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Exome sequencing in bipolar disorder identifies *AKAP11* as a risk gene shared with schizophrenia

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We report results from the Bipolar Exome (BipEx) collaboration analysis of whole-exome sequencing of 13,933 patients with bipolar disorder (BD) matched with 14,422 controls. We find an excess of ultra-rare protein-truncating variants (PTVs) in patients with BD among genes under strong evolutionary constraint in both major BD subtypes. We find enrichment of ultra-rare PTVs within genes implicated from a recent schizophrenia exome meta-analysis (SCHEMA; 24,248 cases and 97,322 controls) and among binding targets of CHD8. Genes implicated from genome-wide association studies (GWASs) of BD, however, are not significantly enriched for ultra-rare PTVs. Combining gene-level results with SCHEMA, *AKAP11* emerges as a definitive risk gene (odds ratio (OR) = 7.06, $P = 2.83 \times 10^{-9}$). At the protein level, AKAP-11 interacts with GSK3B, the hypothesized target of lithium, a primary treatment for BD. Our results lend support to BD's polygenicity, demonstrating a role for rare coding variation as a significant risk factor in BD etiology.

BD is a heritable neuropsychiatric disorder characterized by episodes of mania and, oftentimes, episodes of depression. BD has a lifetime prevalence of 1–2% in the population, often with onset in early adulthood. BD is a chronic condition that affects individuals across their lifespan and is an important source of disease burden worldwide[1]. Meta-analysis of 24 twin studies estimated the broad-sense heritability of BD to be ~67%[2], whereas recent molecular genetic analyses estimated the additive heritable component from common single-nucleotide polymorphisms (SNPs) (minor allele frequency (MAF) > 1%) to be between 17% and 23%[3]. This difference between twin-based heritability estimates of BD and additive heritability tagged by common SNPs indicates that a large fraction of genetic risk is still undiscovered. The discrepancy in variance explained likely originates from a variety of sources, including copy-number variants (CNVs), heterogeneity in phenotype and diagnosis and rare, often deleterious genetic variants of more recent origin[4,5]. Each of these sources of variation are excluded from common variant-based estimates of heritability.

Rare variation, including CNVs and PTVs, has been shown to influence risk for BD, albeit to a lesser degree than other neuropsychiatric illnesses such as schizophrenia and autism spectrum disorders (ASDs)[6,7]. Negative selection on BD would cause high-penetrance alleles to be held at low frequency in the population[8,9]. In a large Swedish birth cohort, males and females with BD have a lower reproductive rate compared to their unaffected siblings (0.75:1 and 0.85:1, respectively)[10]. The reduction in offspring observed in BD, however, is markedly more modest than observed for individuals with schizophrenia (0.23 for males and 0.47 for females) or autism (0.25 for males and 0.48 for females), suggesting that the role of rare variation is likely to be smaller in magnitude, as selection is not acting as strongly on BD in aggregate. These conclusions are tempered by uncertainty about the consistency in reduced fecundity over human evolutionary history. Nevertheless, the interrogation of rare variation in patients with BD will be pivotal in the discovery of variants with high penetrance for BD risk.

Within BD, two clinical subtypes are recognized: bipolar I disorder (BD1) and bipolar II disorder (BD2) (American Psychiatric Association DSM-IV (ref. [11]); World Health Organization ICD-10 (ref. [12])). BD1 diagnosis necessitates at least one manic episode, although at least one depressive episode is usually present. Psychotic symptoms may occur during the manic and/or depressive episodes. In contrast, a BD2 diagnosis requires at least one depressive episode and one hypomanic (but not manic) episode across the lifetime. In addition, the DSM-5 includes schizoaffective disorder bipolar type as a subtype of schizoaffective disorder. Patients with schizoaffective disorder exhibit psychotic symptoms concurrent with a major

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mood episode and depressed mood. For a diagnosis of schizoaffective disorder bipolar type, a manic episode must constitute part of the presentation[13–15]. Despite distinct diagnostic categories, genetic susceptibility for BD from common SNPs has shown strong overlap with schizophrenia (genetic correlation $r_g = 0.70$) and major depressive disorder ($r_g = 0.35$), with BD1 showing preferential overlap with schizophrenia and BD2 with major depressive disorder, reflecting a broad continuum of genetic influence on psychosis and mood disturbance[3].

To date, GWAS meta-analysis of common SNPs has identified 64 independent loci that contribute to BD susceptibility, implicating genes encoding ion channels, neurotransmitter transporters and synaptic and calcium signaling pathways[3,5]. Evidence of rare variation in BD risk, however, remains inconclusive, as sample sizes are substantially smaller than GWASs. Analysis of large rare CNVs (MAF < 1%) in 6,353 patients with BD found CNV enrichment among schizoaffective disorder bipolar type over both controls and other BD diagnoses, suggesting that increased risk among detectable rare CNVs is restricted to individuals with psychotic symptoms[6]. Analysis of whole-exome and genome sequencing of pedigree and case–control cohorts has shown only nominal enrichment among individual genes and candidate gene sets[16–19].

Here, we report results from the BipEx collaboration, the largest whole-exome study of BD to date at the time of analysis, comprising 13,933 patients with BD and 14,422 controls following aggregation, sequencing and quality control (QC).

Results

Curated exome sequence data generation. We combined BD case–control whole-exome sequencing data from 13 sample collections in 6 countries. The aggregated dataset consists of 33,699 individuals, 16,486 of whom have been diagnosed with BD and 17,213 who have no known psychiatric diagnosis (Supplementary Table 1). All sample collections have been previously genotyped for common variant analyses[3]. However, this is the first time that exome sequencing and joint analysis has been performed on these collections. All exome sequencing data were generated using the same library preparation, sequencing platform and joint-calling pipeline (Methods). Following sequencing and joint calling, we ran a series of QC steps to filter out low-quality variants and samples (Supplementary Tables 2 and 3) and restricted the dataset to unrelated individuals of broad continental European ancestry (Methods, Supplementary Note and Supplementary Figs. 1–5). The analysis-ready dataset (Supplementary Table 4) consists of 13,933 individuals with BD (8,238 BD1, 3,446 BD2, 1,288 BD not otherwise specified (which includes disorders with bipolar features that do not meet criteria for any specific BD[11]) and 961 individuals with BD without a finer diagnosis), 277 individuals with schizoaffective disorder and 14,422 controls. We excluded individuals with schizoaffective disorder to obtain more BD-specific results and reduce signals more attributable to schizophrenia.

Significant contribution of rare damaging PTVs to BD risk. To test whether patients with BD carry an excess of damaging coding variants, we analyzed exome-wide burden relative to controls using a logistic regression model controlling for principal components (PCs), sex and overall coding variant burden (Methods). Drawing from previous exome sequencing studies of psychiatric disease[18,20,21], we restricted our analysis to variants with minor allele count (MAC) ≤ 5 (MAF ≅ 0.01%) across the entirety of the dataset. We annotated variants using the Ensembl Variant Effect Predictor (VEP)[22] version 95 and assigned variants to four variant classes: two putatively damaging classes (PTVs and damaging missense variants) and two likely benign annotations (other missense and synonymous variants) (Methods and Supplementary Table 5). After this initial restriction, we observed nominally significant enrichment of damaging missense variation in patients with BD overall and BD2 cases over controls (OR = 1.01, $P = 0.024$ and OR = 1.02, $P = 0.0086$ respectively; Fig. 1b,c), but not of PTVs. However, stepwise filtering of rare PTVs to those not in the nonneurological portion of the Genome Aggregation Database (gnomAD), hereafter referred to as 'ultra-rare variants', and then in constrained genes (defined as pLI ≥ 0.9), showed that case–control PTV enrichment was present once we filtered to high-pLI genes, a finding in line with that from schizophrenia exomes[23] (Fig. 1b,c and Supplementary Fig. 6). This enrichment is consistent across both BD1 and BD2 subtypes (Fig. 1a). A conservative Bonferroni significance threshold (accounting for all analyses in Fig. 1) was set at $P = 0.05/27 \approx 0.0019$. The magnitude of PTV enrichment in BD (OR = 1.11, $P = 5.0 \times 10^{-5}$) was considerably lower than that in schizophrenia (OR = 1.26; Singh et al.[23]), in line with the decreased selective pressure estimated from higher reproductive rates in BD-affected siblings relative to schizophrenia-affected siblings[10].

