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<https://escholarship.org/uc/item/0z48z3xr>

Journal

Nature Genetics, 56(6)

ISSN

1061-4036

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Publication Date

2024-06-01

DOI

10.1038/s41588-024-01771-1

Peer reviewed



Published in final edited form as:

Nat Genet. 2024 June ; 56(6): 1310–1318. doi:10.1038/s41588-024-01771-1.

Improved functional mapping of complex trait heritability with GSA-MiXeR implicates biologically specific gene-sets

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Abstract

While genome-wide association studies (GWAS) are increasingly successful in discovering genomic loci associated with complex human traits and disorders, the biological interpretation of these findings remains challenging. We developed the GSA-MiXeR analytical tool for gene-set analysis (GSA), which fits a model for the heritability of individual genes, accounting for linkage disequilibrium across variants, and allowing the quantification of partitioned heritability and

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Authors Contributions Statement

O.F. conceived the study; O.F., A.A.S., N.P., S.B., and K.O. pre-processed the data; O.F., D.v.d.M., B.A., and E.H. performed all analyses, with conceptual input from G.H., A.A.S., C.d.L., D.P., W.C., D.H., O.B.S., O.A.A. and A.M.D.; O.F. and G.H. drafted the manuscript; all authors contributed to and approved the final manuscript.

Competing Interests Statement

Dr. Anders M. Dale was a Founder of and holds equity in CorTechs Labs, Inc, and serves on its Scientific Advisory Board. He is also a member of the Scientific Advisory Board of Human Longevity, Inc. (HLI), and the Mohn Medical Imaging and Visualization Centre in Bergen, Norway. He receives funding through a research agreement with General Electric Healthcare (GEHC). The terms of these arrangements have been reviewed and approved by the University of California, San Diego in accordance with its conflict-of-interest policies. Dr. Andreassen has received speaker fees from Lundbeck, Janssen, Otsuka, and Sunovion and is a consultant to Cortechs.ai unrelated to the topic of this study. Christiaan de Leeuw is funded by Hoffman-La Roche. The remaining authors have no competing interest.

Code availability

The GSA-MiXeR software (v1.0.0), user tutorial, and all codes required to process input data and generate the major results of this study are available from <https://github.com/precimed/gsa-mixer>⁶². Additionally, the following software packages were involved in performing data analysis: PLINK 1.90 build 20200616, <https://www.cog-genomics.org/plink/1.9/>; MAGMA v1.09b, <https://ctg.cncr.nl/software/magma>; LDAK v5.2, <https://dougsspeed.com/ldak-gbat/>; KING v2.2.5, <https://www.kingrelatedness.com/>; flashpca 2.0, <https://github.com/gabraham/flashpca>; cleansumstats v1.6.0, <https://github.com/BioPsyk/cleansumstats/>; modified sLDSC v2023.05.15, https://github.com/ofrei/ldsc/tree/disable_jackknife; simu v0.9.3 pipeline to simulate synthetic GWAS from genotypes, <https://github.com/precimed/simu>; GWAS pipeline for UK Biobank v2, https://github.com/Nealelab/UK_Biobank_GWAS.

fold enrichment for small gene-sets. We validated the method using extensive simulations and sensitivity analyses. When applied to a diverse selection of complex traits and disorders, including schizophrenia, GSA-MiXeR prioritizes gene-sets with greater biological specificity compared to standard GSA approaches, implicating voltage-gated calcium channel function and dopaminergic signaling for schizophrenia. Such biologically relevant gene-sets, often with less than ten genes, are more likely to provide insights into the pathobiology of complex diseases and highlight potential drug targets.

Introduction

Genome-wide association studies (GWAS) have discovered thousands of genomic loci associated with complex human traits and disorders, highlighting their polygenic nature and the predominance of small individual effects of common genetic variants¹. Gene-set analysis (GSA) has become a powerful tool for understanding the biological implications of GWAS findings². Building upon large databases of gene-sets such as the Molecular Signatures Database (MSigDB)³, Gene Ontology (GO)⁴, Synaptic Gene Ontologies (SynGO)⁵ and others, GSA has provided many prominent findings^{6–8}, implicating the role of biological pathways and yielding relevant tissue- and cell type-specific insights into complex human traits and disorders.

