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Archival Report

Contribution of Rare Copy Number Variants to Bipolar Disorder Risk Is Limited to Schizoaffective Cases

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ABSTRACT

BACKGROUND: Genetic risk for bipolar disorder (BD) is conferred through many common alleles, while a role for rare copy number variants (CNVs) is less clear. Subtypes of BD including schizoaffective disorder bipolar type (SAB), bipolar I disorder (BD I), and bipolar II disorder (BD II) differ according to the prominence and timing of psychosis, mania, and depression. The genetic factors contributing to the combination of symptoms among these subtypes are poorly understood.

METHODS: Rare large CNVs were analyzed in 6353 BD cases (3833 BD I [2676 with psychosis, 850 without psychosis, and 307 with unknown psychosis history], 1436 BD II, 579 SAB, and 505 BD not otherwise specified) and 8656 controls. CNV burden and a polygenic risk score (PRS) for schizophrenia were used to evaluate the relative contributions of rare and common variants to risk of BD, BD subtypes, and psychosis.

RESULTS: CNV burden did not differ between BD and controls when treated as a single diagnostic entity. However, burden in SAB was increased relative to controls (p = .001), BD I (p = .0003), and BD II (p = .0007). Burden and schizophrenia PRSs were increased in SAB compared with BD I with psychosis (CNV p = .0007, PRS p = .004), and BD I without psychosis (CNV p = .0004, PRS $p = 3.9 \times 10^{-5}$). Within BD I, psychosis was associated with increased schizophrenia PRSs (p = .005) but not CNV burden.

CONCLUSIONS: CNV burden in BD is limited to SAB. Rare and common genetic variants may contribute differently to risk for psychosis and perhaps other classes of psychiatric symptoms.

Keywords: Bipolar disorder, Copy number variant, Genetics, Polygenic risk score, Rare variant burden, Schizophrenia

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Classically conceptualized as an episodic mood disorder with alternating periods of mania and depression, the diagnosis of bipolar disorder (BD) encompasses heterogeneous clinical presentations that vary with respect to symptomatology (1,2), comorbidity (3), and longitudinal course (4). There are three diagnoses on the BD spectrum in current classifications of mental illness (5,6): bipolar I disorder (BD I), bipolar II disorder (BD II), and schizoaffective disorder bipolar type (SAB). The criteria for these diagnoses differ from one another—and from clinically related diagnoses such as schizophrenia (SCZ) and major depressive disorder—by nuances in the prominence and timing of manic, depressive, and psychotic symptoms that are subject to change across versions of the same system of classification (5,7,8). The factors determining the combination of symptoms that occur in a given patient remain poorly understood.

BD genetic risk is characterized by many common single nucleotide polymorphisms of small effect across the genome (9), many of which also are implicated in clinically related psychiatric conditions (10,11). The overlap between BD and SCZ is particularly high in this regard, with genetic correlation estimates between the two (r_g = .60–.70) comparable to estimates between BD I and BD II (r_g = .70–.80) (9–12). In contrast, rare variants—in particular, rare copy number variants

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(CNVs)—have not been consistently implicated in risk for BD (13–26), unlike in SCZ, where an increased burden of rare CNVs is well established and recurrent risk CNVs have been identified (20,22,27,28). The largest genome-wide study of rare CNVs in BD to date found no differences in burden between approximately 2600 cases and 8800 controls (13). Smaller studies have been inconsistent (21,22,29). For instance, CNV burden in early-onset BD—a focus of BD CNV studies owing to the increased rare CNV burden in neurodevelopmental disorders (25)—has been found by some (15,16,20,26) but not by others (17,21–23). Specific CNVs implicated in SCZ and neurodevelopmental disorders have been tested for association with BD, and a duplication of 16p11.2 implicated in SCZ (30) was recently reported to be enriched in BD (13). Tested as a set rather than individually, these psychiatric CNVs are not significantly enriched in BD (21,22,26), nor have CNVs in BD consistently been found enriched for particular biological pathways or gene sets (15–17,26). In total, the evidence that rare CNVs contribute to BD risk broadly is limited.

There is mounting evidence suggesting that the common alleles conferring risk to BD and SCZ act at the symptom level (31,32), rooting the clinical similarity of BD and SCZ at least partially in common genetic variation. In contrast, the relative absence of rare CNV burden in BD (13) raises the possibility that this class of variation confers risk to clinical phenomena more commonly associated with SCZ. Such phenomena could include both the nuances in the prominence and timing of psychotic symptoms that formally differentiate SCZ and BD diagnostic criteria (5,6), and nondiagnostic features such as differences in cognitive deficits (33) and clinical course that historically formed the basis for the dichotomization of BD and SCZ (34,35). Profiling rare CNVs and common risk alleles in BD cases stratified by granular clinical data would provide the opportunity to more directly test whether these classes of genetic variation make differential contributions to particular psychiatric traits. To our knowledge, such studies are lacking.

Here, we present results on a genome-wide study of rare CNV burden in 6353 BD cases and 8656 controls. In addition, we compare the relative contribution of rare CNVs and common SCZ risk alleles to risk of psychosis, a clinical phenomenon that differentiates BD subtypes from one another and from SCZ.

METHODS AND MATERIALS

Sample Description

The International Cohort Collection for Bipolar Disorder (ICCBD) includes BD cases and unaffected controls from the Sweden Bipolar Disorder Cohort (SWEBIC), the Bipolar Disorder Research Network (BDRN) in the United Kingdom, and the Genomic Psychiatry Consortium (GPC) from the University of Southern California. Full ICCBD sample descriptions have been previously reported in a genome-wide association study (GWAS) (12). The BDRN controls were collected as part of the Wellcome Trust Case Control Consortium; half were used in a genome-wide CNV burden analysis with a set of BD cases not in the current study (22), and the other half were used in a separate genome-wide CNV analysis (13). The subset of the SWEBIC cases and controls genotyped on the Affymetrix platform were in a previous report of genome-wide CNV burden in BD (20). Genome-wide CNV burden has not been reported before for the GPC cohort or for the SWEBIC cases and controls genotyped on the Illumina platform (45% of ICCBD cases in this study).

