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

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Abstract

Here we report results from the Bipolar Exome (BipEx) collaboration analysis of whole exome sequencing of 13,933 individuals diagnosed with bipolar disorder (BD), matched with 14,422 controls. We find an excess of ultra-rare protein-truncating variants (PTVs) in BD patients among genes under strong evolutionary constraint, a signal evident in both major BD subtypes, bipolar 1 disorder (BD1) and bipolar 2 disorder (BD2). We also find an excess of ultra-rare PTVs within genes implicated from a recent schizophrenia (SCZ) exome meta-analysis (SCHEMA; 24,248 SCZ cases and 97,322 controls) and among binding targets of CHD8. Genes implicated from GWAS of BD, however, are not significantly enriched for ultra-rare PTVs. Combining BD gene-level results with SCHEMA, *AKAP11* emerges as a definitive risk gene (ultra-rare PTVs seen in 33 BD/SCZ cases and 13 controls, OR = 7.06, $P = 2.83 \times 10^{-9}$). At the protein level, AKAP-11 is known to interact with GSK3B, the hypothesized site of action for lithium, a primary treatment for BD. Overall, our results lend further support to the polygenic basis of BD and demonstrate a role for rare coding variation as a significant risk factor in BD aetiology.

Introduction

Bipolar disorder (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 a significant source of disease burden worldwide (*1*). Meta-analysis of 24 twin studies estimated broad-sense heritability of BD to be around 67% (*2*), while recent molecular genetic analyses estimated the additive heritable component from common SNPs (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, 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, particularly copy number variants, have 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*). Similarly, previous studies showed some evidence for the role of rare PTVs in BD risk, but with a more modest effect size compared to ASDs and schizophrenia (*7*). The extent that rare variation may be expected to influence BD susceptibility can be inferred by assessing the degree of natural selection acting on individuals with BD. Specifically, negative selection on BD would cause alleles with high penetrance for BD risk to be kept at a low frequency in the population (*8*, *9*). Evidence for negative selection on BD can be seen in the significantly lower reproductive rate of both males (0.75 to 1) and females (0.85 to 1) with BD compared to their unaffected siblings in a large Swedish birth cohort (*10*). The reproductive rate observed in BD, however, is substantially higher than for individuals with schizophrenia (0.23 for males, 0.47 for females) or autism (0.25 for males, 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 degree to which this reduction in fecundity has been consistent over human history. Nevertheless, the interrogation of rare variation in BD patients will be pivotal in the discovery of variants with high penetrance for BD risk.

Within BD, two clinical subtype classifications are recognized: bipolar I disorder (BD1) and bipolar II disorder (BD2; APA DSM-IV (*11*); WHO ICD-10 (*12*)). BD1 diagnosis includes at least one manic episode and usually at least one depressive episode. 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 mood episode, and depressed mood. To be diagnosed with schizoaffective disorder bipolar type, a manic episode must constitute part of the presentation (*13*–*15*). Despite the 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 (MDD) (r_g = 0.35), with BD1 showing preferential overlap with schizophrenia and BD2 with MDD, reflecting a broad continuum of genetic influence on psychosis and mood disturbance (*3*).

To date, GWAS meta-analysis of common SNPs have identified 64 independent loci that contribute to BD susceptibility, implicating genes encoding ion channels, neurotransmitter transporters, and synaptic and calcium signalling pathways (*3*, *5*). Evidence of rare variation on BD risk, however, remains inconclusive as sample sizes are substantially smaller than GWAS. Analysis of large rare copy number variants (MAF < 1%) in 6,353 BD cases 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 both pedigree and case-control cohorts have shown only nominal enrichment among individual genes and candidate gene sets (*16*–*19*), with none surpassing exome-wide significance.

Here, we report results from the Bipolar Exome (BipEx) collaboration, the largest whole-exome study of BD to date, comprising 13,933 BD cases and 14,422 controls following aggregation, sequencing, and quality control.

Results

Description of exome sequencing data generation, sample cohorts and quality control

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 (See Table S1 and

supplementary materials: sample collections, for a full breakdown by cohort and subtype, and subtype definitions). All of the 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: exome sequencing of the full sample set was performed between July 2017 and September 2018 using Illumina Nextera sample preparation and HiSeqX sequencing. Samples were then jointly processed and run through variant calling using the Genome Analysis ToolKit (GATK), (supplementary materials: sequence data production). Following sequencing and joint calling, we ran a series of quality control steps to filter out low quality variants (Table S2) and samples (Table S3), and restricted the dataset to unrelated individuals of broad continental European ancestry (supplementary materials: exome quality control, Figures S1-5). The analysis-ready high-quality dataset consisting of 13,933 bipolar cases and 14,422 controls is summarised in Table S4. Breaking down by BD subtype, the curated dataset consists of 8,238 BD1, 3,446 BD2, 1,288 BDNOS, 961 BD cases without a finer diagnosis (together encompassing the 13,933 BD cases), and 277 schizoaffective disorder cases. Throughout our analyses, we exclude individuals diagnosed with schizoaffective disorder in order to obtain a more BD specific collection of results and guard against signals more attributable to schizophrenia influencing reported associations.

Significant contribution of rare damaging protein truncating variation to bipolar risk

To test whether BD cases carry an excess of damaging coding variants, we analyzed exome-wide burden relative to controls using a logistic regression model controlling for principal components, sex, and overall coding variant burden (supplementary materials: exome-wide burden analyses). Drawing from previous exome sequencing studies of psychiatric disease (*18*, *20*, *21*), we restricted our analysis to variants with minor allele count (MAC) ≤ 5 across the entirety of the dataset, corresponding to MAF ≤ 0.01%. We annotated variants using the Ensembl Variant Effect Predictor (VEP) (*22*) version 95 with the LOFTEE plugin, and assigned variants to classes of variation. We first defined two putatively damaging classes of coding variation: protein-truncating variants (PTVs) and damaging missense variants (missense variants annotated as ‘probably damaging’ in PolyPhen-2 (*23*) and ‘deleterious’ in SIFT (*24*)). We further defined two annotations which we hypothesized to be likely benign: other missense (the remaining missense variants), and synonymous variants (see supplementary materials: variant annotation and Table S5 for full details). Following this initial restriction we observed nominally significant enrichment of damaging missense variation in BD cases and BD2 cases over controls (OR = 1.01, P = 0.024 and OR = 1.02, P = 0.0086 respectively); Figure 1B,C, but not of PTVs. However, stepwise filtering of rare PTVs to those not in the non-neurological portion of the Genome Aggregation Database (gnomAD), hereafter referred to as ‘ultra-rare variants’, and then in constrained genes (defined as pLI ≥ 0.9), shows that case-control PTV enrichment is present once we filter to high pLI genes, a finding in line with that from schizophrenia exomes (*25*); Figure 1B,C, Figure S6. This enrichment is consistent among both BD1 and BD2 subtypes (Figure 1A). A conservative Bonferroni significance threshold

accounting for multiple testing (for all of the analyses depicted in Figure 1) was set at P = 0.05/27 ≈ 0.0019. While the magnitude of the significant PTV enrichment in BD (OR = 1.11, P = 5.0×10^{-5}) is considerably lower than the latest PTV enrichment in schizophrenia (OR = 1.26; (*25*)), this difference is in line with the decreased selective pressure estimated from higher reproductive rates in BD affected siblings relative to those seen in schizophrenia affected siblings (*10*).

In an attempt to refine the nominally significant damaging missense signal, we sought to further distinguish likely deleterious missense variants from benign missense variants. To do this, we annotated variants with a missense deleteriousness predictor which takes into account regional missense constraint: “Missense badness, PolyPhen-2, and regional Constraint score” (MPC) (*26*). We then identified a subset of missense variants that are highly deleterious (MPC ≥ 2), as suggested by the authors. 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 *p*LI ≥ 0.9 gene) for either BD1, BD2 or BD (Figure S7). This is likely because the MPC ≥ 2 group accounts for a small proportion of the total damaging and benign missense variants annotated by PolyPhen-2 and SIFT. For example, the number of MPC ≥ 2 variants in the data-set following the increasingly stringent filters (MAC ≤ 5, ultra-rare, or ultra-rare at in a *p*LI ≥ 0.9 gene) were 39,000, 23,000 and 5,000 respectively, compared to 360,000, 159,000 and 31,000 for damaging missense variants.

We looked to tease apart the signal of excess ultra-rare PTVs in BD cases compared to controls. We first looked to see if age of first impairment stratifies ultra-rare PTV burden. We evaluated age at first impairment for a subset of 3,134 cases (supplementary materials: age of onset definitions, Table S6), but found no difference in the distribution of ultra-rare PTV burden or carrier status between earlier onset cases compared to older onset cases (minimum *P*-value across 50 Kolmogorov-Smirnov (KS) tests was 0.40, minimum *P*-value across 50 Fisher’s exact tests was 0.067 (supplementary materials: testing for relationship between age of onset and rare variant burden)).

We also assessed whether the presence or absence of psychosis in a subset of 8,017 BD case samples (4,214 with psychosis (comprising 3,152 BD1, 661 BD2, 352 BDNOS, and 49 BD cases without a fine subclassification), 3,803 without psychosis (comprising 1,423 BD1, 1,845 BD2, 505 BDNOS, and 30 BD cases without a fine subclassification)) stratified ultra-rare PTV burden (Table S7, supplementary materials: psychosis definitions). Both case subsets displayed significant enrichment of ultra-rare PTV burden in constrained genes (OR = 1.12, P = 0.0018; OR = 1.16, $P = 6.6 \times 10^{-5}$ for cases with and without psychosis 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 damaging missense (MPC ≥ 2) variation in BD cases, in contrast to schizophrenia (*25*); Figure S7. 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 (*p*LI ≥ 0.9); Figure 1B,C (BD: OR = 1.02, *P* = 0.0018; BD1: OR = 1.02, *P* = 0.014; BD2: OR = 1.03, *P* = 0.0036).

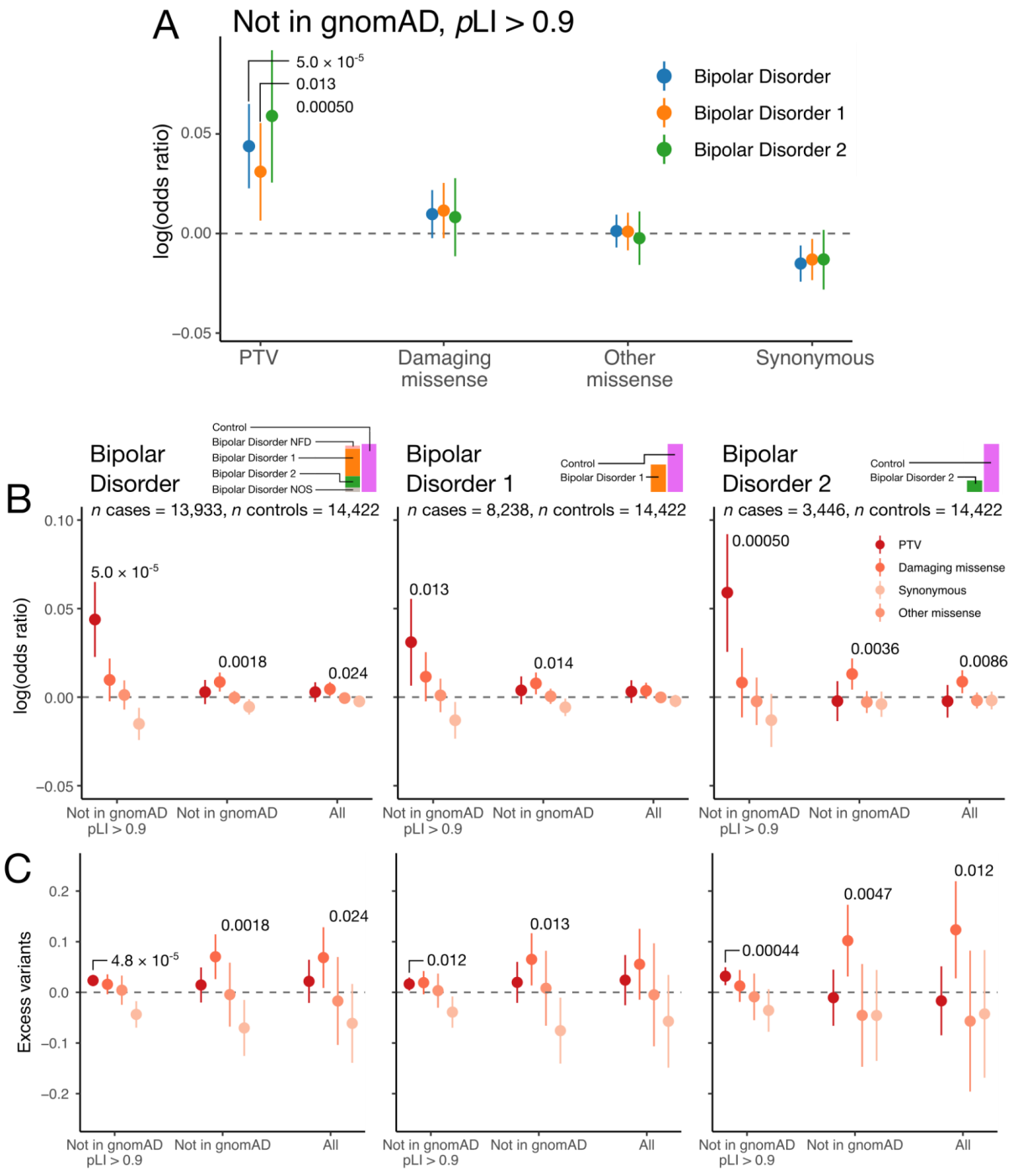

Figure 1: Case-control enrichment of ultra-rare variants, split by case status and consequence category. Panel A displays enrichment in cases over controls in case subsets, according to the legend. In panels B and C, we display case-control enrichment and excess case rare variant burden in increasingly *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 *p*LI ≥ 0.9 gene and not in the non-neurological portion of gnomAD (Not in gnomAD *p*LI ≥ 0.9), to a variant with MAC ≤ 5 (All) according to the *x*-axis labelling. Bars in panels B and C represent the 95% confidence intervals on the logistic and linear regression estimate of the enrichment of the class of

variation labelled on the *x*-axis respectively. Regressions are run as described in supplementary materials: exome-wide burden analyses, and include sex, 10 PCs and total coding burden with the same rarity as covariates. Nominally significant enrichments or excess variants in cases are labelled with the associated *P*-value.

Ultra-rare variant burden in tissues and candidate gene-sets

Beyond exome-wide and constrained gene burden, 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 portal (*27*), we find weak evidence for enrichment of ultra-rare PTVs in 13,372 genes expressed in brain tissues in bipolar cases (OR = 1.01, $P = 0.032$), but not in genes expressed in non-brain 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 ((*28*), Table S8) in collections of genes defined as having the strongest tissue specific expression (Figure 2A, Figure S8). To arrive at these gene-lists, *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 (*28*). For full details see supplementary materials: gene-set variant burden testing. The pattern of enrichment for damaging ultra-rare variation resides predominantly in brain tissues, with the strongest association seen in the Amygdala (OR = 1.03, $P = 3.9 \times 10^{-5}$), a brain region previously found to be reduced in size in BD1 cases (*29*).

We then considered 68 candidate gene-sets either generated or implicated in previous genetic studies of psychiatric disorders (supplementary materials: gene-set enrichment analysis, Figure 2B, Figure S9), and a more strictly defined collection of genes highly expressed in brain in GTEx: those with average expression over two-fold higher in brain tissues than the average across all tissues in GTEx (*30*). With this more stringent brain-enrichment definition (6,630 genes), we saw stronger ultra-rare PTV enrichment in BD cases (OR = 1.04, $P = 2.49 \times 10^{-3}$). Among the 68 candidate gene sets, we observe significant enrichment (multiple test correction set at $P < 3.68 \times 10^{-4}$) of ultra-rare variation in two gene sets in BD cases. For ultra-rare PTVs, we see significant enrichment in SCHEMA genes; FDR < 5% (*25*) (34 genes, OR = 1.89, $P = 4.81 \times 10^{-5}$), and CHD8 binding targets in human brain (*31*) (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 (*32*) (948 genes, OR = 1.07, $P = 3.70 \times 10^{-4}$), and ASD FDR < 10% (*33*) (66 genes, OR = 1.24, $P = 7.25 \times 10^{-4}$), though neither passes multiple testing correction. The enrichment of ultra-rare PTVs in SCHEMA and damaging missense variants in ASD provides 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 (*34*), and schizophrenia and ASD in rare variation (*25*). Notably, we did not observe a rare-variant enrichment of damaging variation in gene sets generated from GWAS of BD of 20,352 cases and 31,358 controls (*3*). Despite this, we do see 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 GWAS (*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 (*25*). We observed ultra-rare PTV enrichment in the top 50 genes, which include the FDR < 5% set (OR = 2.05, $P = 1.25 \times 10^{-8}$), but this significant enrichment disappears when we evaluated 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 (*35*) (OR = 0.867, $P = 0.192$).

Along with a candidate gene-set enrichment analysis approach, we considered a broad-based enrichment analysis using gene-sets derived from large pathway databases including Gene Ontology (GO), REACTOME and KEGG, a total of 1,697 gene-sets (Figure S10). By analyzing excess rare variant burden in such a large collection of gene-lists we sought to elucidate pathways enriched for damaging variation associated with BD in an agnostic manner. We observed significant enrichment of one gene-set after correction for multiple tests: genes involved in the G1/S transition of the mitotic cell cycle (172 genes; OR = 1.46, $P = 1.37 \times 10^{-5}$.

