Retrograde Endocannabinoid Signaling Shows Robust Enrichment in Bipolar Disorder: Insights from Standardized Pathway Analyses

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Abstract

Background and Rationale: Bipolar disorder is among the most heritable psychiatric conditions, yet its polygenic architecture is far from fully accounted for by models that foreground synaptic pruning and calcium signaling. The endocannabinoid system, which plays a well-documented role in modulating synaptic plasticity, stress reactivity, and affective stability, has received comparatively little attention as a source of independent genetic liability. This study set out to test whether standardized endocannabinoid signaling pathways contribute to bipolar disorder risk in their own right.

Methods: The analysis drew on summary statistics from the largest published European-ancestry genome-wide association study of bipolar disorder (O'Connell et al., 2025; effective sample approximately 137,097, after exclusion of UK Biobank and 23andMe participants). Three publicly curated endocannabinoid gene sets were obtained from the Molecular Signatures Database: the Gene Ontology Biological Process Cannabinoid Signaling Pathway (9 genes), the WikiPathways Cannabinoid Receptor Signaling set (29 genes), and the KEGG Retrograde Endocannabinoid Signaling pathway (148 genes). These were benchmarked against a negative control comprising housekeeping genes (182 genes) and a positive control of monoaminergic system genes (101 genes). Partial overlap between sets was retained so as not to distort their biological meaning. Three complementary post-GWAS approaches were applied: competitive gene-set enrichment testing through MAGMA version 1.10, using a window of 35 kilobases upstream and 10 kilobases downstream; partitioned linkage disequilibrium score regression for heritability enrichment, with annotations extended 10 kilobases in each direction and one-tailed testing against a European 1000 Genomes linkage disequilibrium reference; and transcriptome-wide association analysis via S-PrediXcan, employing GTEx version 8 MASHR prediction models across eight brain tissues, with tibial artery included as a peripheral control.

Results: The KEGG and WikiPathways endocannabinoid sets showed consistent enrichment across all three analytic frameworks. In the MAGMA analysis, both survived Bonferroni correction for five tests (KEGG, p = .003; WikiPathways, p = .035). Partitioned linkage disequilibrium score regression yielded the strongest heritability enrichment for the KEGG set (1.61-fold after linkage disequilibrium adjustment; one-tailed p = 1.79 times 10 to the negative third), with the WikiPathways set following at 1.13-fold. In the S-PrediXcan analysis, absolute Z-score distributions were notably elevated for both pathways (WikiPathways, 1.61-fold enrichment, p = 2.48 times 10 to the negative sixth; KEGG, 1.25-fold, p = 2.99 times 10 to the negative fifth). DAGLA, encoding the principal synthetic enzyme for the endocannabinoid 2-arachidonoylglycerol, was implicated repeatedly, including through significantly lower predicted expression in the frontal cortex (Brodmann area 9). The monoaminergic positive control and the smaller Gene Ontology set performed in line with expectations or were likely underpowered, while housekeeping genes showed only modest baseline enrichment.

Conclusions and Implications: Taken together, these convergent results point to polygenic variation within core endocannabinoid signaling machinery as a contributor to bipolar disorder susceptibility that is not reducible to previously emphasised synaptic or calcium-dependent pathways. The findings

sharpen mechanistic accounts of bipolar disorder and lay a biological groundwork for future stratified clinical trials of non-intoxicating endocannabinoid modulators, such as cannabidiol, particularly among individuals whose risk profiles are shaped by endocannabinoid-related polygenic scores. Realising the translational potential of these observations will require larger multi-ancestry investigations that incorporate single-cell transcriptomic and lipidomic data.

Introduction

Bipolar Disorder: Burden, Heritability, and Gaps in Current Genetic Models

Bipolar disorder is a major contributor to global disability. Roughly one to two percent of the population is affected, and the personal, social, and economic toll is considerable [1, 2]. The clinical picture centres on recurring swings between mania or hypomania and depression. For many patients, these mood episodes are compounded by cognitive difficulties, a heightened risk of suicide, and, over time, a decline in daily functioning that can be difficult to reverse [3]. That genes play a substantial part is beyond doubt—twin and family data consistently place heritability between 60 and 90 percent—but working out exactly how thousands of common variants combine to produce the disorder has proved stubbornly difficult [4]. Successive genome-wide association studies, each larger than the last, have now identified dozens of risk loci. Many of these point toward synaptic function, ion-channel biology (voltage-gated calcium channels like CACNA1C being a recurring theme), and broader neurodevelopmental processes [5, 6].

Still, important pieces of the puzzle are missing. The dominant accounts of how genetic risk translates into illness tend to centre on two mechanisms: excessive synaptic pruning during adolescence and dysregulated calcium signaling, both thought to destabilise neural circuits. While there is good evidence for each, neither—alone or in combination—can readily explain some of bipolar disorder's most characteristic features. Many patients show preserved or even superior cognitive abilities before they become unwell. The illness follows an episodic rather than a steady course. And vulnerability to stress seems, paradoxically, to grow worse with each successive episode rather than attenuating [7, 8]. A broader biological framework seems necessary.