Phenotyping Methods

SWEBIC clinical data were derived from three primary sources that used a mixture of semistructured interviews, retrospective chart reviews, and standardized rating scales. BDRN cases were assessed using Schedules for Clinical Assessment in Neuropsychiatry. GPC cases were assessed through a combination of focused direct interviews and data extraction from medical records. On the basis of these data, best-estimate lifetime diagnoses were made according to DSM-IV criteria

Table 1. Sample Characteristics for ICCBD CNV Analyses

						BD I					
Cohort	Ethnicity	Chip	Batch[a]	Cases	Controls	All	Psychosis	No psychosis	BD II	SAB	NOS
SWEBIC	European	Affymetrix 6.0	Wave3	0	499	0	0	0	0	0	0
		Affymetrix 6.0	Wave4	917	1170	538	355	162	114	20	245
		Omni Express	Wave6	1356	1140	597	355	199	500	30	229
				0							
BDRN	European	Combo Chip	–	1084	0	677	389	185	361	34	12
		Omni Express	–	1493	0	991	694	161	426	57	19
		Illumina 1.2M	58BC	0	2428	0	0	0	0	0	0
		Illumina 1.2M	NBS	0	2149	0	0	0	0	0	0
				0							
GPC	European American	Omni Express	–	1503	1270	1030	883	143	35	438	0
ICCBD				6353	8656	3833	2676	850	1436	579	505

BD I, bipolar I disorder; BD II, bipolar II disorder; BDRN, Bipolar Disorder Research Network; CNV, copy number variant; GPC, Genomic Psychiatry Consortium; ICCBD, International Cohort Collection for Bipolar Disorder; NOS, not otherwise specified; SAB, schizoaffective disorder bipolar type; SWEBIC, Sweden Bipolar Disorder Cohort.

[a]CNV analyses including Swedish samples from waves 2 to 4 were previously reported in Bergen *et al*. (20). CNV results for SWEBIC controls from all waves were reported in Marshall *et al*. (27). CNV analyses for bipolar disorder using subsets of the BDRN controls were reported in Grozeva *et al*. (22) and Green *et al*. (13). BDRN cases were included in analyses by Green *et al*. (13).

Table 2. CNV Burden in Bipolar Disorder

				Burden					*p* Value						
CNV Type	Size, kb	Frequency	Events	BD	CON	BD I	BD II	SAB	BD CON	BD I CON	BD II CON	SAB CON	BD I SAB	BD II SAB	BD I BD II
Number of CNVs per Individual															
All	100	<1%	10515	0.698	0.702	0.682	0.683	0.800	.855	.345	.753	.036	.016	.025	1.000
		Singleton	1849	0.125	0.122	0.129	0.114	0.131	.532	.274	.588	.318	.562	.274	.276
	500	<1%	1014	0.070	0.066	0.069	0.054	0.111	.353	.545	.423	.001[a]	3×10^{-4}[a]	7×10^{-4}[a]	.145
		Singleton	270	0.020	0.017	0.019	0.015	0.035	.243	.556	.791	.009	.006	.037	.481
Deletions	100	<1%	3970	0.266	0.264	0.251	0.280	0.323	.778	.193	.418	.099	.010	.146	.055
		Singleton	918	0.067	0.057	0.067	0.065	0.070	.021	.057	.348	.141	.282	.686	.884
	500	<1%	231	0.017	0.014	0.016	0.016	0.022	.112	.308	.553	.024	.098	.117	1.000
		Singleton	107	0.009	0.006	0.008	0.008	0.012	.064	.265	.458	.071	.078	.495	1.000
Duplications	100	<1%	6545	0.433	0.439	0.432	0.403	0.477	.722	.714	.241	.441	.258	.068	.328
		Singleton	1442	0.094	0.098	0.098	0.083	0.103	.570	.933	.201	.677	.719	.319	.259
	500	<1%	783	0.053	0.052	0.052	0.038	0.089	.851	.896	.313	.007	.002	.002[a]	.118
		Singleton	226	0.015	0.015	0.015	0.009	0.034	1.000	1.000	.221	.004	.001[a]	.002[a]	.112
Number of Genes Within CNVs per Individual															
All	100	<1%	10515	1.470	1.517	1.476	1.239	1.579	.664	.728	.056	.869	.600	.016	.068
		Singleton	1849	0.333	0.303	0.346	0.272	0.285	.366	.209	.519	.773	.440	.751	.172
	500	<1%	1014	0.402	0.329	0.416	0.227	0.580	.091	.058	.332	.023	.102	.004	.030
		Singleton	270	0.124	0.082	0.120	0.088	0.169	.053	.088	.877	.059	.369	.221	.401
Deletions	100	<1%	3970	0.365	0.318	0.359	0.329	0.437	.056	.179	.783	.026	.393	.062	.592
		Singleton	918	0.132	0.088	0.142	0.107	0.173	.010	.003	.447	.008	.702	.151	.387
	500	<1%	231	0.091	0.048	0.097	0.062	0.161	.010	.007	.560	.001[a]	.241	.054	.449
		Singleton	107	0.052	0.021	0.052	0.033	0.131	.015	.015	.542	7×10^{-4}[a]	.018	.106	.597
Duplications	100	<1%	6545	1.105	1.199	1.117	0.910	1.142	.285	.373	.018	.843	.864	.106	.054
		Singleton	1442	0.271	0.277	0.279	0.208	0.259	.868	.953	.130	.798	.817	.256	.118
	500	<1%	783	0.312	0.281	0.319	0.165	0.420	.449	.365	.194	.185	.254	.009	.021
		Singleton	226	0.106	0.080	0.103	0.056	0.167	.245	.276	.470	.052	.257	.033	.155
Total Distance (kb) Covered by CNVs per Individual															
All	100	<1%	10515	388	372	391	349	467	.100	.100	.531	.001[a]	.003	.001[a]	.095
		Singleton	1849	372	327	381	314	424	.068	.064	.718	.124	.425	.083	.150
	500	<1%	1014	935	911	942	839	984	.602	.570	.549	.403	.627	.127	.313
		Singleton	270	1075	955	1171	869	930	.345	.157	.556	.918	.352	.602	.175
Deletions	100	<1%	3970	274	256	275	258	324	.055	.094	.896	.005	.083	.029	.444
		Singleton	918	322	289	327	286	378	.213	.236	.944	.110	.453	.185	.463
	500	<1%	231	954	938	973	777	1196	.837	.715	.292	.151	.235	.041	.274
		Singleton	107	1069	1052	1162	813	1237	.899	.539	.374	.520	.821	.085	.213
Duplications	100	<1%	6545	379	368	380	337	469	.352	.397	.414	.012	.010	.003	.133
		Singleton	1442	389	344	399	303	500	.149	.138	.458	.063	.137	.002[a]	.083
	500	<1%	783	923	893	928	850	922	.588	.601	.796	.781	.957	.543	.491
		Singleton	226	1096	946	1179	881	910	.317	.191	.799	.899	.442	.666	.262