To attempt to refine the nominally significant damaging missense signal, we sought to further distinguish likely deleterious missense variants from benign missense variants. We annotated variants with a missense deleteriousness predictor, MPC (missense badness, PolyPhen-2 and regional constraint) score[24], which takes into account regional missense constraint, and identified a highly deleterious subset of missense variants (MPC ≥ 2), as recommended[24]. However, upon restriction to this subset of missense variants, we did not observe a significant burden of enrichment at either of the three levels of filtering (MAC ≤ 5, ultra-rare, or ultra-rare in a pLI ≥ 0.9 gene) for either BD1, BD2 or BD (Supplementary Fig. 7). This is likely because the MPC ≥ 2 group accounts for a small proportion of missense variants (Methods).

To try to tease apart the signal of excess ultra-rare PTVs in patients with BD over controls, we examined whether age of first impairment or presence of psychosis stratified ultra-rare PTV burden (Methods). We found no difference in the distribution of ultra-rare PTV burden or carrier status between earlier-onset cases and older-onset cases (Supplementary Table 6; $n = 3{,}134$, minimum P value across 50 Kolmogorov–Smirnov tests was 0.40, minimum P value across 50 Fisher's exact tests was 0.067). Patients with BD with and without psychosis (Supplementary Table 7) displayed significant enrichment of ultra-rare PTV burden in constrained genes ($n = 4{,}214$, OR = 1.12, $P = 0.0018$; $n = 3{,}803$, OR = 1.16, $P = 6.6 \times 10^{-5}$, respectively). There was no significant difference in excess ultra-rare PTV burden between individuals with and without psychosis; a logistic regression of ultra-rare PTV burden in constrained genes on psychosis status was not significant when controlling for BD case status ($P = 0.42$).

Restricting to missense variants, we do not observe a significant signal of enrichment of ultra-rare MPC ≥ 2 variation in patients with BD, in contrast to schizophrenia[23] (Supplementary Fig. 7). However, we did observe nominally significant enrichment of ultra-rare damaging missense variation across both BD subtypes when not filtering to loss-of-function intolerant genes (pLI ≥ 0.9) (Fig. 1b,c; BD: OR = 1.02, $P = 0.0018$; BD1: OR = 1.02, $P = 0.014$; BD2: OR = 1.03, $P = 0.0036$).

Ultra-rare variant burden in tissues and candidate gene sets. Biologically and empirically informed gene sets can refine our understanding of how ultra-rare PTVs confer risk for BD and generate potential biological hypotheses for follow-up analyses. Using the Genotype-Tissue Expression (GTEx) portal[25], we found weak evidence for enrichment of ultra-rare PTVs in 13,372 genes expressed in brain tissues in patients with BD (OR = 1.01, $P = 0.032$), but not in genes expressed in nonbrain tissues (23,450 genes, OR = 1.00, $P = 0.15$). To examine tissue-specific enrichment more broadly, we tested for enrichment of ultra-rare PTVs in 43 GTEx tissues (Finucane et al.[26]; Supplementary Table 8) in tissue-specific expression gene sets (Fig. 2a, Supplementary Fig. 8 and Methods).

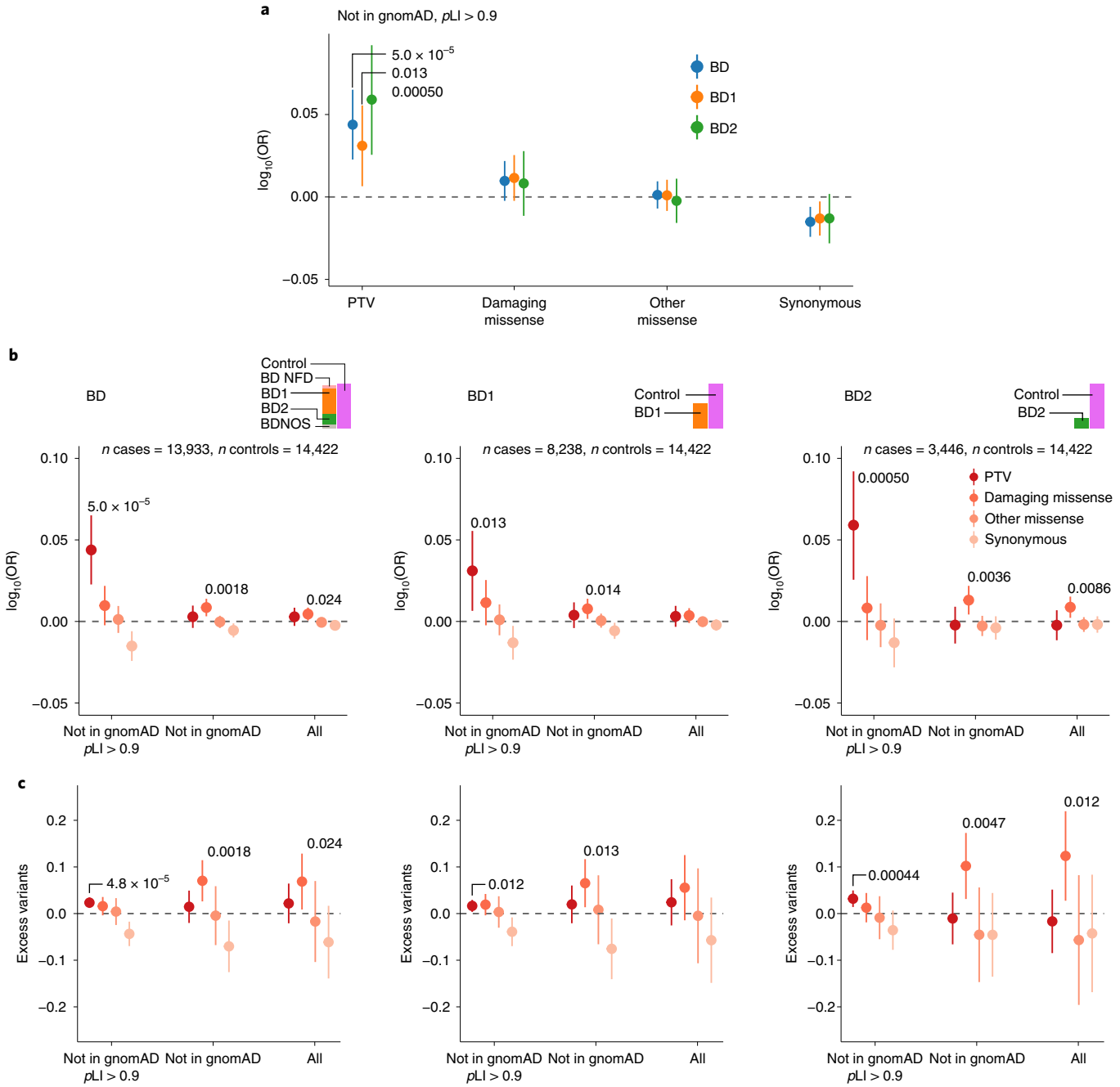

Fig. 1 | Case–control enrichment of ultra-rare variants, split by case status and consequence category. a, Enrichment in cases over controls ($n = 14{,}422$) in case subsets (BD, $n = 13{,}933$; BD1, $n = 8{,}238$; BD2, $n = 3{,}446$) according to the legend. The midpoint displays the logistic regression estimate. Bars show the 95% confidence intervals on the logistic regression estimate of the enrichment of the class of variation labeled on the *x* axis. **b**,**c**, Case–control enrichment and excess case rare-variant burden in increasingly a priori damaging variant subsets using logistic and linear regression, respectively. Consequence categories are stratified by rarity; moving from left to right, the putatively damaging nature of the variants reduces from dark red to pink according to the legend, and the rarity reduces from a variant with MAC ≤ 5 in a pLI ≥ 0.9 gene and not in the nonneurological portion of gnomAD (not in gnomAD pLI ≥ 0.9) to a variant with MAC ≤ 5 (All) according to the *x* axis labeling. Midpoints and bars display the logistic regression estimates and associated 95% confidence intervals of the enrichment of the class of variation labeled on the *x* axis (**b**) or linear regression estimates on excess variants in cases and associated 95% confidence intervals for the class of variation labeled on the *x* axis (**c**). Regressions are run as described in Methods (Exome-wide burden) and include sex, ten PCs and total coding burden with the same rarity as covariates. Nominally significant enrichments or excess variants in cases are labeled with the unadjusted associated two-sided *P* value computed using a Wald test.