Gene-based analysis approach

To boost power for gene discovery, we again restricted to ultra-rare variants and tested for enrichment of putatively damaging classes of variation: PTVs (Table S5) and damaging missense variants (supplementary materials: gene-based analysis approach; Table S5). Throughout, we use Fisher's exact tests in each gene to test for case-control enrichment (supplementary materials: gene-based analysis approach, Figures S11-15). Associated *Q*-values for Fisher's exact test statistics in BipEx were evaluated using the Benjamini and Hochberg adjustment (*36*) applied to all genes with at least 10 ultra-rare PTVs across cases and controls. We found that enrichment in constrained genes remains significant after excluding the top 20 BD-risk associated genes in BipEx (OR = 1.07; $P = 0.00313$) with *p*LI ≥ 0.9 (Table S9).

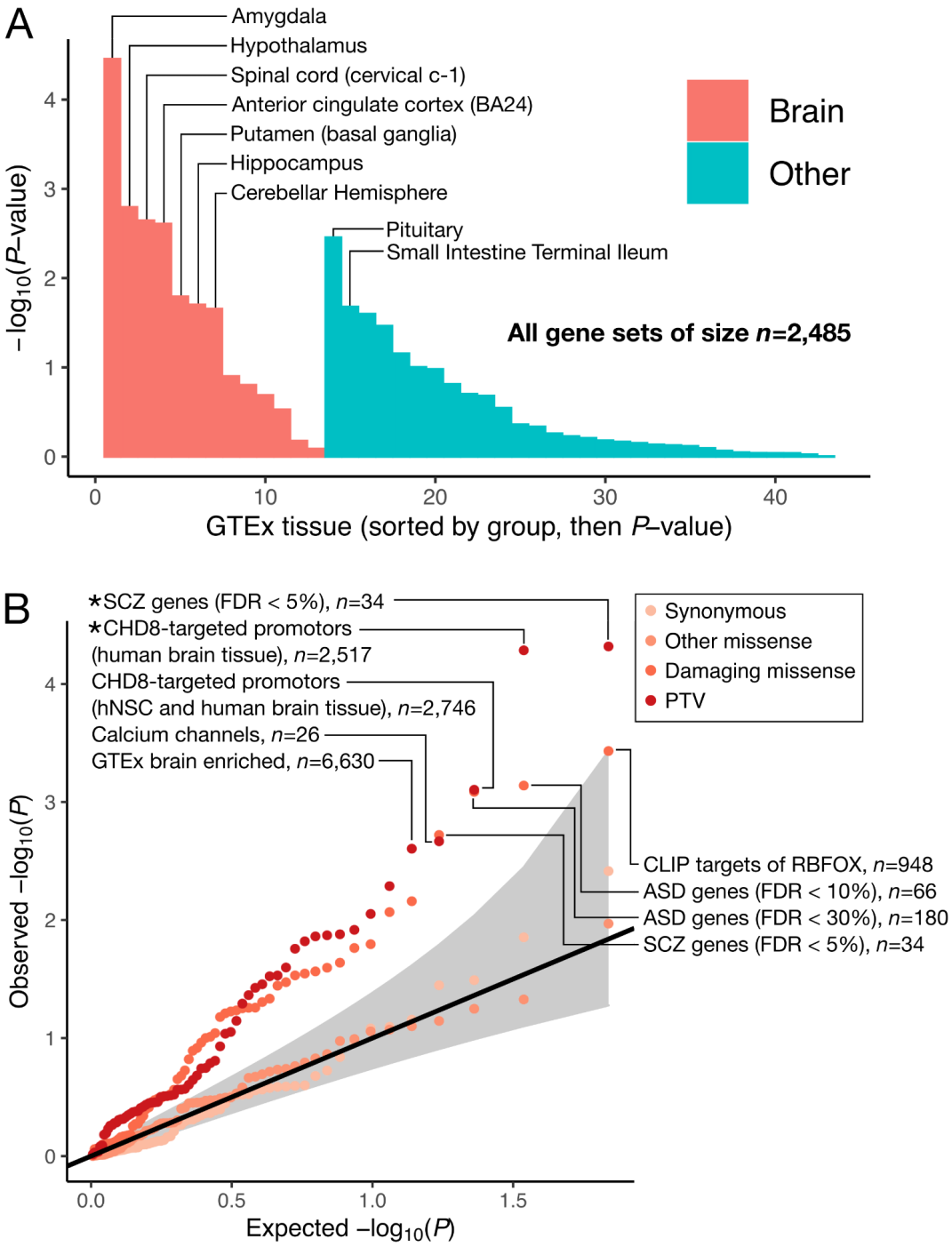

Figure 2: Biological insights from bipolar case-control whole-exome sequencing data. A. Enrichment of ultra-rare PTVs in BD cases over controls in tissue-specific expression gene-sets. Gene-sets are defined in (*28*) in detail. Bars are ordered by *P*-value, first for brain tissue and then for other tissues. B. Enrichment of ultra-rare variants in targeted 68 gene-sets taken from the literature (*25*, *37*). Top PTV and damaging missense gene-sets are labelled, and annotated with the number of genes in each gene-set. Classes of variants tested in each gene-set are coloured according to the legend. Gene-sets surpassing Bonferroni test correction are labelled with an asterisk.

AKAP11 implicated by ultra rare protein truncating variants

In our primary analysis, no gene surpassed exome-wide significance (set at $P < 2.14 \times 10^{-6}$ for 23,321 tests; dotted line in Figure 3). We do, however, begin to observe deviation from the null in the collection of tests of ultra rare PTV enrichment in BD cases, particularly in BD1 (Figure S16). This deviation was not observed for BD2 (Figure S17) despite the genome-wide enrichment of the PTV signal (Figure 1B,C), and is 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 sought to determine whether there is evidence of a shared signal of enrichment of ultra-rare PTVs in BD and schizophrenia cases. Due to overlap in controls between SCHEMA and BipEx, we analysed an ultra-rare variant count data-set which excluded these controls, and meta-analysed the data (supplementary materials: combining SCHEMA and BipEx data in meta-analysis). 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 10 *P*-values before and after meta-analysis with SCHEMA counts (Table 1 and Table S10). The combined analysis in BD and schizophrenia cases reveals one exome-wide significant gene, *AKAP11* ($P = 2.83 \times 10^{-9}$), and one gene which almost attains exome-wide significance, *ATP9A* ($P = 5.36 \times 10^{-6}$).

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 (LOEUF = 0.3, *p*LI = 0.98), and is highly expressed in the brain (cerebellar hemisphere: 38.54 median TPM;, frontal cortex (BA9): 31.52 median TPM (*27*)). Additionally, AKAP-11 has been shown to interact with GSK3B, the hypothesized target of lithium therapy (*38*–*40*). Therefore, we gathered all available lithium response information for carriers of *AKAP11* PTVs among the BD cases (supplementary materials: lithium response). Of the eleven cases for which lithium response information was available, seven reported a good response (of which five were in SWEBIC cohort and reported 'complete response, recovered', and two were in the Cardiff collection and reported that lithium helped stabilise their moods), and four did not respond well to lithium. Of the poor responders, three were in the London cohort, and one was in the SWEBIC cohort. While the percent of good responders in *AKAP11* PTV carriers (63.6%) is marginally elevated relative to the background response rate in available BD cases (52%), the sample size is far too small to form any robust conclusions from the data.

AKAP11 does not appear to be a prominent risk gene for autism (*41*, *42*). Furthermore, to our knowledge, there is no signal of enrichment in *AKAP11* in other neurodevelopmental disorders at current sample sizes. *AKAP11* is not present in a collection of 'developmental disorder genes' curated to be associated with developmental disorders (*43*); https://decipher.sanger.ac.uk/ddd/ddgenes), the autism sequencing consortium (ASC) analysis

(*41*), or Epi25 study (*44*). Furthermore, expression of *AKAP11* tends to occur later in development (Figure S18).

We also examined ultra-rare PTV variant counts in the Bipolar Sequencing Consortium (BSC) (*18*) exome sequence data (supplementary materials: external validation with the BSC exome data, Table S11). Non-zero count data were available for seven of the top ten genes exhibiting differences in ultra-rare PTV counts between BD cases and controls as measured by *P*-value in the BipEx dataset. Of these, one was enriched for ultra-rare PTVs in controls (FREM2) in BipEx, and did not display control enrichment in the BSC data. The remaining six displayed case enrichment in BipEx. In four out of these six genes (including *AKAP11* and *ATP9A*), we observed further case enrichment (Table S12) in the BSC data.

	BD (BipEx)					SCZ (SCHEMA)				Combined	
Gene	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}

Table 1: BipEx and SCHEMA case-control counts of the top ten most significant genes in the BipEx gene-based analysis. Case and control columns denote the count of ultra-rare PTVs in the gene in the respective dataset. *P*-values are determined using Fisher's exact and CMH tests for BipEx and SCHEMA (supplementary materials: gene-based analysis approach) respectively, and meta-analysed weighting by effective sample size. *Q*-values for Fisher's exact test statistics in BipEx were evaluated using the Benjamini and Hochberg adjustment (*36*) applied to all genes with at least 10 ultra-rare PTVs across cases and controls. BipEx: BD case count 13,933, control count 14,422. SCHEMA: schizophrenia case count 24,248, control count 91,960. 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/control counts differ across genes.

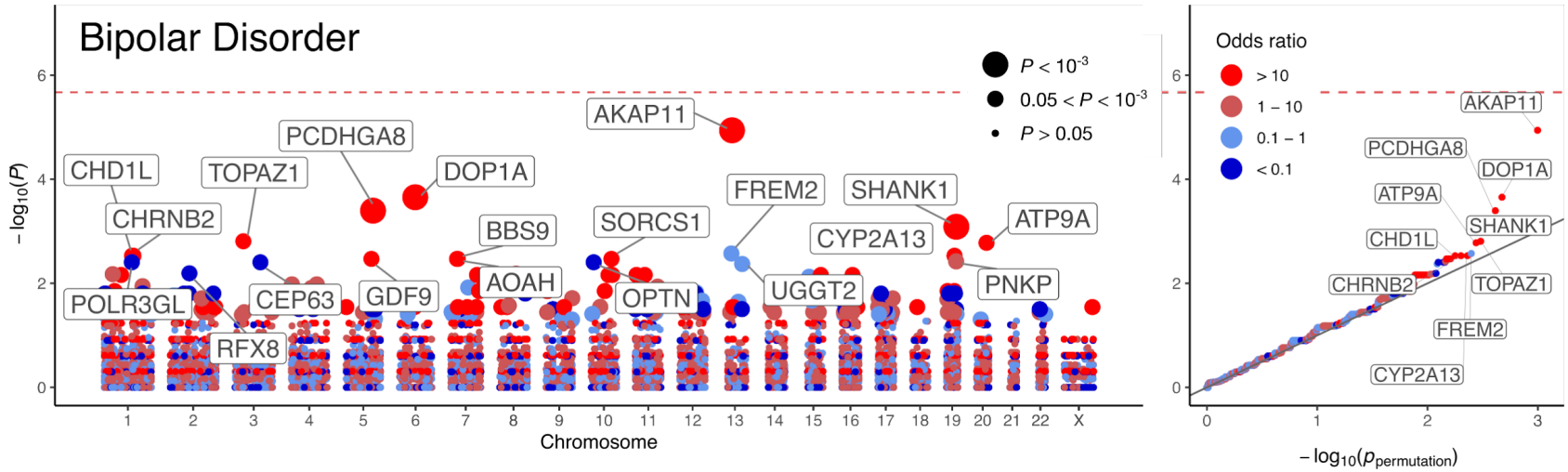

Figure 3: Results of the analysis of ultra-rare PTVs in 13,933 cases and 14,422 controls. Gene-based Manhattan and QQ plot for BD (comprising BD1, BD2 and BDNOS). -$\log_{10}$ *P*-values obtained via Fisher's exact tests are plotted against genetic position for each of the analysed genes. In the QQ plots, observed -$\log_{10}$ *P*-values are plotted against permutation *P*-values according to the procedure described in the supplementary materials: gene-based analysis approach. Points are coloured 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 labelled, respectively. Points in the Manhattan plot are sized according to *P*-value as displayed in the legend.

Discussion

In the largest BD exome study to date, ultra rare PTVs in constrained genes are significantly enriched in BD cases. 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 pLI ≥ 0.9 (Table S9). This reflects the highly polygenic genetic architecture of BD, a property shared with schizophrenia (*25*), and suggests that the majority of genes involved in BD risk will require larger sample sizes to be discovered. Furthermore, in BD cases, 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 reveals 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 bipolar disorder (*45*). 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 more than one hundred different substrates, thus affecting a myriad of signalling pathways (*6*, *45*, *46*). With this in mind, we looked to determine the efficacy of lithium treatment in BD cases harboring an ultra-rare PTV in *AKAP11*. Of the eleven individuals with treatment data available, seven displayed a good response to lithium treatment, in line with the notion that the effects of disrupting AKAP-11 may be partially rescued by lithium therapy. However, the ultra-rare PTV carrier sample size is currently too low to draw robust conclusions regarding lithium treatment response.

Beyond PTV enrichment in constrained genes, 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 given the signal of association seen for rare (MAC ≤ 5; MAF ≈ 2×10^{-5}) missense variation in schizophrenia cases is mainly within constrained missense regions (MPC ≥ 2) (*25*). Because BD2 displays a stronger correlation of common variant effects with major depression than BD1, and BD1 is more correlated with schizophrenia than BD2, there is a chance that this missense signal is capturing something distinct to mood disorders relative to psychotic disorders. However, we should be cautious not to read too much into differences in ultra-rare damaging

missense enrichment across the BD subtypes; the number of BD2 samples (*n* = 3,446) in the BipEx dataset is less than half that of BD1 (*n* = 8,238), and confidence intervals around the damaging missense enrichment overlap (Figure 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 (Figure S17, Figure S9). As with PTV enrichment, we expect to see a refinement of the putatively damaging missense signal as sample sizes increase.

Despite sequencing 13,933 BD cases, we did not observe any BD specific risk genes surpassing exome-wide significance. In contrast, the 24,248 schizophrenia cases analyzed in SCHEMA yielded 10 significant risk genes. When we compare 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 in BD to achieve comparable statistical power to discover individual risk genes. Moreover, we now see meaningful convergence of gene overlap for schizophrenia from the common and rare end of the allele frequency spectrum, in large part through larger exome sample sizes as well as fine-mapping of GWAS loci (*25*). The genetic overlap from common and rare variation in BD, however, remains uncertain. The BSC examined 3,987 BD case exomes (*18*), and found suggestive enrichment in 165 genes implicated in BD GWAS (OR = 1.9, $P = 6.0 \times 10^{-4}$), but we did not replicate this finding in our current sample (OR = 0.9, $P = 0.40$). Prior to SCHEMA, evidence of common and rare gene overlap in schizophrenia was modest (*20*, *21*, *37*). As sample sizes increase for both common and rare variation analyses in BD, we expect to see a slow but steady convergence of genes identified through common and rare variant analyses, as seen in schizophrenia. .

In summary, ultra-rare PTVs in constrained genes are significantly enriched in BD patients 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.

Data availability

We display all of our results, from the variant and gene level in a browser available at https://bipex.broadinstitute.org. A detailed summary of phenotype curation, and QC, including additional plots is available at https://astheeggeggs.github.io/BipEx/. Whole Exome Sequence data generated under this study are hosted on and shared with the collaborating study groups via the controlled access Terra platform (https://app.terra.bio). The Terra environment, created

by the Broad Institute, contains a rich system of workspace functionalities centered on data sharing and analysis. Requests for access to the controlled datasets are managed by data custodians at the Broad Institute and sent to sample contributing investigators for approval.

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 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'.

Code availability

Code used to perform QC, analysis, and creation of plots is provided at github.com/astheeggeggs/BipEx.

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Competing interests

B.M.N. is a member of the scientific advisory board at Deep Genomics and RBNC and consultant for Camp4 Therapeutics, Takeda Pharmaceutical, and Biogen. D.S.P. was an employee of Genomics plc. All the analyses reported in this paper were performed as part of D.S.P.'s employment at the Analytic and Translational Genetics Unit, Department of Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA, and Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, Massachusetts, USA. C-Y.C. is an employee of Biogen. F.D. is an employee of Sheppard Pratt. A.L. and E.A.S. are now employees of Regeneron. All other authors declare no competing interests.

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Exome sequencing in bipolar disorder reveals *AKAP11* as a risk gene shared with schizophrenia: Supplementary Materials

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Sample collections

The BipEx cohort aggregates 13 separate sample collections, involving 21 primary investigators across 6 separate countries. We performed careful quality control steps to variants and samples, detailed in the Exome QC section. The aggregated dataset consists of 39,617 individuals, 16,486 of which have been diagnosed with bipolar disorder, and 17,213 with no known psychiatric diagnosis. Of the remaining individuals, 5,483 have a schizophrenia diagnosis (which we use as positive controls in the rare variant burden analyses), 87 have a separate psychiatric diagnosis, and 32 lack phenotypic information. Full details of PI sample contributions prior to curation of sequence data are provided in Table S1. Following curation, the case and control count is displayed in Table S4. Breakdown of bipolar cases with age of onset information for age of first impairment is provided in Table S6, and a breakdown of sample sizes with psychosis information is displayed in Table S7. Sample collection and phenotype tables are also available at https://astheeggeggs.github.io/BipEx/qc.html.