Burden metrics are presented for the following groups (*n*): BD treated as a single diagnostic entity (6353), CON (8656), BD I (3833), BD II (1436), and SAB (579). Three classes of CNV burden were assessed, indicated by the description above the dotted lines. Burden was compared between cases and controls as well as between case subtypes. The *p* values are two sided, uncorrected for multiple testing, and based on 10,000 permutations testing for relative burden between the two groups. CNV type, size, and frequency refer to the filters applied for the test being reported. Events refers to the total number of CNVs observed in the groups being compared for the specified parameters. Singletons are those CNVs that occur once within the full ICCBD case cohort when filtered for those > 100 kb in size.

BD, bipolar disorder; BD I, bipolar I disorder; BD II, bipolar II disorder; CNV, copy number variant; CON, controls; SAB, schizoaffective disorder bipolar type.

[a]*p* Value surpassing study-wide significance.

and key clinical variables were rated. The intersite reliability of diagnoses was assessed using Fleiss's kappa statistic for multiple raters (κ = .72 for the primary diagnostic variable). Full descriptions of the approaches used in the phenotyping of the ICCBD cohorts have been reported previously (12,36) (see Supplement). For some analyses in this report, clinical variables beyond case-control status were included from all three ICCBD sites, including age of onset, history of psychosis, and

family history. Age of onset was defined as the age at which first symptoms, impairment, or diagnosis occurred. Psychosis was defined as the lifetime presence of hallucinations or delusions. Family history was defined as having any family member with any psychiatric diagnosis. For each variable, a set of standardized numerical values was derived and site investigators harmonized datasets according to these metrics. This was necessary to facilitate analysis across sites that used different phenotyping approaches.

Genotyping and Ancestry Covariates

Sample collection and genotyping procedures for the ICCBD have previously been reported (12). In brief, for all ICCBD sites, DNA was extracted from peripheral blood samples that had been collected and stored at −20°C. Samples were then genotyped at the Broad Institute (Cambridge, MA), and genotypes were called using either Birdsuite (Affymetrix, Santa Clara, CA) or BeadStudio (Illumina, San Diego, CA). Ancestry covariates were derived from the genotyping data through multidimensional scaling analysis on genome-wide identity-by-descent distances calculated for all pairs of individuals. Quality control procedures implemented to derive the genotype calls used are detailed in an earlier GWAS of this cohort (12).

CNV Calling and Quality Control

Rare CNVs were identified using the Birdseye program in Birdsuite (37). Only subjects who passed quality control filters in an earlier GWAS of the same individuals (12) were considered for CNV analyses. CNVs were excluded if any of the following criteria were met: logarithm of the odds ratio (OR) score < 10, number of probes < 10, probe density of < 1 per 20 kb, frequency in ICCBD $> 1\%$, or location within a region known to contain common CNVs or large genomic gaps (e.g., centromeres). If in a given individual the distance between two CNVs was less than 20% of their combined size, they were considered artificially split by the calling algorithm and combined into a single event. For the BDRN cohort, only genomic regions covered in both cases and controls were retained, in order to reduce batch effects resulting from cases and controls being genotyped on different Illumina arrays (see Supplement and Supplemental Figure S1). Subjects were removed for having a total CNV number > 2 SDs different from the mean number of CNVs in the cohort (prior to applying filters for CNV frequency). Unless otherwise specified, burden analyses were restricted to autosomal CNVs > 100 kb. Two events were considered equivalent for the purposes of defining frequency if one overlapped the other by at least 50%. Quality control checks were performed separately for the SWEBIC Affymetrix, SWEBIC Illumina, BDRN, and GPC cohorts (Table 1). In the context of burden analyses, we use the term CNV to refer to the combined set of deletions and duplications, and a singleton CNV was defined as any event that occurred once in the full ICCBD case-control cohort without consideration of whether the event was a deletion or a duplication. Singleton deletions and duplications were defined after first filtering the dataset for that type of event. As such, not all singleton deletions and duplications are in the singleton CNV group.