Enrichment of damaging ultra-rare variation resides predominantly in brain tissues, with the strongest association in the amygdala (OR = 1.03, $P = 3.9 \times 10^{-5}$), a brain region found to be reduced in size in BD1 cases[27].

We considered 68 candidate gene sets generated or implicated in previous genetic studies of psychiatric disorders (Fig. 2b and Supplementary Fig. 9) and a stricter definition of highly brain expressed (average expression over twofold higher in brain tissues

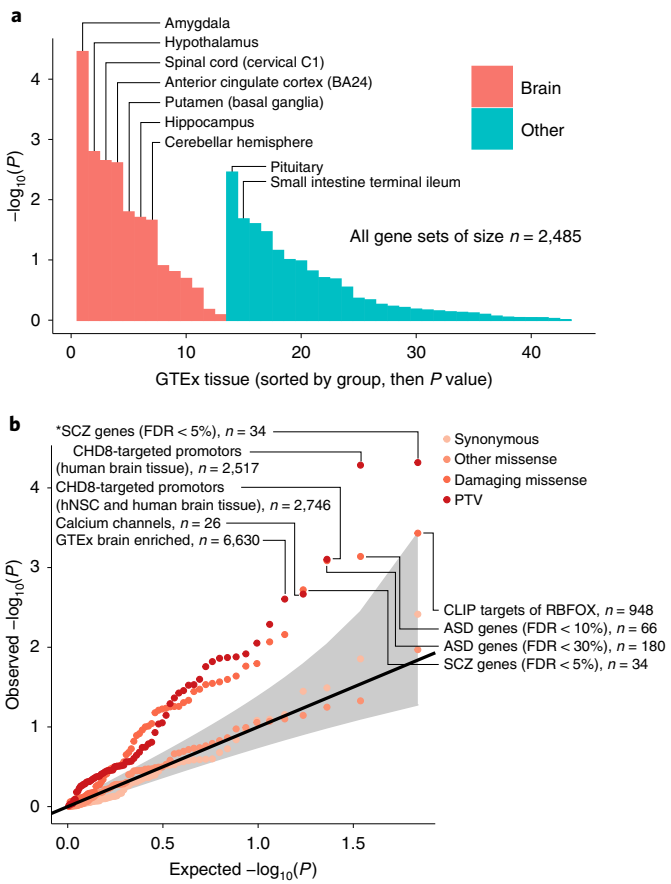

Fig. 2 | Biological insights from bipolar case–control whole-exome sequencing data. a, Enrichment of ultra-rare PTVs in BD cases over controls in tissue-specific expression gene sets. We ran logistic regressions of case status on ultra-rare PTV burden in tissue-specific expression gene sets. Logistic regressions were performed as described in the Supplementary Note. Two-sided *P* values were obtained via Wald tests. Gene sets are defined in Finucane et al.[26] in detail. Bars are ordered by *P* value, first for brain tissue and then for other tissues. No nominally significant association was enriched in controls over BD cases. **b**, Enrichment of ultra-rare variants in targeted 68 gene sets taken from the literature[23,43]. We ran logistic regressions of case status on ultra-rare variant burden in classes of variation labeled in the legend and display the expected against observed two-sided unadjusted −log(*P* values). Logistic regressions were performed as described in Methods. Two-sided *P* values were obtained via Wald tests. Top PTV and damaging missense gene sets are labeled and annotated with the number of genes in each gene set. The 95% confidence interval under the null is shown in gray. Classes of variants tested in each gene set are colored according to the legend. Gene sets surpassing Bonferroni test correction are labeled with an asterisk. CLIP, crosslinking and immunoprecipitation; hNSC, human neuronal stem cells; SCZ, schizophrenia.

than the average across all GTEx[28] tissues). Using this more stringent definition (6,630 genes), we saw stronger ultra-rare PTV enrichment in patients with BD (OR = 1.04, $P = 2.49 \times 10^{-3}$). Among the 68 candidate gene sets, we observed significant enrichment ($P < 0.05/68 \approx 3.68 \times 10^{-4}$) of ultra-rare PTV variation in two gene sets in patients with BD, including SCHEMA genes (false discovery rate (FDR) < 5% (ref. [23]) (34 genes, OR = 1.89, $P = 4.81 \times 10^{-5}$) and CHD8 binding targets in human brain[29] (2,517 genes, OR = 1.09, $P = 5.18 \times 10^{-5}$). For ultra-rare damaging missense variants, the strongest gene set enrichment was in genes targeted by RBFOX[30] (948 genes, OR = 1.07, $P = 3.70 \times 10^{-4}$), and ASD FDR < 10%[31] (66 genes, OR = 1.24, $P = 7.25 \times 10^{-4}$), though neither passed multiple testing correction.

Enrichment of ultra-rare PTVs in SCHEMA and damaging missense variants in ASD provided further evidence of convergence of shared signal across psychiatric and neurodevelopmental disorders in the ultra-rare end of the allele frequency spectrum, mirroring the overlapping genetic risk for schizophrenia and BD observed in common variation[32] and schizophrenia and ASD in rare variation[23].

We did not observe rare-variant enrichment of damaging variation in gene sets generated from a GWASs of BD of 20,352 cases and 31,358 controls[3]. However, we did find a nominally significant (OR = 1.69, $P = 0.00215$) signal of enrichment of ultra-rare PTVs in calcium channel genes (26 genes), in line with significant common variant signals of enrichment in targets of calcium channel blockers determined from BD GWASs[5].

To investigate the overlapping rare-variant signal with schizophrenia further, we considered four distinct gene sets, each with 50 genes, ordered by *P* value in SCHEMA[23]. We observed ultra-rare PTV enrichment in the top 50 genes, which included the FDR < 5% set (OR = 2.05, $P = 1.25 \times 10^{-8}$), but not in the less significant genes in SCHEMA (genes 51–100; OR = 1.01, $P = 0.932$, genes 101–150; OR = 1.07, $P = 0.481$, genes 151–200; OR = 1.06, $P = 0.703$). We also did not observe enrichment of ultra-rare PTVs in the recently fine-mapped schizophrenia genes published by the Psychiatric Genetics Consortium[33] (OR = 0.867, $P = 0.192$).

To seek to elucidate pathways enriched for damaging variation in patients with BD in an agnostic manner, we performed an enrichment analysis using gene sets derived from large pathway databases, including Gene Ontology, REACTOME and KEGG (1,697 gene sets; Supplementary Fig. 10). We observed significant ($P < 0.05/1697 \approx 2.95 \times 10^{-5}$) enrichment of one gene set: genes involved in the G1/S transition of the mitotic cell cycle (172 genes; OR = 1.46, $P = 1.37 \times 10^{-5}$).

***AKAP11* implicated by ultra-rare protein truncating variants.** To boost power for gene discovery, we again restricted to ultra-rare variants and tested for enrichment of putatively damaging classes of variation, PTVs and damaging missense variants (Methods and Supplementary Figs. 11–15). Enrichment in constrained genes remains significant after excluding the top 20 BD risk-associated genes in BipEx with $pLI \geq 0.9$ (OR = 1.07; $P = 0.00313$; Supplementary Table 9).

In our primary analysis, no gene surpassed exome-wide significance ($P < 0.05/23{,}321 \approx 2.14 \times 10^{-6}$, Fig. 3). However, we began to observe deviation from the null in the collection of tests of ultra-rare PTV enrichment in patients with BD, particularly for BD1 (Fig. 3 and Supplementary Fig. 16). This deviation was not observed for BD2 (Supplementary Fig. 17) despite the genome-wide enrichment of the PTV signal (Fig. 1b,c), likely due to the reduced power of Fisher's exact tests in BD2 case counts ($n = 3{,}446$). The strongest case–control association we observed was with *AKAP11* ($P = 1.15 \times 10^{-5}$, $Q = 2.02 \times 10^{-2}$ in BD, $P = 5.30 \times 10^{-6}$, $Q = 5.77 \times 10^{-3}$ in BD1).

