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Archival Report

Cross-Disorder Analysis of Genic and Regulatory Copy Number Variations in Bipolar Disorder, Schizophrenia, and Autism Spectrum Disorder

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ABSTRACT

BACKGROUND: We aimed to determine the similarities and differences in the roles of genic and regulatory copy number variations (CNVs) in bipolar disorder (BD), schizophrenia (SCZ), and autism spectrum disorder (ASD).

METHODS: Based on high-resolution CNV data from 8708 Japanese samples, we performed to our knowledge the largest cross-disorder analysis of genic and regulatory CNVs in BD, SCZ, and ASD.

RESULTS: In genic CNVs, we found an increased burden of smaller (<100 kb) exonic deletions in BD, which contrasted with the highest burden of larger (>500 kb) exonic CNVs in SCZ/ASD. Pathogenic CNVs linked to neurodevelopmental disorders were significantly associated with the risk for each disorder, but BD and SCZ/ASD differed in terms of the effect size (smaller in BD) and subtype distribution of CNVs linked to neurodevelopmental disorders. We identified 3 synaptic genes (*DLG2*, *PCDH15*, and *ASTN2*) as risk factors for BD. Whereas gene set analysis showed that BD-associated pathways were restricted to chromatin biology, SCZ and ASD involved more extensive and similar pathways. Nevertheless, a correlation analysis of gene set results indicated weak but significant pathway similarities between BD and SCZ or ASD (r = 0.25–0.31). In SCZ and ASD, but not BD, CNVs were significantly enriched in enhancers and promoters in brain tissue.

CONCLUSIONS: BD and SCZ/ASD differ in terms of CNV burden, characteristics of CNVs linked to neurodevelopmental disorders, and regulatory CNVs. On the other hand, they have shared molecular mechanisms, including chromatin biology. The BD risk genes identified here could provide insight into the pathogenesis of BD.

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Although bipolar disorder (BD), schizophrenia (SCZ), and autism spectrum disorder (ASD) have traditionally been considered separate disease entities, they share some common behavioral characteristics and cognitive deficits. Genetic epidemiological studies have suggested the presence of shared genetic factors among these and other psychiatric disorders (1–3). In line with this, rare copy number variations (CNVs) at multiple loci have been identified as shared risk

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factors for SCZ and ASD (4–10). Gene set analyses of genes affected by CNVs have implicated common molecular mechanisms in the pathogenesis of SCZ and ASD (e.g., synapse function, fragile X mental retardation protein [FMRP] targets, chromatin regulation) (4–6,9,11). On the other hand, BD-associated CNVs and genes are currently limited, and molecular mechanisms resulting from CNVs to BD are less clear (12–17).

There are several limitations in published CNV studies. First is the use of single nucleotide polymorphism arrays that cannot reliably detect small CNVs (<50 kb), which outnumber CNVs of a larger size. The detection of such small CNVs may reveal BD-associated CNVs and genes. Second, although there is growing evidence of a cross-disorder effect of CNVs (18–21), there have been few studies of cross-disorder CNV analysis that simultaneously include all three disorders (BD, SCZ, and ASD). These analyses could also reveal similarities and differences in the roles of CNVs among the three disorders. Third, CNVs affecting noncoding regulatory elements have not been fully explored despite emerging evidence for their involvement in human diseases (22–24). Large consortia such as Encyclopedia of DNA Elements (ENCODE) and Roadmap Epigenomics Projects have identified enhancer or promoter regions in brain tissue (25,26). Therefore, it is expected that the role of regulatory CNVs in psychiatric disorders may be clarified.

In the present study, we conducted the largest known cross-disorder analysis of genic and regulatory CNVs in BD, SCZ, and ASD based on high-resolution CNV data from 8708 individuals in a Japanese population. We found an increased burden of smaller (<100 kb) exonic deletions in BD, which was in contrast to the highest burden of larger (>500 kb) exonic CNVs in SCZ and ASD. Pathogenic CNVs linked to neurodevelopmental disorders (NDDs) were associated with the risk of each disorder, but BD and SCZ/ASD differed in terms of the characteristics of NDD-CNVs. Whereas gene set analysis showed that BD-associated pathways were restricted to chromatin biology, SCZ and ASD involved more extensive and similar pathways. Nevertheless, a correlation analysis of gene set results showed weak but significant similarities between BD and SCZ/ASD. Finally, in SCZ and ASD, CNVs were significantly enriched in enhancers and promoters in brain tissue.

METHODS AND MATERIALS

Participants

We studied 8903 Japanese individuals, including 1843 BD cases (42.2% bipolar I disorder, 53.9% bipolar II disorder, and 4.0% unknown subtype), 1236 ASD cases, 3111 SCZ cases, and 2713 psychiatrically normal controls (Table S1 in the Supplement). Whereas all BD cases have not been previously analyzed in CNV studies, some of the samples (2519 SCZ, 1132 ASD cases, 2110 controls) were included in our previous study (9). Disorders in cases were diagnosed according to DSM-5 criteria for BD, SCZ, and ASD. Controls were selected from the general population and had no history of mental disorders based on responses to questionnaires or self-reporting. More of the participants' characteristics are provided in Supplemental Methods in the Supplement.

This study was approved by the ethics committee of Nagoya University and each participating institute. Written informed consent was obtained from all participants.

Array Comparative Genomic Hybridization

We performed CNV analysis using two types of array comparative genomic hybridization (aCGH): NimbleGen 720K Whole-Genome Tiling array (Roche NimbleGen) and Agilent SurePrint G3 Human CGH 400K (Agilent Technologies). CNV calls were made with Nexus Copy Number 9.0 (BioDiscovery) using the Fast Adaptive States Segmentation Technique 2 algorithm. The following $\log_2$ ratio thresholds were set to detect CNVs in the NimbleGen and Agilent arrays: 10–500 kb: −0.6 (deletion) and 0.4 (duplication), >500 kb: −0.4 (deletion) and 0.3 (duplication). The significance threshold to adjust the sensitivity of the segmentation algorithm was set at 1×10^{-6}, and at least 3 contiguous probes were required for CNV calls. A noise-reduction algorithm for aCGH data was used for the systematic correction of artifacts caused by GC content and fragment length (27).

In terms of quality control (QC), scores were calculated for each sample based on the statistical variance of the probe-to-probe log ratios. Lower QC scores indicated better-quality results. We excluded samples with QC scores >0.2, gender mismatch, and excessive autosomal CNV calls (subject QC). Next, we excluded CNV calls <10 kb; those with low probe density (<1 probe/30 kb), >70% overlap with segmental duplications, >10% overlap with CpG islands, and call p value $>1 \times 10^{-10}$; and those on the Y chromosome. Finally, we filtered out common CNVs (≥1% of our total samples). Large CNVs can be split by CNV-calling algorithms. To overcome this issue, adjacent CNV calls were merged using a custom script. We merged the adjacent CNVs of the same type (i.e., deletion or duplication) if they occurred in a single individual and the gap was <50% of the entire length of the newly merged CNV. We performed all statistical analyses based on rare (<1%) CNVs. All genomic locations are given in hg18 coordinates. Gene annotation was based on GENCODE Release 35. We evaluated the accuracy of CNVs identified by aCGH using a quantitative real-time polymerase chain reaction (TaqMan copy number assays) (Applied Biosystems), as previously described (28).