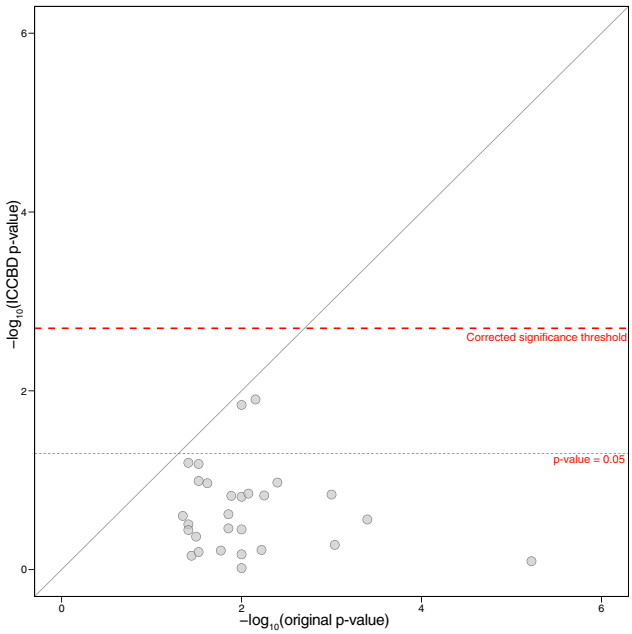

Figure 1. Replication of previous reports of copy number variant burden in bipolar disorder. Curation of literature on copy number variant burden in bipolar disorder identified 36 instances where nominal association ($p < .05$) was reported. We were able to test 28 of these in the International Cohort Collection for Bipolar Disorder (ICCBD). Plotted here are *p* values in previous reports (x-axis) compared with the same test performed in the ICCBD cohort (y-axis). There were four tests for which nominal significance was observed in the ICCBD data: 1) singleton deletions > 100 kb in cases compared with controls, 2) proportion of individuals with a singleton deletion > 100 kb in cases compared with controls, 3) singleton deletions > 100 kb in early onset cases compared with controls, and 4) proportion of individuals with a singleton deletion > 100 kb in early onset cases compared with controls. None of these observations surpassed multiple test correction for the 27 tests we followed up in our data.

CNV Burden Tests

For our primary CNV burden tests, we defined CNV burden in three ways: the number of CNVs occurring per individual (the CNV number), the number of genes overlapped by CNVs per individual (the CNV gene count), and the combined genomic length of all CNVs. We elected to focus on these three classes of burden because there is no clear class of burden most relevant to BD and these classes significantly differ between SCZ cases and controls (27). We stratified CNVs by three types: deletions only, duplications only, and deletions and duplications (or CNVs); by two sizes: > 100 kb and > 500 kb; and by two frequencies: singletons (a frequency of 6.7×10^{-5}) and those occurring in less than 1% in the ICCBD. This led to 36 tests between each of seven pairs of phenotypes we compared: 1) BD cases with controls, 2) BD I cases with controls, 3) BD II cases with controls, 4) SAB cases with controls, 5) BD I cases with BD II cases, 6) BD I cases with SAB cases, and 7) BD II cases with SAB cases. Thus, a total of 252 tests comprised our primary assessment of CNV burden.

Previous studies of CNV burden in BD have reported significant results for tests where the definition of burden fell outside

Table 3. Follow-up to Previous Reports of Increased Copy Number Variant Burden in BD

Test Parameters					Original Report				ICCBD		
Comparison	Freq	Type	Burden Definition	Size, kb	Authors	*N*	Ratio	*p* Value	*N*	Ratio	*p* Value
BD/CON	<1%	CNV	Gene set enrichment: "psychological disorders"	≥100	Zhang *et al.*, 2009 (15)	998/1001	NR	6×10^{-6}	6353/8656	0.90	.806
BD/CON	<1%	CNV	Gene set enrichment: "behavior (learning)"	≥100	Zhang *et al,* 2009 (15)	998/1001	NR	6×10^{-3}	6353/8656	1.04	.603
BD/CON	<1%	CNV	Number of CNVs per individual	≥100	McQuillin *et al.*, 2011 (21)[a]	500/500	0.82	.0360	6353/8656	1.01	.700
BD/CON	<1%	CNV	Number of CNVs per individual	≥1000	Grozeva *et al.*, 2013 (23)	1650/10259	0.58	.0100	6353/8656	0.84	.962
BD/CON	<1%	CNV	Number of de novo CNVs per individual	≥10	Malhotra *et al.*, 2011 (16)	185/426	4.78	.0090	NA	NA	NA
BD/CON	<1%	CNV	Number of de novo CNVs per individual	≥10	Georgieva *et al.*, 2014 (17)	768/3208	2.15	.0007	NA	NA	NA
BD/CON	<1%	DEL	Number of genes hit by CNVs per individual	≥500	Bergen *et al.*, 2012 (20)[b]	834/2087	2.12	.0390	5436/6987	1.73	.064
BD/CON	<1%	DEL	Number of CNVs per individual	≥100	Grozeva *et al.*, 2010 (22)	1697/2806	0.83	.0100	6353/8656	1.01	.355
BD/CON	<1%	DEL	Number of CNVs per individual	≥1000	Grozeva *et al.*, 2013 (23)	1650/10259	0.44	.0300	6353/8656	1.45	.102
BD/CON	<1%	DEL	Number of CNVs per individual	100–200	Grozeva *et al.*, 2010 (22)	1697/2806	0.83	.0300	6353/8656	0.98	.636
BD/CON	<1%	DEL	Number of CNVs per individual	200–500	McQuillin *et al.*, 2011 (21)[a]	500/500	0.64	.0390	6353/8656	1.04	.312
BD/CON	SING	CNV	Number of genes hit by CNVs per individual	≥100	Bergen *et al.*, 2012 (20)[b]	834/2087	1.37	.0320	5436/6987	1.00	.428
BD/CON	SING	DEL	Average size of genic CNVs	NR	Priebe *et al.*, 2012 (26)	882/872	1.90	.0140	6353/8656	1.04	.346
BD/CON	SING	DEL	Proportion of individuals with a CNV	≥100	Zhang *et al.*, 2009 (15)	998/1001	1.33	.0070	6353/8656	1.17	.012
BD/CON	SING	DEL	Number genes hit by CNVs per individual	≥100	Bergen *et al.*, 2012 (20)[b]	834/2087	1.93	.0100	5436/6987	1.24	.153
BD/CON	SING	DEL	Number of CNVs per individual	≥100	Zhang *et al.*, 2009 (15)	998/1001	1.38	.0100	6353/8656	1.17	.014
BD/CON	SING	DEL	Number of CNVs per individual	≥100	Bergen *et al.*, 2012 (20)[b]	834/2087	1.35	.0130	5436/6987	1.12	.150
BD/CON	SING	DEL	Total size of genic CNVs	NR	Priebe *et al.*, 2012 (26)	882/872	1.84	.0140	6353/8656	1.07	.240
BD/SCZ	<1%	CNV	Number of de novo CNVs per individual	≥100	Georgieva *et al.*, 2014 (17)	768/1115	0.51	.0150	NA	NA	NA
BD/SCZ	<1%	DEL	Number of exonic CNVs per individual	≥1000	Green *et al.*, 2016 (13)	2591/6882	0.40	.0009	NA	NA	NA
BD/SCZ	<1%	DUP	Number of exonic CNVs per individual	500–1000	Green *et al.*, 2016 (13)	2591/6882	0.80	.0450	NA	NA	NA
EO/CON	<1%	CNV	Proportion of individuals with a genic CNV	NR	Priebe *et al.*, 2012 (26)	291/872	1.26	.0009	2235/8656	0.96	.529
EO/CON	<1%	CNV	Number of de novo CNVs per individual	≥10	Malhotra *et al.*, 2011 (16)	107/426	6.22	.0060	NA	NA	NA
EO/CON	<1%	DUP	Proportion of individuals with a genic CNV	NR	Priebe *et al.*, 2012 (26)	291/872	1.34	.0004	2235/8656	0.97	.275
EO/CON	<1%	DUP	Number of genic CNVs per individual	NR	Priebe *et al.*, 2012 (26)	291/872	NR	.0240	2235/8656	1.00	.108
EO/CON	SING	CNV	Proportion of individuals with a CNV	≥100	Zhang *et al.*, 2009 (15)	NR/1001	1.17	.0390	2235/8656	1.05	.361
EO/CON	SING	CNV	Number of genic CNVs per individual	NR	Priebe *et al.*, 2012 (26)	291/872	NR	.0450	2235/8656	1.14	.251
EO/CON	SING	DEL	Average size of genic CNVs	NR	Priebe *et al.*, 2012 (26)	291/872	2.65	.0056	2235/8656	1.20	.148