Given the strong overlap in common variant risk between BD and schizophrenia, we looked for a shared signal of enrichment of ultra-rare PTVs in BD and schizophrenia cases. Due to overlap in controls between SCHEMA and BipEx, we meta-analyzed an ultra-rare variant count data that excluded these controls in SCHEMA (Methods). To avoid the schizophrenia ultra-rare PTV case–control enrichment signal overwhelming the BD signal when presenting results, we first sorted on *P* value in the primary gene-based BD analysis and displayed the top ten *P* values before and after meta-analysis with SCHEMA (Table 1 and Supplementary Table 10). The combined analysis in BD and schizophrenia cases revealed one exome-wide significant gene, *AKAP11* ($P = 2.83 \times 10^{-9}$), and one gene that almost attained exome-wide significance, *ATP9A* ($P = 5.36 \times 10^{-6}$).

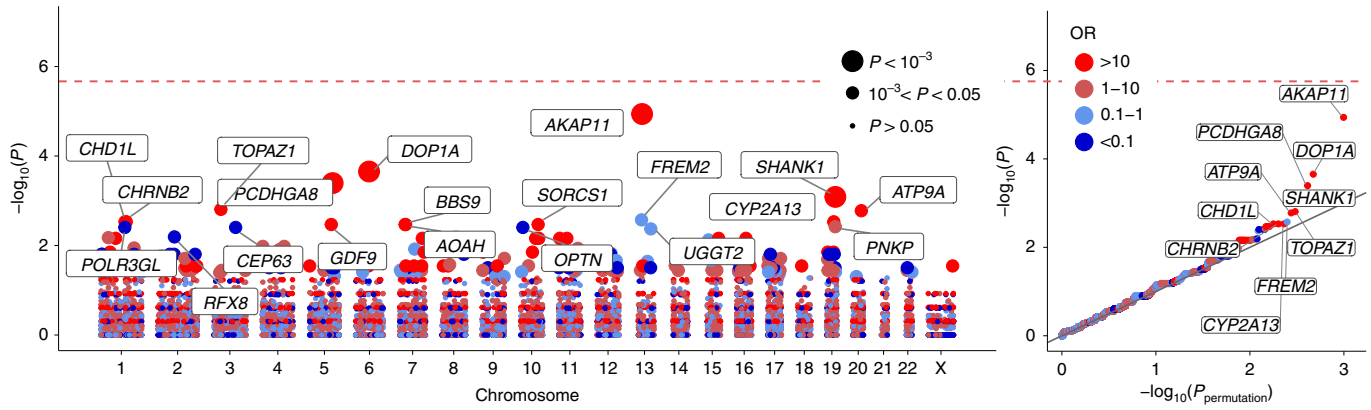

Fig. 3 | Results of the analysis of ultra-rare PTVs in 13,933 cases and 14,422 controls. Gene-based Manhattan and quantile-quantile (QQ) plot for BD (comprising BD1, BD2 and BD not otherwise specified) showing −log10 *P* values obtained via two-sided Fisher's exact tests are plotted against genetic position for each of the analyzed genes. In the QQ plots, observed −log10 *P* values are plotted against permutation *P* values according to the procedure described in Methods. Points are colored according to the discrete scale displayed in the legend. In the Manhattan plot and QQ plot, the gene symbols of the top 20 and top 10 genes by *P* value are labeled, respectively. Points in the Manhattan plot are sized according to *P* value as displayed in the legend.

The top gene hit, *AKAP11* (the gene encoding A-kinase anchoring protein 11 (AKAP-11, also known as AKAP220)) has only a single isoform, is under evolutionary constraint (loss-of-function observed/expected upper bound fraction = 0.3, *p*LI = 0.98) and is highly expressed in the brain (cerebellar hemisphere, 38.54 median transcripts per million; frontal cortex (BA9), 31.52 median transcripts per million[25]). Additionally, AKAP-11 has been shown to interact with GSK3B, the hypothesized target of lithium therapy[34–36]. We gathered available lithium response data for carriers of *AKAP11* PTVs among patients with BD; of the 11 patients for whom lithium response information (Methods) was available, 7 patients reported a good response and 4 patients did not respond well to lithium. Although the percentage of good responders in *AKAP11* PTV carriers (63.6%) was marginally elevated relative to the background response rate in available patients with BD (52%), the sample size was far too small to form any robust conclusions from the data.

To our knowledge and at the time of analysis, there was no signal of enrichment in *AKAP11* in other neurodevelopmental disorders at current sample sizes; *AKAP11* does not appear to be a prominent risk gene for autism[37,38] or epilepsy[39] and is not present in a collection of curated 'developmental disorder genes'[40]. Furthermore, expression of *AKAP11* tends to occur later in development (Supplementary Fig. 18).

We examined ultra-rare PTV variant counts in the Bipolar Sequencing Consortium (BSC)[18] exome sequencing data (Methods and Supplementary Table 11). Non-zero count data were available for seven of the top ten gene associations (Table 1). One, enriched for ultra-rare PTVs in controls (*FREM2*) in BipEx, did not display control enrichment in the BSC data. The remaining six displayed case enrichment in BipEx, with four (including *AKAP11* and *ATP9A*) displaying further case enrichment in the BSC data (Supplementary Table 12).

Discussion

In this large BD exome study, ultra-rare PTVs in constrained genes are significantly enriched in patients with BD. In fact, enrichment in constrained genes remains significant even after excluding the top 20 BD risk-associated genes (OR = 1.07; $P = 0.00313$) with *p*LI ≥ 0.9 (Supplementary Table 9). This reflects the highly polygenic genetic architecture of BD, a property shared with schizophrenia[23], and suggests that the majority of genes involved in BD risk will require larger sample sizes to be discovered. Furthermore, in patients with BD, ultra-rare PTVs are significantly enriched in schizophrenia risk genes identified in the SCHEMA consortium, suggesting that rare variation in these genes is not specific to schizophrenia pathophysiology; overlap in risk for schizophrenia and BD is now evident in both rare and common variation. Finally, combining our results with data from SCHEMA revealed strong evidence that haploinsufficiency in *AKAP11* confers risk for both BD and schizophrenia, but this does not appear to be the case for early-onset neurodevelopmental disorders.

AKAP11 codes for the AKAP-11 protein (also known as AKAP220), one of a family of scaffolding proteins that bind to the regulatory subunit of the protein kinase A (PKA). These anchoring proteins confine PKA to discrete locations in the cell to target specific substrates for phosphorylation and dephosphorylation. In particular, GSK3B is bound by AKAP-11. GSK3B is hypothesized to be the target of lithium, the primary treatment for BD[41]. By binding to GSK3B, AKAP-11 mediates PKA-dependent inhibition of GSK3B. PKA inhibits the activity of GSK3B bound to AKAP-11 more strongly than GSK3B in general, and thus, modifications to AKAP-11 have the potential to affect downstream pathways. *GSK3B* is one of two paralogous genes (*GSK3A* and *GSK3B*) that encode a serine/threonine protein kinase, glycogen synthase kinase 3. The primary known function of this protein is phosphorylation of over 100 substrates, affecting myriad signaling pathways[6,41,42].

We see early evidence of enrichment in ultra-rare damaging missense variation, particularly within BD2. This enrichment is evident outside of missense constrained regions (as defined by MPC ≥ 2), which is perhaps surprising, as the signal of association seen for rare (MAF ≈ 2×10^{-5}) missense variation in schizophrenia cases is mainly within constrained missense regions (MPC ≥ 2)[23]. Because BD2 displays a stronger correlation of common variant effects with major depression than BD1 and BD1 is more correlated with schizophrenia than BD2, this missense signal may be capturing something distinct to mood disorders relative to psychotic disorders. However, we should be cautious not to overinterpret differences in ultra-rare damaging missense enrichment across BD subtypes; the BD2 sample count ($n = 3{,}446$) was less than half that of BD1 ($n = 8{,}238$), and the confidence intervals overlapped (Fig. 1). Furthermore, attempts to refine this exome-wide signal to individual genes or targeted gene sets did not result in any significant signals of association after correcting for multiple testing (Supplementary Figs. 9 and 17). We expect to see a refinement of the putatively damaging missense signal as sample sizes increase.