Some of the popular analytical tools for GSA analysis of GWAS data are MAGMA⁹, Fisher's exact (hypergeometric) test¹⁰, and the stratified linkage disequilibrium (LD) score regression (sLDSC)¹¹. MAGMA implements a two-stage approach, where GWAS p-values of single nucleotide polymorphisms (SNPs) are first aggregated into gene-level p-values, which are further tested for association with predefined gene-sets. The hypergeometric test evaluates whether the genes implicated in GWAS are over-represented within predefined gene-sets. Both tools implement competitive GSA¹², testing the null hypothesis that the genes in question are no more strongly associated with the phenotype than other genes. A limitation to both approaches is that they only provide a measure of statistical significance ("enrichment p-value"), which largely depends on the sample size of the GWAS and on the size of a gene-set. Such enrichment p-values do not constitute a biologically meaningful measure of enrichment magnitude, limiting our ability to compare the relative strength of enrichment across or within traits. Importantly, enrichment p-values correlate with gene-set size, tending to be more significant for larger gene-sets, thus limiting our ability to discover more informative biological pathways, with greater specificity to a given phenotype.

The sLDSC method quantifies enrichment by partitioning heritability across genomic regions. Specifically, such partitioning allows one to compute a trait's "fold enrichment" score for a genomic region. This is given by the ratio of the trait's heritability attributed to the region (as estimated by the model) to the heritability of the region estimated from a baseline model which assumes that heritability is uniformly distributed across the genome. The sLDSC method uses GWAS summary statistics to quantify fold enrichments in functional categories. For example, regions conserved in mammals have a disproportionately large contribution to heritability for many traits and disorders¹¹. However, for smaller annotations, it has been shown that block jackknife-based significance testing used in

sLDSC does not always control type 1 error for annotations with approximately 0.5% of SNPs or less¹³. Furthermore, sLDSC is based on an infinitesimal model with additional simplifying assumptions about the distribution of genetic effects with respect to minor allele frequency (MAF) and LD¹⁴, which in certain cases has been shown to bias fold enrichment estimates¹⁵. Finally, the sLDSC method has so far been applied only to tissue- and cell-type-specific annotations, but not to gene ontologies, both due to computational challenges (such as excessive memory usage and runtime) and algorithmic failures (such as non-invertible matrices arising in block jackknife-based significance testing).

Here, building on a previously developed MiXeR framework^{16–19}, we introduce GSA-MiXeR, a method for competitive GSA which quantifies fold enrichment of partitioned heritability attributed to each gene-set. Its efficient design and implementation, enabled by stochastic gradient-based log-likelihood optimization²⁰, allow GSA-MiXeR to jointly model more than 18,000 genes in a single model, while accounting simultaneously for the trait's polygenicity, MAF- and LD-dependency of genetic effects, and differential enrichment of functional categories, thus taking into account the unique genetic architecture of each trait. We evaluated the accuracy of GSA-MiXeR's fold enrichment estimates in extensive simulations using real UK Biobank genotypes. We also performed replication analysis using independent GWAS for discovery and replication, which confirmed that ranking gene-sets according to fold enrichment (GSA-MiXeR estimate) often results in a more stable order of gene-sets compared to ranking based on enrichment p-values from MAGMA, even though GSA-MiXeR promoted smaller gene-sets of potentially higher biological relevance. We also performed extensive sensitivity analyses, testing the robustness of the GSA-MiXeR method against a misspecified model of the genetic architecture and out-of-sample LD information.

To demonstrate GSA-MiXeR's performance with real phenotypic data, we applied it to the latest GWAS for schizophrenia⁸, as well as to a selection of diverse human traits and disorders^{21–35}. In our main analysis, we report GSA-MiXeR results for significant gene-sets first identified by MAGMA. This shows how the addition of fold enrichments enhances the biological interpretation of GSA results, allowing comparisons of gene-set effect size within and across phenotypes. In schizophrenia, GSA-MiXeR reveals that gene-sets related to calcium channel function have greater fold enrichment than larger gene-sets related to post-synaptic functioning. In a subsequent exploratory analysis of schizophrenia without filtering on significant MAGMA associations, we demonstrate that GSA-MiXeR implicates other highly enriched and biologically plausible gene-sets, including biological processes related to dopaminergic neurotransmission, pointing to potential key biological pathways perturbed in schizophrenia, and supporting prior hypotheses of schizophrenia pathogenesis³⁶. When applied to 20 additional phenotypes, GSA-MiXeR tended to prioritize small, biologically relevant gene-sets.