The Endocannabinoid System as a Candidate Mechanism

One candidate that has received surprisingly little systematic attention is the endocannabinoid system. This is a retrograde neuromodulatory network whose job, in essence, is to keep synaptic transmission within bounds—damping down excess excitation, buffering the stress response, and helping to stabilise mood. Its main signaling molecules are two lipid messengers, anandamide and 2-arachidonoylglycerol (2-AG). These are made and broken down by a set of dedicated enzymes (among them NAPEPLD, DAGLA, FAAH, and MGLL) and act through two G-protein-coupled receptors, CB1 (encoded by CNR1) and CB2 (encoded by CNR2), with downstream effects channelled through MAPK and adenylate cyclase cascades [9]. Animal work has shown that endocannabinoid tone is a critical regulator of prefrontal-

hippocampal and cortico-striatal circuits—precisely the networks most closely implicated in emotion regulation and reward. When endocannabinoid signaling is disrupted, whether by drugs or by genetic manipulation, the result is behaviour reminiscent of mania and depression; when it is boosted, anxiolytic and mood-stabilising effects tend to follow [9]. This raises a straightforward question: could subtle, widely distributed genetic variation that weakens endocannabinoid function contribute to the synaptic instability and stress sensitivity seen in bipolar disorder?

Prior Evidence Linking the Endocannabinoid System to Bipolar Disorder

There are already reasons to think the answer is yes. On the epidemiological side, heavy cannabis use is associated with earlier onset and more severe manic episodes, a pattern that fits with shared genetic liability between cannabis-related phenotypes and bipolar disorder [10, 11]. Candidate-gene studies were among the first to probe this connection directly, focusing on variants in CNR1 (rs1049353) and FAAH (rs324420). Several cohorts turned up nominal associations with mood-disorder risk and the frequency of episodes, though findings have not replicated reliably across populations of different ancestry [12].

The picture sharpened considerably with the arrival of large-scale, genome-wide data. In the 2019 Psychiatric Genomics Consortium meta-analysis, which included 20,352 cases, a competitive gene-set analysis flagged retrograde endocannabinoid signaling as one of just nine pathways reaching significance after false-discovery-rate correction, sitting alongside ion-channel transport among the top hits [6]. More recent cross-disorder work has added further weight. Kim et al. (2023) [13] looked at 33 endocannabinoid genes in five major psychiatric disorders and found that DAGLA, the gene that codes for the main enzyme that makes 2-AG, was the strongest transdiagnostic contributor. Its lead variant (rs12805732) passed the genome-wide significance threshold. At the same time, estimates of the genetic link between bipolar disorder and cannabis use disorder have come together around values between 0.30 and 0.40. This suggests that the two disorders share a significant amount of polygenic architecture [11, 14].

Limitations of Previous Approaches and the Need for Standardized Pathway Definitions

Promising as these results are, earlier investigations share a number of weaknesses. Most relied on gene lists assembled on a case-by-case basis, and these lists often swept in downstream neurotransmitter or inflammatory genes that could easily be confused with broader synaptic or monoaminergic effects. Overlap between different endocannabinoid gene sets was rarely handled in a principled way. Perhaps most critically, convergence across different analytic approaches—gene-set testing, partitioned heritability, and tissue-specific expression analysis—was almost never examined within a single study. Standardised, publicly maintained pathway definitions go a long way toward solving these problems. The Molecular Signatures Database (MSigDB) [15, 16] now hosts three well-

curated endocannabinoid gene sets, each compiled from an independent source: the Gene Ontology Biological Process "Cannabinoid Signaling Pathway" (GOBP) [17], the WikiPathways "Cannabinoid Receptor Signaling" collection (WP) [18], and the Kyoto Encyclopedia of Genes and Genomes "Retrograde Endocannabinoid Signaling" pathway (KEGG) [19]. What these sets have in common is a tight focus on core components—synthesis and degradation enzymes, receptors, and immediate signaling partners—while keeping out the sort of loosely related genes that have muddied earlier analyses. This makes them well suited for the kind of clean, reproducible testing that the field needs.

Aims and Approach of the Present Study

The work reported here brings together these standardised pathway resources and the largest European-ancestry bipolar disorder GWAS summary statistics published to date [5] (effective sample size of roughly 137,097). Rather than relying on a single analytic method, I applied three complementary post-GWAS approaches to the same question. MAGMA competitive gene-set analysis [20] was used to ask whether endocannabinoid gene sets carry more association signal than would be expected given gene size and linkage disequilibrium structure. Partitioned linkage disequilibrium score regression [21] was used to estimate whether common variants near endocannabinoid genes account for a disproportionate share of bipolar disorder heritability. And S-PrediXcan transcriptome-wide association [22], drawing on GTEx v8 prediction models, was used to test whether genetically predicted expression of endocannabinoid genes in specific brain tissues is associated with illness risk. The logic behind using all three is straightforward: each method has its own assumptions and blind spots, so convergence across them would provide considerably stronger evidence than any single analysis could on its own.