Table 1 | BipEx and SCHEMA case–control counts of the top ten most significant genes in the BipEx gene-based analysis

Gene	BD (BipEx)					Schizophrenia (SCHEMA)				Combined	
	Case count $n=13{,}933$	Control count $n=14{,}422$	*P* value	*Q* value	OR	Case count $n=24{,}248$	Control count $n=91{,}960$	*P* value	OR	OR	Meta *P* value
AKAP11	16	0	1.15×10^{-5}	2.02×10^{-2}	∞	17	13	2.02×10^{-5}	5.60	7.06	2.83×10^{-9}
DOP1A	15	1	2.22×10^{-4}	1.95×10^{-2}	15.54	19	43	1.47×10^{-1}	1.59	2.11	1.44×10^{-4}
PCDHGA8	11	0	4.02×10^{-4}	2.36×10^{-1}	∞	6	44	2.19×10^{-1}	0.54	0.99	3.38×10^{-3}
SHANK1	10	0	8.19×10^{-4}	3.60×10^{-1}	∞	4	4	4.43×10^{-1}	2.90	6.99	9.71×10^{-3}
TOPAZ1	12	1	1.56×10^{-3}	5.48×10^{-1}	12.43	2	3	6.67×10^{-1}	0.93	3.93	2.51×10^{-3}
ATP9A	9	0	1.66×10^{-3}	-	∞	15	11	6.96×10^{-4}	4.08	5.46	5.36×10^{-6}
FREM2	4	19	2.67×10^{-3}	5.77×10^{-1}	0.22	22	92	5.48×10^{-1}	0.83	0.65	3.80×10^{-2}
CHD1L	11	1	2.95×10^{-3}	5.77×10^{-1}	11.39	16	73	5.99×10^{-1}	0.82	1.01	4.57×10^{-2}
CHRNB2	11	1	2.95×10^{-3}	5.77×10^{-1}	11.39	2	17	5.54×10^{-1}	0.52	1.88	3.04×10^{-2}
CYP2A13	11	1	2.95×10^{-3}	6.68×10^{-1}	11.39	13	28	6.30×10^{-1}	1.29	2.27	4.61×10^{-2}

Case and control columns denote the count of ultra-rare PTVs in the gene in the respective dataset. Two-sided *P* values are determined using Fisher's exact and Cochran–Mantel–Haenszel (CMH) tests for BipEx and SCHEMA (Methods), respectively, and meta-analyzed weighting by effective sample size. Two-sided *Q* values for Fisher's exact test statistics in BipEx were evaluated using the Benjamini–Hochberg adjustment[44] applied to all genes with at least ten ultra-rare PTVs across cases and controls. BipEx: 13,933 BD cases and 14,422 controls; SCHEMA: 24,248 schizophrenia cases and 91,960 controls. The SCHEMA OR is the estimated OR averaged over strata, whereas the combined OR is the simple OR calculated by combining the BipEx and SCHEMA cases and controls. Note that differential coverage across exome sequencing platforms and whole- genome sequencing means that case and control counts differ across genes.

Despite sequencing 13,933 patients with BD, we did not observe any BD-specific risk genes surpassing exome-wide significance. In contrast, the analysis of 24,248 patients with schizophrenia in SCHEMA yielded ten significant risk genes. When comparing the observed ultra-rare PTV enrichment among constrained genes in our current sample (OR = 1.11) to SCHEMA (OR = 1.26), we estimate that roughly double the case sample size of schizophrenia is needed to achieve comparable statistical power to discover individual BD risk genes. We now see convergence of gene overlap for schizophrenia from the common and rare end of the allele frequency spectrum, in large part through increased exome sample sizes[23]. Genetic overlap between common and rare variation in BD, however, remains uncertain. The BSC examined exomes of 3,987 individuals with BD[18] and found suggestive enrichment in 165 genes implicated in BD GWAS (OR = 1.9, $P=6.0\times10^{-4}$), but we did not replicate this finding (OR = 0.9, $P=0.40$). Before SCHEMA, convergence of genes implicated in schizophrenia by common and rare variation was modest[20,21,43]. As BD sample sizes increase in both common and rare variation analyses, we expect a similar convergence of genes implicated in BD.

In summary, ultra-rare PTVs in constrained genes are significantly enriched in BD cases over controls, a result firmly established in schizophrenia and other early-onset neurodevelopmental disorders. We are beginning to see promising signals among individual genes, despite none surpassing exome-wide significance for BD alone. We observe that shared risk for BD and schizophrenia is present in both common and damaging ultra-rare variation. Our top gene, *AKAP11*, shows shared evidence of risk for BD and schizophrenia, increasing our confidence that we are discovering true risk factors underlying psychiatric disease. Overall, the current evidence suggests gene discovery in BD is on a similar trajectory to schizophrenia, where increased sample sizes and further collaborative efforts will inevitably lead to biologically meaningful risk genes and pathways underlying BD risk.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41588-022-01034-x.

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Methods

Sequence data production. Exome sequencing was performed at the Broad Institute of MIT and Harvard from July 2017 to September 2018. Processing included sample QC using the picogreen assay to measure for sample volume, concentration and DNA yield. Sample library preparation was carried out using Illumina Nextera, followed by hybrid capture using Illumina rapid capture enrichment of a 37-Mb target. Sequencing was performed on HiSeqX instruments to 150-bp paired reads. Sample identification checking was carried out to confirm all samples. Sequencing was run until hybrid selection libraries met or exceeded 85% of targets at 20×, comparable to ~55× mean coverage. Data for each sample were demultiplexed, aggregated into a BAM file and processed through a pipeline based on the Picard 2.19 suite of software tools. The BWA aligner mapped reads onto the human genome build 38 (GRCh38). SNPs and insertions/deletions were joint called across all samples using the Genome Analysis Toolkit[45] HaplotypeCaller package version 4.0.10 to produce a version 4.2 variant callset file. Variant call accuracy was estimated using the Genome Analysis Toolkit variant quality score recalibration approach[46].

QC. We performed a series of hard filters on genotype and variant metrics (Supplementary Table 2), followed by a collection of hard filters on sample metrics (Supplementary Table 3). We confirmed genotype sex with reported sex, removed related individuals and restricted analysis to samples of continental European ancestry where we had sufficient sample size and balanced case–control counts (Supplementary Table 3) using random forest classifiers. Finally, we filtered based on a second collection of sample and variant hard filters (Supplementary Tables 2 and 3). Final curated sample counts, split by cohort, are provided in Supplementary Table 4. We used Hail 0.2 and PLINK 1.9 to perform all QC steps, in combination with R (4.0.2) scripts for data filtering and plotting. Data were manipulated in R using data.table (1.13.0) and dplyr (1.0.1), random forest classifiers were trained using the randomForest (4.6) library and plotting was performed using a ggplot2 (3.3.2) and the add-on packages ggsci (2.9) and ggExtra (0.9). Full details are provided in the Supplementary Note.