As we used two types of aCGH, it is important to control for batch effects in statistical analyses. To this end, we included array type as a covariate in all analyses for SCZ and ASD. The aCGH for BD cases was performed using Agilent arrays only. Therefore, statistical analyses for BD cases versus controls were performed based on the CNV data from Agilent arrays (1818 BD cases and 1847 controls).

Genome-wide Burden Analysis

We performed burden analyses across a range of CNV sizes (<100 kb, 100–500 kb, >500 kb) and CNV types (deletion, duplication, deletion+duplication). The burden of CNVs was measured as the number of rare exonic CNVs. Exonic CNVs were defined as overlapping with any exon of a gene. Statistical tests were performed using a logistic regression model to predict case-control status by the number of rare exonic CNVs along with array type and sex as a covariate. One-sided

empirical *p* values were calculated based on 100,000 permutations, swapping case-control status. The *p* values were adjusted for multiple testing using Bonferroni correction.

CNVs Linked to Neurodevelopmental Disorders

We examined whether NDD-CNVs are significantly associated with risk for BD, SCZ, and ASD. We preselected 307 NDD-linked loci (265 risk genes and 42 CNV loci) (Tables S2a and S2b in the Supplement). The NDD-linked genes were selected based on the NDD-gene databases (e.g., Developmental Brain Disorder Gene Database, denovo-db, Gene4Denovo, and SFARI database) and findings in previous literature (29–32). Their associations with NDDs are supported by strong genetic evidence (e.g., identification of de novo loss-of-function variants in multiple patients and significant association in a large-scale case-control study). The NDD-linked CNV loci were selected from our previous study (9). Then, we identified pathogenic or likely pathogenic CNVs in these loci according to the American College of Medical Genetics guidelines (33,34). Further details are provided in Supplemental Methods in the Supplement.

Next, we performed association analyses in 3 ways. First, we examined the associations of all NDD-CNVs combined. Second, we tested the associations of each subtype of NDD-CNVs with at least 5 observations. Third, we tested the associations of individual NDD-CNVs with at least 5 observations.

Statistical analyses were conducted using Firth's bias-reduced logistic regression model, in which case versus control status was regressed on NDD-CNVs along with array type and sex as a covariate. We calculated one-sided empirical *p* values based on 100,000 permutations, swapping case-control status. The empirical significance values obtained via permutation are robust to data with sparse cell counts (35). The *p* values were adjusted for multiple testing using Bonferroni correction.

Gene Set Analysis

To identify biological pathways underlying the pathogenesis of each disorder, we tested for the enrichment of rare exonic CNVs in gene sets relative to all rare exonic CNVs. Specifically, we used a logistic regression model, in which case versus control status was regressed on the number of genes within a given gene set that were intersected by rare exonic CNVs, with adjustment for covariates, including array type, sex, total length of rare CNVs, and number of rare CNVs. This method is robust against not only batch effects, but also case-control differences in total length of rare CNVs, number of rare CNVs, and systematic differences in gene size (35). The enrichment in cases was reported as one-sided empirical *p* values using 100,000 permutations, swapping case-control status. Multiple-testing correction was performed separately for each gene set group and CNV type using the Benjamini-Hochberg false discovery rate (36). Gene sets were considered significant if the Benjamini-Hochberg false discovery rate was $< .05$.

The following gene sets were used in this study (shown in Table S3): 1) functional gene sets previously associated with SCZ/ASD, 2) mouse gene sets, 3) synapse gene sets from SynGO release 1.1, and 4) Gene Ontology (GO) gene sets. The functional gene sets contain synaptosome and postsynaptic density genes from Genes2Cognition, FMRP target genes (37,38), and chromatin-related genes (39,40). The mouse gene sets include 11 sets of human orthologs of mouse genes whose disruption results in neurobehavioral and nervous system abnormalities (41). The SynGO gene sets are evidence-based, expert-curated sets of synapse biology (42). We analyzed 59 SynGO gene sets with at least 30 genes. The GO gene sets (size 150–500 genes) were taken from the Molecular Signatures Database version 7.2 C5 collection (43).

Correlation of Biological Pathways

To quantify the similarity of biological pathways among BD, SCZ, and ASD, we calculated correlation coefficients based on GO gene set results in all pairwise combinations of CNV types and disorders. To reduce the bias owing to the non-independent nature of the gene sets, we removed gene sets that had an overlap coefficient {[size of (A intersect B)]/[size of (minimum (A, B))]} of >0.5 with regard to other gene sets, resulting in 295 gene sets for the analysis. The *z* score for each GO gene set was calculated from two-sided *p* values and odds ratios (ORs) using the following equation: $z = \text{sign}(\ln(\text{OR})) \times \left|\Phi^{-1}(p/2)\right|$, where Φ^{-1} is the inverse cumulative distribution function of the normal distribution. Therefore, the *z* score was positive for gene sets where the possession of CNVs increased the risk of disease and negative for gene sets where the possession of CNVs decreased the risk of disease. Pearson's correlation coefficient among the three disorders was calculated by using the z scores. The *p* values for the correlation coefficients were adjusted for multiple testing using Bonferroni correction. To compare the magnitude of the two correlations, we used the R package cocor (44), which is suitable for the comparison of coefficients calculated from two dependent groups that share a variable in common.

CNVs in Regulatory Elements

We examined whether case CNVs were enriched in promoters, enhancers, and topologically associating domain (TAD) boundaries in brain regions. TAD boundaries are regions bordering TADs that regulate gene expression by restricting interactions of *cis*-regulatory sequences to their target genes. These regulatory elements were taken from 2 sources: 1) enhancer regions in the prefrontal cortex, H3K27ac (histone H3 acetylation at lysine 27) peaks in the prefrontal, temporal, and cerebellar cortex, and TAD boundaries in the adult dorsolateral prefrontal cortex from the PsychENCODE website (http://resource.psychencode.org/), and 2) enhancer and promoter regions in 10 types of brain tissues from Reg2Map: HoneyBadger2 (https://personal.broadinstitute.org/meuleman/reg2map/HoneyBadger2_release/). H3K27ac peaks are active enhancer regions.

For the statistical analysis, we used a logistic regression model, in which case versus control status was regressed on overlap length (one unit: 1 kb) with regulatory elements, with adjustments for array type, sex, and total length of rare CNVs. One-sided empirical *p* values were calculated based on 100,000 permutations, swapping case-control status. The *p*

values were adjusted for multiple testing using Bonferroni correction.

RESULTS

Identification of CNVs

Of 8903 samples, 8708 (1818 BD cases, 3014 SCZ cases, 1205 ASD cases, and 2671 controls) passed QC (Figure 1; Table S1 in the Supplement). We obtained 25,654 rare (<1%) CNVs from all participants. The CNV characteristics are summarized in Table S4 in the Supplement. The median CNV size was 53.1 kb, and 69% and 48% were <100 kb and <50 kb, respectively. We validated 97.6% (661 of 677) of tested CNVs (Table S5 in the Supplement). For the smallest class of CNVs (10–50 kb), the validation rate was 97.0%.