Results

Overview of the methods

GSA-MiXeR implements two heritability models: a full model, where an individual contribution of each gene to the heritability of a complex trait is estimated from GWAS summary statistics; and a baseline model of heritability distribution that is irrespective of

individual genes. Fold enrichment attributed to a gene-set is then computed as the ratio of its heritability from the full over the baseline models. The parameters of both models and their respective standard errors (SEs) are estimated from the trait's GWAS summary statistics (z-scores and p-values) plugged into a comprehensive likelihood function which accounts for LD correlations between SNPs. The likelihood function is also used to compute Akaike information criterion (AIC) values quantifying available evidence for the enrichment of individual genes and gene-sets. Further details are presented in Methods.

Simulation studies

To evaluate the accuracy of the GSA-MiXeR's fold enrichment estimates and SEs, we conducted simulations by synthesizing a quantitative trait and its respective GWAS summary statistics using real UK Biobank genotypes (N=337,145 subjects and M=12,926,669 variants after quality control). We simulated 9 distinct scenarios (see Methods and Supplementary Table 1 for an overview) of genetic architectures to validate the sensitivity of the fold enrichment estimates to the underlying assumptions of the GSA-MiXeR model. In simulations without enrichment, total heritability ($h^2=0.1, 0.4, \text{ or } 0.7$) of the trait was uniformly distributed across the genic regions. To simulate enrichment, a certain number of genes (1, 3, 10, 30, or 100) received a 3-, 10- or 30-fold enrichment in heritability attributed to those genes. We used boundaries of real protein-coding genes in these simulations and selected an enriched subset of genes at random. Then we fitted two models: the baseline model with two variance parameters, allowing for different effect size variance in genic regions versus non-genic regions; and the full model, where, in addition to the baseline model, each gene was modelled with its own effect size variance parameter. The estimated fold enrichment of a gene-set was calculated as a ratio of its heritability estimate from the full model versus the baseline model (see Methods).

The results (Fig. 1a–d, Supplementary Fig. 1, and Supplementary Tables 1–2) show that GSA-MiXeR clearly differentiated between scenarios with 1-, 3-, 10-, and 30-fold enrichment, with estimated fold enrichment of 1.04, 2.67, 8.61, and 23.88, respectively, as measured by an average estimate across all simulated scenarios with a given true fold enrichment. This indicates that GSA-MiXeR estimates are somewhat conservative (biased downwards). However, for the least challenging simulated scenarios, the estimates were more accurate. For example, in six simulated scenarios with sufficiently large gene-sets (10, 30, 100), sufficiently powered GWAS summary statistics (corresponding to $h^2=0.4$ and 0.7 in simulations performed with N=337,145 subjects), and the “base” genetic architecture (causal variants uniformly distributed across protein-coding genes), the average fold enrichment estimate from GSA-MiXeR was 1.00, 2.90, 10.75, and 29.35, compared to the expected enrichment of 1, 3, 10, and 30, respectively. These results show that GSA-MiXeR gives accurate results if the underlying assumptions of its model are met, and if the region that is being tested for enrichment has a sufficiently powered signal in the GWAS summary statistics. The SEs generally matched the standard deviation across simulations (Supplementary Table 3); however, for genes with close to zero heritability estimate the hessian-based SE of the σ_g^2 parameter was insufficiently robust and was truncated at 5 times the respective point estimate. For further sensitivity analysis using simulated data see Supplementary Information.

We performed additional simulations to compare GSA-MiXeR and MAGMA's ability to rank enriched gene-sets (Fig. 1e–g, Supplementary Fig. 2, Supplementary Table 4). For each of the previously conducted simulations, we generated 999 additional gene-sets, each with the same number of genes as the enriched gene-set. We then computed the position of the truly enriched gene-set on the list, sorted either according to GSA-MiXeR fold enrichment or according to MAGMA p-values. Both methods correctly rank the most enriched gene-sets at the 1st position if there was sufficient signal in the data, e.g., in a scenario with 100 genes and $h^2=0.7$. For the “base” genetic architecture, GSA-MiXeR outperforms MAGMA in the less-powered scenarios. For instance, in simulations with 10x fold enrichment, 10 genes, and $h^2=0.4$, the average rank of the truly enriched gene-set was 1.1 for GSA-MiXeR, and 22.7 for MAGMA. Simulations with other scenarios of genetic architecture show that GSA-MiXeR's advantage in ranking accuracy over MAGMA depends on how well the underlying assumptions about the trait's genetic architecture are met (Supplementary Information). The individual genes were generally ranked at approximately the 100-th position or higher by both MiXeR and MAGMA, indicating that the error of estimating gene-level fold enrichment currently appears to be too large to make reliable inferences at the level of individual genes.