Variant annotation. We used VEP[22] version 95 with the LOFTEE plugin to annotate variants against GRCh38 using hail, including SIFT[47] and PolyPhen-2 scores[48], according to the GENCODE v19 reference. The configuration file is available in Google cloud (gs://hail-us-vep/vep95-GRCh38-loftee-gcloud.json). In addition, we annotated with version 2.1.1 gnomAD site annotations[49] and MPC scores[24] after lifting the genome coordinates over to GRCh38. MPC is an aggregate score that uses ExAC to identify subgenic regions that are depleted of missense variation in combination with existing metrics to create a composite predictor. Finally, we annotated with Combined Annotation Dependent Depletion version 1.4[24,50], and we annotated constraint using the gnomAD loss-of-function metrics table from release 2.1.1 (ref. [49]). We then processed the VEP annotated consequences, and we defined variant-specific consequences and gene annotations as the most severe consequence of a canonical transcript on which that variant lies. We then assigned variants (where possible) to four distinct consequence classes (PTV, missense, synonymous and noncoding) as defined in Supplementary Table 5. We then subdivided missense variants into 'damaging missense' if the PolyPhen-2 prediction was 'probably damaging' and the SIFT prediction was 'deleterious' and 'other missense' otherwise. Note that the MPC ≥ 2 group accounted for a small proportion of the total damaging and benign missense variants annotated by PolyPhen-2 and SIFT (e.g., the numbers of MPC ≥ 2 variants in BipEx following the increasingly stringent filters (MAC ≤ 5, ultra-rare, or ultra-rare and in a *p*LI ≥ 0.9 gene) are 39,000, 23,000 and 5,000, respectively, compared to 360,000, 159,000 and 31,000 for damaging missense variants).

Exome-wide burden. We ran a series of logistic regressions to test for an association between putatively damaging rare variation and case status, and linear regressions to test for an association between case status and excess burden of damaging variation using base-R and logistf (1.23) in R (4.0.2). Both tests resulted in near identical *P* values; the motivation here was to ascertain two effect-size parameters of rare-variant burden. We then sought to focus on more recent mutations by restricting to rare variation not present in the nonneurological portion of the gnomAD database, and we performed the same collection of association tests. Furthermore, we leveraged evolutionary constraint models to enrich for deleterious variation by testing for enrichment of missense variation with MPC ≥ 2 (representing the top ~3.9% pathogenicity of missense variation[24] and restricting our PTV enrichment tests within genes most likely to be loss-of-function intolerant (*p*LI ≥ 0.9).

Throughout, we tested for a signal of enrichment of synonymous and other missense as a negative control to confirm that our burden model was well calibrated. For each collection of regressions, we included sex, ten PCs and overall burden of MAC ≤ 5 variants in the dataset following the imposed restrictions. All regressions were robust to incorporation of the overall burden covariate; the overall observed patterns did not change if we controlled for overall coding burden or did not control for overall burden.

To ensure that the results in the full dataset were not driven by artifacts introduced by jointly analyzing multiple cohorts or residual population structure, we ran burden tests within each location and meta-analyzed these results. We observed consistent results across the cohorts, and we estimated that ORs and excess burden between the joint analysis and meta-analysis were roughly equivalent.

Multiple studies have shown enrichment of rare damaging coding variation in schizophrenia cases over controls[20,21]. As a positive control, we considered the schizophrenia cases in BipEx and tested for enrichment of ultra-rare PTVs in loss-of function intolerant (*p*LI ≥ 0.9) genes and replicated this result (OR = 1.28, $P = 1.9 \times 10^{-10}$).

Age of onset. Three definitions for age of onset were available for subsets of the data: age at first symptoms, age at first diagnosis and age at first impairment. In each case, two distinct age encodings were used: (1) <18 years, 18–40 years and ≥40 years and (2) <12 years, 12–24 years and ≥24 years. Onset definitions were defined differently dependent on cohort and included data from clinical instruments, algorithms using clinician notes and telephone interviews with patients. Detailed age-of-onset information split by cohort is provided in the Supplementary Note.

To test for an association between age of onset and burden of rare damaging variation, we first restricted to the class of variation with the strongest signal for excess in cases over controls, PTVs. We considered only 'age at first impairment' (Supplementary Table 6), as phenotypic data using this definition were available for the largest number of samples (3,677). We further split the age of first impairment categories into five discrete age bins across the two age encodings: <12 years, 12–18 years, 18–24 years, 24–40 years and >40 years. We tested all ten possible 'younger bin' versus 'older bin' pairs across this partition to check for differences in MAC ≤ 5 PTV burden, MAC ≤ 5 not in gnomAD PTV burden and MAC ≤ 5 not in nonneurological gnomAD PTV in *p*LI ≥ 0.9 burden using Kolmogorov–Smirnov tests. We also used Fisher's exact tests to test for an association between carrier status for the damaging rare PTV categories between the younger and older bins. All tests were performed in R (4.0.2).

Psychosis definitions. Psychosis was defined by a lifetime history of hallucinations or delusions. Presence of psychosis was evaluated differently across cohorts based on available data as follows:

Boston, USA: validated natural language processing-based algorithm run on clinical notes[51,52].

Cardiff, UK: SCAN interview[53] and case records. Definite evidence of lifetime presence of psychotic symptoms and lifetime presence of individual OPCRIT[53,54] psychotic symptoms.

London, UK: OPCRIT[53,54] interview, with lifetime presence of psychotic symptoms as defined by questions 52, 54, 55 and 57–77 of the OPCRIT checklist detailed in the DNA polymorphisms in mental illness bipolar affective disorder questionnaire.

Stockholm, Sweden: Swedish Bipolar Cohort Collection:

St. Göran bipolar project: Affective Disorder Evaluation (ADE) question, "Any psychotic disorder?"

BipoläR and HDR: During a structured telephone interview that research nurses conduct, 'Have you ever lost touch with reality (that is have heard or seen things that others have not seen) or experienced things that you later realized were not real?' was asked. Patients were defined as having psychosis if the answer to this question was clear-cut 'yes' and not having psychosis if doubtful.

Gene set burden testing. For each gene set, we tested for ultra-rare variant enrichment of the following classes of variation: PTV, damaging missense, other missense and synonymous. For each gene set, we regressed case status on ultra-rare burden of each variant class in that gene set using logistic regression, including ultra-rare coding burden in the gene set (sum of ultra-rare burden of PTVs, damaging missense, other missense and synonymous variants in the gene set), sex and PCs 1–10 as covariates. The resulting logistic regression performed for each (gene set, variant class) pair is case status ~ $\text{burden}_{g,c}$ + $\text{burden}_{g,\text{coding}}$ + sex + PC1 + PC2 +... PC10, where $\text{burden}_{g,c}$ is the count of ultra-rare variants of variant class *c* in gene set *g* for the sample and $\text{burden}_{g,\text{coding}}$ is the total number of ultra-rare coding variants in the gene set for the sample.

GTEx tissue-specific gene sets are defined in Finucane et al.[26]. Briefly, *t*-statistics for specific expression in each of the focal tissues were determined for each gene; these were then ranked and the top 10% of *t*-statistics defined the collection of genes 'specifically expressed' in that tissue. For full details, see Supplementary Note.

Gene-based analysis. To increase power for gene discovery, we filtered down to variants not present in the nonneurological portion of the gnomAD dataset[49], and we further enriched for pathogenic variants by restricting our analysis to variants with MAC ≤ 5. We then examined case–control enrichment of PTVs or damaging missense variants (missense variants annotated as 'probably damaging' in PolyPhen and 'deleterious' in SIFT). We further restricted our analysis to the coding exons within the target intervals of the Illumina capture to reduce potential for artifacts that could potentially be induced due to differential coverage across batches in any padded target interval, using synonymous and other missense ultra-rare variants in each gene as the negative control (Supplementary Figs. 11 and 12).

Throughout, we use Fisher's exact tests in each gene. We considered a CMH test, using the strata defined by broad geographic location (Supplementary Table 13). We used a permutation approach to determine the null distribution of test statistics throughout our gene-based analysis, and we evaluated QQ plots of synonymous and other missense ultra-rare variants to ensure that tests are well calibrated (Supplementary Figs. 11 and 12). We used Fisher's exact tests in our primary analysis, as tests showed the strongest power and also had well-calibrated QQ plots across annotation categories (Supplementary Figs. 11–15). To determine *Q* values we apply the Benjamini and Hochberg adjustment[44] to Fisher's exact test *P* values for genes with at least ten ultra-rare PTVs across cases and controls. We excluded genes with fewer than ten ultra-rare PTVs in the BipEx dataset to guard against incorrect *P*-value adjustment using the Benjamini–Hochberg procedure. Conservative *Q* values occur when applying the Benjamini–Hochberg correction to discrete test statistics with low counts due to the null distribution of *P* values not following a uniform distribution under the null.