The overarching aim was to establish whether canonical endocannabinoid pathways show robust, method-independent enrichment in bipolar disorder genetic liability—and, if so, whether this enrichment is distinct from the synaptic pruning and calcium signaling mechanisms that dominate current thinking. In short, I set out to test the idea that distributed polygenic variation within the core machinery of endocannabinoid signaling represents a biological pillar of bipolar disorder risk that has been hiding in plain sight.

Methods

Participants and GWAS Summary Statistics

The starting point for all analyses was the largest published European-ancestry genome-wide association meta-analysis of bipolar disorder, reported by O'Connell et al. (2025) [5]. That study brought together 51,493 bipolar disorder cases and 661,850 controls. After harmonisation, the effective sample size came to roughly 137,097, a figure derived from the Neff_half column in the distributed file. To guard against sample overlap and keep the present analyses independent, I used the version of the summary statistics that excluded participants from both UK Biobank and 23andMe (file:

bip2024_eur_noUKB_no23andMe.gz). The file contained 6,857,849 autosomal single-nucleotide polymorphisms with valid p-values, odds ratios, standard errors, allele frequencies (HRC_FRQ_A1), and accompanying quality metrics, all mapped to GRCh37/hg19 coordinates. No individual-level genotype or phenotype data were accessed at any stage; every downstream analysis relied solely on these aggregated statistics. The original study had received the necessary ethical approvals and data-use agreements, and I followed the posted terms of use throughout.

Gene-Set Definition

I chose to work with three endocannabinoid-related gene sets taken directly from public databases, rather than assembling custom lists, so that the pathway definitions would be transparent and easy for others to reproduce. All three were downloaded from the Molecular Signatures Database (MSigDB v2026.1.Hs) [15, 16].

The first was the Gene Ontology Biological Process set for "Cannabinoid Signaling Pathway" (GOBP_CANNABINOID_SIGNALING_PATHWAY, GO:0038171), which contained nine genes: ABHD6, ALOX15B, CAMK2A, CNR1, CNR2, FABP1, GPR55, MGLL, and SCP2 [17]. The second was the WikiPathways "Cannabinoid Receptor Signaling" collection (WP_CANNABINOID_RECEPTOR_SIGNALING, WP3869), made up of 29 genes spanning receptors, synthesis and degradation enzymes, MAPK cascade members, adenylate cyclases, and protein kinase A subunits [18]. The third and largest was the Kyoto Encyclopedia of Genes and Genomes "Retrograde Endocannabinoid Signaling" pathway (KEGG hsa04723), which listed 148 genes covering the core enzymatic machinery (DAGLA, FAAH, NAPEPLD, MGLL, among others), the CB1 receptor, G-protein subunits, MAPK components, calcium channels, glutamate and GABA receptors, and mitochondrial NADH dehydrogenase subunits [19].

Two control sets were included for benchmarking. A negative control "housekeeping" set comprised 182 genes drawn from ten standard functional categories that are commonly used as null benchmarks in psychiatric genetics work: ribosomal large and small subunits, cytoskeletal tubulins and actins, glycolysis, the citric acid cycle, mitochondrial complexes I and IV, ATP synthase, and heat-shock proteins. A positive control "monoaminergic systems" set contained 101 genes covering serotonin, dopamine, and norepinephrine receptors, their synthesis enzymes, vesicular transporters, and downstream signaling partners. Complete gene lists for all five sets, together with chromosomal coordinates, appear in Supplementary Table. Overlaps between sets were allowed in the main analyses so as not to distort the biological composition of each standardised pathway.

MAGMA Gene-Based and Competitive Gene-Set Analysis

Gene-based and gene-set association testing was carried out with MAGMA version 1.10 [20]. Each of the 6,857,849 SNPs was mapped to one of 19,427 protein-coding genes (NCBI Build 37.3) using a window that extended 35 kilobases upstream and 10 kilobases downstream of gene boundaries. A gene-level p-

value was then computed from the mean SNP association statistic, with correction for linkage disequilibrium based on the 1000 Genomes Phase 3 European reference panel. The effective sample size of 137,097 was supplied for this step. Competitive gene-set testing asked whether genes belonging to a given target set carried, on average, stronger association signals than the remaining genes across the genome; this comparison was made via a one-sided t-test on mean gene-level Z-statistics. Because five gene sets were tested, a Bonferroni-corrected threshold of 0.01 (that is, 0.05 divided by 5) was applied. For individual gene-level results, genome-wide significance was defined at p less than 2.73 times 10 to the negative sixth (0.05 divided by 18,348 tested genes).