Table 3. Continued

Test Parameters					Original Report				ICCBD		
Comparison	Freq	Type	Burden Definition	Size, kb	Authors	*N*	Ratio	*p* Value	*N*	Ratio	*p* Value
EO/CON	SING	DEL	Proportion of individuals with a CNV	≥100	Zhang *et al.*, 2009 (15)	NR/1001	1.58	.0010	2235/8656	1.22	.145
EO/CON	SING	DEL	Number of CNVs per individual	≥100	Zhang *et al.*, 2009 (15)	NR/1001	1.54	.0040	2235/8656	1.25	.106
EO/CON	SING	DEL	Total size of genic CNVs	NR	Priebe *et al.*, 2012 (26)	291/872	2.60	.0084	2235/8656	1.19	.141
EO/CON	SING	DUP	Proportion of individuals with a genic CNV	NR	Priebe *et al.*, 2012 (26)	291/872	1.45	.0170	2235/8656	0.98	.613
EO/CON	SING	DUP	Number of genic CNVs per individual	NR	Priebe *et al.*, 2012 (26)	291/872	NR	.0100	2235/8656	0.97	.675
FAM/CON	<1%	DUP	Number of genic CNVs per individual	≥500	Malhotra *et al.*, 2011 (16)	107/426	1.83	.0300	3072/8656	1.09	.066
SPOR/CON	<1%	CNV	Number of de novo CNVs per individual	≥10	Malhotra *et al.*, 2011 (16)	78/426	4.22	.0190	NA	NA	NA
SPOR/FAM	<1%	CNV	Number of de novo CNVs per individual	≥10	Georgieva *et al.*, 2014 (17)	318/50	0.31	.0390	NA	NA	NA

Listed are all findings identified from manual curation of literature on rare CNV burden in BD with a *p* value below .05 in the original report. Details of the test performed in the original report that was reproduced in the ICCBD are described in the test parameter fields and include the phenotypes compared, the definition of burden, and the filters applied for CNV frequency, type, and size. Ratios were calculated as the burden in the first phenotype in the comparison field relative to the second phenotype. When the original report did not specify the CNV size studied, all CNVs > 100 kb were included in the ICCBD. Original reports where the test included either SCZ cases or BD trios could not be followed up in the ICCBD but are included in this table so as to consolidate all of the previously significant findings in rare CNV studies of BD. BD cases and controls in Bergen *et al.* (20) are part of the ICCBD, so for these follow-up tests only the ICCBD samples not in the original report were used. The controls in Grozeva *et al.* (22) comprise half of the BDRN controls in the ICCBD; because there was no case overlap between these studies, all of these controls were included in the follow-up test in the ICCBD. The cases in Green *et al.* (13) are the BDRN cases in the ICCBD, although these findings were not followed up in the ICCBD because they involved SCZ cases. EO was defined as under 21 years of age in Priebe *et al.* (26) and under 18 years of age in Zhang *et al.* (15) and the ICCBD. Family history in Malhotra *et al.* (16) was defined as having a relative with BD (BD I, BD II, or SAB), schizophrenia, autism, major depressive disorder, or intellectual disability; in the ICCBD, it was defined as having a family member with any psychiatric history. Reported *p* values for the ICCBD are one sided from using 10,000 permutations to test for enrichment in the direction observed in the original report.