Application to real phenotypic data

We applied GSA-MiXeR to the latest GWAS of schizophrenia³⁷, as well as a selection of 20 traits and disorders representing a variety of different human phenotypes (Table 1), including somatic disorders, quantitative biophysical and biochemical measures, and mental traits. In our main analysis, we first identified all enriched gene-sets with MAGMA p-values below 0.05 after Bonferroni correction. Next, we re-ranked gene-sets based on GSA-MiXeR fold enrichment and compared them to the original MAGMA p-value-based ranking. We also compared fold enrichment and partitioned heritability estimated by GSA-MiXeR within and across phenotypes.

When applied to schizophrenia (Fig. 2a–b, Supplementary Table 5), the top ten GSA-MiXeR gene-sets had on average 236 genes, with an average fold enrichment of 2.49 (root mean square error (RMSE) 0.50) (Supplementary Table 5), versus an average of 620 genes and fold enrichment of 1.53 (RMSE 0.22) for the top ten MAGMA gene-sets. Interestingly, among the significant MAGMA gene-sets, the top four gene-sets after ordering based on GSA-MiXeR enrichment for schizophrenia were related to voltage-gated calcium channels and membrane depolarization (Fig. 2a), which are more specific biological processes compared to the larger synaptic and post-synaptic gene-sets prioritized by MAGMA. Furthermore, voltage-gated calcium channels gene-sets were approximately ten times more fold enriched than other MAGMA identified gene-sets (mean fold enrichment = 4.05 (RMSE 0.78) vs 1.33 (RMSE 0.09), respectively), with a marked drop in fold enrichment to the next most fold enriched gene-set (intrinsic component of post-synaptic membrane, fold enrichment 1.58 (SE 0.16)). The larger enrichment was partly attributable to the *CACNA1C* gene which was the most enriched gene in all related gene-sets. To evaluate heterogeneity of gene fold enrichments within implicated gene-sets, we performed a leave-one-gene-out analysis, showing that the average fold enrichment across voltage-gated calcium channels gene sets reduced from 4.05 (RMSE 0.78) to 2.89 (RMSE 0.85) after

exclusion of the *CACNA1C* gene (Supplementary Table 5), but the gene-sets remained enriched. The purpose of the leave-one-gene-out analysis is to evaluate heterogeneity of gene fold enrichments within implicated gene-sets, rather than to estimate uncertainty of the enrichment estimates.

In an exploratory analysis we considered all gene-sets with a positive value of GSA-MiXeR's Akaike Information Criterion (AIC) (see Methods). For schizophrenia this implicated 36 gene-sets (Fig. 3, Supplementary Table 7), with an average size of approx. 8 genes and fold enrichment of 7.28 (RMSE 3.41). These included several gene-sets related to dopaminergic neurotransmission, such as “dopamine neurotransmitter receptor activity” and “dopamine binding”³⁸, as well as “interneuron migration”³⁹ and “forebrain neuron fate commitment”, none of which were identified by MAGMA. However, the enrichment in all of the top ten gene-sets besides “forebrain neuron fate commitment” was largely driven by the *DRD2* gene, rather than by the joint effect of the entire gene-set. After exclusion of *DRD2*, the average enrichment of these nine gene-sets reduced from 14.9 (RMSE 6.59) to 0.91 (RMSE 0.83) as opposed to 8.53 (SE 2.66) to 6.22 (2.26) for “forebrain neuron commitment” after exclusion of its most enriched gene “*SATB*” (Supplementary Table 7). This suggests large heterogeneity in enrichment across genes within highly enriched gene-sets, further demonstrated by QQ plots constrained to genetic variants within 10 kilobases (KB) up/down of each gene (Supplementary Fig. 3). This revealed “null” QQ plots for a subset of genes, while other genes – particularly *DRD2* and *CACNA1C* – showed strong enrichment. Further, despite the high fold enrichments of the gene-sets discussed here, it is important to note that they individually contribute to less than 0.5% of schizophrenia's heritability, further emphasizing its broad polygenic architecture.