Genome-wide Burden Analysis

The results of genome-wide burden analysis are shown in Figure 2 and Table S6 in the Supplement. In BD, we found an increased burden of smaller (<100 kb) exonic deletions (OR = 1.14, $p_{corrected}$ = .034). By contrast, SCZ and ASD showed the highest burden of larger (>500 kb) exonic CNVs (deletion+duplication, SCZ: OR = 1.27, ASD: OR = 1.49, $p_{corrected} < .01$).

CNVs Linked to Neurodevelopmental Disorders

In our sample, we identified 432 NDD-CNVs according to the American College of Medical Genetics guidelines (Figure 3A; Table S7). A significant association was found between NDD-CNVs and each disorder (BD: OR = 2.9, SCZ: OR = 3.7, ASD: OR = 4.2, $p_{corrected} < 1 \times 10^{-4}$) (Figure 3B; Table S8 in the Supplement). Figure 3C shows the percentage of individuals with each subtype of NDD-CNVs: 1) risk gene–disrupting CNVs, 2) large recurrent CNVs, 3) large nonrecurrent CNVs, and 4) sex chromosome aneuploidies. In the association analysis for BD, only the risk gene–disrupting CNVs were significant (OR = 3.6, $P_{corrected} = 1.0 \times 10^{-4}$), whereas three subtypes were significant in SCZ and ASD (Figure 3C; Table S9 in the Supplement).

For individual NDD-CNVs, 12 showed at least nominally significant associations (Table 1; Table S10 in the Supplement). They included CNVs at three synaptic genes (*DLG2*, *PCDH15*, and *ASTN2*) associated with BD (Figure S1 in the Supplement). Five of 12 NDD-CNVs survived Bonferroni correction for multiple comparisons ($p_{corrected} < .05$): *DLG2* CNV in BD and *DLG2* CNV, 22q11.2 deletion, 1q21.1 deletion, and 47,XXX/47,XXY in SCZ.

Gene Set Analysis

In 6 functional gene sets previously associated with SCZ and ASD, we found a significant enrichment of CNVs in chromatin organization and chromatin modification in BD (Figure 4A; Table S11a). In SCZ and ASD, we confirmed a significant enrichment in all 6 gene sets, except for synaptosome in ASD. In mouse gene sets and synapse gene sets, no significant enrichment was observed in BD, whereas many sets were significant in SCZ and ASD. In mouse gene sets, we found 4 significant sets common to SCZ and ASD: abnormal brain development, abnormal nervous system development, abnormal central nervous system synaptic transmission, and abnormal learning/memory/conditioning (Figure 4B; Table S11b).

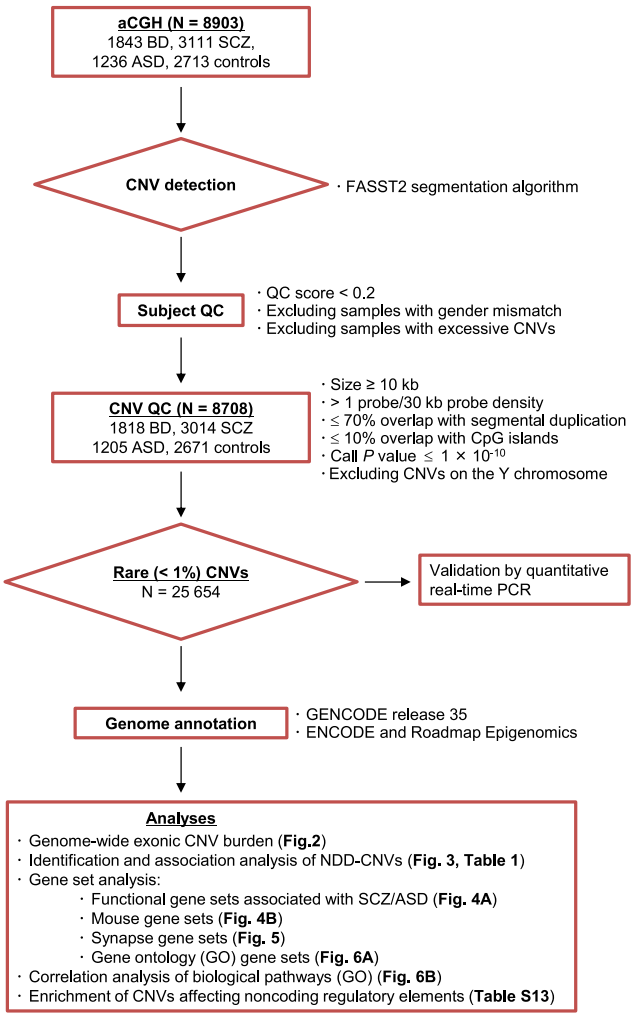

Figure 1. CNV analysis workflow. aCGH, array comparative genomic hybridization; ASD, autism spectrum disorder; BD, bipolar disorder; CNV, copy number variation; NDD, neurodevelopmental disorder; PCR, polymerase chain reaction; QC, quality control; SCZ, schizophrenia.

In synapse gene sets, we identified 4 significant sets common to SCZ and ASD: process in the synapse, synaptic vesicle exocytosis, postsynaptic membrane, and integral component of postsynaptic membrane (Figure 5; Table S11c). In terms of cellular components, both presynapse and postsynapse were significant in SCZ and ASD. The gene sets with the largest effect sizes were regulation of synaptic vesicle exocytosis in SCZ and postsynaptic membrane in ASD.

In the GO gene sets, we found 1, 352, and 100 significant gene sets in BD, SCZ, and ASD, respectively (Table S11d). Among them, 81 sets were common to SCZ and ASD, and one set (covalent chromatin modification) was common to all three disorders. As shown in Figure 6A, significant gene sets were