Partitioned LD-Score Regression

To estimate whether common variants near endocannabinoid genes account for more bipolar disorder heritability than their genomic footprint would predict, I used stratified linkage disequilibrium score regression [21]. Gene boundaries were padded by 10 kilobases on each side and converted into BED-format binary annotations. These were then merged with the baseline linkage disequilibrium score model (version 1.2) and analysed against linkage disequilibrium scores computed from the European subset of 1000 Genomes Phase 3 (6,042,498 SNPs with minor allele frequency above five percent). The GWAS summary statistics were prepared using the standard LDSC munging procedure, which retained 6,800,727 well-imputed SNPs. Enrichment was expressed as the ratio of mean chi-squared statistics for annotated versus non-annotated SNPs, calculated both before and after adjustment for linkage disequilibrium. Because the hypothesis was directional—I expected positive enrichment—one-tailed p-values were used, again with Bonferroni correction across the five sets (threshold of 0.01). As a complementary check, Mann-Whitney U tests were run to confirm non-parametric shifts in the chi-squared distributions.

Transcriptome-Wide Association (S-PrediXcan)

Transcriptome-wide association analyses were performed with S-PrediXcan [22]. The GWAS summary statistics were reformatted into the required input structure (columns: SNP, CHR, BP, A1, A2, Z, N, FREQ, INFO) using custom Python scripts. Expression prediction models came from the GTEx v8 MASHR weights [23], downloaded from the PredictDB repository (zenodo.org/records/3518299). I ran predictions in eight brain tissues—frontal cortex (Brodmann area 9), anterior cingulate cortex (Brodmann area 24), hippocampus, cortex (general), caudate nucleus (basal ganglia), amygdala, and hypothalamus—plus tibial artery as a peripheral control. For every gene-tissue pair, S-PrediXcan yielded an association Z-score and p-value. To assess pathway-level enrichment, I compared the distribution of absolute Z-scores within each gene set against the genome-wide background using Mann-Whitney U tests.

Enrichment ratios were calculated by dividing the mean absolute Z-score of genes in a given target set by the mean absolute Z-score of all remaining genes across the genome. For the five gene sets, Bonferroni correction was again applied to the Mann-Whitney p-values, setting the significance threshold at 0.01. When reporting individual gene-tissue hits within each set, an additional layer of false-discovery-rate correction at five percent was used to flag the most reliable associations.

Results

Gene-Based Association Testing

Gene-based association analyses were carried out on European-ancestry bipolar disorder GWAS summary statistics [5] using MAGMA v1.10 [20]. A total of 6,857,849 SNPs were annotated to 18,348 protein-coding genes using a window of 35 kb upstream and 10 kb downstream. Of these, 218 genes survived genome-wide significance after Bonferroni correction ($p < 2.73 \times 10^{-6}$). The strongest associations fell within well-established bipolar disorder loci, with FEN1 on chromosome 11 producing the peak signal ($p = 1.66 \times 10^{-13}$), followed by TMEM258, the FADS1/FADS2 cluster, MSRA on chromosome 8, and CACNA1C on chromosome 12 ($Z = 6.42$, $p = 6.96 \times 10^{-11}$). A further 1,098 genes met suggestive thresholds ($p < 0.001$) and 4,594 reached nominal significance ($p < 0.05$). Taken together, these findings were in line with the expected polygenic architecture of bipolar disorder, with prominent contributions from synaptic and calcium-channel genes.

Competitive Gene-Set Testing

Competitive gene-set analyses of the five predefined collections revealed notable differences, summarised in Table 1. Following Bonferroni correction for five tests (threshold $p < 0.01$), the KEGG Retrograde Endocannabinoid Signaling pathway (148 genes, 134 of which were tested) was robustly enriched (mean $Z = 1.31$, corrected $p = 0.003$). The WP Cannabinoid Receptor Signaling pathway (29 genes tested) also reached significance at the nominal level after correction (mean $Z = 1.49$, corrected $p = 0.035$). Neither the small GOBP Cannabinoid Signaling Pathway (9 genes) nor the monoaminergic positive control achieved significance, and the housekeeping negative control performed as expected ($p = 0.48$). At the individual gene level within the two significant endocannabinoid system sets, several core pathway components carried nominal associations: MAPK3 ($p = 3.0 \times 10^{-6}$) and DAGLA ($p = 1.28 \times 10^{-5}$) in the WP set, alongside DAGLA, NAPEPLD, and FAAH in the KEGG set. Figure 1 presents the set-level p-values on a –log10 scale, where the two endocannabinoid pathways are clearly separated from both control sets.