Among the 20 other phenotypes that were analyzed, thirteen were associated with significantly enriched gene-sets as estimated by MAGMA (Table 1, Supplementary Table 6), while all phenotypes had at least one gene-set with a positive GSA-MiXeR AIC value (Table 1, Supplementary Table 8; see also Supplementary Information for more extended description of the results). Overall, the gene-sets identified by MAGMA had a mean size of 400 genes, versus a mean size of 9 genes for gene-sets implicated by GSA-MiXeR. The average fold-enrichments were 3.5 (RMSE 1.42) and 10.3 (RSME 4.83), respectively. Additionally, GSA-MiXeR enabled cross-trait comparisons of gene-set enrichments. Across all 21 phenotypes there was large variation in the mean fold enrichment, ranging from 2.85 (RMSE 0.80) for educational attainment to 31.27 (RMSE 11.55) for heart failure. This was to some extent influenced by the number of gene-sets implicated by GSA-MiXeR for each phenotype, varying from a single gene-set for alcohol consumption, heart failure and hospitalized COVID, to 161 gene-sets for type 2 diabetes, 202 for systolic blood pressure, and 405 for height (Table 1, Supplementary Table 8). Differences in the number of gene-sets identified likely reflect the statistical power of the input GWAS as well as the extent to which the current set of Gene Ontology gene-sets corresponds to the biological mechanisms underlying each phenotype. Nevertheless, there was a trend for biochemical measures and somatic disorders, such as glycosylated haemoglobin and ulcerative colitis, to exhibit higher fold enrichments than complex mental phenotypes such as educational attainment and cognition.

We also compared GSA-MiXeR and LDAK-GBAT⁴⁰ estimates of heritability for individual genes (Supplementary Table 9–10 and Supplementary Fig. 9). For a subset of 36 gene-sets implicated in the exploratory GSA-MiXeR analysis of schizophrenia, we also estimated fold enrichment with the stratified LDSC⁴¹ method (Supplementary Table 11 and Supplementary Fig. 10). The results from the different methods generally correlate well. There are certain discrepancies in the estimates for individual genes and gene-sets, which likely can be attributed to the differing methodology compounded by large uncertainty in the estimates. For example, LDAK-GBAT analyses all genes independently from each other, while GSA-MiXeR applies a joint model. Similarly, sLDSC uses an additive model across all categories (in this case 75 functional annotations plus additional categories for the gene-set and its genes), while GSA-MiXeR's heritability model separately accounts for functional annotations and genes, joining them as two factors in a multiplicative model.

Replication analysis

In our analysis of real GWAS data, we first used MAGMA to filter on significantly associated gene-sets, followed by GSA-MiXeR to re-rank these gene-sets. Here, we present the results of an independent replication analysis using either MAGMA p-values or GSA-MiXeR fold enrichments to rank gene-sets. As a discovery sample, we used half of the UK Biobank panel for the height phenotype and half of the Psychiatric Genomics Consortium (PGC) schizophrenia sub-studies. The remaining subjects or studies were used as replication data sets. Using independently obtained results for MAGMA and GSA-MiXeR from the discovery and replication samples, we computed the fraction of top-N gene-sets in the discovery samples that also appeared within the top-N gene-sets in the replication samples, allowing N to be 10, 20, 50 or 100 gene-sets. Further, we investigated how the replication rate depended on gene-set size, by computing the replication rate for a stratum of gene-sets with up to 25 genes per set, and a second stratum of gene-sets with more than 25 genes per set.

The results are presented in Fig. 4 (for schizophrenia) and in Supplementary Fig. 7 (for height). GSA-MiXeR and MAGMA methods had a comparable replication rate when evaluated on all gene-sets, or in a stratum with more than 25 genes. The biggest differences were observed in the stratum of up to 25 genes, where we consistently observed better replication rates for GSA MiXeR ranking, compared to MAGMA ranking, both for schizophrenia and for height. Overall, the results indicate that replication of GSA-MiXeR-based ranking is equivalent to MAGMA-based ranking for all gene-sets and outperforms MAGMA-based ranking for smaller gene-sets. We also demonstrated that GSA-MiXeR may potentially improve the accuracy of polygenic risk scores in terms of Nagelkerke pseudo-R² (Supplementary Fig. 8, also Supplementary Information), however further evaluation of this approach is a subject of future work.