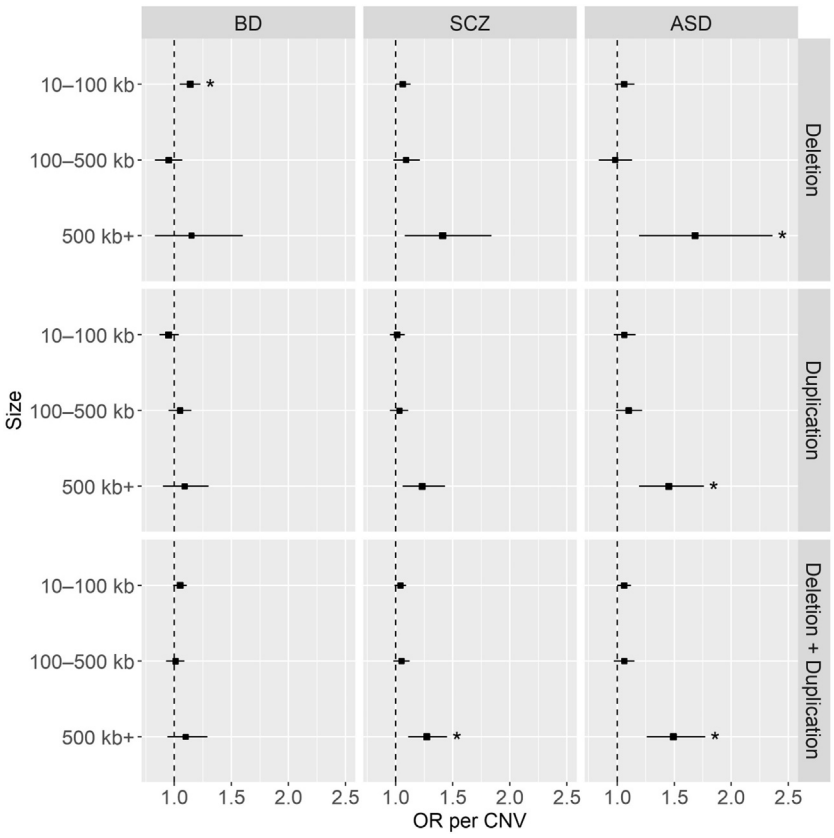

Figure 2. Genome-wide CNV burden. Forest plots show OR estimates and 95% confidence intervals for exonic CNV burden (CNV number) in the three disorders. Asterisks denote a significant enrichment of CNVs ($p_{corrected} < .05$). ASD, autism spectrum disorder; BD, bipolar disorder; CNV, copy number variation; OR, odds ratio; SCZ, schizophrenia.

broadly classified into 14 biological pathways, 11 of which were common to SCZ and ASD.

Correlation of Biological Pathways

We calculated correlations of the GO gene set results in all pairwise combinations of CNV types and disorders. In deletion+duplication, we found significant correlations among the three disorders. SCZ and ASD showed the highest degree of correlation ($r = 0.48$, $p_{corrected} = 3.0 \times 10^{-17}$), followed by BD and ASD ($r = 0.31$, $p_{corrected} = 1.2 \times 10^{-6}$), and then BD and SCZ ($r = 0.25$, $P_{corrected} = 3.7 \times 10^{-4}$) (Figure 6B; Table S12 in the Supplement). The correlation coefficient between SCZ and ASD was significantly higher than that between BD and ASD ($p = .0072$) or between BD and SCZ ($p = .0002$). In deletion or duplication, we observed significant correlations among the three disorders except for the nonsignificant correlation between SCZ and BD in deletion.

CNVs in Noncoding Regulatory Elements

In SCZ and ASD, but not BD, CNVs were significantly ($p_{corrected} < .05$) enriched in enhancers and promoters in brain regions from HoneyBadger2 and PsychENCODE (Tables S13a and S13b in the Supplement). In most cases, deletions were significant in SCZ, whereas duplications were significant in ASD.

DISCUSSION

We conducted the largest known ($N = 8708$) cross-disorder analysis of CNVs in BD, SCZ, and ASD. The strengths of our study are as follows: 1) the use of a high-quality and high-resolution CNV dataset (validation rate >97%, approximately 50% of CNVs <50 kb), 2) analyses of a highly homogeneous Japanese population, and 3) systematic evaluation of both genic and regulatory CNVs. Although two types of aCGH were used, we considered the batch effect to be limited for 2 reasons. First, we included array type as a covariate in all analyses for SCZ and ASD. Second, all analyses of BD cases versus controls were performed based on data from Agilent arrays.

We found an increased burden of smaller (<100 kb) exonic deletions in BD, in contrast to the highest burden of larger (>500 kb) exonic CNVs in SCZ/ASD. Whereas an increased burden of large CNVs has been reported in SCZ/ASD (6,45,46), the finding in BD is a novel observation and suggests that CNVs <100 kb may play an important role in BD. Interestingly, a study showed an increased burden of small (<100 kb) deletions in major depressive disorder, which was primarily in enhancer regions (47). Therefore, BD is similar to major depressive disorder in terms of the increased burden of small deletions, but different from major depressive disorder in terms of the direct effect on genes rather than regulatory elements.