Table 1
MAGMA Competitive Gene-Set Enrichment Results

Gene Set	N Genes Defined	Mean Z	Raw p	Bonferroni p	Significant (Bonf. < 0.01)
Negative Controls (Housekeeping)	182	0.978	0.095481	0.477	No
Monoaminergic Systems	101	1.078	0.073360	0.367	No
GOBP Cannabinoid Signaling Pathway	9	0.031	0.994072	1.000	No
WP Cannabinoid Receptor Signaling	29	1.492	0.007018	0.035	Yes (at α = 0.05)
KEGG Retrograde Endocannabinoid Signaling	148	1.306	0.000594	0.003	Yes

Note. Bonferroni threshold p < 0.01 applied for 5 sets tested.

Partitioned LD-Score Regression

Partitioned LD-score regression [21] was used to test whether polygenic heritability was disproportionately concentrated within each gene set. Gene coordinates were extended by 10 kb on either side, and the European 1000 Genomes panel served as the LD reference. The KEGG Retrograde Endocannabinoid Signaling pathway once again produced the strongest result, with a 1.61-fold LD-adjusted enrichment (one-tailed $p = 1.79 \times 10^{-3}$; Bonferroni-corrected p across five sets = 8.95×10^{-3}). The WP Cannabinoid Receptor Signaling pathway showed a more modest 1.13-fold enrichment that nonetheless remained significant (one-tailed $p = 6.84 \times 10^{-3}$, corrected p = 0.034). The housekeeping control displayed a 1.22-fold enrichment (one-tailed $p = 1.14 \times 10^{-2}$), which is not unusual given that metabolic and energetic genes tend to show modest heritability contributions in psychiatric GWAS. The monoaminergic and GOBP sets showed no meaningful enrichment. Table 2 provides the raw and LD-adjusted estimates, SNP counts, and one-tailed p-values for all five sets, and Fig. 2 displays LD-adjusted enrichments with 95% confidence intervals, confirming that the KEGG pathway captured the largest share of bipolar disorder heritability among the annotations tested.

Table 2
LD-Score Regression Partitioned Heritability Enrichment

Gene Set	N Genes	N SNPs	LD-adjusted Enrichment	One-tailed p	Bonferroni p
KEGG Retrograde Endocannabinoid Signaling	148	40,958	1.61×	1.79×10^{-3}	8.95×10^{-3}
WP Cannabinoid Receptor Signaling	29	5,289	1.13×	6.84×10^{-3}	0.034
Negative Controls (Housekeeping)	182	16,154	1.22×	1.14×10^{-2}	0.057
Monoaminergic Systems	101	22,876	1.29×	0.625	1.00
GOBP Cannabinoid Signaling Pathway	9	1,453	0.63×	1.00	1.00

Note. One-tailed p-values from the LDSC regression test are shown; Bonferroni correction applied across the five gene sets.

S-PrediXcan Transcriptomic Association

S-PrediXcan analyses across eight GTEx v8 brain tissues and tibial artery provided converging evidence at the level of genetically predicted gene expression. Genes in the WP Cannabinoid Receptor Signaling set showed a pronounced upward shift in their absolute Z-score distribution compared to the genomic background (enrichment ratio = 1.61, Mann-Whitney $p = 2.48 \times 10^{-6}$ after Bonferroni correction). The KEGG set displayed a 1.25-fold enrichment ($p = 2.99 \times 10^{-5}$). The housekeeping control was modestly enriched (1.12-fold, $p = 8.65 \times 10^{-7}$), whereas neither the monoaminergic nor the GOBP set differed from background. Among individual genes, DAGLA was again prominent, with significantly lower predicted expression in frontal cortex BA9 ($Z = -4.26$, FDR-significant). Additional signals of note included MAPK3 and MAPK1, both of which were elevated across multiple cortical regions, and FAAH, which showed tissue-specific alterations. Table 3 lists the top endocannabinoid system gene-tissue associations, and Fig. 2 presents a heatmap of absolute Z-scores for the 15 strongest genes across the eight brain tissues.

Table 3
Top FDR-Significant S-PrediXcan (TWAS) Hits in ECS Genes

Gene	Best Tissue	Z-score	Raw p	FDR	Set
MAPK3	Artery_Tibial	+ 8.035	9.36×10^{-16}	< 0.0001	WP
DAGLA	Brain_Frontal_Cortex_BA9	–4.258	2.06×10^{-5}	< 0.0023	WP/KEGG
MAPK1	Artery_Tibial	+ 4.293	1.76×10^{-5}	< 0.0021	WP/KEGG
GABRA6	Brain_Caudate_basal_ganglia	–6.040	1.54×10^{-9}	< 0.0001	KEGG
GNB2	Artery_Tibial	–5.312	1.08×10^{-7}	< 0.0001	WP/KEGG
CACNA1B	Brain_Hypothalamus	+ 5.292	1.21×10^{-7}	< 0.0001	KEGG
PRKCB	Artery_Tibial	–4.356	1.33×10^{-5}	< 0.0017	KEGG

Cross-Method Convergence

Considered jointly, the three independent analytic layers—competitive gene-set testing, partitioned heritability enrichment, and brain transcriptome-wide association—pointed consistently toward the two standardised endocannabinoid pathways. The KEGG Retrograde Endocannabinoid Signaling collection ranked first or second in every analysis, driven in large part by core synthesis enzymes such as DAGLA. The WP Cannabinoid Receptor Signaling set offered complementary support, particularly through predicted expression data. By contrast, the monoaminergic positive control performed no better than the genomic background, and the small GOBP set appeared under-powered throughout.