PI	Location	BD	BD1	BD2	BDNOS	SAD	SCZ	Other	Unknown	Controls	Total
Andreas Reif	Wurzburg, GER	7	216	159	15	0	0	0	14	414	825
Andrew McQuillin Hugh Gurling	London, UK	228	1,309	372	0	157	1,595	0	0	1,203	4,864
Robert Yolken Faith Dickerson	Baltimore, USA	8	117	9	5	0	0	8	0	126	273
Danielle Posthuma	Amsterdam, NED	0	0	0	0	0	0	0	1	948	949
David St Clair	Aberdeen, UK	0	0	0	0	0	564	0	1	331	896
Derek Morris Aiden Corvin	Dublin, IRE	0	180	0	0	11	29	3	0	9	232
Douglas Blackwood	Edinburgh, UK	401	368	114	2	6	304	0	0	64	1,259
Fernando Goes	Baltimore, USA	0	241	0	0	0	0	0	0	0	241
Jordan Smoller	Boston, USA	361	2,122	390	576	52	0	0	0	3,498	6,999
Michael O Donovan	Cardiff, UK	0	0	0	0	11	2,986	1	0	0	2,998
Michael Owen	Cardiff, UK	0	0	0	0	0	0	0	0	1,106	1,106
Mikael Landén	Stockholm, SWE	138	2,364	1,753	905	1	0	0	0	761	5,922
Nancy Pedersen	Stockholm, SWE	0	0	0	0	0	0	0	0	4,780	4,780
Nick Craddock Arianna Di Florio Ian Jones Lisa Jones James Walters	Cardiff, UK	85	1,518	772	67	57	4	17	0	0	2,520
Roel Ophoff	Utrecht, NED	1	1,032	169	10	21	1	58	16	663	1,971
Rolf Adolfsson	Umea, SWE	0	320	149	3	0	0	0	0	459	931
Willem Ouwehand	Cambridge, UK	0	0	0	0	0	0	0	0	2,851	2,851
Total		**1,229**	**9,787**	**3,887**	**1,583**	**316**	**5,483**	**87**	**32**	**17,213**	**39,617**

Table S1: Detailed summary of subtype sample contributions across PIs and geographies. BD=BD without a fine subclassification, BD1=bipolar I disorder, BD2=bipolar II disorder, BDNOS=bipolar disorder not otherwise specified, SAD=schizoaffective disorder, BD+SAD=bipolar disorder and schizoaffective disorder combined, SCZ=schizophrenia, other=other unspecified case, unknown=unknown case status.

Cohort descriptions and bipolar subtype definitions

Aberdeen, UK

PI: David St Clair

All participants self-identified as born in the British Isles (95% in Scotland). All schizophrenia cases met the DSM-IV (*1*) and International Classification of Diseases 10th edition (ICD-10) (*2*) criteria for schizophrenia. Diagnosis was made by Operational Criteria Checklist (OPCRIT) (*3*, *4*). All case participants were outpatients or stable in-patients. Detailed medical and psychiatric histories were collected. Controls were volunteers recruited through general practices in Scotland. Practice lists were screened for potentially suitable volunteers by age and sex and by exclusion of subjects with major mental illness or use of neuroleptic medication. Volunteers who replied to a written invitation were interviewed using a short questionnaire to exclude major mental illness in individuals themselves and first degree relatives. All cases and controls gave informed consent. The study was approved by both local and multiregional academic ethical committees.

Amsterdam, NED

PI: Danielle Posthuma

Controls taken from the NESCOG study, described previously (*5*). NESCOG contains both a general population and family-based sample of which closely related individuals were excluded. Data were collected on cognitive tasks, behavioral conditions, life events, personality and environmental factors. To correct for undiagnosed attention deficit hyperactivity disorder (ADHD) status, participants scoring over three standard deviations above the mean on the Conners' Adult ADHD Rating Scale (CAARS) (*6*), or the Attention Problems scale of the Young Adult Self Report (YASR) (*7*) were excluded. To correct for autism spectrum disorder (ASD) status, participants scoring over three standard deviations above the mean on the Autism Quotient (AQ) (*8*) were removed.

Baltimore, USA

PIs: Faith Dickerson, Robert Yolken

Samples were collected as part of a larger study about infectious agents and immune factors in serious mental illness. Psychiatric participants were recruited at a large psychiatric health system and non-psychiatric controls from the same geographic region. The diagnosis of psychiatric and non-psychiatric participants was confirmed with a structured clinical interview (*9*, *10*) based on DSM-IV (*1*). All participants provided written informed consent. The study was approved by the IRB of the institution where the study was performed and included a data sharing agreement.

PI: Fernando Goes

Cases represented independent probands from a European-American family sample that was collected at Johns Hopkins University from 1988-2010. Families had at least 2 additional relatives with a major mood disorder (defined as bipolar disorder type 1, bipolar type 2 or recurrent major depressive disorder). Diagnostic interviews were performed using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version and the Diagnostic Instrument for Genetics Studies. All cases underwent best-estimate diagnostic procedures. Diagnoses were based on DSM-III and DSM-IV (*1*) criteria. Probands from this sample have been previously studied in family based linkage and exome studies (*11*, *12*).

Boston, USA

PI: Jordan Smoller

Cases and controls were collected as part of the International Cohort Collection for Bipolar Disorder (ICCBD), (*13*, *14*). The Massachusetts General Hospital site of the ICCBD collected DNA from cases and controls by linking discarded blood samples to de-identified electronic health record (EHR) data. Cases and controls were identified by deriving EHR-based phenotyping algorithms applied to the Partners Healthcare Research Patient Data Registry (RPDR), described in detail previously (*15*). Bipolar subtypes were defined by Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) (*1*). Regular expression rules were used to extract mention by clinician in an inpatient or outpatient note or ICD-9/DSM-IV (*1*, *16*, *17*) code indicating Bipolar type. Full details of the algorithm are provided in (*14*).

Cambridge, UK

PI: Willem Ouwehand

Controls taken from the Wellcome Trust case control consortium (WTCCC) described elsewhere (*18*).

Cardiff, UK

PIs: Nick Craddock, Arianna Di Florio, Ian Jones, Lisa Jones, James Walters

Cases were all over the age of 17 years, living in the UK and of European descent. Cases were recruited via systematic and not systematic methods as part of the Bipolar Disorder Research Network project (www.bdrn.org), provided written informed consent and were interviewed using a semi-structured diagnostic interview, the Schedules for Clinical Assessment in Neuropsychiatry. Based on the information gathered from the interview and case notes review, best-estimate lifetime diagnosis was made according to DSM-IV. Inter-rater reliability was formally assessed using 20 randomly selected cases (mean κ Statistic = 0.85). In the current study we included cases with a lifetime diagnosis of DSM-IV bipolar disorder or schizoaffective disorder, bipolar type. The BDRN study has UK National Health Service (NHS) Research Ethics Committee approval and local Research and Development approval in all participating NHS Trusts/Health Boards. All subjects gave written informed consent.

PIs: Michael O Donovan, Michael Owen

The schizophrenia case sample included European ancestry schizophrenia cases recruited in the British Isles and has been described previously (*19*). All cases gave written informed consent to. The study was approved by the Multicentre Research Ethics Committee in Wales and Local Research Ethics Committees from all participating sites. The control sample used the WTCCC sample described elsewhere, (*18*) but included similar numbers of individuals from the 1958 British Birth Cohort and a panel of consenting blood donors (UK Blood Service). Additional controls, held by Cardiff University, were recruited from the UK National Blood Transfusion Service. They were not specifically screened for psychiatric illness. All control samples were from participants who provided informed consent (*20*).

Dublin, IRE

PIs: Derek Morris, Aiden Corvin

Samples were collected as part of a larger study of the genetics of psychotic disorders in the Republic of Ireland, under protocols approved by the relevant IRBs and with written informed consent that permitted repository use. Cases were recruited from Hospitals and Community psychiatric facilities in Ireland by a psychiatrist or psychiatric nurse trained to use the SCID (*21*, *22*). Diagnosis was based on the structured interview supplemented by case note review and collateral history where available. All diagnoses were reviewed by an independent reviewer. Controls were ascertained with informed consent from the Irish GeneBank and represented blood donors who met the same ethnicity criteria as cases. Controls were not specifically screened for psychiatric illness.

Edinburgh, UK

PI: Douglas Blackwood

This sample comprised Caucasian individuals contacted through the inpatient and outpatient services of hospitals in South East Scotland. A BD1 diagnosis was based on an interview with the patient using the SADS-L (*23*) supplemented by case note review and frequently by information from medical staff, relatives and caregivers. Final diagnoses, based on DSM-IV criteria were reached by consensus between two trained psychiatrists. Ethnically-matched controls from the same region were recruited through the South of Scotland Blood Transfusion Service. Controls were not directly screened to exclude those with a personal or family history of psychiatric illness. The study was approved by the Multi-Centre Research Ethics Committee for Scotland and patients gave written informed consent for the collection of DNA samples for use in genetic studies.

London, UK

PIs: Andrew McQuillin, Hugh Gurling

The UCL sample comprised Caucasian individuals who were ascertained and received clinical diagnoses of bipolar 1 disorder according to UK National Health Service (NHS) psychiatrists at interview using the categories of ICD10. In addition bipolar subjects were included only if both parents were of English, Irish, Welsh or Scottish descent and if three out of four grandparents were of the same descent. All volunteers read an information sheet approved by the Metropolitan Medical Research Ethics Committee who also approved the project for all NHS hospitals. Written informed consent was obtained from each volunteer. The UCL control subjects were recruited from London branches of the National Blood Service, from local NHS family doctor clinics and from university student volunteers. All control subjects were interviewed with the SADS-L (*23*) to exclude all psychiatric disorders.

Stockholm, SWE

PI: Mikael Landén

SWEBIC (Swedish Bipolar Cohort Collection), SWE

Data in SWEBIC combines phenotypic data from three routes of collection:

1. The St. Göran Bipolar Project cohort (**SBP**).
2. SWEBIC samples recruited from the Swedish National quality assurance register for bipolar disorders (**BipoläR**).
3. SWEBIC samples recruited from the Swedish Hospital Discharge Register (**HDR**).

SBP: DSM-IV-criteria was evaluated by psychiatrists or residents in psychiatry using a Swedish version of the Affective Disorder Evaluation (ADE) employed in the Systematic Treatment Enhancement Program for Bipolar Disorder (STEP-BD) study (*21*) which includes the Structured Clinical Interview for DSM Disorders (SCID) (*21*, *22*) module for affective disorders.
BipoläR: Diagnostic phenotyping was made by registering physicians in the QA-register according to DSM-IV criteria.
HDR: Bipolar disorder cases were selected based on a validated algorithm using ICD-codes with a positive predictive value of 0.92 (*24*) Bipolar subdiagnoses (BD1, BD2, BDNOS) were made by trained research nurses using a structured telephone interview.

PI: Nancy Pederson

Controls were sourced from the LifeGene Biorepository at the Karolinska Institute, described in detail previously (*25*).

Umea, SWE

PI: Rolf Adolfsson

Bipolar disorder outpatients at the Affective Unit at the Psychiatric Clinic at the University Hospital (Umeå, Sweden) were enrolled in this study between 1998 and 2007 (*26*). Patients were characterised clinically in a number of ways, including the MINI (*27*), the Family Interview for Genetic Studies (FIGS), the Diagnostic Interview for Genetic Studies (DIGS) (*28*), and the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (*23*). Final subtype diagnoses were evaluated in line with the DSM-IV-TR (*29*) and determined through consensus of two

research psychiatrists. Controls in the data set were a randomly sampled subset of the ‘Betula study’ chosen to be representative of the population of the region.

Utrecht, NED
PI: Roel Ophoff
The case sample consisted of inpatients and outpatients recruited through psychiatric hospitals and institutions throughout the Netherlands. Cases with DSM-IV bipolar disorder, determined after interview with the SCID-I (*22*) were included in the analysis. Controls were collected in parallel at different sites in the Netherlands and were volunteers with no psychiatric history after screening with the Mini-International Neuropsychiatric Interview (MINI) (*30*, *31*). Ethical approval was provided by UCLA, the University Medical Center Utrecht, and local ethics committees and all participants gave written informed consent.

Wurzburg, GER
PI: Andreas Reif
Cases were recruited from consecutive admissions to psychiatric in-patient units at the University Hospital Würzburg. All cases received a lifetime diagnosis of BD according to the DSM-IV criteria using a consensus best-estimate procedure based on all available information, including semi-structured diagnostic interviews using the Association for Methodology and Documentation in Psychiatry (*29*, *32*), medical records and the family history. In addition, the OPCRIT (*3*, *4*) system was used for the detailed polydiagnostic documentation of symptoms. Control subjects were healthy participants who were recruited from the community of the same region as cases (*33*). They were of Caucasian descent and fluent in German. Exclusion criteria were manifest or lifetime DSM-IV axis I disorder, severe medical conditions, intake of psychoactive medication as well as alcohol abuse or abuse of illicit drugs. Absence of DSM-IV axis I disorder was ascertained using the German versions of the MINI (*27*). IQ was above 85 as ascertained by the German version of the Culture Fair Intelligence Test 2. Study protocols were reviewed and approved by the ethical committee of the Medical Faculty of the University of Würzburg. All subjects provided written informed consent.

Sequence data production

Exome Sequencing and Alignment

Exome sequencing was performed at the Broad Institute of Harvard and MIT 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 37Mb target. Sequencing was performed on HiSeqX instruments to 150bp 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 20x, comparable to ~55x mean coverage. Data delivery per sample includes a demultiplexed, aggregated into a BAM file and processed through a pipeline based on the Picard suite of software tools. The BWA aligner mapped reads onto the human genome build 38 (GRCh38). Single nucleotide polymorphism and insertions/deletions were joint called across all samples using Genome Analysis Toolkit (GATK) (*34*) 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 (*35*).

Exome Quality Control

Throughout, to perform quality control and a subset of the downstream analyses, we made use of Hail, an open-source, general-purpose, Python-based data analysis library with a particular focus on the analysis of large-scale genetic data (website: https://www.hail.is, GitHub: https://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. Unless otherwise stated, all of the data curation and quality control steps were performed in hail 0.2.
Briefly, we perform a series of hard-filters on genotype and variant metrics (Table S2), followed by a collection of hard-filters on sample metrics (Table S3). We confirm genotype sex with reported sex, remove related individuals, and restrict analysis to samples of continental European ancestry where we have sufficient sample size and balanced case-control counts (Table S3). Finally we filter based on a second collection of sample and variant hard-filters (Tables S2-3).

Initial Hard Filters

A series of quality control (QC) steps were run to clean and curate the sequence data. We first apply a collection of genotype filters, removing genotypes according to the following criteria: If homozygous reference, remove if at least one of the following is true: phred-scaled genotype quality (GQ) < 20, depth (DP) < 10. If heterozygous, remove if at least one of the following is true: (reference allele depth + alternative allele depth) divided by total depth < 0.8, alternative allele depth divided by total depth < 0.2, reference genotype quality < 20, depth < 10. If homozygous alternate, remove if at least one of the following is true: alternative allele depth divided by total depth < 0.8, reference genotype quality < 20, depth < 10. We then apply a series of initial variant filtering steps: removing sites with more than 6 alleles, that fail variant quality score recalibration (VQSR) (*34*, *35*), lie within a low complexity region (LCR) of the genome (*36*), or fall over 50 base pairs outside the ICE exome sequencing target intervals. In addition, we perform a series of empirically derived genotype call rate filters (set at 0.97) and remove sites that become invariant after applying this filter (Table S2). As an initial pass to remove low quality and contaminated samples, we filter out samples with call rate < 0.93, free-mix contamination > 0.02 (*37*), chimeric read percentage > 0.015, mean read depth < 30x or mean genotype quality < 55 (Table S3, Figure S1).