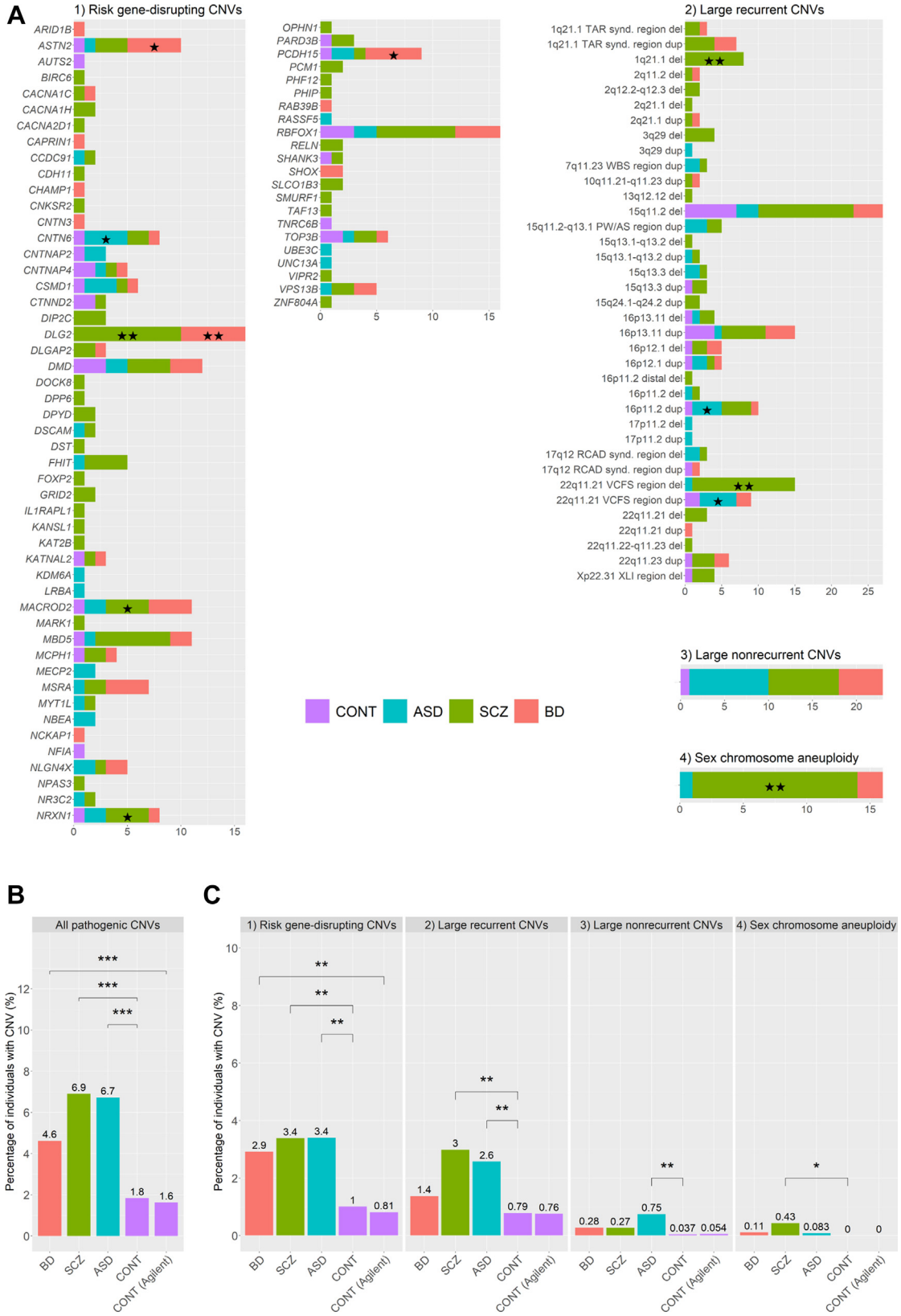
A
1) Risk gene-disrupting CNVs
ARID1B
ASTN2
AUTS2
BIRC6
CACNA1C
CACNA1H
CACNA2D1
CAPRIN1
CCDC91
CDH11
CHAMP1
CNKSR2
CNTN3
CNTN6
CNTNAP2
CNTNAP4
CSMD1
CTNND2
DIP2C
DLG2
DLGAP2
DMD
DOCK8
DPP6
DPYD
DSCAM
DST
FHIT
FOXP2
GRID2
IL1RAPL1
KANSL1
KAT2B
KATNAL2
KDM6A
LRBA
MACROD2
MARK1
MBD5
MCPH1
MECP2
MSRA
MYT1L
NBEA
NCKAP1
NFIA
NLGN4X
NPAS3
NR3C2
NRXN1
0 5 10 15
OPHN1
PARD3B
PCDH15
PCM1
PHF12
PHIP
RAB39B
RASSF5
RBFOX1
RELN
SHANK3
SHOX
SLCO1B3
SMURF1
TAF13
TNRC6B
TOP3B
UBE3C
UNC13A
VIPR2
VPS13B
ZNF804A
0 5 10 15
2) Large recurrent CNVs
1q21.1 TAR synd. region del
1q21.1 TAR synd. region dup
1q21.1 del
2q11.2 del
2q12.2-q12.3 del
2q21.1 del
2q21.1 dup
3q29 del
3q29 dup
7q11.23 WBS region dup
10q11.21-q11.23 dup
13q12.12 del
15q11.2 del
15q11.2-q13.1 PW/AS region dup
15q13.1-q13.2 del
15q13.1-q13.2 dup
15q13.3 del
15q13.3 dup
15q24.1-q24.2 dup
16p13.11 del
16p13.11 dup
16p12.1 del
16p12.1 dup
16p11.2 distal del
16p11.2 del
16p11.2 dup
17p11.2 del
17p11.2 dup
17q12 RCAD synd. region del
17q12 RCAD synd. region dup
22q11.21 VCFS region del
22q11.21 VCFS region dup
22q11.21 del
22q11.21 dup
22q11.22-q11.23 del
22q11.23 dup
Xp22.31 XLI region del
0 5 10 15 20 25
3) Large nonrecurrent CNVs
0 5 10 15 20
4) Sex chromosome aneuploidy
0 5 10 15
CONT
ASD
SCZ
BD
B
All pathogenic CNVs
Percentage of individuals with CNV (%)
0 2 4 6 8 10 12
***
***
***
4.6
6.9
6.7
1.8
1.6
BD
SCZ
ASD
CONT
CONT (Agilent)
C
1) Risk gene-disrupting CNVs
2) Large recurrent CNVs
3) Large nonrecurrent CNVs
4) Sex chromosome aneuploidy
Percentage of individuals with CNV (%)
0 2 4 6 8 10
**
**
**
2.9
3.4
3.4
1
0.81
**
**
1.4
3
2.6
0.79
0.76
**
0.28
0.27
0.75
0.037
0.054
*
0.11
0.43
0.083
0
0
BD
SCZ
ASD
CONT
CONT (Agilent)

Table 1. NDD-CNVs With at Least Nominally Significant Associations With Each Disorder

Diagnosis	NDD-CNVs	Frequency		Number of CNVs		OR (95% CI)	*p* Value	$p_{corrected}$
		Cases	Controls	Cases	Controls			
SCZ	*DLG2* CNV	0.0033	0	10	0	19.1 (2.4, 2460)	.00002	.0005
SCZ	22q11.21 (VCFS region) del	0.0046	0	14	0	21.8 (2.9, 2792)	.00017	.0043
SCZ	1q21.1 del	0.0027	0	8	0	15.6 (1.9, 2021)	.00017	.0043
SCZ	47,XXX/47,XXY	0.0043	0	13	0	20.9 (2.7, 2681)	.00026	.0065
BD	*DLG2* CNV	0.0033	0	6	0	13.7 (1.6, 1789)	.00062	.016
ASD	*CNTN6* CNV	0.0033	0.00037	4	1	8.1 (1.2, 90.7)	.0031	.078
ASD	16p11.2 dup	0.0033	0.00037	4	1	7.5 (1.3, 77.6)	.0034	.085
ASD	22q11.21 (VCFS region) dup	0.0041	0.00075	5	2	5.8 (1.3, 34.5)	.0049	.12
BD	*PCDH15* CNV	0.0028	0.00054	5	1	3.8 (0.76, 37)	.019	.48
BD	*ASTN2* CNV	0.0028	0.00054	5	1	3.8 (0.76, 37)	.02	.5
SCZ	*NRXN1* CNV	0.0013	0.00037	4	1	3.5 (0.63, 35.2)	.041	1
SCZ	*MACROD2* CNV	0.0013	0.00037	4	1	3.4 (0.61, 33.8)	.041	1

Of NDD-CNVs, 12 showed at least nominally significant associations; 5 of them were significant after Bonferroni correction for multiple comparisons ($p_{corrected} < .05$).

ASD, autism spectrum disorder; BD, bipolar disorder; CNV, copy number variation; del, deletion; dup, duplication; NDD, neurodevelopmental disorder; OR, odds ratio; SCZ, schizophrenia; VCFS, velocardiofacial syndrome.

We found that NDD-CNVs increased the risk for BD as well as SCZ/ASD. The effect size in BD (OR = 2.9) was lower than that in SCZ/ASD (OR = 3.7–4.2), which is consistent with the notion that the contribution of CNVs to BD is smaller than that to SCZ/ASD (16). To our knowledge, this is the first evidence for an association between NDD-CNVs and BD. There are two reasons for this. First, the use of high-resolution aCGH enabled us to identify small CNVs, which are difficult to detect reliably with single nucleotide polymorphism arrays. Second, we carefully evaluated the pathogenicity of CNVs, including intragenic duplications according to the American College of Medical Genetics guidelines (33,34).

The subtype distribution of NDD-CNVs differed between BD and SCZ/ASD. Both risk gene–disrupting CNVs and large recurrent CNVs were significant in SCZ/ASD, but only the former was significant in BD. This is consistent with the results of CNV burden analysis, as the majority of risk gene–disrupting CNVs were smaller (<100 kb) exonic deletions. This also implies that risk gene–disrupting CNVs point to strong risk genes for BD as well as SCZ/ASD, as described below.