We tested for an excess of ultra-rare variation (MAC ≤ 5 and not present in the nonneurological portion of the gnomAD dataset) in each gene using both Fisher's exact and CMH tests for each phenotype. For each gene, each sample was assessed for carrier status for each of the following consequence classes: synonymous, other missense, damaging missense and PTV (Supplementary Table 5); individuals harboring at least one copy in the consequence class under analysis were counted as carriers. These counts were then taken through to define 2 × 2 and 2 × 2 × 6 contingency tables for Fisher's exact and CMH tests, respectively, using location as strata (Supplementary Table 13). To ensure that our tests were well calibrated, we randomly permuted case labels (within stratum for CMH) for each gene and reran the test 20 times across all genes and keep track of the summation of the ordered vectors of *P* values up to that permutation, before taking an average at the last permutation. This vector of length |*n* genes| then defines our expected distribution of *P* values. Fisher's exact test *P* values and OR for carrier status are displayed in the gene results tables on the browser (https://bipex.broadinstitute.org).

To ensure that our tests were robust, we performed a series of checks to see if the Fisher's exact (Supplementary Figs. 11 and 12) and CMH test results showed an elevated false-positive rate. In both tests, we observed the expected null *P*-value distribution in the collection of gene-based tests when analyzing synonymous and 'other missense' variants with MAC ≤ 5 not in gnomAD nonneurological. To further test calibration of the test statistic, we filtered to genes where we are well powered to detect differences between BD cases and controls. We examined case–control enrichment of synonymous ultra-rare variants in genes with an allele count of >20 and >50 and compared observed *P* value to the uniform expectation (Supplementary Fig. 15). In each examination, we did not observe inflation of the test statistic. All tests were performed in R (4.0.2).

Meta-analyzing SCHEMA and BipEx. To examine the extent of shared ultra-rare PTV signal between BD and schizophrenia, we ran separate Fisher and CMH tests for BipEx and SCHEMA separately and meta-analyzed the results using weighted *Z* scores, weighing by effective sample sizes. Fisher's exact and CMH two-sided *P* values were halved and converted to signed *Z* scores using the OR to define the sign. Weighted *Z* scores were then evaluated as follows:

$$Z = \frac{\sum_{i=1}^{m} w_i Z_i}{\sqrt{\sum_{i=1}^{m} w_i^2}},$$

where $w_i = \sqrt{N_{eff,i}}$, $N_{eff,i} = 4Np_{case,i}\,(1 - p_{case,i})$, and $p_{case,i}$ is the case proportion in the i^{th} cohort. Associated *P* values were then evaluated. As the UK and Ireland controls were present in BipEx and SCHEMA, these controls were excluded from the SCHEMA *Z* score in the meta-analysis.

Lithium response. For the BipolāR and HDR subsets of the SWEBIC (Swedish Bipolar Cohort Collection), during a structured telephone interview that research nurses conducted, patients prescribed lithium for at least 12 months were asked, 'What do you think of the effect (of lithium)? Do not consider side effects.' Patients were partitioned according to the following response options: nonresponder (none or very doubtful effect), partial responder 'Doubtless effect of treatment but additional temporary or continuous treatment needed' or good responder (complete response, recovered).For Cardiff, UK, patients were partitioned according to the following criteria: no evidence of response, subjective good response (upon interview, patients reported that lithium helped stabilize their moods), objective evidence for beneficial response (clear reduction in number and/or severity of episodes following introduction of lithium prophylaxis (can only be rated if at least three episodes of illness have occurred before lithium prophylaxis and lithium response has been observed for at least 3 years)) or objective evidence for excellent response to lithium prophylaxis (i.e., frequency of episodes reduced to <10% of frequency after lithium prophylaxis and/or two or more episodes of illness occurring within weeks of cessation of lithium (can only be rated if at least three episodes of illness have occurred before lithium prophylaxis and lithium response has been observed for at least 5 years)).

External validation. To externally check our gene-based PTV results, we obtained PTV counts from the BSC. Specifically, rare variant counts within the top ten genes defined by *P* value in the Fisher's exact tests of enrichment of ultra-rare PTVs in the data were provided by the BSC. To harmonize BSC data with BipEx, we used annotation definitions defined in Supplementary Table 5. We then generate MAC ≤ 5 counts for each gene in the BSC data. The addition of the BSC dataset has some limitations. Frameshift indels were not called for a subset of the cohorts, reducing power to detect an association. Among the BSC cohorts that called indels, only the Rarebliss dataset provided indel calls. Furthermore, library preparation, sequencing platform and variant calling differed across the BSC cohorts (Supplementary Table 11).

Ethics statement. IRB approvals and study consent forms from each of the sample contributing organizations were sent to the Broad Institute before samples were sequenced and analyzed. Contributing organizations included University of Aberdeen, Trinity College Dublin, University of Edinburgh, University College London, Cardiff University, University of Cambridge, Vrije Universitat Amsterdam, University College of Los Angeles, Universitats Klinikum Frankfurt, Massachusetts General Hospital, Johns Hopkins University, Karolinska Institute, LifeGene Biorepository at Karolinska Institute and Umea University.

All ethical approvals are on file at the Massachusetts General Brigham (formerly Partners) IRB office amended to protocol 2014P001342, title 'Molecular Profiling of Psychiatric Disease'.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

We display all of our results, from the variant and gene level, in a browser available at https://bipex.broadinstitute.org. Phenotype curation and QC are available at https://astheeggeggs.github.io/BipEx/. Data are available under the following EGS study accession numbers: EGAS00001005838, EGAS00001005841, EGAS00001005842, EGAS00001005843, EGAS00001005844, EGAS00001005845, EGAS00001005851, EGAS00001005852, EGAS00001005853, EGAS00001005854, EGAS00001005855, EGAS00001005856, EGAS00001005857, EGAS00001005858, EGAS00001005859 and EGAS00001005860. WES data generated under this study are also hosted via the Terra platform (https://app.terra.bio). The Terra environment, created by the Broad Institute, contains a system of workspace functionalities centered on data sharing and analysis. To gain access via Terra, please contact the corresponding authors directly. The GnomAD database can be accessed at gnomad.broadinstitute.org. We used the following pathway databases: Gene Ontology (geneontology.org), KEGG (https://www.genome.jp/kegg) and REACTOME (reactome.org/). GTEx tissue-specific enrichment gene sets are available at data.broadinstitute.org/alkesgroup/LDSCORE/LDSC_SEG_ldscores/.

Code availability

The code used to perform QC, analysis and plot creation is available at github.com/astheeggeggs/BipEx. Data were manipulated using Hail 0.2 and R (4.0.2) using data.table (1.13.0) and dplyr (1.0.1), and plotted with ggplot2 (3.3.2), ggsci (2.9), ggExtra (0.9), ggrepel (0.8.2), RColorBrewer (1.1–2) and gridExtra (2.3) in R (4.0.2).

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Author contributions

B.M.N. initiated the project. A.R., A.M.M., A.M., N.B., D.C., R.Y., F.D., D.P., D.S.C., D.W.M., A.C., D.B., F.S.G., J.W.S., M.C.O., M.J.O., M.L., N.L.P., N.C., A.D.F., I.J., L.J., J.W., R.O., M.P.M.B., R.S.K. and R.A. led sample recruitment of the BipEx cohort collection. P.Z., F.S.G., W.W., E.A.S., X.J., C.S., N.R., L.S. and A.E.L. led sample recruitment and curation of the BSC cohorts. X.J., L.S. and A.E.L. curated sample genetic and phenotypic data in the BSC collection. S.B.C. managed sample collections, exome sequencing and data hosting. D.S.P. processed and curated exome sequence data, with assistance from D.P.H., T.S. and B.M.N. D.S.P. processed and curated phenotype data in coordination with A.V. and N.B.F. D.S.P. performed the core analyses in close coordination with B.M.N. and D.P.H. The main findings were interpreted by D.S.P., D.P.H. and B.M.N. M.S. and N.A.W created the bipex.broadinstitute.org browser. A.L. performed gene-based analyses in the BSC dataset. D.S.P. drafted the manuscript in close coordination with D.P.H. and B.M.N., with editing assistance from C.C., A.D.F., A.M., N.A.W., I.J., L.J., L.S., W.W., R.Y., R.O., L.J., M.L., C.-Y.C., D.C., A.V. and F.D. D.S.P., A.D.F., D.C., N.B.F., L.J., R.O., S.B.C., D.P.H. and B.M.N. addressed and responded to reviewer comments.