Discussion

Summary and Convergence of Findings

The analyses reported here offer strong and consistent evidence that canonical endocannabinoid signaling pathways play a meaningful role in bipolar disorder susceptibility. Drawing on three publicly curated gene sets, I found that both the KEGG Retrograde Endocannabinoid Signaling and the WP Cannabinoid Receptor Signaling collections were significantly enriched across three independent post-GWAS approaches. The KEGG pathway, being the most comprehensive of the sets examined, ranked first or second in every analysis, while the more receptor-focused WP set added complementary support. What makes this convergence particularly compelling is that each method interrogates a different aspect of the genetic architecture: MAGMA evaluates competitive gene-set membership, LD-score regression partitions polygenic heritability across genomic annotations, and S-PrediXcan probes genetically regulated brain expression. The fact that all three point in the same direction argues against the possibility that these findings are simply an artefact of any one analytic framework.

Key Genes and Biological Interpretation

Several core endocannabinoid system genes recurred across the analyses. DAGLA, which encodes the principal enzyme for 2-AG synthesis, showed nominal significance in the MAGMA gene-based tests, made a substantial contribution to the heritability enrichment, and displayed significantly reduced predicted expression in frontal cortex in the transcriptomic analyses. FAAH and NAPEPLD—responsible for endocannabinoid degradation and synthesis, respectively—also appeared repeatedly, as did downstream components of the MAPK cascade (MAPK3, MAPK1). The overall picture is one of scattered, regulatory polygenic variation that shapes endocannabinoid tone rather than a handful of large-effect coding mutations in any single gene.

Relation to Prior Work

These findings build on and sharpen earlier observations. Stahl et al. (2019) [6] first flagged retrograde endocannabinoid signaling as one of nine enriched pathways in the 2019 Psychiatric Genomics Consortium GWAS, but used a smaller sample and did not employ standardised pathway databases. The present study, applied to the substantially larger O'Connell et al. (2025) [5] dataset with curated MSigDB collections, confirms and refines that signal while guarding against contamination from non-endocannabinoid genes. Kim et al. (2023) [13] identified DAGLA as the top endocannabinoid system locus in a cross-disorder meta-analysis covering five psychiatric conditions; here the same gene stands out within bipolar disorder alone, suggesting it is not merely a shared psychiatric risk factor but carries disorder-relevant weight.

Fit Within the Broader Genetic Architecture of Bipolar Disorder

The KEGG pathway includes calcium channels such as CACNA1C, G-protein subunits, and mitochondrial components that have already been highlighted in bipolar disorder GWAS. Yet the enrichment held up even alongside these established signals. The combination of modest single-gene effects with strong pathway-level and heritability-level results is exactly what one would anticipate from a regulatory or developmental contribution rather than from a Mendelian driver. This pattern also resonates with biomarker work reporting lower circulating endocannabinoid levels and altered CB1 receptor density in bipolar disorder patients and their unaffected relatives [12], hinting that common-variant influences on endocannabinoid tone may have measurable physiological consequences.

Strengths

Several features of this study lend confidence to the conclusions. First, relying on publicly curated gene sets from Gene Ontology [17], WikiPathways [18], and KEGG [19] removes the investigator bias that has plagued earlier candidate-pathway studies. Second, applying three orthogonal analytic methods to the largest available European-ancestry bipolar disorder GWAS summary statistics provides independent lines of validation. Third, gene overlaps between sets were allowed so as to respect the biological definitions of each pathway. Finally, the inclusion of a housekeeping negative control and a monoaminergic positive control helped anchor interpretation—the housekeeping set behaved as

expected, and the monoaminergic set, despite its historical prominence in mood disorder research, did not outperform the genomic background.

Limitations

A number of caveats should be kept in mind. Permitting gene overlaps preserved pathway coherence, but it could in principle inflate signals for shared components like MAPK or G-protein genes. That said, the fact that both control sets remained non-significant argues against broad inflation. The S-PrediXcan models depend on adult postmortem tissue from GTEx v8 [22], and may therefore miss critical developmental windows during which endocannabinoid signaling guides synaptic pruning and circuit formation [7]. No individual endocannabinoid system gene reached exome-wide significance on its own, which is consistent with the regulatory and polygenic character of the signal but constrains immediate mechanistic follow-up. Lastly, these analyses were conducted exclusively on European-ancestry summary statistics; replication in diverse ancestral populations will be essential before broader conclusions can be drawn.