We found a nominally significant association of 12 NDD-CNVs, 5 of which survived correction for multiple testing: CNVs at *DLG2* in SCZ and BD and 22q11.21 deletion, 1q21.1 deletion, and 47,XXX/47,XXY in SCZ. Deletions at *DLG2* were previously associated with SCZ and BD (11,48). *DLG2* plays a critical role in the molecular organization of multiprotein complexes in the postsynaptic density at excitatory synapses. Moreover, 47,XXX/47,XXY has been associated with SCZ, ASD, and BD (49), but we found a specific and strong association with SCZ (OR = 20.9, $p_{corrected}$ = .0065). We also found a nominally significant association between BD and two synaptic genes, *PCDH15* (OR = 3.8, p = .019) and *ASTN2* (OR = 3.8, p = .020). *PCDH15* is responsible for Usher syndrome, characterized by retinitis pigmentosa and congenital deafness. About 20% of patients with Usher syndrome also receive a diagnosis of a psychiatric disorder (50). Neurons derived from induced pluripotent stem cells of patients with BD with *PCDH15* deletion exhibit abnormalities in dendrite and synapse formation (51). Rare CNVs at *ASTN2* were identified in patients with BD, ASD, and attention-deficit hyperactivity disorder (17,52). All of the deletions identified in patients with BD in our study affected multiple isoforms of *ASTN2*. *ASTN2* plays an important role in the modulation of synaptic strength by the trafficking and degradation of synaptic proteins (53). Taken together, these findings suggest that synaptic dysfunction is of pathogenic relevance to BD.

The results of gene set analysis implicated chromatin modification and organization in BD pathogenesis. Consistent with this, changes in histone modification and DNA methylation were detected in postmortem brain tissue from patients with BD (54,55). The involvement of chromatin modification is suggested based on the clinical efficacy of the mood stabilizer valproic acid. Valproic acid, a histone deacetylation inhibitor, causes chromatin remodeling and gene expression change (56).

In synapse gene sets, both presynapse and postsynapse were associated with the pathogenesis of SCZ and ASD. In terms of presynapse, synaptic vesicle exocytosis was implicated in both disorders. This process is essential for the maintenance of neurotransmission, and its dysregulation in SCZ/ASD has been suggested in studies of human brain tissue

Figure 3. NDD-CNVs. **(A)** Number of NDD-CNVs identified in this study. Stars indicate a significant association between the CNV and disorder (*$p_{uncorrected} < .05$; **$p_{corrected} < .05$). *DLG2* CNVs were significantly associated with both SCZ and ASD at $p_{corrected} < .05$. **(B)** Percentage of patients carrying NDD-CNVs. Frequencies of NDD-CNVs were significantly higher in each disorder compared with controls (***$p_{corrected} < .0001$). As BD cases were analyzed by Agilent arrays only, statistical analyses for BD cases vs. controls were performed based on the data from Agilent arrays. **(C)** Percentage of patients carrying each subtype of NDD-CNVs: risk gene–disrupting CNVs, large recurrent CNVs, large nonrecurrent CNVs, and sex chromosome aneuploidies. *$p_{corrected} < .01$; **$p_{corrected} < .001$. ASD, autism spectrum disorder; BD, bipolar disorder; CNV, copy number variation; CONT, controls; del, deletion; dup, duplication; NDD, neurodevelopmental disorder; PW/AS, Prader-Willi/Angelman syndrome; RCAD synd, renal cysts and diabetes syndrome; SCZ, schizophrenia; TAR synd, thrombocytopenia–absent radius syndrome; VCFS, velocardiofacial syndrome; WBS, Williams-Beuren syndrome; XLI, X-linked ichthyosis.