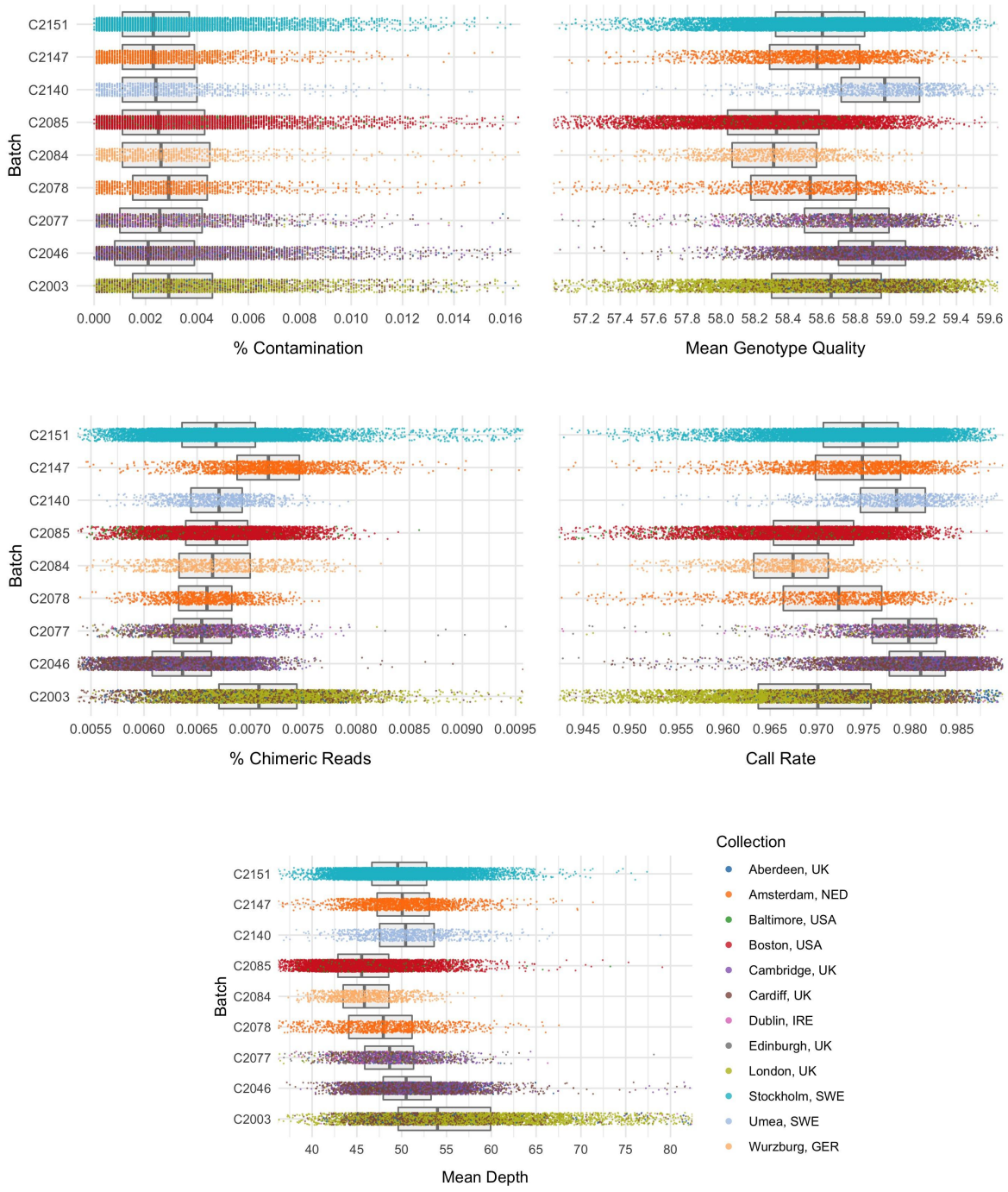

Figure S1: Distributions of variant metrics following restriction to variants passing VQSR, lying outside low-complexity regions and inside the padded (50bp) ICE target intervals, and prior to the initial hard sample filters (call rate > 0.93, FREEMIX contamination (%) < 0.02, percentage chimeras < 0.015, mean depth > 30, mean genotype quality > 55). In each plot, jittered scatters display the distribution for each sequencing batch, coloured according to sample collection. Boxplots behind the scatter display the median and interquartile range for each sequencing batch.

Sex Imputation and Relatedness

To confirm participant sex, filter out related samples, and calculate principal components, we extracted high quality common variants (allele frequency between 0.01 to 0.99 with high call rate (> 0.98)) and LD-prune to pseudo-independent SNPs using `--indep 50 5 2` in PLINK (*38*, *39*). Using autosomal markers (49,366 SNPs), we determine relatedness within the sample, and iteratively prune out samples until no pair exhibited $\hat{\pi} > 0.2$ to ensure that first and second degree relatives are filtered out. When reported sex does not match genotyped sex, it may signal potential sample swaps in the data. Using the *F*-statistic for each sample using the subset of the non-pseudo autosomal region on chromosome X (1275 SNPs), we identify and remove samples where reported sex information is not confirmed in the sequence data (Figure S2).

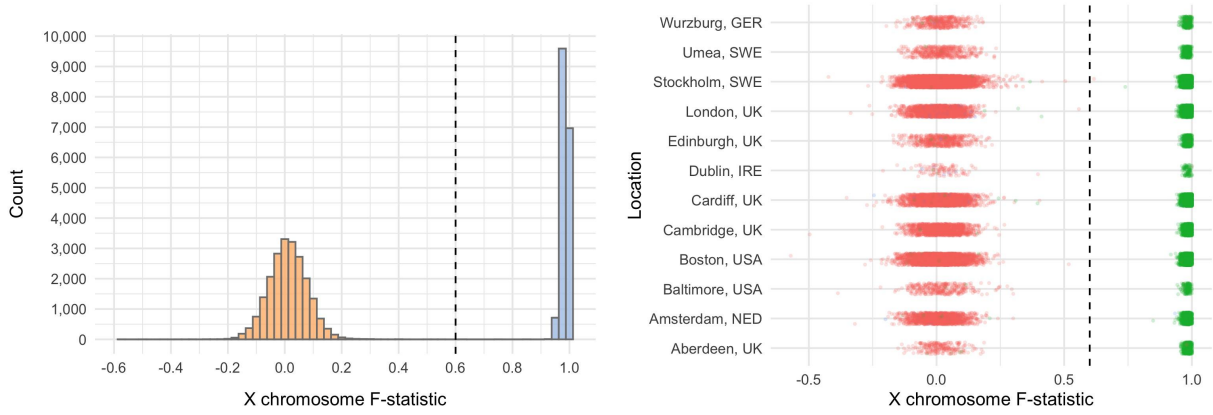

Figure S2: Histogram and scatterplots of X chromosome *F*-statistic by collection. Samples lying to the left and right of the dashed line were called as female and male respectively.

Principal component based population inference

We then merged the remaining samples with the 1000 Genomes phase 3 dataset (*40*), and computed principal components (PCs) using the LD pruned autosomal variants (49,366 SNPs). To ensure adequate case-control matching, we removed samples outside of the continental European population (EUR) using a random forest classifier trained on the EUR subset of 1000 Genomes (Figure S3), retaining samples with probability > 0.95 of being European according to the classifier. Additionally, we removed Ashkenazi Jewish samples by running principal components analysis (PCA) on samples recruited in the United States and identifying a distinct Ashkenazi Jewish cluster. Using this labelling, we trained another random forest classifier and removed additional Ashkenazi Jewish samples from downstream analysis, again using a hard cutoff of probability > 0.95 of belonging to the main European cluster (Table S3).

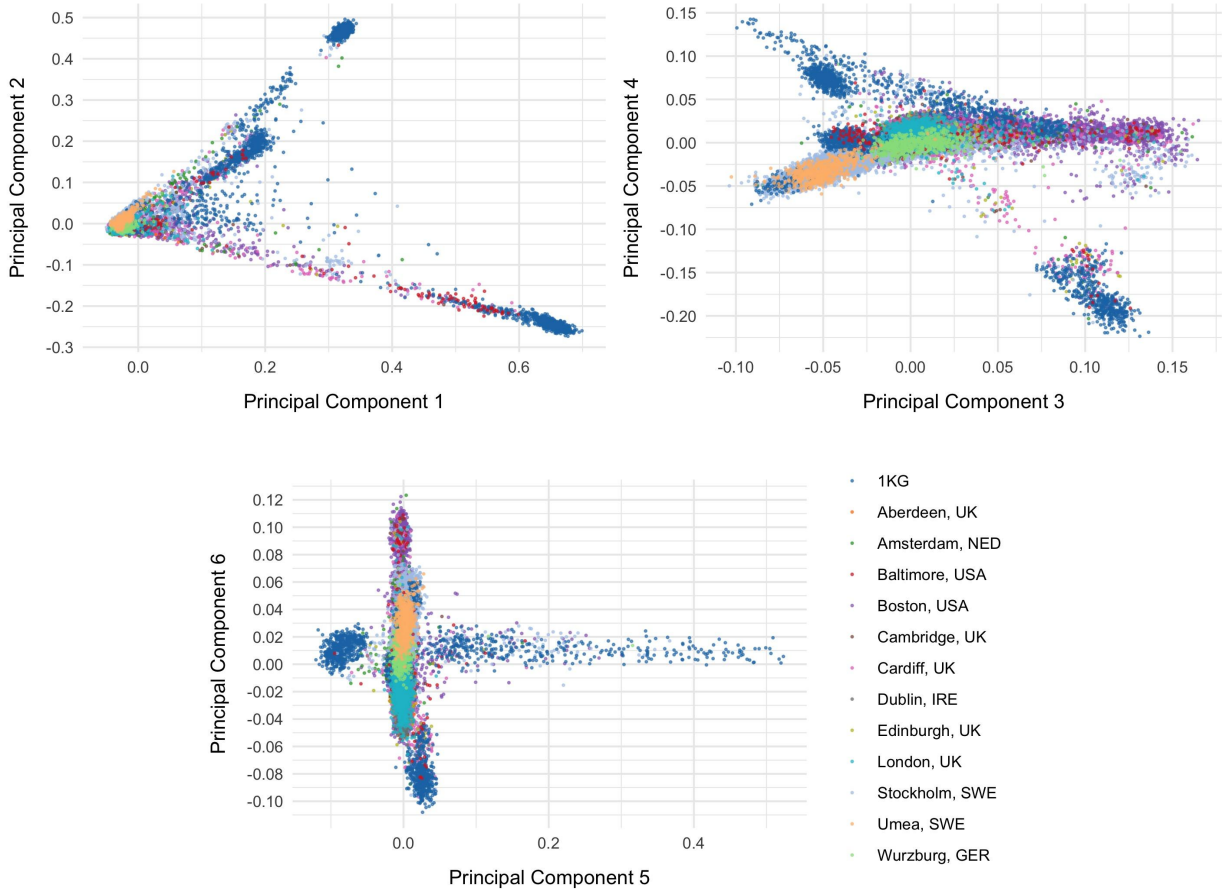

Figure S3: Scatterplots of principal components of BipEx samples together with 1000 Genomes samples. Points are coloured according to sample collection, with 1000 Genomes samples coloured in blue. 1000 Genomes super-populations labels were used to train a random forest classifier.

Final Hard Filters

For our second round of variant and sample filtering, we filter out variants based on call rate (BD call rate < 0.97, control call rate < 0.97, overall call rate < 0.97), difference in call rate between bipolar cases and controls (> 0.02), and remove variants not in Hardy-Weinberg equilibrium ($P < 10^{-6}$); Table S2. After restricting to these high quality variants (Figure S4), we perform a final set of sample filters to finalise the quality controlled data. We evaluate a collection of sample metrics and remove samples falling outside three standard deviations of the sequencing batch mean (Ti/Tv, Het/HomVar, Insertion/Deletion ratios) or cohort location (as defined by recruitment centre; *n* singletons) mean (Table S3, Figure S4). The resultant dataset consisting of 28,355 bipolar disorder cases and controls across 12 locations in Europe and the United States is summarised in Table S4. Following our QC pipeline, average heterozygote allele balance was 0.484, with 1.52% of samples lying below 0.3, and Ti/Tv became comparable between sequencing batches and sample collection. Further, average sample Ti/Tv within the targeted exome region was ~3.1 (rather than the 50bp padded), in line with expectation for populations of European ancestry (Figure S5).

Full details, code and all files required to run our pipeline are available at github.com/astheeggeggs/BipEx.

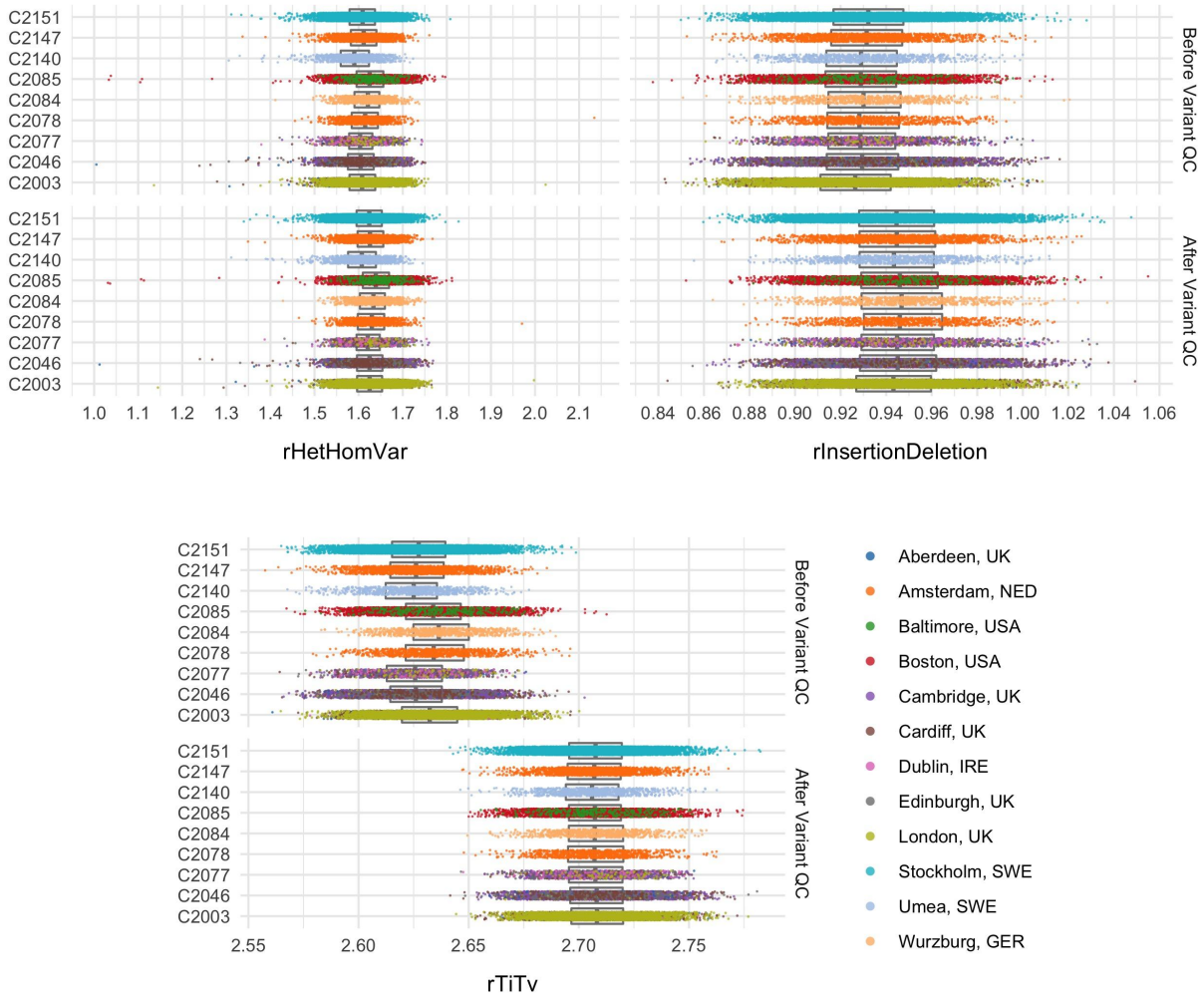

Figure S4: Distributions of variant metrics before and after the second set of empirically derived hard variant filters (BD call rate > 0.97, control call rate > 0.97, overall call rate > 0.97), difference in call rate between bipolar cases and controls (< 0.02), and remove variants not in Hardy-Weinberg equilibrium ($p > 10^{-6}$). In each plot, jittered scatters display the distribution for each sequencing batch, coloured according to sample collection. Boxplots behind the scatter display the median and interquartile range for each sequencing batch. Points shown are following variants hard-filters and prior to removal of variants with metrics outside 3 sds of the sequencing batch mean.

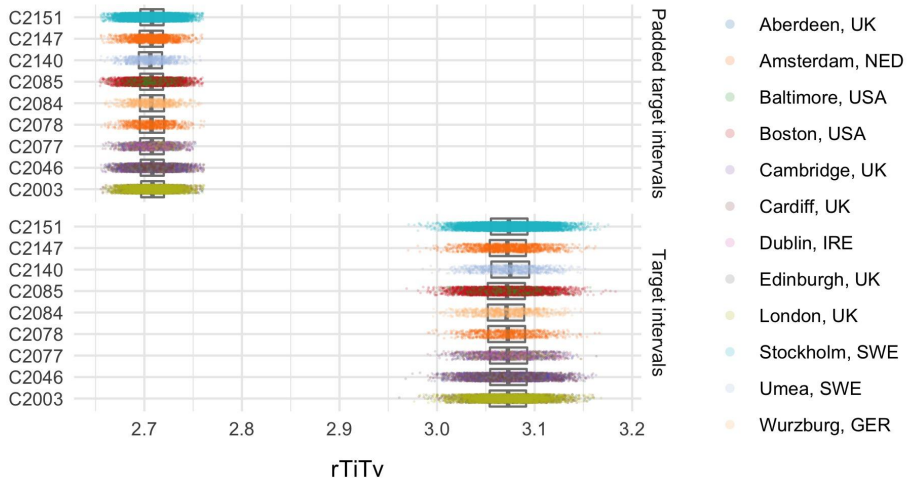

Figure S5: TiTv before and after further restriction to Target intervals with no padding. In each plot, jittered scatters display the distribution for each sequencing batch, coloured according to sample collection. Boxplots behind the scatter display the median and interquartile range for each sequencing batch.