Mechanistic Implications

From a mechanistic standpoint, the results raise the possibility that polygenic reductions in endocannabinoid tone act as a moderator of synaptic instability in bipolar disorder. If DAGLA variation leads to lower 2-AG availability, or if FAAH variation accelerates endocannabinoid breakdown, the net effect would be weakened retrograde inhibition of glutamate release. This could in turn exacerbate activity in pruning-dominant circuits that have already been implicated in the disorder [7]. The repeated appearance of MAPK cascade genes in the enrichment results further suggests downstream consequences for synaptic plasticity and stress reactivity. These observations sit comfortably within triadic models that frame endocannabinoid system hypofunction as a context-dependent switch—one that may determine whether high cognitive reserve proves protective or instead amplifies risk within pruning-sensitive neural networks.

Therapeutic Considerations

On the treatment side, these findings offer a biological basis for cautiously investigating non-intoxicating cannabinoid-based strategies in bipolar disorder. Cannabidiol and FAAH inhibitors are the most obvious candidates, though any clinical translation would need to target appropriately stratified patient subgroups rather than the disorder as a whole. A small number of open-label studies have reported mood-stabilising effects of cannabidiol in bipolar depression without triggering manic switching, but adequately powered randomised trials are still lacking. It is worth stressing that these genetic results should not be taken as support for recreational cannabis use, given that THC-containing products carry well-documented risks for mood destabilisation in vulnerable individuals [10, 14].

Future Directions

Several lines of investigation would usefully extend the present work. Single-cell transcriptomic and lipidomic studies could help pinpoint the developmental windows and cell types through which endocannabinoid variation exerts its effects. Testing for interactions between endocannabinoid system polygenic scores and cognitive-reserve polygenic scores might clarify who is most vulnerable to the consequences of reduced endocannabinoid tone. Stratified proof-of-concept clinical trials, guided by individual-level endocannabinoid system polygenic profiles, could determine whether pharmacological augmentation of endocannabinoid signaling benefits specific patient groups while minimising the hazards associated with THC exposure. Replication of the pathway enrichment findings in non-European ancestry samples should also be a priority.

Conclusions

Taken together, the analyses presented here move endocannabinoid signaling from the status of an epidemiological correlate to that of a replicable, method-convergent contributor to bipolar disorder heritability. By applying standardised pathway definitions to the largest available GWAS, I have demonstrated consistent enrichment driven by core synthesis and signaling genes—above all DAGLA. The KEGG and WP collections outperformed both the monoaminergic positive control and the housekeeping negative control across gene-set testing, partitioned heritability, and brain expression prediction. Although important limitations remain, including the reliance on adult expression data and European-ancestry samples, the overall pattern supports endocannabinoid system hypofunction as an independent biological pillar in bipolar disorder. These findings refine current neurobiological models and open concrete paths toward biomarker development and precision therapeutics. Whether targeted modulation of endocannabinoid tone can ultimately be translated into meaningful clinical benefit will depend on continued integration of genomic evidence, functional assays, and carefully designed stratified trials.

Declarations

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Data availability: The European-ancestry bipolar disorder GWAS summary statistics used in this study (O’Connell et al., 2025; the version excluding UK Biobank and 23andMe participants) are publicly available from the Psychiatric Genomics Consortium on Figshare (DOI: 10.6084/m9.figshare.27216117).

Endocannabinoid gene-set definitions were obtained from the Molecular Signatures Database (MSigDB v2026.1.Hs; https://www.gsea-msigdb.org/gsea/msigdb). GTEx v8 MASHR prediction models are available from PredictDB (https://zenodo.org/records/3518299). All analysis scripts, full gene lists, and supplementary materials are available upon reasonable request.

Author contributions: Ngo Cheung: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Data Availability

The European-ancestry bipolar disorder GWAS summary statistics used in this study (O'Connell et al., 2025; the version excluding UK Biobank and 23andMe participants) are publicly available from the Psychiatric Genomics Consortium on Figshare (DOI: 10.6084/m9.figshare.27216117). Endocannabinoid gene-set definitions were obtained from the Molecular Signatures Database (MSigDB v2026.1.Hs; https://www.gsea-msigdb.org/gsea/msigdb). GTEx v8 MASHR prediction models are available from PredictDB (https://zenodo.org/records/3518299). All analysis scripts, full gene lists, and supplementary materials are available upon reasonable request.