Discussion

Here, we present GSA-MiXeR, a method for competitive gene-set enrichment analysis that applies stochastic gradient-based optimization for maximum likelihood estimation from GWAS summary statistics. We show that GSA-MiXeR can accurately model the

contribution of more than 18,000 genes to SNP-heritability of complex polygenic traits, while controlling for polygenicity, MAF- and LD-dependent genetic architecture, and functional categories. This enables the robust estimation of gene-set fold enrichment alongside partitioned heritability, thus estimating the magnitude of the enrichment, which was previously unfeasible due to computational and methodological limitations. While future work improving the validity of current gene-set definitions is required to fully exploit the power of GSA-MiXeR and GSA more generally, we show through real data from schizophrenia and 20 other traits how GSA-MiXeR, building on current GO gene-sets, enhances the biological interpretation of GWAS findings.

Applying GSA-MiXeR to simulated and real data, we show its capability to reorder gene-sets in a way that promotes smaller gene-sets while yielding an equivalent or higher replication rate compared to current standards in the field. When applied to sufficiently powered GWAS, GSA-MiXeR provides an unbiased estimate of fold enrichments even for small gene-sets with 10 genes or less. GSA-MiXeR's ability to estimate fold enrichment of a gene-set, rather than its statistical significance (p-value), represents a key advance in GSA, allowing direct comparison of the relative biological effect of implicated gene-sets. This facilitates the promotion of smaller gene-sets with more specific and well-defined functions that can be more readily interrogated in downstream experimental analysis.

When applied to schizophrenia, we demonstrate how the estimation of gene-set fold enrichments can enhance MAGMA-based GSA. GSA-MiXeR showed that gene-sets related to voltage-gated calcium channels were approximately ten times more fold-enriched than other larger gene-sets identified by MAGMA. This supports previous evidence indicating a role for voltage-gated calcium channels in schizophrenia, which has been hypothesized to affect schizophrenia risk through neurodevelopmental processes including hippocampal neurogenesis⁴². GSA-MiXeR's potential for novel mechanistic insights was further illustrated in our exploratory analysis without filtering on significant MAGMA associations. Further, despite recent advances in schizophrenia GWAS^{8,43}, results from GSA analyses have not yet mapped closely onto the primary clinical theories of schizophrenia pathoetiology³⁶. It is therefore striking that the top two most fold-enriched gene-sets were related to dopaminergic neurotransmission, the leading theory of schizophrenia pathogenesis⁴⁴⁻⁴⁶. In addition, GSA-MiXeR implicated several other relevant gene-sets including “interneuron migration” which may affect parvalbumin-positive GABAergic interneurons that have been implicated in schizophrenia pathogenesis⁴⁷, and “forebrain neuron fate commitment”⁴⁸. It is also worth noting that enrichment in the dopamine-related gene-sets appears to be driven largely by a single gene, *DRD2*. By contrast, the high enrichment of the gene-sets related to voltage-gated calcium channels and “forebrain neuron fate commitment” appears to be a property of multiple genes in each gene-set. When applied to 20 diverse human phenotypes, GSA-MiXeR also tended to prioritize smaller gene-sets of high biological relevance.

Previous methods, such as LDAK-GBAT⁴⁰ and h2gene⁴⁹, have estimated heritability attributed to individual genes, identifying individual genes that significantly contribute to trait's heritability, but without considering gene-set and without computing fold enrichment over a baseline. Existing methods also estimate disease heritability mediated by the cis

genetic component of gene expression levels and its enrichment across gene-sets (MES⁵⁰ and GCSC⁵¹ methods), leveraging expression imputation from genetic data⁵². While these methods found both overall evidence of expression-mediated heritability and specific gene-sets showing disease-relevant enrichment, they have only accounted for a small fraction of estimated SNP-heritability. This can be either due to insufficient accuracy of gene expression imputation potentially related to low sample size of expression quantitative trait loci datasets for trait-relevant tissues and cell types, or due to other causal pathways beyond gene expression contributing to shaping genotype-phenotype associations. GSA-MiXeR complements these methods by estimating the fraction of the total SNP-based heritability attributed to a gene-sets without relying on gene expression data. Other methods, such as AI-MiXeR¹⁸, RSS-E⁵³, and RSS-NET⁵⁴ have also computed enrichment in certain regions of the genome. However, they do not build a comprehensive model including all known human genes, a key advantage of GSA-MiXeR. To achieve this, the GSA-MiXeR model incorporates several parameters describing the distribution of additive genetic effects, fitting those parameters from the GWAS summary statistics. Furthermore, GSA-MiXeR evaluates fold enrichment of a gene-set versus a comprehensive baseline model, which accounts for differential enrichment of functional categories yet without gene-specific effects – an enrichment model which cannot be implemented using a fixed-effects approach⁵⁵.