Filter	Variants	%
Variants with < 7 alleles	37,344,246	100.0
Failing VQSR	100,742	0.3
In LCRs	1,215,218	3.3
Outside padded target interval	27,119,165	72.6
Invariant sites after initial variant and genotype filters	3,117,961	8.3
Invariant sites after sample filters	1,051,421	2.8
Overall variant call rate < 0.97	737,072	2.0
Overall variant case call rate < 0.97	716,709	1.9
Overall variant control call rate < 0.97	743,659	2.0
Difference between case and control variant call rate < 0.02	232,341	0.6
Variants failing HWE filter ($P < 10^{-6}$)	1,083,479	2.9
Variants remaining after all filters	**5,104,759**	**13.7**

Table S2: Summary of variant filters. Moving down through the rows of the table, we move through QC filters described in the methods section. Full details and code are provided at astheeggeggs.github.io/BipEx.

Filter	Samples	Bipolar cases	Controls	%
Initial samples in vcf	39,618	16,486	17,212	100.0
Unable to obtain both phenotype and sequence information	2	-	-	0.0
Unknown phenotype	32	-	-	0.1
Low coverage or high contamination	133	72	54	0.3
Sample call rate < 0.93	185	124	53	0.5
% FREEMIX contamination > 0.02	268	146	104	0.7
% chimeric reads > 0.015	152	49	100	0.4
Mean DP < 30	20	5	12	0.1
Mean GQ < 55	56	28	25	0.1
Samples with sex swap	238	147	52	0.6
Related samples for removal	1,716	792	688	4.3
PCA based filters	2,880	1,120	1,422	7.3
Within batch Ti/Tv ratio outside 3 standard deviations	100	50	42	0.3
Within batch Het/HomVar ratio outside 3 standard deviations	150	66	58	0.4
Within batch Insertion/Deletion ratio outside 3 standard deviations	93	31	48	0.2
Within location *n* singletons outside 3 standard deviations	443	151	236	1.1
Samples after final sample filters	**33,527**	**13,933**	**14,422**	**84.6**

Table S3: Summary of sample filters. Moving down through the rows of the table, we move through QC filters described in the methods section. Full details and code are provided at astheeggeggs.github.io/BipEx.

Final sample counts for analysis

Following data curation and quality control, the resultant composition of the samples by collection and bipolar subtype is summarised in Table S4.

Location	BD	BD1	BD2	BDNOS	SAD	BD total	Controls	BD total and controls
Aberdeen, UK	0	0	0	0	0	0	322	322
Amsterdam, NED	1	951	155	9	19	1,116	1,359	2,475
Baltimore, USA	3	254	6	4	0	267	41	308
Boston, USA	248	1,503	279	404	31	2,434	2,544	4,978
Cambridge, UK	0	0	0	0	0	0	2,656	2,656
Cardiff, UK	64	1,301	681	62	65	2,108	1,006	3,114
Dublin, IRE	0	150	0	0	11	150	7	157
Edinburgh, UK	298	317	94	2	6	711	58	769
London, UK	212	1,169	350	0	144	1,731	1,082	2,813
Stockholm, SWE	128	2,095	1,595	791	1	4,609	4,530	9,139
Umea, SWE	0	297	141	3	0	441	426	867
Wurzburg, GER	7	201	145	13	0	366	391	757
Total	**961**	**8,238**	**3,446**	**1,288**	**277**	**13,933**	**14,422**	**28,355**

Table S4: Detailed summary of subtype sample contributions across locations following variant and sample QC. BD=BD without a fine subclassification, BD1=bipolar I disorder, BD2=bipolar II disorder, BDNOS=bipolar disorder not otherwise specified, SAD=schizoaffective disorder, BD total = BD+BD1+BD2_BDNOS, BD total and controls=BD total+controls (excluding SAD).

Variant annotation

We use the Ensembl Variant Effect Predictor (VEP) (*41*) version 95 with the loftee plugin to annotate variants against GRCh38 using hail, including SIFT (*42*) and Polyphen2 scores (*43*), according to the GENCODE v19 reference. The configuration file available in google cloud: gs://hail-us-vep/vep95-GRCh38-loftee-gcloud.json. In addition, we annotate with version 2.1.1 gnomAD site annotations (*44*) and MPC scores (*45*) after lifting the genome coordinates over to GRCh38. MPC is an aggregate score which uses ExAC to identify sub-genic regions that are depleted of missense variation in combination with existing metrics to create a composite predictor. Finally, we annotate with Combined Annotation Dependent Depletion (CADD) version 1.4 (*45*, *46*), and annotate constraint using the gnomAD loss of function (LOF) metrics table from release 2.1.1 (*44*). We then process the VEP annotated consequences, and define variant specific consequences and gene annotations as the most severe consequence of a canonical transcript on which that variant lies. We then assign variants (where possible) to four distinct consequence classes: protein truncating variant (PTV), missense, synonymous, and non-coding as defined in Table S5. We then subdivide missense variants into ‘damaging missense’ if both the polyphen prediction is ‘probably damaging’ and the SIFT prediction is ‘deleterious’, and ‘other missense’ otherwise.

Consequence class	**VEP consequences**
PTV	Transcript ablation, splice acceptor variant, splice donor variant, stop gained, frameshift variant.
Missense	Stop lost, start lost, transcript amplification, inframe insertion, inframe deletion, missense variant, protein altering variant, splice region variant.
Synonymous	Incomplete terminal codon variant, stop retained variant, synonymous variant.
Non-coding	Coding sequence variant, mature miRNA variant, 5’ UTR variant, 3’ UTR variant, non-coding transcript exon variant, intron variant, NMD transcript variant, non-coding transcript variant, upstream gene variant, downstream gene variant , TFBS ablation, TFBS amplification, TF binding site variant, regulatory region ablation, regulatory region amplification, feature elongation, regulatory region variant, feature truncation, intergenic variant.

Table S5: Consequence classes defined based VEP annotation.

Exome-wide burden analyses

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. Note that both tests will result in near identical *P*-values; the motivation here is to ascertain two effect size parameters of rare-variant burden. We then sought to hone in on more recent mutations by restricting to rare variation not present in the non-neurological portion of the gnomAD database, and perform 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 score ≥ 2 (representing the top ~3.9% pathogenicity of missense variation (*45*), and restricting our PTV enrichment tests within genes most likely to be loss-of-function intolerant (*p*LI ≥ 0.9).

Throughout, we test 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 include sex, ten PCs and overall burden of MAC ≤ 5 variants in the dataset following the imposed restrictions (e.g. not in gnomAD non-neurological). In each case, 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 artefacts introduced by jointly analysing multiple cohorts or residual population structure, we also ran burden tests within each location and meta-analysed these results. We observed consistent results across the cohorts, and found that estimated odds ratios and excess burden between the joint analysis and meta-analysis were roughly equivalent.

Schizophrenia as a positive control for damaging rare burden analysis

In the case of schizophrenia, multiple studies have shown enrichment of rare damaging coding variation in cases over controls (*47*, *48*). As a positive control, we considered the subset of schizophrenia cases in the BipEx cohort and tested for enrichment of putatively damaging variation in these 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 definitions

Three definitions for age of onset were available for subsets of the data and considered for analysis: age at first symptoms, age at first diagnosis, and age at first impairment. In each case, two distinct age encodings were used:

1. < 18; 18-40; 40+.
2. < 12; 12-24; 24+.

Cardiff, UK
Age at first symptoms: SCAN (*3*) interview and case records; age of first clinically significant symptoms due to affective/psychotic illness was used to define encodings 1 and 2.
Age at first impairment: SCAN (*3*) interview and case records; age of first clinically significant impairment due to affective/psychotic illness was used to define encodings 1 and 2.

Boston, USA
Age of diagnosis: A regular expression algorithm extracting mention by clinician in an inpatient or outpatient note (*14*, *49*). Age of onset must be explicitly mentioned by a physician in a clinical note. Results were used to define encoding 1.

London, UK
Age of first impairment. OPCRIT (*3*, *4*) question 4 of the DPIM BPAD questionnaire (github.com/astheeggeggs/BipEx/DPIM_BPAD.docx): age of onset, defined as the earliest age at which medical advice was sought for psychiatric reasons or at which symptoms began to cause subjective distress or impair functioning, provided to the nearest year. Age was used to define encodings 1 and 2.

Stockholm, SWE
SWEBIC (Swedish Bipolar Cohort Collection), SWE
SBP
Age at first symptoms: Age at first sign of psychiatric disorder as recorded in the ADE
Age at first diagnosis: Age at first contact with healthcare professionals for mental health issues as recorded in ADE.
BipoläR:
Age at first symptoms: Age at first signs of mental health problems or psychiatric disorder as recorded in the QA-register stratified by < 8 years of age, 8-11 yrs, 12-17 yrs, 18-24 yrs, > 24 years of age;
Age at first diagnosis: Question at telephone interview: “How old were you at your first contact with health care professionals due to mental health issues / a psychiatric disorder?”
HDR:
Age at first symptoms: Age at first signs of mental health problems / psychiatric disorder as recorded in the telephone interview stratified by < 8 years of age, 8-11 yrs, 12-17 yrs, 18-24 yrs, > 24 years of age.

Age at first diagnosis: Question at telephone interview: “How old were you at your first contact with health care professionals due to mental health issues / a psychiatric disorder?”

Age at first diagnosis exists as actual age but was divided according to encodings 1 and 2.
Age at first symptoms is provided according to encoding 2.

Data on age of first impairment was not collected in the Swedish cohort collection. However, a subset of Swedish data (The St. Göran Project, **SBP**) contains information on, ‘age at first health care contact for any psychiatric problem’, which herein was considered to indicate age of first impairment, and was divided according to encodings 1 and 2.

Testing for relationship between age of onset and rare variant burden

To test for an association between age of onset (see ‘age of onset definitions’ for full details) and burden of rare damaging variation, we first restricted our attention to the class of variation with the strongest signal for excess in cases over controls: PTVs. We considered only ‘age at first impairment’ (Table S6) as this was the definition with the largest amount of available data: 3,677 in both encoding 1 and 2. Using these two encodings, we further split the age of first impairment categories into five discrete bins: < 12, 12-18, 18-24, 24-40 and > 40. We tested all 10 possible ‘younger bin’ vs ‘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 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.

Location	Age First Impairment <12	Age First Impairment 12-24	Age First Impairment >24	Total	Age First Impairment <18	Age First Impairment 18-40	Age First Impairment >40	Total
Cardiff, UK	80	824	404	1,308	469	782	57	1,308
London, UK	78	978	752	1,808	446	1,188	174	1,808
Stockholm, SWE	26	256	279	561	135	355	71	561
Total	**184**	**2,058**	**1,435**	**3,677**	**1,050**	**2,325**	**302**	**3,677**

Table S6: Age of onset ‘age of first impairment’ data. We have data split according to two encodings as described in ‘age on onset definitions’ in three of the BipEx cohorts.

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.

Boston, USA
Validated Natural language processing based algorithm run on clinical notes (*14*, *49*).

Cardiff, UK
SCAN interview (*3*) and case records. Definite evidence of lifetime presence of psychotic symptoms and lifetime presence of individual OPCRIT (*3*, *4*) psychotic symptoms.

London, UK
OPCRIT (*3*, *4*) interview: lifetime presence of psychotic symptoms as defined by questions 52, 54, 55, 57-77 of the OPCRIT checklist detailed in the DNA polymorphisms in mental illness (DPIM) bipolar affective disorder (BPAD) questionnaire (github.com/astheeggeggs/BipEx/DPIM_BPAD.docx).

Stockholm, SWE
SWEBIC (Swedish Bipolar Cohort Collection), SWE
SBP: ADE question: any psychotic disorder?
BipoläR and HDR: During a structured telephone interview that research nurses conducted, “have you ever lost touch with reality (i.e. 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.

Before restricting to samples with high quality sequence data

	Psychosis					No psychosis					
	Bipolar Disorder					Bipolar Disorder					
Location	**BD**	**BD1**	**BD2**	**BDNOS**	**SAD**	**BD**	**BD1**	**BD2**	**BDNOS**	**SAD**	**BD total**
Boston, USA	13	438	82	100	34	6	105	27	34	0	805
Cardiff, UK	40	994	74	6	54	12	247	488	30	0	1,891
London, UK	12	869	86	0	128	6	343	226	0	9	1,542
Stockholm, SWE	0	1,349	497	315	0	0	742	1,142	510	0	4,555
Wurzburg, GER	0	47	11	1	0	7	169	148	14	0	397
Total	**65**	**3,697**	**750**	**422**	**216**	**31**	**1,606**	**2,031**	**588**	**9**	**9,190**

Following restriction to samples with high quality sequence data

	Psychosis					No psychosis					
	Bipolar Disorder					Bipolar Disorder					
Location	**BD**	**BD1**	**BD2**	**BDNOS**	**SAD**	**BD**	**BD1**	**BD2**	**BDNOS**	**SAD**	**BD total**
Boston, USA	9	303	54	66	22	6	74	19	23	0	554
Cardiff, UK	29	842	65	6	51	11	216	438	28	0	1,635
London, UK	11	770	79	0	118	6	317	219	0	9	1,402
Stockholm, SWE	0	1,193	453	279	0	0	659	1,034	442	0	4,060
Wurzburg, GER	0	44	10	1	0	7	157	135	12	0	366
Total	**49**	**3,152**	**661**	**352**	**191**	**30**	**1,423**	**1,845**	**505**	**9**	**8,017**

Table S7: Breakdown of psychosis diagnosis information across BipEx cohorts available in the phenotype data, and following destruction to the analysis ready dataset. BD=BD without a fine subclassification, BD1=bipolar I disorder, BD2=bipolar II disorder, BDNOS=bipolar disorder not otherwise specified, SAD=schizoaffective disorder, BD+SAD=bipolar disorder and schizoaffective disorder combined, SCZ=schizophrenia, other=other unspecified case, unknown=unknown case status.

Gene-set variant burden testing

For each gene-set, we tested for ultra-rare variant enrichment of the following classes of variation:

- PTV
- Damaging missense
- Other missense
- Synonymous

To do this, for each gene-set of interest, we regressed case status on ultra-rare (MAC ≤ 5, not in non-neurological portion of gnomAD) burden of each variant class in that gene-set using logistic regression. We included the following covariates as possible confounders in each regression analysis:

- Ultra-rare coding burden in the gene set (the sum of ultra-rare burden of PTVs, damaging missense, other missense, and synonymous variants in the gene-set)
- Sex
- PCs 1-10

The resulting logistic regression performed for each (gene-set, variant class) pair is then:

$$case\ status\ \sim burden_{g,c}\ +\ burden_{g,coding}\ +\ sex\ +\ PC1\ +\ PC2\ +\ \ldots +\ PC10,$$

where $burden_{g,c}$ is the count of ultra-rare variants of variant class c in gene-set $g$for the sample, and $burden_{g,coding}$ is the total number of ultra-rare coding (any variant annotated as either PTV, damaging missense, other missense, or synonymous) variants in the gene-set for the sample.

For example, consider PTVs in calcium channel genes. For each sample, we count the number of ultra-rare PTVs that individual harbors within the calcium channel genes, and define this quantity as their 'ultra-rare PTV burden' ($burden_{Ca\ channels,\ PTV}$).

We included overall ultra-rare coding burden in the gene-set ($burden_{g,coding}$) as a covariate as it ensured that any signal was significant above overall rare coding differences between cases and controls in the analysed gene set. All cohorts were analysed together.

Following the observation of enrichment of brain expressed genes in the initial gene-set analysis, we sought to refine the signal. In the collection of GTEx tissue specific gene-sets defined by (*50*), a subset of 13 are brain regions (italicised in Table S8). We tested for enrichment of MAC ≤ 5 PTVs in these GTEx gene-sets defined as having the strongest tissue

specific expression using logistic regression, again controlling for 10 PCs, sex and MAC ≤ 5 coding burden.

Gene-lists were arrived at in the following manner, summarised in Figure 1 of Finucane *et al. (50)*:

For each gene and tissue, construct a design matrix *X,* where rows are samples taken from either the tissue of interest, or outside the larger tissue category. For example, in the case of any of the brain regions, expression data for the gene under all other brain regions are excluded from rows of *X.* As a concrete example, consider Hippocampus and some gene *g*. The first column of *X* is set to '1' for each hippocampus sample, and '-1' for non-brain samples. Other columns are covariates: age and sex. *Y* is the expression of gene *g* in the tissue. The model *Y* ~ *X* is then fit using ordinary least squares and a *t*-statistic evaluated for the first term:

$$t = \frac{\left(X^{\top}X\right)^{-1}X^{\top}Y[0]}{\sqrt{MSE\cdot\left(X^{\top}X\right)^{-1}[0,0]}},$$

where MSE is the mean squared error of the fitted model:

$$MSE = \frac{1}{N}\left(Y - X\left(X^{\top}X\right)^{-1}X^{\top}Y\right)^{\top}\left(Y - X\left(X^{\top}X\right)^{-1}X^{\top}Y\right),$$

where N is the number of rows of X. This then provides a *t*-statistic for each gene in the current tissue of interest. Finally, the top 10% of genes in these lists was defined as the tissue-specific gene-set for each of the tissues.

The 43 tested genesets are available for download at https://data.broadinstitute.org/alkesgroup/LDSCORE/LDSC_SEG_ldscores/. Results are displayed in Figure 2A.

