of 16p11.2 lesions found in autism**. *Proc Natl Acad Sci U S A* 2011, **108**:17076-17081.

84. Portmann T, Yang M, Mao R, Panagiotakos G, Ellegood J, Dolen G, Bader PL, Grueter BA, Goold C, Fisher E *et al.*: **Behavioral abnormalities and circuit defects in the basal ganglia of a mouse model of 16p11.2 deletion syndrome**. *Cell Rep* 2014, **7**:1077-1092.

85. • Arbogast T, Ouagazzal AM, Chevalier C, Kopanitsa M, Afinowi N, Migliavacca E, Cowling BS, Birling MC, Champy MF, Reymond A *et al.*: **Reciprocal effects on neurocognitive and metabolic phenotypes in mouse models of 16p11.2 deletion and duplication syndromes**. *PLoS Genet* 2016, **12**:e1005709.
This paper describes newly engineered mouse models carrying a deletion or a duplication of the region homologous to human 16p11.2. Comparing the same CNV models with different backgrounds, the authors showed that genetic background affects social behavioral phenotypes.

86. Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S, Reymond A, Sun M, Sawa A, Gusella JF *et al.*: **KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant**. *Nature* 2012, **485**:363-367.

87. Escamilla CO, Filonova I, Walker AK, Xuan ZX, Holehonnur R, Espinosa F, Liu S, Thyme SB, Lopez-Garcia IA, Mendoza DB *et al.*: **Kctd13 deletion reduces synaptic transmission via increased RhoA**. *Nature* 2017, **551**:227-231.

88. Lin GN, Corominas R, Lemmens I, Yang X, Tavernier J, Hill DE, Vidal M, Sebat J, Iakoucheva LM: **Spatiotemporal 16p11.2 protein network implicates cortical late mid-fetal brain development and KCTD13-Cul3-RhoA pathway in psychiatric diseases**. *Neuron* 2015, **85**:742-754.

89. Blizinsky KD, Diaz-Castro B, Forrest MP, Schurmann B, Bach AP, Martin-de-Saavedra MD, Wang L, Csernansky JG, Duan J, Penzes P: **Reversal of dendritic phenotypes in 16p11.2 microduplication mouse model neurons by pharmacological targeting of a network hub**. *Proc Natl Acad Sci U S A* 2016, **113**:8520-8525.

90. de Anda FC, Rosario AL, Durak O, Tran T, Graff J, Meletis K, Rei D, Soda T, Madabhushi R, Ginty DD *et al.*: **Autism spectrum disorder susceptibility gene TAOK2 affects basal dendrite formation in the neocortex**. *Nat Neurosci* 2012, **15**:1022-1031.

91. Ultanir SK, Yadav S, Hertz NT, Oses-Prieto JA, Claxton S, Burlingame AL, Shokat KM, Jan LY, Jan YN: **MST3 kinase phosphorylates TAO1/2 to enable Myosin Va function in promoting spine synapse development**. *Neuron* 2014, **84**:968-982.

92. Yadav S, Oses-Prieto JA, Peters CJ, Zhou J, Pleasure SJ, Burlingame AL, Jan LY, Jan YN: **TAOK2 kinase mediates PSD95 stability and dendritic spine maturation through Septin7 phosphorylation**. *Neuron* 2017, **93**:379-393.

93. Ramirez-Solis R, Liu P, Bradley A: **Chromosome engineering in mice**. *Nature* 1995, **378**:720-724.

94. Kraft K, Geuer S, Will AJ, Chan WL, Paliou C, Borschiwer M, Harabula I, Wittler L, Franke M, Ibrahim DM *et al.*: **Deletions, inversions, duplications: engineering of structural variants using CRISPR/Cas in mice**. *Cell Rep* 2015, **10**:833-839.

95. Sittig LJ, Carbonetto P, Engel KA, Krauss KS, Barrios-Camacho CM, Palmer AA: **Genetic background limits generalizability of genotype–phenotype relationships**. *Neuron* 2016, **91**:1253-1259.

96. •• Tai DJ, Ragavendran A, Manavalan P, Stortchevoi A, Seabra CM, Erdin S, Collins RL, Blumenthal I, Chen X, Shen Y *et al.*: **Engineering microdeletions and microduplications by targeting segmental duplications with CRISPR**. *Nat Neurosci* 2016, **19**:517-522.
Using CRISPR/Cas9 system, the authors generated CNVs of 16p11.2 and 15q13.3 in iPSCs.

97. Liu XS, Wu H, Ji X, Stelzer Y, Wu X, Czauderna S, Shu J, Dadon D, Young RA, Jaenisch R: **Editing DNA methylation in the mammalian genome**. *Cell* 2016, **167** 233-247.e217.