BD, bipolar disorder; BD I, bipolar I disorder; BD II, bipolar II disorder; CNV, copy number variant; CON, controls; DEL, deletion; DUP, duplication; EO, early-onset bipolar disorder; FAM, bipolar disorder with a family history of psychiatric illness; Freq, frequency; ICCBD, International Cohort Collection for Bipolar Disorder; *N*, number of individuals for the groups listed in the comparison field; NA, not applicable; NR, not reported; SAB, schizoaffective disorder bipolar type; SCZ, schizophrenia; SING, singleton; SPOR, sporadic bipolar disorder (i.e., no family history of psychiatric illness); SWEBIC, Sweden Bipolar Disorder Cohort.

[a]Sample sizes approximated from earlier studies of the same cohort.

[b]This study included the ICCBD SWEBIC Affymetrix cohort; thus, our replication test excluded this cohort.

the scope of these 252 tests. Manual curation of the literature identified 34 nominal associations at a *p* value of less than .05 in the original report. We were able to follow up 27 of these in the ICCBD (for the other 7, the original study included either SCZ cases or BD parent–child trios), of which 21 were not in our primary 252 tests. For these tests, if applicable, we excluded ICCBD samples overlapping those in the original report.

We also tested ICCBD CNVs (size > 100 kb, frequency < 1%) for enrichment of three sets of CNVs previously identified in studies of BD, SCZ, or neurodevelopmental disorders. The BD CNV set (16 deletions and 14 duplications) was composed of autosomal de novo CNVs reported in three previous studies of BD trios (16,17,24). The SCZ CNV set (11 deletions and 8 duplications) was composed of autosomal CNVs with suggestive evidence for association in a meta-analysis of more than 20,000 SCZ cases and 20,000 controls (27). The neurodevelopmental CNV set (27 deletions and 18 duplications) was from a list curated for a previous report (17) after removing those overlapping the SCZ set. For a CNV in the test set to be considered overlapping with an ICCBD CNV, the ICCBD CNV was required to cover at least 50% of the test CNV and be of the same CNV type (i.e., deletion or duplication).

All tests were performed using permutation in PLINK (38) while controlling for genotyping platform and ICCBD site. Significance was evaluated using 10,000 permutations. The 252 tests in the primary assessment were two sided with the exception of six tests that had previously been reported as significant. A one-sided test in the direction of the association reported in the original article was used for these six tests as well as for the additional 21 tests following up previous associations and the three tests of CNV sets.

Multiple Test Correction for CNV Burden Tests

In the genome-wide CNV burden analyses described above, there are a total 276 tests (252 tests in our primary assessment of CNV burden, 21 tests of previous associations, and three tests of CNV sets). The empirical tests performed in PLINK as described above were controlled for multiple testing using the false discovery rate estimation method of

Benjamini–Hochberg (39) implemented in R using the p.adjust() function. Using a false discovery rate of 5%, tests with empirical p values less than .002 were considered study-wide significant.

Contribution of CNV Burden and SCZ Polygenic Risk Scores to Psychosis

Following results from our primary burden analyses, we analyzed CNV burden and loading of common SCZ risk alleles in BD I and SAB cases. BD II was excluded from these analyses to remove effects resulting from known differences in polygenic loading of SCZ alleles across BD subtypes (12). For these analyses, burden was defined as the number of CNVs > 500 kb and present in less than 1% of the study sample. We focused on this particular burden class because it was the only class in our primary 252 tests where an increase was seen in SAB compared with controls, BD I, and BD II (see Results). For these analyses, burden was tested using logistic regression, which returned similar results to permutation but allowed us to include in the model continuously distributed ancestry covariates and facilitated the calculation of ORs for CNV burden (27). In the regression model, we used phenotype status as the dependent variable and CNV burden as an independent predictor variable. The OR was calculated as the exponential of the logistic regression coefficient, and OR > 1 represents increased risk for the affected phenotype in the model, which was designated to be the phenotype more clinically similar to SCZ. Using a similar regression model, we carried out polygenic scoring analyses (40). Quantitative polygenic risk scores (PRSs) were computed for all cases based on the set of single nucleotide polymorphisms with p values less than .50 in the second SCZ GWAS from the Psychiatric Genomics Consortium (41). PRS analyses excluded ICCBD samples present in the Psychiatric Genomics Consortium studies. We calculated the proportion of variance explained (Nagelkerke's R^2) by SCZ PRSs by subtracting the Nagelkerke's R^2 attributable to covariates alone from the Nagelkerke's R^2 for PRSs plus covariates. Effect sizes for both CNV burden and SCZ PRSs were calculated as a t statistic that is the ratio of the coefficient of the burden or PRS variable and its standard error from a generalized linear regression model equation. Because studies of SCZ have consistently demonstrated higher CNV burden in cases compared with controls (27,28), cases were stratified by clinical dimensions related to SCZ (i.e., psychosis), and one-sided statistical tests were used in evaluating for higher rates in groups with the more SCZ-like phenotype.

Power Calculations

We calculated power for tests of CNV burden in BD compared with controls as well as between subtypes of BD stratified by psychosis. Specifically, calculations were performed for the three primary classes of burden assessed in BD compared with controls and for the one class of burden assessed in our analyses of psychosis. Effect sizes ranging from 1 to 2.5 (by increments of 0.01) were used in the power calculations. To account for the possibility of allele frequency differences between cohorts, the effect size in the power calculation was divided by the standard error from the burden test.