Competing interests

B.M.N. is a member of the scientific advisory board at Deep Genomics and Neumora and a consultant for Camp4 Therapeutics, Takeda Pharmaceutical and Biogen. A.M.M. has received speaker fees from Illumina and Janssen and research grant support from The Sackler Trust. M.L. has received speakers fees from Lundbeck Pharmaceuticals. M.J.O., M.C.O. and J.W. have received a research grant from Takeda Pharmaceuticals outside the scope of the present study. F.S.G. has received a research grant from Janssen Pharmaceuticals outside the scope of the present study. C.-Y.C. is an employee of Biogen. F.D. is an employee of Sheppard Pratt. A.E.L. and E.A.S. are now employees of Regeneron. X.J. is now an employee of Genentech. All other authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41588-022-01034-x.

Correspondence and requests for materials should be addressed to Duncan S. Palmer or Benjamin M. Neale.

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Software and code

Policy information about availability of computer code

Data collection	Data delivery per sample includes a demultiplexed, aggregated into a BAM file and processed through a pipeline based on the Picard 2.19 suite of software tools. The BWA aligner mapped reads onto the human genome build 38. Single nucleotide polymorphism and insertions/deletions were joint called across all samples using Genome Analysis Toolkit (GATK) HaplotypeCaller package version 4.0.10 to produce a version 4.2 variant callset file (VCF). Variant call accuracy was estimated using the GATK Variant Quality Score Recalibration (VQSR) approach.
Data analysis	To perform Exome quality control (QC) we use PLINK 1.9 and a combination of scripts in hail 0.2, an open-source, general-purpose, Python-based data analysis library with a particular focus on the analysis of large-scale genetic data (website: www.hail.is, GitHub: github.com/hail-is/hail). We made use of the general purpose hail framework to create our own methods, as well as take advantage of the functionality that has been rewritten to enable fast and scalable analysis of large exome and genome sequencing projects. This was combined with R (4.0.2) scripts for data filtering and plotting. Data was manipulated in R using data.table (1.13.0) and dplyr (1.0.1), and plotted using a ggplot2 (3.3.2) and add on packages ggsci (2.9) and ggExtra (0.9). Random Forest classifiers were trained using the randomForest (4.6) library in R. All QC scripts are available at github.com/astheeggeggs/BipEx/tree/master/scripts_BipEx/QC_BipEx. Analysis of curated data was also performed in hail 0.2 and R. Regressions were performed using base-R and logistf (1.23) in R (4.0.2). Data was manipulated in R using data.table (1.13.0) and dplyr (1.0.1), and using built-in functions in hail. In R, results were plotted using ggplot2 (3.3.2) and add on packages ggsci (2.9), ggExtra (0.9), ggrepel (0.8.2), RColorBrewer (1.1-2), and gridExtra (2.3). All Hail and R analysis scripts are available at github.com/astheeggeggs/BipEx/tree/master/scripts_BipEx/analysis_BipEx.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

We display all of our results, from the variant and gene level in a browser available at https://bipex.broadinstitute.org. Once validated and approved, data will be available under the following EGS study accession numbers: EGAS00001005838, EGAS00001005841, EGAS00001005842, EGAS00001005843, EGAS00001005844, EGAS00001005845, EGAS00001005851, EGAS00001005852, EGAS00001005853, EGAS00001005854, EGAS00001005855, EGAS00001005856, EGAS00001005857, EGAS00001005858, EGAS00001005859, and EGAS00001005860. GnomAD database: gnomad.broadinstitute.org. Pathway databases: Gene Ontology: geneontology.org, KEGG: www.genome.jp/kegg, REACTOME: reactome.org. GTEx tissue specific enrichment gene-sets: data.broadinstitute.org/alkesgroup/LDSCORE/LDSC_SEG_ldscores/.

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Life sciences study design

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Sample size	Case and control counts following quality control following sequencing and QC evaluation: 13,933 bipolar disorder cases, 14,422 controls. We obtained as many samples as possible. The sample size achieved represents a ~249% increase over the largest bipolar disorder whole-exome sequencing study to date. There is no minimum effect size that we are trying to capture - we hope to identify the smallest significant effect size that we can with the available samples.
Data exclusions	We remove data to filter out poor quality DNA and low quality sequencing data. To do this, we filter using established metrics on the sample, variant, and genotype level. Variants failing VQSR, in low complexity regions, outside padded exome target capture, with low call rate <0.97, differential call rate between cases and controls, or out of Hardy-Weinberg equilibrium were removed. Samples lacking case/control information, with low coverage, high contamination, low average sequencing, or mismatched sex were removed. We also removed samples to ensure there are no related samples in the final dataset, and remove samples lying in ancestral space where we have low case and control counts (those samples that are not non-Finnish European).
Replication	One replication study was performed in collaboration with the bipolar exome sequencing consortium (BSC). Of the 6 genes in Table 1 (10 genes) displaying case enrichment and with available non-zero count data from the BSC, 4 replicated direction of effect (including AKAP11 and ATP9A) but not to genome-wide significance due to sample size. The ultra-rare PTV burden result replicated the finding in Ganna et al. Am J Hum Genet. 2018 Jun 7; 102(6): 1204–1211.
Randomization	The current work was not an experimental study, so did not require randomization into experimental groups.
Blinding	The current work was not an experimental study, so did not require blinding to group allocation during data collection or analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
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Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Following sample exclusions described in Table S3, Sex: 11,313 males, 17,042 females. Continental genotypic ancestry: European. By cohort, sample counts are: Aberdeen, UK: 844, Amsterdam, NED: 2,569, Baltimore, USA: 312, Boston, USA: 5,009, Cambridge, UK: 2,656, Cardiff, UK: 5,685, Dublin, IRE: 197, Edinburgh, UK: 1,046, London, UK: 4,433, Stockholm, SWE: 9,140, Umea, SWE: 867, Wurzburg, GER: 769. Diagnoses: 13,933 bipolar disorder cases, 14,422 controls. Psychosis information was available for 8,017 bipolar disorder cases. 4,214 showed symptoms of psychosis, 3,803 did not show symptoms of psychosis. Sample age data was not available.
Recruitment	Case samples were recruited from outpatients or stable in-patients from hospitals, from community psychiatric facilities by a psychiatrist or psychiatric nurse, collected by linking discarded blood samples to de-identified electronic health record data, through consecutive admissions to psychiatric in-patient hospital units. Controls were volunteers recruited through general practices, from non-psychiatric hospital units, control bio-repositories, blood-donors, and by linking discarded blood samples to de-identified electronic health record data. Diagnosic criteria across cohorts may differ, and some cohort controls are screened negative which may introduce a slight bias. Although differences between diagnostic systems the resultant differences in allocation of case status between systems is low/absent at least for BD1 and as such, unlikely to introduce bias to our results. Further, apart from the Boston cohort and a subcohort of the Stockholm, SWE samples, all cohorts were assessed using a validated diagnostic instrument. For these two cohorts which used algorithms, their concordance with psychiatrist diagnosis was high, keeping bias to a low level.
Ethics oversight	IRB approvals and study consent forms from each of sample contributing organizations were sent to the Broad Institute before samples were sequenced and analyzed. Contributing organizations include: University of Aberdeen, Trinity College Dublin, University of Edinburgh, University College London, Cardiff University, University of Cambridge, Vrije Universitat Amsterdam, University College of Los Angeles, Universitats Klinikum Frankfurt, Massachusetts General Hospital, Johns Hopkins University, Karolinska Institute, LifeGene Biorepository at Karolinska Institute, and Umea University. All ethical approvals are on file at the Massachusetts General Brigham (MGB), formerly Partners, IRB office amended to protocol #2014P001342, title: 'Molecular Profiling of Psychiatric Disease'.

Note that full information on the approval of the study protocol must also be provided in the manuscript.