Tissue	Tissue category	Broad tissue category	Number of samples
Bladder	Bladder	Other	11
Kidney Cortex	Kidney	Other	32
Minor Salivary Gland	Salivary Gland	Other	57
Brain Substantia nigra	*Brain*	CNS	63
Brain Spinal cord (cervical c-1)	*Brain*	CNS	70
Brain Amygdala	*Brain*	CNS	72
Brain Anterior cingulate cortex (BA24)	*Brain*	CNS	84
Small Intestine Terminal Ileum	Small Intestine	Digestive	88
Brain Hippocampus	*Brain*	CNS	94
Brain Hypothalamus	*Brain*	CNS	96
Brain Putamen (basal ganglia)	*Brain*	CNS	97
Pituitary	Pituitary	Endocrine	103
Spleen	Spleen	Blood/Immune	104
Brain Cerebellar Hemisphere	*Brain*	CNS	105
Brain Frontal Cortex (BA9)	*Brain*	CNS	108
Brain Nucleus accumbens (basal ganglia)	*Brain*	CNS	113
Brain Cortex	*Brain*	CNS	114
Brain Caudate (basal ganglia)	*Brain*	CNS	115
Liver	Liver	Liver	119
Brain Cerebellum	*Brain*	CNS	125
Artery Coronary	Blood Vessel	Cardiovascular	133
Adrenal Gland	Adrenal Gland	Endocrine	145
Colon Sigmoid	Colon	Digestive	149
Esophagus Gastroesophageal Junction	Esophagus	Digestive	153
Pancreas	Pancreas	Other	171
Stomach	Stomach	Digestive	193
Heart Atrial Appendage	Heart	Cardiovascular	194
Colon Transverse	Colon	Digestive	196
Breast Mammary Tissue	Breast	Other	214
Heart Left Ventricle	Heart	Cardiovascular	218
Artery Aorta	Blood Vessel	Cardiovascular	224
Adipose Visceral (Omentum)	Adipose Tissue	Adipose	227
Esophagus Muscularis	Esophagus	Digestive	247
Skin Not Sun Exposed (Suprapubic)	Skin	Other	250
Esophagus Mucosa	Esophagus	Digestive	286
Nerve Tibial	Nerve	Other	304
Lung	Lung	Other	319
Thyroid	Thyroid	Endocrine	322
Artery Tibial	Blood Vessel	Cardiovascular	332
Adipose Subcutaneous	Adipose Tissue	Adipose	350
Skin Sun Exposed (Lower leg)	Skin	Other	357
Whole Blood	Blood	Blood/Immune	393
Muscle Skeletal	Muscle	Musculoskeletal/connective	430

Table S8: GTEx tissue information for analysed (*50*) GTEx gene-sets. The 43 tested genesets are available at data.broadinstitute.org/alkesgroup/LDSCORE/LDSC_SEG_ldscores/.

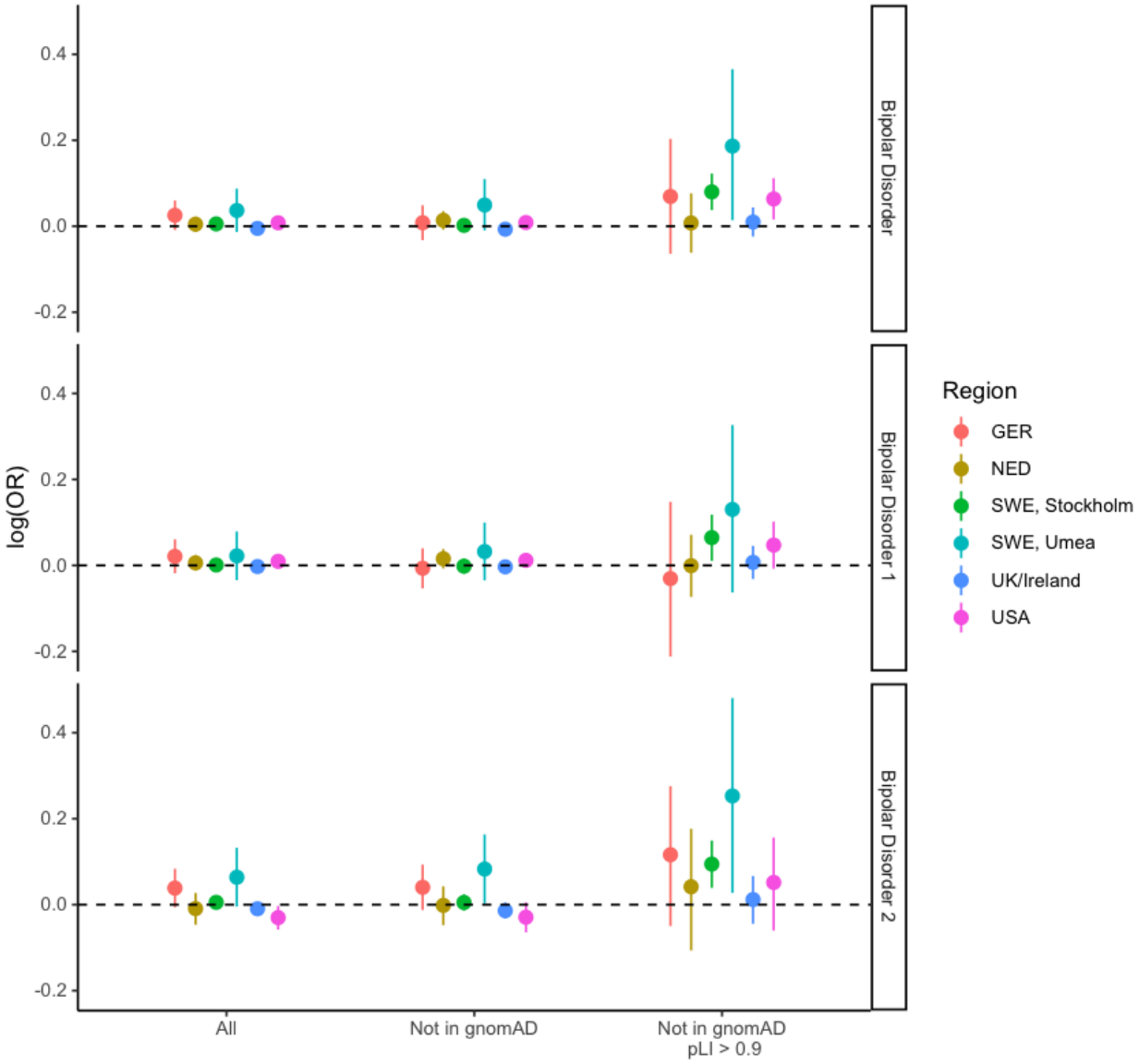

Figure S6: Case-control enrichment of PTVs, split by case status and consequence category. We display case-control enrichment of PTVs in increasingly *a priori* damaging variant subsets, split by region, using logistic regression. Consequence categories are stratified by rarity: the rarity increases from PTVs with MAC ≤ 5 (All), to PTVs with MAC ≤ 5 in a *p*LI ≥ 0.9 gene and not in the non-neurological portion of gnomAD (not in gnomAD *p*LI ≥ 0.9), according to the x-axis labelling. Bars represent the 95% confidence intervals on the estimate of the enrichment labelled on the x-axis respectively in the region coloured according to the legend. Regressions are run as described in supplementary materials: exome-wide burden analyses, and include sex, 10 PCs and total MAC ≤ 5 coding burden as covariates.

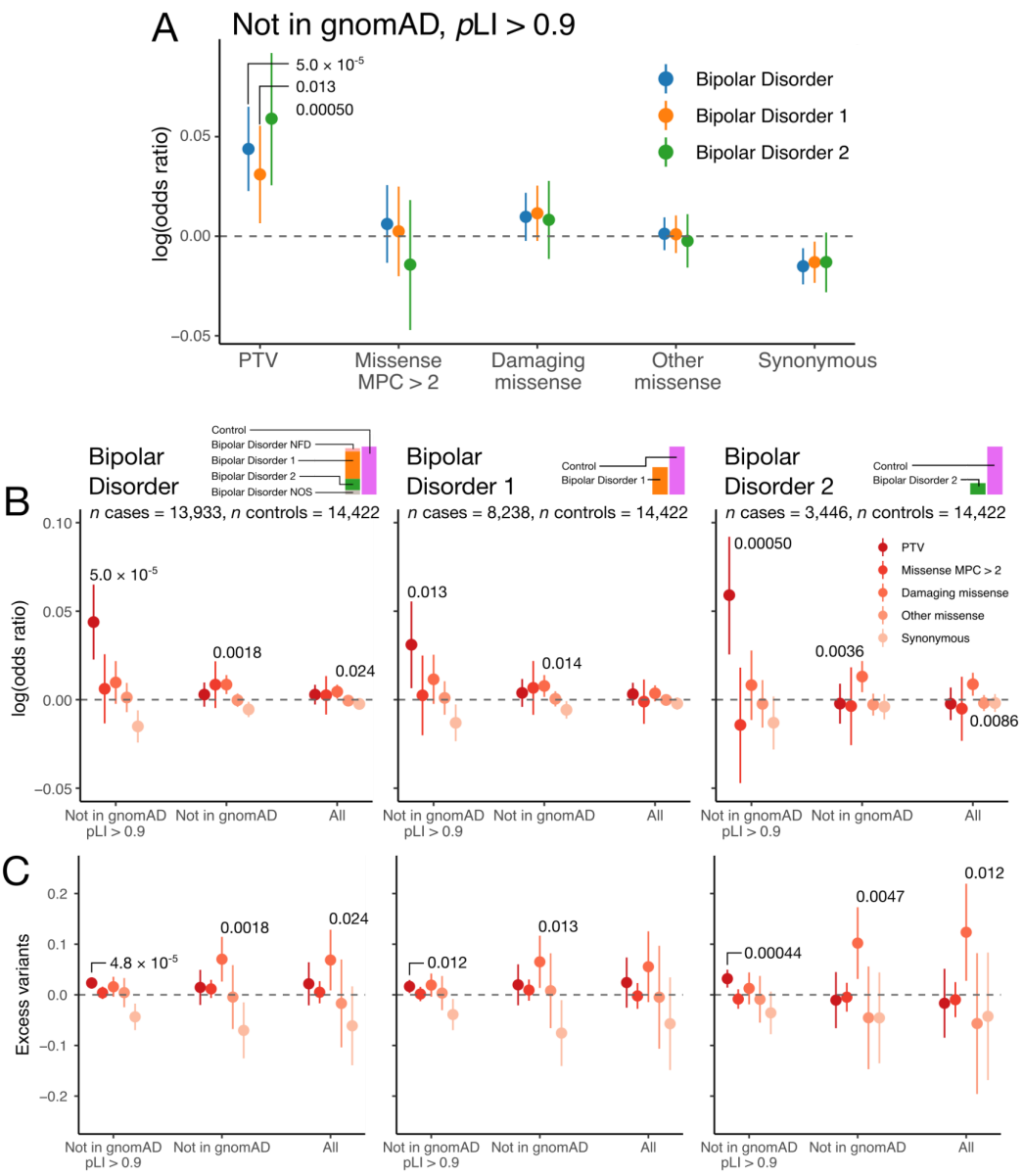

Figure S7: Case-control enrichment of ultra-rare variants, split by case status and consequence category. Panel A displays enrichment in cases over controls in case subsets, according to the legend. In panels B and C, we display case-control enrichment and excess case rare variant burden increasingly *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 *p*LI ≥ 0.9 gene and not in the non-neurological portion of gnomAD (Not in gnomAD *p*LI ≥ 0.9), to a variant with MAC ≤ 5 (All) according to the *x*-axis labelling. Bars in panels B and C represent the 95% confidence intervals on the logistic and linear regression estimate of the enrichment of the class of variation labelled on the *x*-axis respectively. Regressions are run as described in supplementary materials: exome-wide burden analyses, and include sex, 10 PCs and total MAC ≤ 5 coding burden as covariates. Nominally significant enrichments or excess variants in cases are labelled with the associated *P*-value.

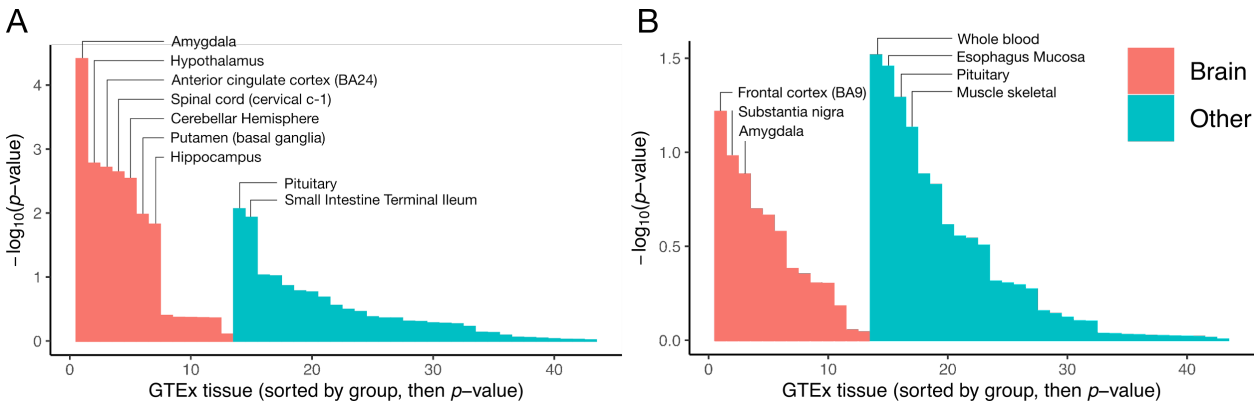

Figure S8: Enrichment of ultra-rare PTVs in BD1 and BD2 cases over controls in tissue-specific expression genesets. Gene-sets are defined in (*50*) in detail. Bars are ordered first by whether they are a brain-tissue, and then by *P*-value. A. displays the results for BD1, B. displays the results for BD2.

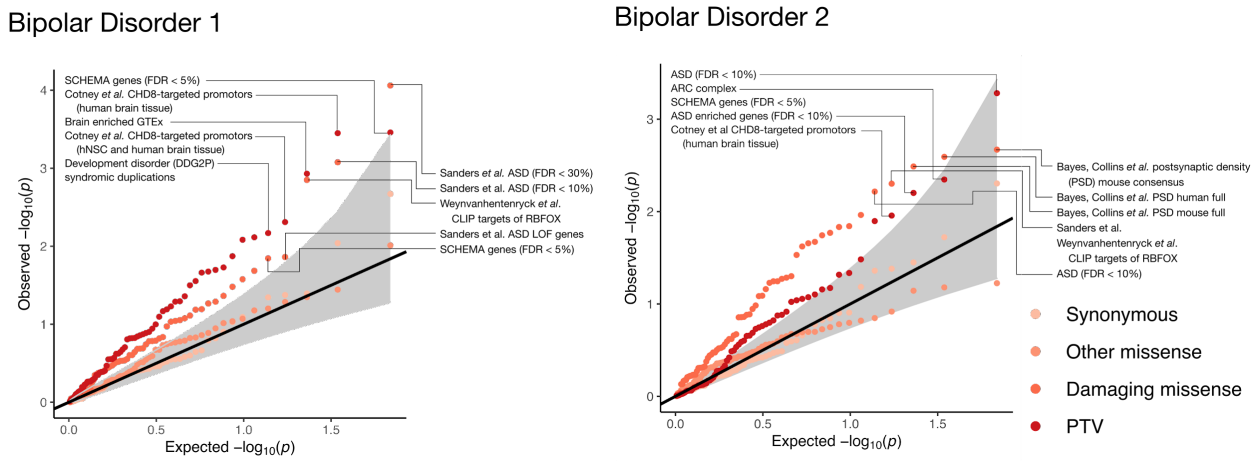

Figure S9: Enrichment of ultra-rare variants in targeted 68 gene-sets taken from the literature. The left plot shows enrichment in BD1, the right plot shows enrichment in BD2. Top PTV and damaging missense gene-sets are labelled. Classes of variants tested in each gene-set are coloured according to the legend.

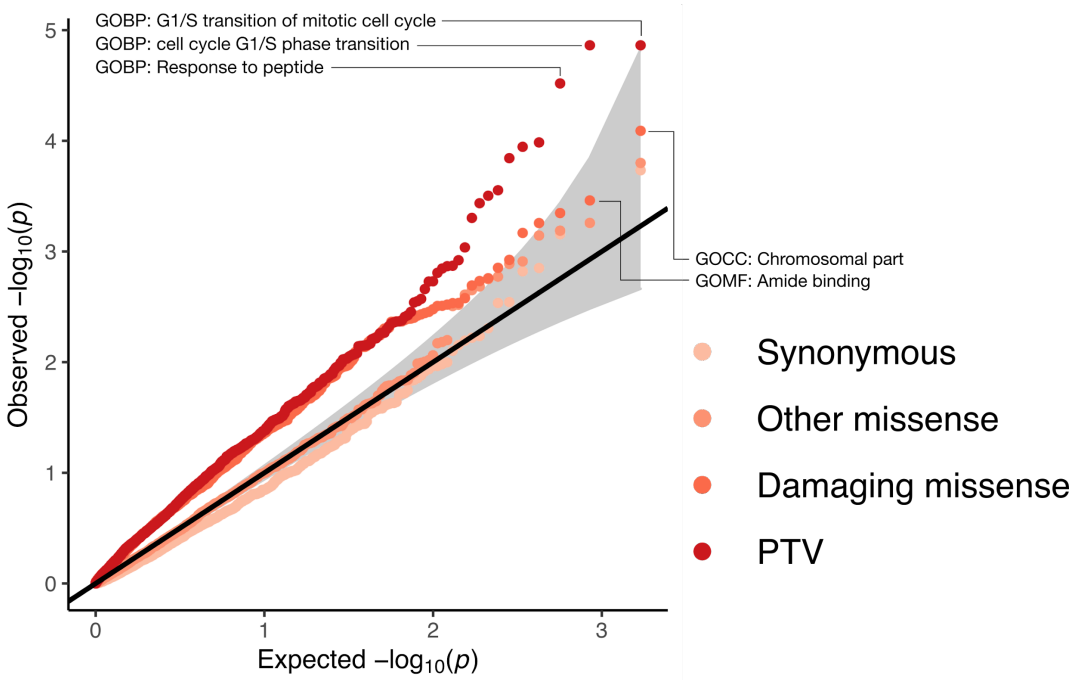

Figure S10: QQ plot of *P*-values testing for enrichment of ultra-rare variants in 1,697 gene-sets taken from derived from large pathway databases including Gene Ontology (GO), REACTOME and KEGG). Top PTV and damaging missense gene-sets are labelled. Classes of variants tested in each gene-set are coloured according to the legend.