RESULTS

CNV Burden in BD

We assessed genome-wide differences in rare CNV burden between 6353 BD cases and 8656 controls (Table 1). After initial filters for size (>100 kb) and frequency (occurring in <1% of ICCBD individuals), we observed 10,515 CNVs (3970 deletions and 6545 duplications). No difference in the CNV number was found between cases and controls (case rate = .698, control rate = .702, p = .86). This was true for both deletions (case rate = .266, control rate = .264, p = .78) and duplications (case rate = .433, control rate = .439, p = .72). Similarly, no differences were observed between cases and controls with respect to the number of genes hit or the combined genomics length of all CNVs (Table 2 and Supplemental Table S1). We calculated power to detect differences in these three burden classes across a range of effect sizes (see Methods and Materials). Assuming effect sizes reported for SCZ (27), power of 100% was attained to identify differences between BD and controls for the number of genes hit and the combined genomic length of all CNVs per individual, while 33% power was attained for the number of CNVs per individual. Following previous literature showing that rarer and larger CNVs carry increased burden for neuropsychiatric illness (28), we further filtered CNVs by size (>500 kb) and frequency (those that occur once in the 15,009 ICCBD individuals, a frequency of 6.7×10^{-5}). No burden in these classes was observed below our study-wide p-value threshold (Table 2 and Supplemental Table S1). Similarly, manual curation of the literature identified 21 additional associations of BD and CNV burden ($p < .05$ in the initial report) that we followed up here (Methods and Materials), none of which withstood correction for multiple tests (Figure 1 and Table 3). Sets of CNVs previously implicated in neuropsychiatric disorders (Methods and Materials) were also not enriched for deletions or duplications in BD compared with controls.

BD is a heterogeneous disorder clinically, and a previous report of common variation in this cohort found evidence for genetic heterogeneity between clinical subtypes of BD (12). This information, combined with CNV burden being a well-established component of SCZ genetic architecture (27), led us to hypothesize that increased CNV burden may be present in the BD subtypes most clinically similar to SCZ. To test this hypothesis, we first sought to determine whether CNV burden differed between BD subtypes (BD I n = 3833, BD II n = 1436, SAB n = 579) and controls (n = 6383) as well as between BD subtypes and one another. Increased burden was seen in SAB compared with controls in all three of the primary burden classes evaluated as well as compared with both BD I and BD II (Table 2). For one burden class, number of CNVs with size > 500 kb and frequency < 1%, SAB had higher burden compared with controls (p = .001), BD I ($p = 3 \times 10^{-4}$) (Figure 2A), and BD II ($p = 7 \times 10^{-4}$). Therefore, we elected to focus downstream CNV analyses on this class of burden.

Contribution of CNV Burden and SCZ PRSs to Psychosis in BD

SCZ is the archetypal psychotic illness in current psychiatric classification systems (5), and increased CNV burden is a well-

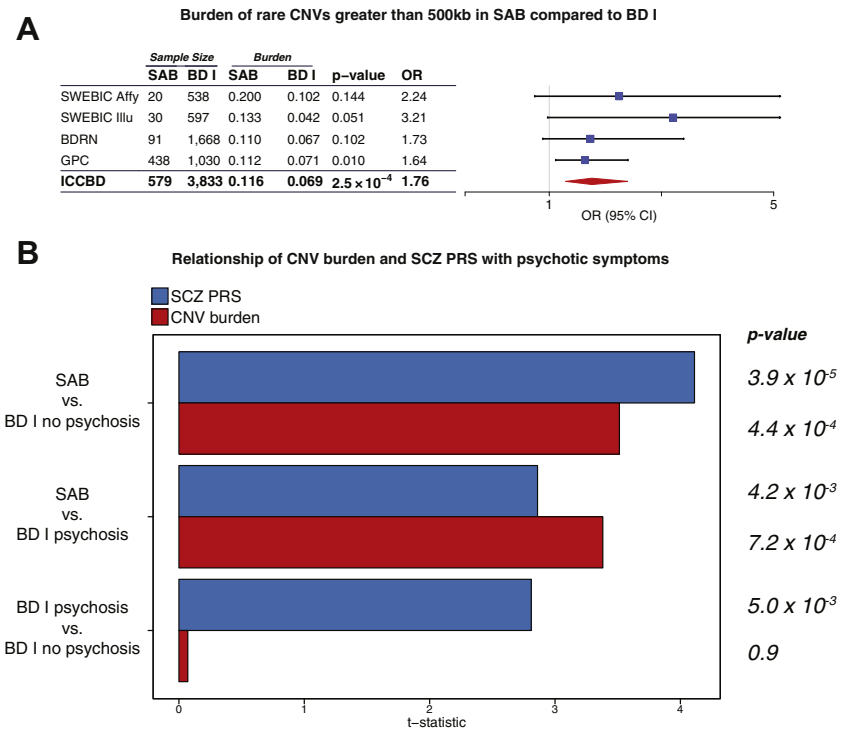

Figure 2. Burden of rare copy number variants (CNVs) (frequency $< 1\%$) $>$ 500 kb in schizoaffective disorder bipolar type (SAB) compared with bipolar I disorder (BD I). **(A)** Forest plot of CNV burden partitioned by site of collection, with the full International Cohort Collection for Bipolar Disorder (ICCBD) sample at the bottom. CNV burden is calculated by combining CNV deletions and duplications. The *p* values presented here for burden tests used a logistic regression model predicting SAB–BD I status by CNV burden along with covariates. The odds ratio (OR) is the exponential of the logistic regression coefficient, and OR > 1 predicts increased SAB risk. **(B)** Comparison of BD and SAB with one another with respect to polygenic risk scores (PRSs) and CNV burden. Regression analyses were performed of phenotype (stratified by history of psychosis) on PRSs derived from a previous genome-wide association study for schizophrenia (SCZ) (blue [upper bars]) and burden of CNVs with frequency less than 1% and size $>$ 500 kilobases (red [lower bars]). Multidimensional scaling components, study site, and gender were used as covariates. The *t* statistic plotted on the x-axis is the ratio of the coefficient of the PRS or CNV burden variable and its standard error from the generalized linear model regression equation. The direction of the plotted bars indicates higher CNV burden or PRSs in the phenotype listed first in the y-axis label. The *p* values for whether PRSs or CNV burden differed significantly between phenotypes are shown at the far right. The Nagelkerke's R^2 values for the corresponding PRS comparisons were .004 for SAB vs. BD I with psychosis, .011 for SAB vs. BD I without psychosis, and .003 for BD I with psychosis vs. BD I without psychosis. Affy, Affymetrix; BDRN, Bipolar Disorder Research Network; CI, confidence interval; GPC, Genomic Psychiatry Consortium; Illu, Illumina; SWEBIC, Sweden Bipolar Disorder Cohort.