The GSA-MiXeR tool has some limitations. First, it was not feasible to provide formal p-values due to technical reasons and difficulties in defining the null-hypothesis (see Supplementary Information), thus GSA-MiXeR relies on the MAGMA tool to pre-filter the set of gene-sets for the most conservative analysis. Our exploratory analysis (without pre-filtering by MAGMA) selects gene-sets based on AIC criteria, which does not allow for multiple testing correction; we however confirmed that ranking gene-sets according to GSA-MiXeR fold enrichment is at least as stable as ranking according to conservatively defined MAGMA p-values; additionally, all estimates have SEs to evaluate their uncertainty. Second, SEs are derived from the likelihood function, and may in some cases be not well calibrated, particularly for genes with close to zero heritability estimate. Real-data analysis with GSA-MiXeR is unlikely to be affected by this due to filtering genes on a positive AIC value, which implies sufficient curvature of the log-likelihood around the MLE point and justifies hessian-based SEs estimation. Applying resampling approaches, such as bootstrap of jackknife, may further improve robustness of the SEs, however for GSA-MiXeR setting these techniques should resample individual subjects rather than SNPs, i.e. resampling should be implemented as an integral part of the GWAS analysis, leading to multiple instances of the GWAS summary statistics which can be later used to assess the uncertainty of model parameters. Third, GSA-MiXeR is based on certain assumptions, such as modeling enrichment through varying the effect sizes rather than varying the density of causal variants; we validated through sensitivity analysis that violation of these assumptions does not lead to significant biases. Finally, GSA-MiXeR does not handle continuous-valued genomic annotations as implemented in extended sLDSC⁴¹. In the future we are planning to extend GSA-MiXeR analysis to cover genes located on non-autosomes (including sex chromosomes and mitochondrial DNA), and provide a reference panel for GSA-MiXeR analysis for non-European populations.

To conclude, GSA-MiXeR estimates fold enrichment and implicates gene-sets with higher biological specificity than current standards. The gene-sets promoted by GSA-MiXeR can provide insights into the pathobiology of complex diseases, with the potential for identifying new drug targets and the development of pathway-specific polygenic risk scores. This may help to advance the classification, diagnosis, and treatment of complex polygenic disorders.

Methods

Individual level data from the UK Biobank, Haplotype Reference Consortium (HRC), and the Thematically Organized Psychosis (TOP) study were analyzed under ethical approvals from Norwegian Regional Committees for Medical and Health Research Ethics (REC; ref. 2009/2485), Data Inspectorate (ref. 03/02051), and The Norwegian Directorate of Health (ref. 05/5821). The use of summary statistics for genetic analysis was evaluated by REC South-East Norway and found that no additional ethical approval was required because no individual data were used (REC #2011/1980).

GSA-MiXeR's full and baseline models

Under the assumptions of a simple additive genetic model, the contribution of the genotype to the phenotype is modeled as a sum of contributions across genetic variants: $y_k = \sum_{i=1}^{\bar{M}} g_{ik} \beta_i + e_k$, where y_k is a quantitative phenotype or disease liability of the k -th individual; g_{ik} is the number of reference alleles for the i -th variant (centered to zero mean, but not variance standardized), where the index i runs over the \bar{M} variants in the reference panel; β_i is per-allele effect size (known also as an additive genetic effect of allele substitution). The parameters β_i and e_k are scaled so that phenotype y has zero mean and unit variance.

Following previous work^{16–19}, GSA-MiXeR builds on a causal mixture model⁵⁶ with a spike-and-slab prior distribution $\beta_i \sim (1 - \pi_1) \delta_0 + \pi_1 N(0, \sigma_i^2)$, where δ_0 is the probability mass at zero (Dirac delta function), $N(\mu, \sigma^2)$ is a normal distribution with mean μ and variance σ^2 , π_1 gives the prior probability of a variant having a non-zero effect size, and the corresponding effect size variance σ_i^2 is allowed to vary across genetic variants. To reduce the number of effective parameters being optimized, the full GSA-MiXeR model parametrizes σ_i^2 based on functional categories, genes, allele frequency, and LD score as follows:

$$\sigma_{i,full}^2 = (\sigma_{\lambda,0}^2 + \sum_{p=1}^{N_A} [i \in A_p] \sigma_{\lambda,p}^2) (\sigma_{G,0}^2 + \sum_{q=1}^{N_G} [i \in G_q] \sigma_{G,q}^2) H_i^S L_i^\ell. \quad (1)$$