Gene-based analysis approach

In order to increase power for gene discovery, we filter down to variants not present in the non-neurological portion of the gnomAD dataset (*44*), and we further enriched for pathogenic variants by restricting our analysis to variants with MAC ≤ 5. We then examine case-control enrichment of PTVs (transcript ablation, splice acceptor variant, splice donor variant, stop gained and frameshift variant; Table S5) or damaging missense variants (missense variants (Table S5) 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 artefacts which 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 (Figure S11-12).

Throughout, we use Fisher's exact tests in each gene. We considered a Cochran–Mantel–Haenszel (CMH) test, using the strata defined by broad geographic location. We use a permutation approach to determine the null distribution of test statistics throughout our gene based analysis, and evaluate QQ plots of synonymous and other-missense ultra-rare variants to ensure that tests are well-calibrated (Figure S11-12). 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 (Figure S11-15). To determine *Q*-values we apply the Benjamini and Hochberg adjustment (*51*) to Fisher's exact test *P*-values for genes with at least 10 ultra-rare PTVs across cases and controls. We exclude genes with less than 10 ultra-rare PTVs in the BipEx dataset to guard against incorrect *P*-value adjustment using the Benjamini and Hochberg procedure. Conservative *Q*-values occur when applying the Benjamini and 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.

CMH and Fisher's exact test for gene based tests

We tested for an excess of ultra-rare variation (MAC ≤ 5 and not present in the non-neurological portion of the gnomAD dataset) in each gene using both Fisher's exact and Cochran-Mantel-Haenszel (CMH) tests for each phenotype. Given that we did not observe excess burden in missense variants with high MPC (>3 or >2) in bipolar cases over controls exome wide (in contrast to schizophrenia; (*52*)), we did not test a weighted summation of counts across consequence categories. For each gene, each sample was assessed for carrier status for each of the following consequence classes: synonymous, other missense, damaging missense, and PTV (Table S5); individuals harbouring 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, see below. 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 odds-ratio for carrier status are displayed in the gene results tables on the browser: bipex.broadinstitute.org.

Location stratum	Cohort
UK/Ireland	Aberdeen, UK Cambridge, UK Cardiff, UK Dublin, IRE Edinburgh, UK London, UK
Germany	Wurzburg, GER
USA	Baltimore, USA Boston, USA
Netherlands	Amsterdam, NED
Sweden, Stockholm	Stockholm, SWE
Sweden, Umea	Umea, SWE

Robustness of gene-based analysis

To ensure that our tests were robust, we performed a series of checks to see if the Fisher's exact (Figures S11-12), and Cochran-Mantel–Haenszel (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 analysing synonymous and 'other-missense' variants with MAC ≤ 5 not in gnomAD non-neurological. 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 (Figure S15). In each, we did not observe inflation of the test statistic.

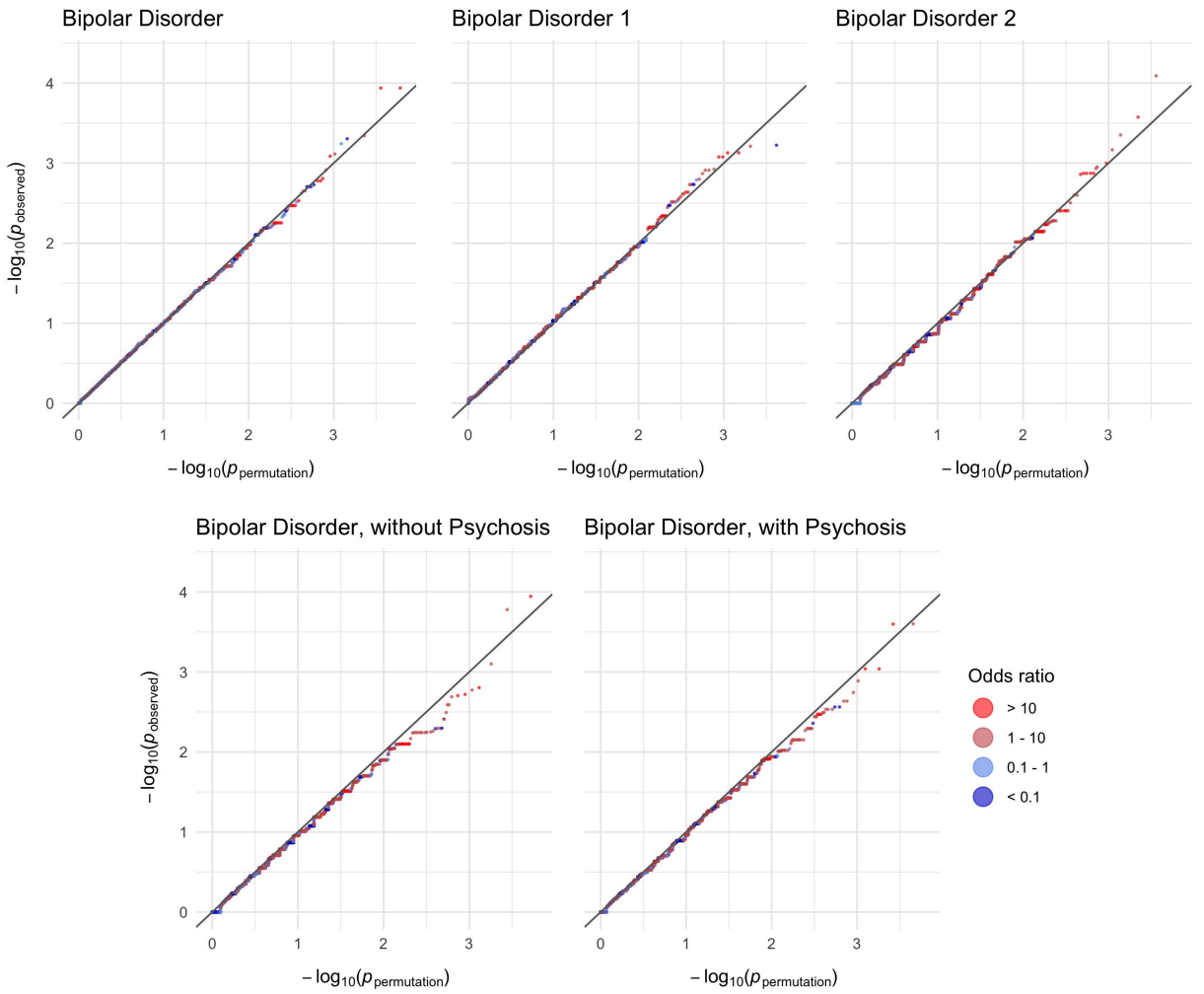

Figure S11: MAC ≤ 5 not in gnomAD non-neurological synonymous variants in 13,933 cases and 14,422 controls: QQ plots. Observed -log10 *P*-values are plotted against permutation *P*-values according to the procedure described in the methods; gene-based analysis. Points are coloured according to the discrete scale displayed in the legend.

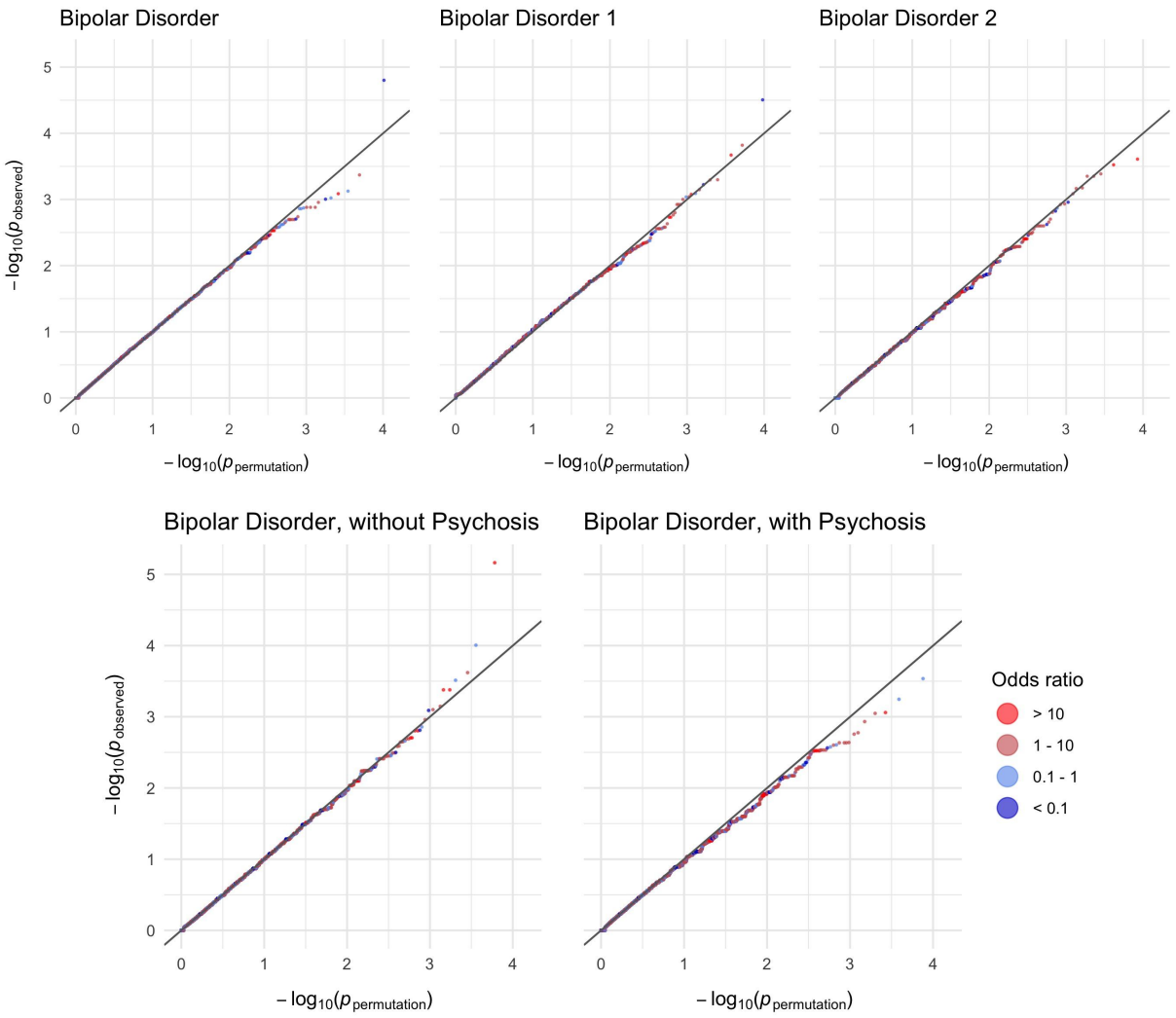

Figure S12: MAC ≤ 5 not in gnomAD non-neurological other missense variants in 13,933 cases and 14,422 controls: QQ plots. Observed -log10 *P*-values are plotted against permutation *P*-values according to the procedure described in the methods; gene-based analysis. Points are coloured according to the discrete scale displayed in the legend.

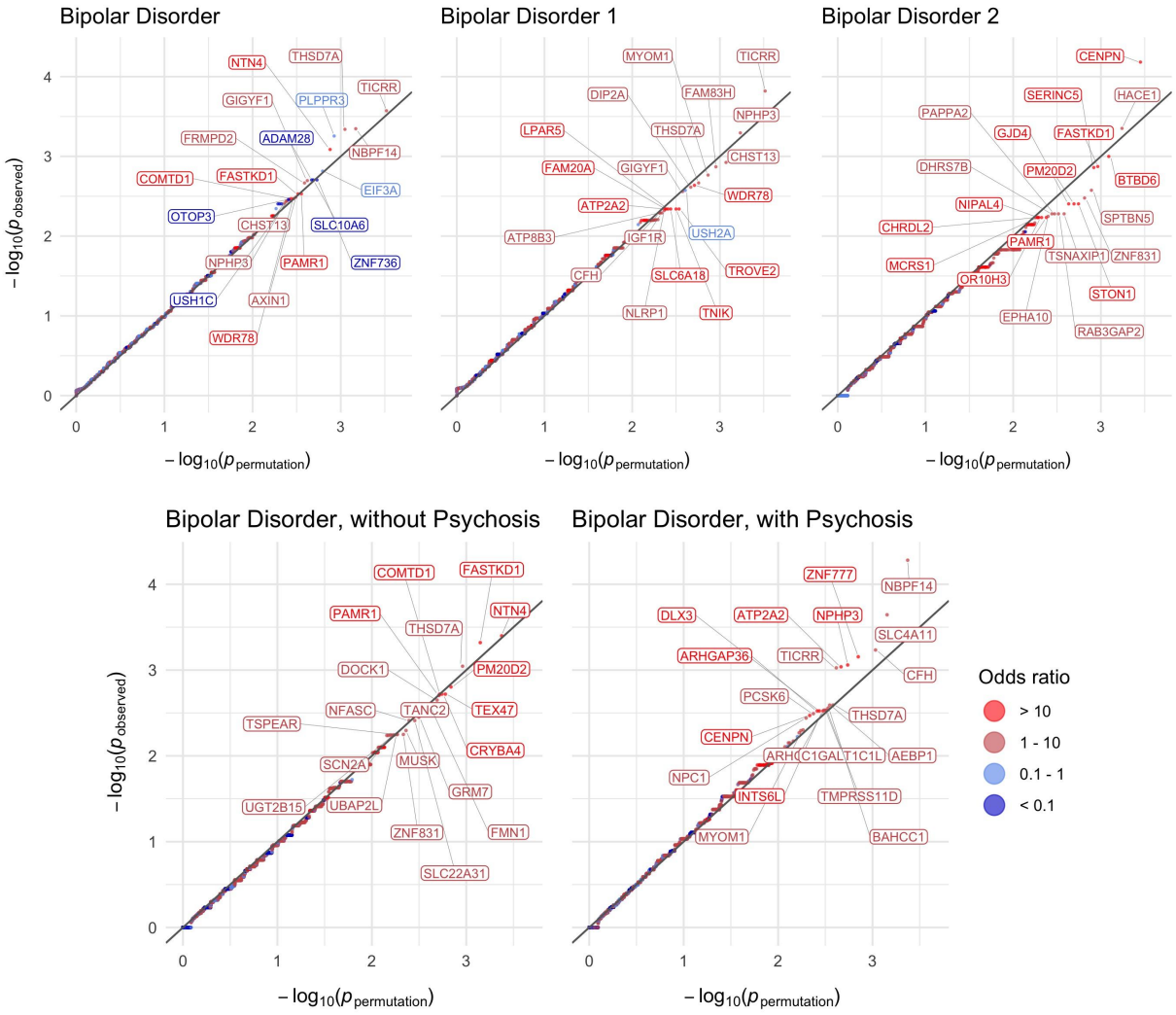

Figure S13: MAC ≤ 5 not in gnomAD non-neurological damaging missense variants in 13,933 cases and 14,422 controls: QQ plots. Observed -log10 *P*-values are plotted against permutation *P*-values according to the procedure described in the methods; gene-based analysis. Points are coloured according to the discrete scale displayed in the legend. In each panel, the gene symbols of the top 20 genes by *P*-value are labelled.

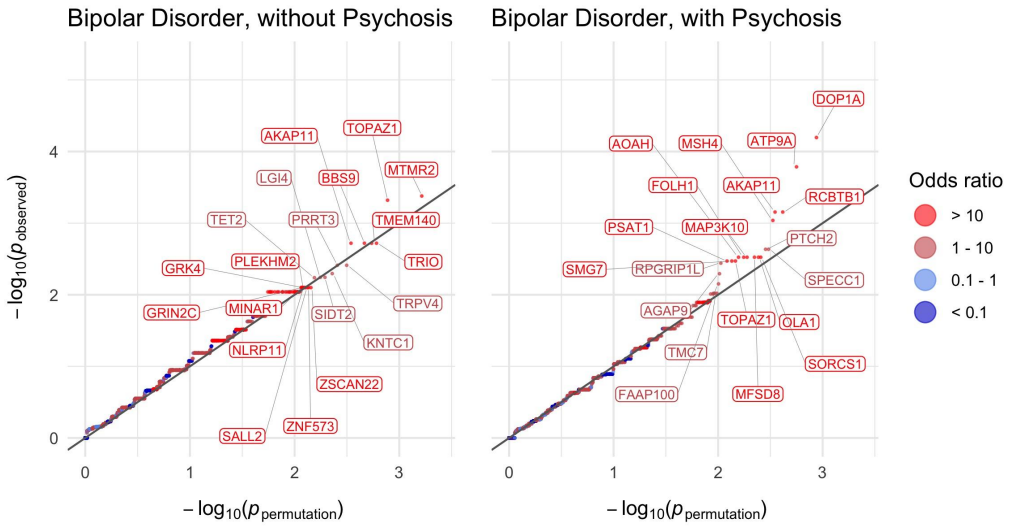

Figure S14: MAC ≤ 5 not in gnomAD non-neurological PTVs in 13,933 cases and 14,422 controls: QQ plots. Observed -log10 *P*-values are plotted against permutation *P*-values according to the procedure described in the methods; gene-based analysis. Points are coloured according to the discrete scale displayed in the legend. In each panel, the gene symbols of the top 20 genes by *P*-value are labelled.