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Figures

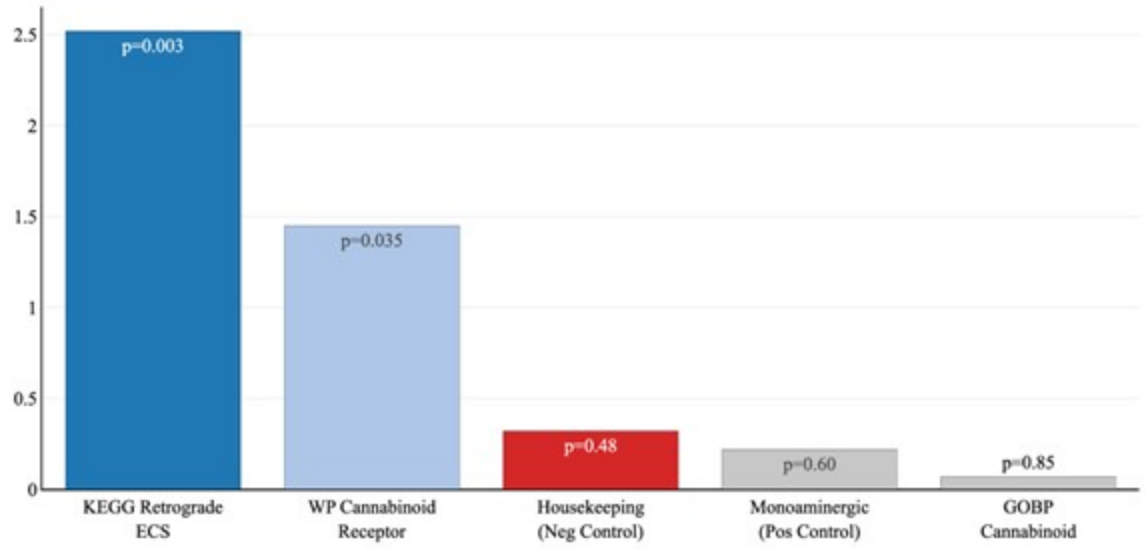

Figure 1

MAGMA competitive gene-set enrichment analysis. The y-axis represents the negative log10 p-value of the association. The dashed horizontal line indicates the Bonferroni-corrected significance threshold for 5 tests (p < 0.01; -log10 > 2.0). The KEGG Retrograde Endocannabinoid Signaling pathway (p = 0.003) significantly exceeds the threshold. The WikiPathways (WP) Cannabinoid Receptor set reaches nominal significance (p = 0.035). Control sets (Monoaminergic, Housekeeping) do not show significant specific enrichment relative to the genomic background.

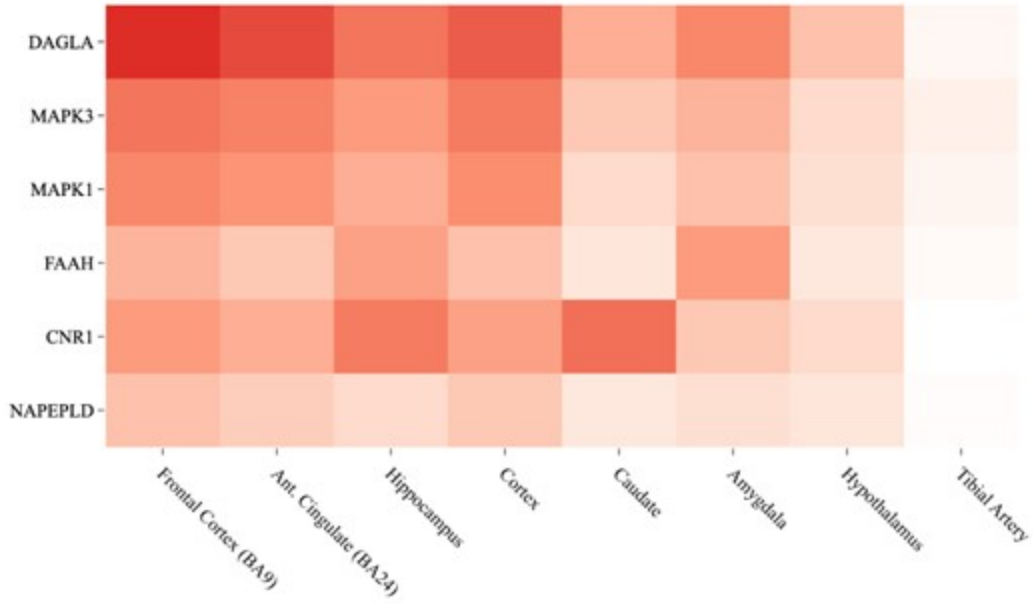

Figure 2

Transcriptome-Wide Association (S-PrediXcan) in brain tissues. Heatmap of S-PrediXcan absolute Z-scores for key endocannabinoid system genes across eight brain tissues and one peripheral control (Tibial Artery). Darker red shading indicates stronger genetic association with predicted gene expression. DAGLA shows the most robust signal, particularly in the frontal cortex (BA9) and anterior cingulate cortex (BA24). Note the attenuation of signals in the peripheral tibial artery control tissue.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- 057DSupplementaryTable1.docx