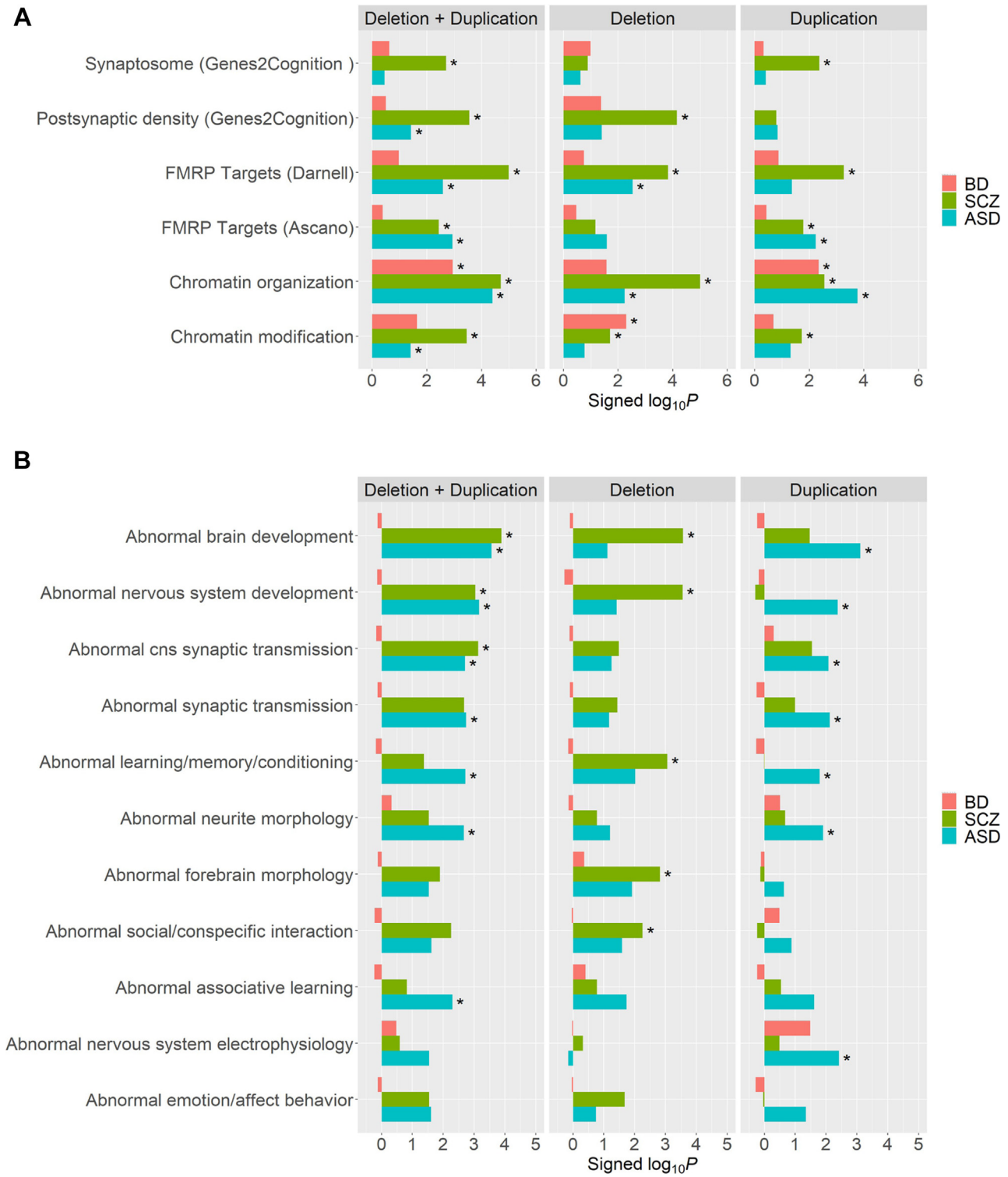

Figure 4. Results of gene set analysis (functional gene sets and mouse gene sets). **(A)** Functional gene sets previously associated with ASD and SCZ: synaptosome and postsynaptic density genes from Genes2Cognition; FMRP target genes from two independent datasets (37,38); chromatin organization and modification genes from previous literature (39,40). Signed $\log_{10}p$ on the horizontal axis represents the $-\log_{10}$ of the p value multiplied by the sign (ln(OR)). Asterisks denote a significant enrichment of CNVs in the gene set (Benjamini-Hochberg false discovery rate $< .05$). **(B)** Mouse gene sets of human orthologs of mouse genes whose disruption causes neurobehavioral and nervous system abnormalities. Asterisks denote a significant enrichment of CNVs (Benjamini-Hochberg false discovery rate $< .05$). ASD, autism spectrum disorder; BD, bipolar disorder; CNV, copy number variation; SCZ, schizophrenia.

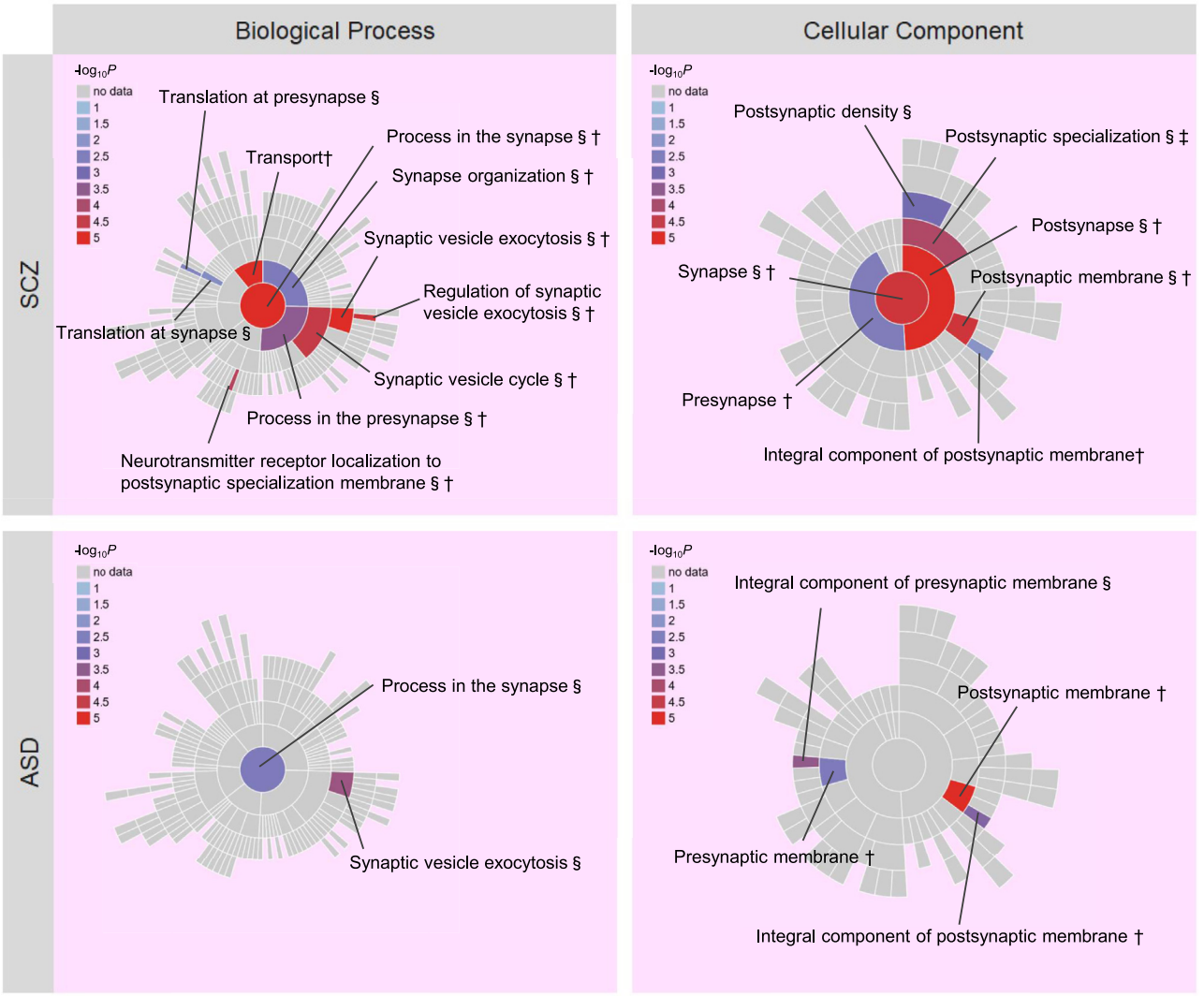

Figure 5. Results of gene set analysis (synapse gene sets: SynGO). In SCZ and ASD, significant gene sets (Benjamini-Hochberg false discovery rate $<$ .05) are visualized in sunburst plots. Plots of bipolar disorder are omitted from the figure because no gene sets were significant. Sunburst plots are a representation of tree structures for biological processes and cellular components. Inner rings of the plot are parent terms of more specific child terms in the outer rings. Color is coded according to *p* values. §Significant in the analysis of deletion+duplication; †significant in the analysis of deletion; ‡significant in the analysis of duplication. ASD, autism spectrum disorder; SCZ, schizophrenia.

and animal models (57,58). In terms of postsynapse, postsynaptic membrane was significant in SCZ/ASD. Alterations of postsynaptic membrane proteins are supported by genetic findings of SCZ/ASD–associated genes (e.g., *NLGN*, *GPHN*) and proteome analysis of brain tissue from patients (59,60).

GO gene set analysis replicated previous findings that SCZ and ASD involve more extensive and similar biological pathways (9,61) (Figure 6A). Among others, substantial overlap was seen in the DNA/chromatin integrity pathway, which includes DNA replication, repair, recombination, and chromatin biology. Experimental evidence supports that dysregulation of these pathways can causally contribute to the pathogenesis (62–64). The defects of the DNA/chromatin integrity pathway may also underlie an increased genome-wide burden of rare or de novo variants in these disorders.