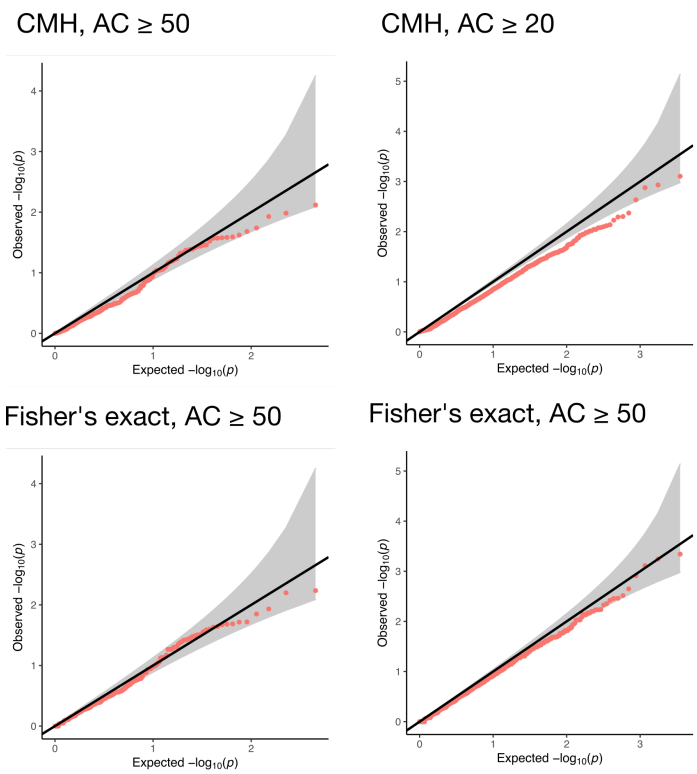

Figure S15: MAC ≤ 5 not in gnomAD non-neurological synonymous variants in 13,933 cases and 14,422 controls: QQ plots for BD in genes with ultra-rare synonymous counts about 20 and 50 across BD cases and controls. Observed -log10 *P*-values are plotted against expected *P*-values using a uniform distribution. The first and second rows show *P*-values obtained via a CMH and Fisher's exact test respectively. The first and second columns restrict to genes with at least 20 and at least 50 individuals across cases and controls harbouring an ultra-rare PTV respectively.

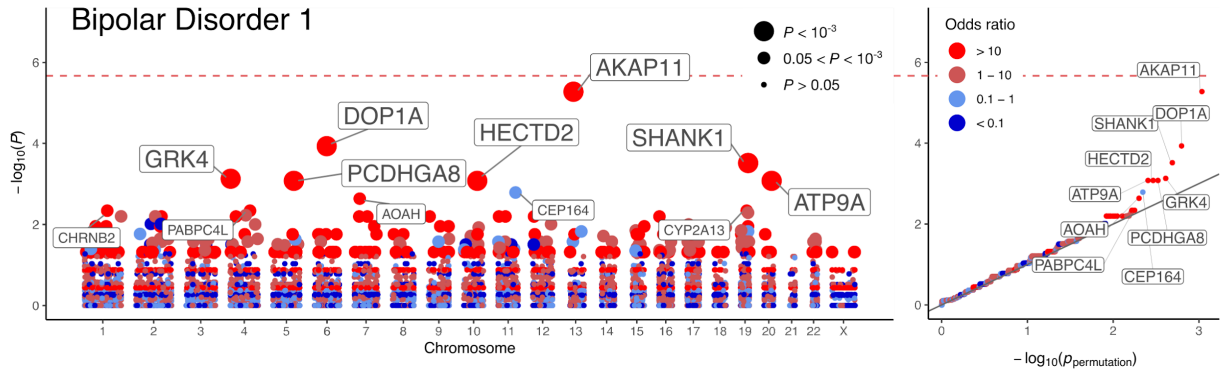

Figure S16: Results of the analysis of ultra-rare PTVs in BD1: 8,238 cases and 14,422 controls. Gene based Manhattan and associated QQ plot for BD1. -$\log_{10}$ *P*-values obtained via Fisher's exact tests are plotted against genetic position for each of the analysed genes. In the QQ plots, observed -$\log_{10}$ *P*-values are plotted against permutation *P*-values according to the procedure described in the supplementary materials: gene-based analysis approach. Points are coloured according to the discrete scale displayed in the legend. In the Manhattan plot and QQ plot, the gene symbols of top genes by *P*-value are labelled. Points in the Manhattan plot are sized according to *P*-value as displayed in the legend.

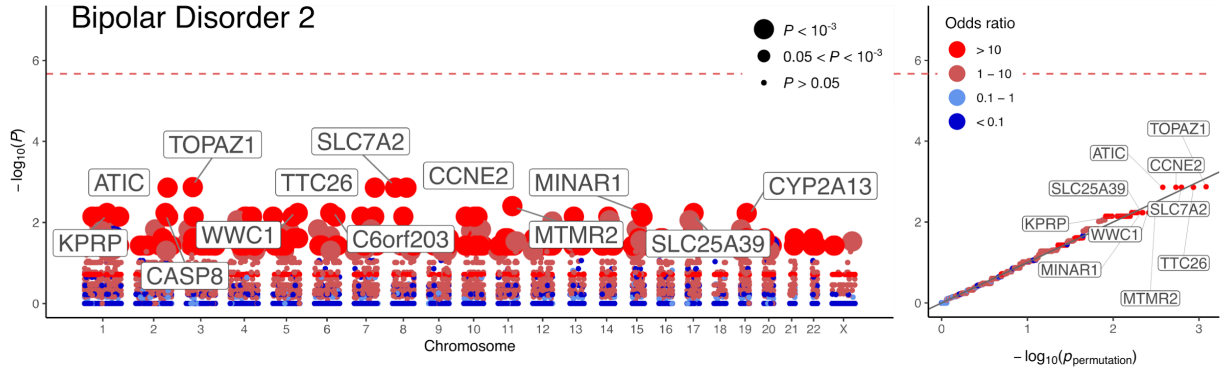

Figure S17: Results of the analysis of ultra-rare PTVs in BD2: 3,446 cases and 14,422 controls. Gene based Manhattan and associated QQ plot for BD1. -$\log_{10}$ *P*-values obtained via Fisher's exact tests are plotted against genetic position for each of the analysed genes. In the QQ plots, observed -$\log_{10}$ *P*-values are plotted against permutation *P*-values according to the procedure described in the supplementary materials: gene-based analysis approach. Points are coloured according to the discrete scale displayed in the legend. In the Manhattan plot and QQ plot, the gene symbols of top genes by *P*-value are labelled. Points in the Manhattan plot are sized according to *P*-value as displayed in the legend.

Combining SCHEMA and BipEx data in meta-analysis

To examine the extent of shared ultra-rare PTV signal between BD and SCZ we ran separate Fisher and CMH tests for BipEx and SCHEMA separately and meta-analysed 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*-score were then evaluated:

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

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 as controls for the SCHEMA study, these controls were excluded from the analysis.

Gene	*P*-value
AKAP11	1.15×10^{-5}
DOP1A	2.22×10^{-4}
SHANK1	8.19×10^{-4}
TOPAZ1	1.56×10^{-3}
ATP9A	1.66×10^{-3}
WWP1	6.52×10^{-3}
HECTD2	6.91×10^{-3}
PSAP	1.41×10^{-2}
RAP1GDS1	1.41×10^{-2}
USP24	1.41×10^{-2}
SPHKAP	1.57×10^{-2}
CACNA1B	1.93×10^{-2}
ANKFY1	1.95×10^{-2}
SCN3A	1.95×10^{-2}
SMG7	1.95×10^{-2}
DNAJC14	2.86×10^{-2}
EXOC3	2.86×10^{-2}
PHIP	2.86×10^{-2}
SBNO1	3.14×10^{-2}
ZFYVE9	3.14×10^{-2}

Table S9: Top 20 genes with *p*LI ≥ 0.9 as measured by gene-based test *P*-value.

	BD (BipEx)				SCZ (SCHEMA)				Combined	
Gene	**Case count BD/BD1/BD2** BD *n* = 13,933 BD1 *n* = 8,238 BD2 *n* = 3,446	**Control count** *n* = 14,422	***P*-value**	**OR**	**Case count** *n* = 24,248	**Control count** *n* = 91,960	***P*-value**	**OR**	**OR**	**Meta *P*-value**
AKAP11	16/12/2	0	1.15×10^{-5}	∞	17	13	2.02×10^{-5}	5.60	7.06	2.83×10^{-9}
DOP1A	15/11/2	1	2.22×10^{-4}	15.54	19	43	1.47×10^{-1}	1.59	2.11	1.44×10^{-4}
PCDHGA8	11/7/1	0	4.02×10^{-4}	∞	6	44	2.19×10^{-1}	0.54	0.99	3.38×10^{-3}
SHANK1	10/8/1	0	8.19×10^{-4}	∞	4	4	4.43×10^{-1}	2.90	6.99	9.71×10^{-3}
TOPAZ1	12/6/5	1	1.56×10^{-3}	12.43	2	3	6.67×10^{-1}	0.93	3.93	2.51×10^{-3}
ATP9A	9/7/2	0	1.66×10^{-3}	∞	15	11	6.96×10^{-4}	4.08	5.46	5.36×10^{-6}
FREM2	4/3/1	19	2.67×10^{-3}	0.22	22	92	5.48×10^{-1}	0.83	0.65	3.80×10^{-2}
CHD1L	11/6/2	1	2.95×10^{-3}	11.39	16	73	5.99×10^{-1}	0.82	1.01	4.57×10^{-2}
CHRNB2	11/7/1	1	2.95×10^{-3}	11.39	2	17	5.54×10^{-1}	0.52	1.88	3.04×10^{-2}
CYP2A13	11/7/4	1	2.95×10^{-3}	11.39	13	28	6.30×10^{-1}	1.29	2.27	4.61×10^{-2}

Table S10: BipEx and SCHEMA case-control counts of the top ten most significant genes in the BipEx BD main gene-based analysis. Case and control columns denote the count of ultra-rare PTVs in the gene in the respective dataset. *P*-values are determined using Fisher's exact and CMH tests for BipEx and SCHEMA (supplementary materials: gene-based analysis approach) respectively, and meta-analysed weighting by effective sample size. BipEx: BD case count 13,933, control count 14,422. SCHEMA: schizophrenia case count 24,248, control count 91,960. 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.

Lithium response

Stockholm, SWE
SWEBIC (Swedish Bipolar Cohort Collection), SWE
SBP: Not available.
BipoläR and HDR: During a structured telephone interview that research nurses conducted, patients who had been on lithium for at least 12 months were asked the following question: “What do you think of the effect (of lithium)? Do not consider side effects.” Patients were partitioned according to the following response options.

0: Non-responder ‘None or very doubtful effect’.
1: Partial-responder ‘Doubtless effect of treatment but additional temporary or continuous treatment needed’.
2: Good-responder ‘Complete response, recovered’.

Cardiff, UK

0. No evidence of response.
1. Subjective good response - upon interview, patients reported that lithium helped stabilise their moods.
2. Objective evidence for beneficial response, i.e., clear reduction in number and/or severity of episodes following introduction of lithium prophylaxis. (Can only be rated if at least 3 episodes of illness have occurred before lithium prophylaxis and lithium response has been observed for at least 3 years).
3. Objective evidence for excellent response to lithium prophylaxis, i.e., frequency of episodes reduced to < 10% of frequency after lithium prophylaxis and/or 2 or more episodes of illness occurring within weeks of cessation of lithium. (Can only be rated if at least 3 episodes of illness have occurred before lithium prophylaxis and lithium response has been observed for at least 5 years).

External validation with the BSC exome data

To externally check our gene-based PTV results, we obtained PTV counts from the Bipolar sequencing consortium (BSC) (http://metamoodics.org/bsc/consortium/). 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 harmonise the BSC data with BipEx, we used annotation definitions defined in Table S5. We then generate MAC ≤ 5 counts for each gene in the BSC data. Full details of the exome or whole genome sequencing platform for each cohort is summarised in Table S11. The addition of the BSC data set has some limitations. Primarily, 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.

Study	Ethnicity	Sequencing platform	Library Preparation	Variant calling	BD	Controls	Total
BRIDGES	US-Caucasian	HiSeq 2500 (WGS)	-	GotCloud analysis pipeline (*41*, *53*)	1,712	1,844	3,556
RareBLISS	US-Caucasian	HiSeq 2000/2500	Nimblegen SeqCap EZ Exome	GATK (*34*)	961	1,039	2,000
Sweden	Swedish-Caucasian	HiSeq 2000/2500	Agilent SureSelect Human All Exon v2	GATK (*34*)	831	1,956	2,787
KPNC-EUR	US-Caucasian	HiSeq 2000/2500	Nimblegen SeqCap EZ Exome	GATK (*34*)	192	192	384
KPNC-AFR	US-African American	HiSeq 2000/2500	Nimblegen SeqCap EZ Exome	GATK (*34*)	96	95	191
KPNC-LAT	US-Latino	HiSeq 2000/2500	Nimblegen SeqCap EZ Exome	GATK (*34*)	98	100	198
KPNC-EAS	US-East Asian	HiSeq 2000/2500	Nimblegen SeqCap EZ Exome	GATK (*34*)	97	96	193
Total					**3,987**	**5,322**	**9,309**

Table S11: Summary of BSC sample data.

	BipEx					BSC	
Gene	**Case count BD/BD1/BD2** BD *n*= 13,933 BD1 *n* = 8,238 BD2 *n* = 3,446	**Control count** *n*=14,422	***P*-value**	***Q*-value**	**OR**	**Case count** *n*=3,987	**Control count** *n*=5,322
AKAP11	16/12/2	0	1.15×10^{-5}	2.02×10^{-2}	∞	1	0
DOP1A	15/11/2	1	2.22×10^{-4}	1.95×10^{-2}	15.54	0	1
PCDHGA8	11/7/1	0	4.02×10^{-4}	2.36×10^{-1}	∞	3	6
SHANK1	10/8/1	0	8.19×10^{-4}	3.60×10^{-1}	∞	1	0
TOPAZ1	12/6/5	1	1.56×10^{-3}	5.48×10^{-1}	12.43	1	0
ATP9A	9/7/2	0	1.66×10^{-3}	-	∞	2	1
FREM2	4/3/1	19	2.67×10^{-3}	5.77×10^{-1}	0.22	3	3
CHD1L	11/6/2	1	2.95×10^{-3}	5.77×10^{-1}	11.39	0	0
CHRNB2	11/7/1	1	2.95×10^{-3}	5.77×10^{-1}	11.39	0	0
CYP2A13	11/7/4	1	2.95×10^{-3}	6.68×10^{-1}	11.39	0	0

Table S12: BipEx and BSC case-control counts of the top ten most significant genes in the BipEx BD primary gene-based analysis. Case and control columns denote the count of ultra-rare PTVs in the gene of interest with MAC ≤ 5 in the respective dataset.

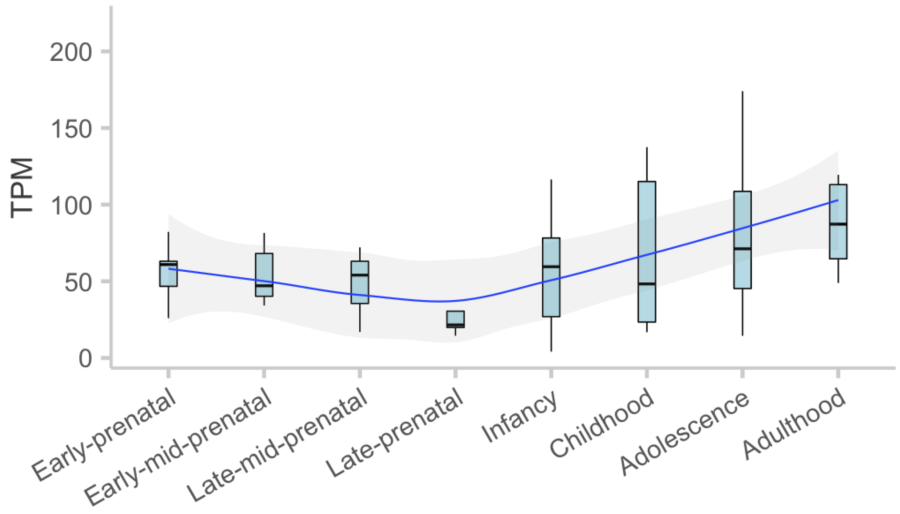

Figure S18: Temporal expression of AKAP11 in the human brain. Expression in four prenatal and four postnatal periods derived from whole-brain tissue in BrainSpan are displayed. The expression values plotted are in transcript-per-million (TPM). In each boxplot, the blue box encloses the interquartile range, with a horizontal line denoting the median. Best fit lines and confidence-intervals across the *x*-axis are overlaid.

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