established component of its genetic architecture (27,28). Psychosis is also a prominent component of BD, and the diagnostic criteria differentiating BD subtypes (e.g., BD I, SAB) from one another and from SCZ relate to the co-occurrence of psychosis with mania (5,6). The observed CNV burden in SAB, a diagnosis that requires most of the criteria of SCZ to be met, being absent in BD as a whole, prompted inquiry into whether CNV burden contributes to psychosis or to nondiagnostic clinical phenomena that differentiate SAB from other BD subtypes and whether the same pattern is seen for common SCZ risk alleles. We stratified the ICCBD cases by the prominence of psychotic symptoms, correlating psychosis risk with both the CNV burden and SCZ PRS (12,32). Cases were stratified into SAB ($n = 579$), BD I with psychosis ($n = 2676$), and BD I without psychosis ($n = 850$). CNV burden was increased in SAB compared with BD I with and without psychosis (SAB rate = .116; BD I with psychosis rate = .069, $p = 7.21 \times 10^{-4}$; BD I without psychosis rate = .067, $p = 4.42 \times 10^{-4}$), but no difference was observed between BD I with psychosis and BD I without psychosis ($p = .88$) (Figure 2B and Supplemental Figure S2). SCZ PRSs were higher in SAB compared with BD I with psychosis (Nagelkerke's $R^2 = .004$, $p = .004$) and in BD I with psychosis compared with BD I without psychosis (Nagelkerke's $R^2 = .003$, $p = .005$) (Figure 2B). We calculated the power to detect differences in CNV burden between these cohorts across a range of effect sizes (Supplemental Figure S3). At the effect size observed in the comparison of SAB with controls (OR = 1.58), a nominally significant difference could be detected with 81% power between SAB and BD I with psychosis, 53% power between SAB and BD I without psychosis, and 84% between BD I with psychosis and BD I without psychosis.

DISCUSSION

We observed no differences in the genome-wide burden of rare large CNVs in a cohort of 6353 BD cases and 8656 controls. Furthermore, we did not find strong support for any previously reported BD CNV burden associations despite reproducing original analyses with respect to phenotypes compared and the cutoffs for CNV size and frequency used in quality control procedures. Taken together, the case-control analyses presented here confirm in a well-powered cohort that rare CNV burden is not a feature of BD when treated as a single diagnostic entity.

Individuals with a diagnosis of BD comprise a clinically heterogeneous group, and the lack of CNV burden when BD is treated as a single diagnostic entity does not preclude a role of CNV burden in the pathogenesis of subsets of cases. Specifically, we hypothesized that this may be the case for individuals who present with psychotic symptoms in the absence of a major mood episode given the known CNV burden in SCZ (27,28) and the clinical overlap between SCZ and BD. Indeed, we found that cases with SAB, who by definition experience psychosis in both the presence and absence of mania, have higher rates of large rare CNVs compared with controls and other BD subtypes. The class of burden with the strongest signal genome-wide in SCZ

compared with controls is the number of genes hit by deletions per individual (27). We observed this to also be the case in SAB compared with controls (Table 2).

The diagnostic criteria differentiating BD I with psychosis, SAB, and SCZ from one another relate to the prominence and timing of psychotic symptoms. Through deeper analyses comparing SAB and BD I, however, we found that CNV burden was unrelated to the presence of psychosis. This was in contrast to SCZ PRSs, which were increased in the phenotypes characterized by more prominent psychosis. Taken together, these results suggest that common variants may contribute to psychotic symptoms, whereas rare CNVs may contribute to dimensions of illness that differentiate psychotic illnesses from one another. One possibility in this regard is that CNVs may influence risk for cognitive deficits, which are more prominent in SCZ compared with BD (33). Another possibility is that CNV burden increases risk for spontaneous psychosis (i.e., the psychoses of SCZ and SAB) but not for psychosis secondary to severe mental stress, which some argue is the mechanism underlying psychosis during mania. Alternatively, it is possible that compared with the persistent psychosis seen in SAB, the psychoses of mania and/or depression are rated less reliably. Future studies with deeper phenotyping should aim to test these and other hypotheses.

This study has important limitations. Diagnostic misclassification of SCZ cases with SAB is possible and, while unlikely, could account for the observed PRS and CNV results. For some of these analyses, sample size is an important consideration, and we emphasize that these findings must be followed up in larger cohorts. If replicated, they would provide support for the notion that different classes of genetic variants contribute to different classes of symptomatology in mood and psychotic syndromes. It might then be fair to inquire whether the higher CNV burden in SCZ compared with BD may be evidence not that they comprise two biologically distinct disease entities, but rather that clinicians are more likely to diagnose SCZ when a particular clinical phenomenon is present (e.g., cognitive deficits, spontaneous psychosis). These unresolved questions highlight the need for a multiscale approach to the study of mental illness, whereby integrating high-dimensional molecular and clinical data from each patient at the scale that GWASs have shown can be achieved may facilitate the development of a data-driven taxonomy.

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