Correlation analysis of gene set results showed not only strong pathway similarities (r = 0.48) between SCZ and ASD, but also weak but significant similarities (r = 0.25–0.31) between BD and SCZ/ASD (Figure 6B). This provides evidence for a shared genetic basis among these disorders, which is consistent with findings from epidemiological studies (1–3). Analyses of genome-wide association study data have reported a high genetic correlation (r = 0.7) between SCZ and BD, but a small correlation between ASD and SCZ (r = 0.21) or between ASD and BD (r = 0.18) (65,66). While the calculation method for correlation differs from that in the present study, it is possible that the cross-disorder effects of common variants (single nucleotide polymorphisms) and rare variants (rare CNVs) may be different. Common variants may have strong cross-disorder effects on SCZ and BD, whereas

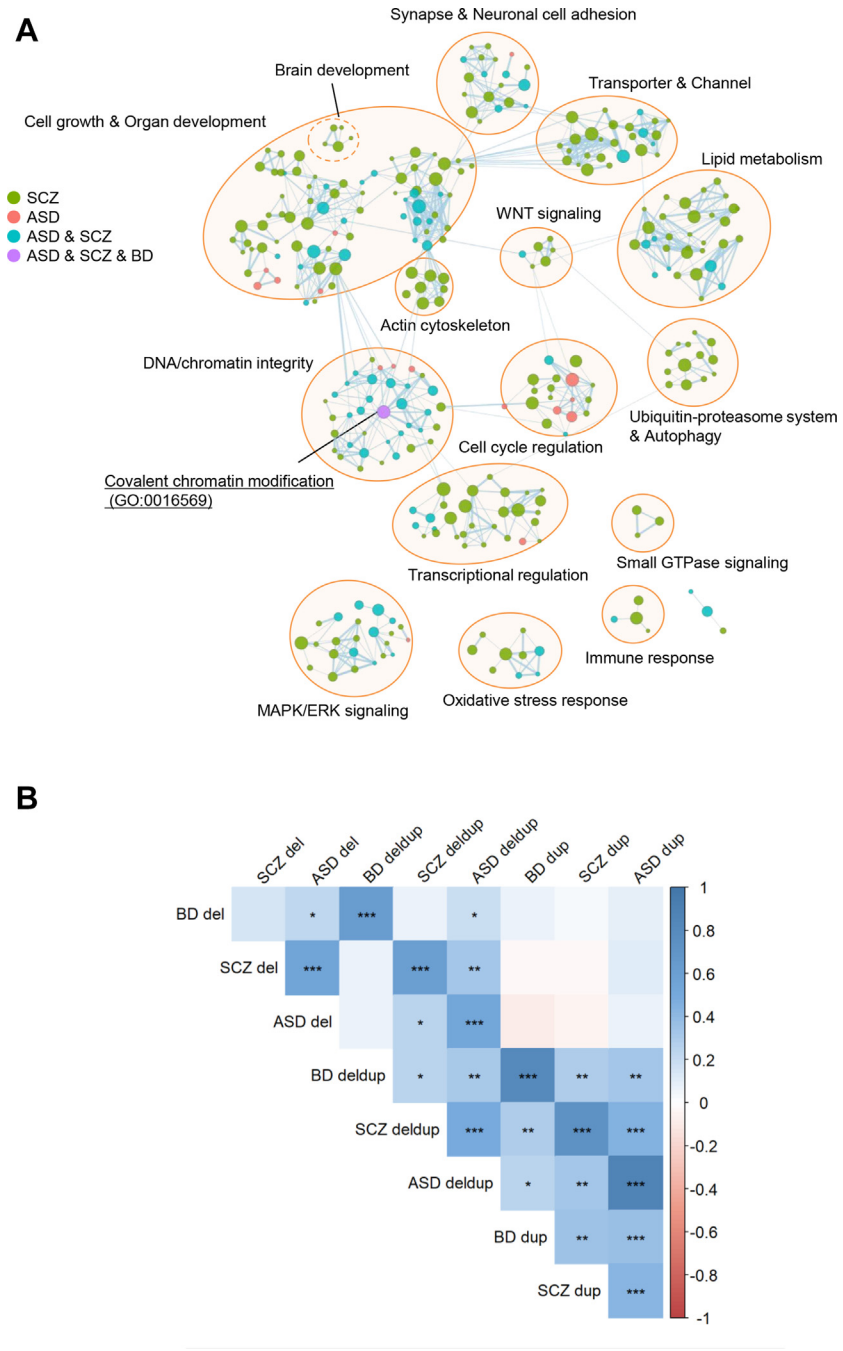

Figure 6. Results of GO gene set analysis and correlation analysis. **(A)** Nodes represent significant gene sets (Benjamini-Hochberg false discovery rate $<$ .05) and are color-coded by diagnosis. These nodes can be broadly classified into 14 biological pathways: DNA/chromatin integrity, transcriptional regulation, cell cycle regulation, synapse/neuronal cell adhesion, transporter/channel, MAPK/ERK signaling, small GTPase signaling, Wnt signaling, cell growth/organ development, actin cytoskeleton, oxidative stress response, ubiquitin-proteasome system/autophagy, immune response, and lipid metabolism. SCZ and ASD share 11 biological pathways, particularly the DNA/chromatin integrity pathway. The purple node is GO:0016569 covalent chromatin modification, which was significant in all 3 disorders. Node size and edge thickness are proportional to the gene set size and the number of genes overlapping between gene sets, respectively. **(B)** Correlation of GO gene set results among the three disorders. Pairwise correlations of the *z* score for each GO gene set were calculated for each copy number variation type and diagnosis. The color of each box indicates the magnitude of the correlation. Correlations significantly different from zero after Bonferroni correction for all pairs of tests are marked with asterisks. $^{*}p_{corrected} < .05$; $^{**}p_{corrected} < .0001$; $^{***}p_{corrected} < .00000001$. ASD, autism spectrum disorder; BD, bipolar disorder; del, deletion; deldup, deletion+duplication; dup, duplication; GO, Gene Ontology; GTPase, guanosine triphosphatase; MAPK/ERK, mitogen-activated protein kinase/extracellular signal-regulated kinase; SCZ, schizophrenia.

rare variants may have strong cross-disorder effects on SCZ and ASD.

In SCZ and ASD, CNVs were significantly enriched in enhancers and promoters in brain tissue. As CNVs in these noncoding regulatory elements affect gene expression (67), they may be implicated in risk through the dysregulation of brain-expressed genes. Previous studies have reported that variants in these regulatory elements play a role in the risk for psychiatric disorders (47,68,69). With some exceptions, deletions in SCZ and duplications in ASD were enriched in regulatory elements (Tables S13a and S13b in the Supplement), which suggests that the effect of these CNVs on gene expression may be reversed in both disorders. While there is strong evidence that SCZ and ASD share genetic commonality, there is also evidence that they have opposite genetic bases (e.g., SCZ is associated with 22q11.2 deletion, whereas ASD is associated with 22q11.2 duplication) (70).

In conclusion, BD and SCZ/ASD differ in terms of CNV burden, characteristics of NDD-CNVs, and regulatory CNVs. On the other hand, they have shared molecular mechanisms,

including chromatin biology. The BD risk genes identified in the present study could provide insight into the pathogenesis of BD.

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