Here, the H_i^S term allows the modeling of architectures dependent on allele frequency, the term $H_i = 2f_i(1 - f_i)$ denotes heterozygosity of the i -th variant (f_i denotes minor allele frequency of the i -th variant), and the S parameter controls the effect size distribution along the allele frequency spectrum. Similarly, the L_i^ℓ term allows the modeling of LD-dependent genetic architectures, where L_i denotes the LD score of the i -th variant and

the ℓ parameter controls the effect size distribution with respect to LD score. Each of the other two multiplicative factors in equation (1) controls the differential enrichment across functional categories and across genes, respectively. Index p runs across functional categories $\{A_1, A_2, \dots, A_{N_A}\}$, where parameter $\sigma_{A,p}^2$ represents the contribution of the p -th functional category to the variance of the i -th genetic variant (if that variant belongs to the category, as indicated by $[i \in A_p]$ term in equation (1), where square brackets maps true or false predicate to 1 or 0 – a notation known as Iverson Bracket). If genetic variant i belongs to multiple categories, the variances of those categories are added together. Similarly, index q runs across genes $\{G_1, G_2, \dots, G_{N_G}\}$, each contributing its own effect size variance $\sigma_{G,q}^2$ for genetic variants that belong to G_q . Parameters $\sigma_{A,0}^2$ and $\sigma_{G,0}^2$ allow for non-zero variance σ_i^2 for genetic variants that do not belong to any functional category or genes.

For the baseline GSA-MiXeR model, we used a single effect size variance parameter, σ_G^2 , for all SNPs that belong to any gene, regardless of which gene they belong to:

$$\sigma_{i,base}^2 = \left(\sigma_{A,0}^2 + \sum_{p=1}^{N_A} [i \in A_p] \sigma_{A,p}^2 \right) \left(\sigma_{G,0}^2 + [i \in G] \sigma_G^2 \right) H_i^S L_i^\ell,$$

where $G = \bigcup_{q=1}^{N_G} G_q$ is a union of all genes. The main results were reported with full and baseline models based on $N_A = 75$ functional annotations and $N_G = 18,201$ protein-coding genes. However, for the sensitivity analysis (Supplementary Figure 4) we allowed a full model where variance parameters $\sigma_{G,q}^2$ were modeled at the level of gene-sets, rather than at the level of individual genes. In this scenario heritability model $\sigma_{i,full}^2$ is still defined by the equation (1), however, each G_q corresponded to one of $N_G = 10,475$ gene-sets.

Heritability enrichment

The following expression defines SNP-based heritability for a phenotype standardized to a unit variance:

$$h_{SNP}^2 = \text{Var}\left(\sum_i g_i \beta_i\right) = \sum_i \text{Var}(g_i \beta_i) + 2 \sum_{i < j} \text{Cov}(g_i \beta_i, g_j \beta_j).$$

Following the h2gene approach⁴⁹, we employ the assumption that there is zero covariance between causal effects of different variants. This enables the partitioning of heritability across variants and, thereby, the definition of heritability of a gene-set G as

$$h_{full}^2(G) = \sum_{i \in G} \pi_1 H_i \sigma_{i,full}^2 \text{ and } h_{base}^2(G) = \sum_{i \in G} \pi_1 H_i \sigma_{i,base}^2,$$

where heterozygosity factor H_i converts variance σ_i^2 defined in the units of per-allele effects into variance expressed in the units of standardized genotypes. Fold enrichment of heritability can be computed as a ratio of $h_{full}^2(G)/h_{base}^2(G)$. However, we observed that fitting one parameter for each gene may lead to a slight inflation of heritability estimates, an issue that did not manifest itself when fitting the baseline model. To account for this, we define fold enrichment based on the fraction of total heritability attributed to the gene-set as:

$$\text{enrich}(G) = \frac{h^2(G)_{full}/h_{full}^2}{h^2(G)_{base}/h_{base}^2} . \quad (2)$$

Log-likelihood computation and its gradients

Let z_j be the GWAS z-score of j -th variant out of in total M variants tested for association. We assume that association is driven by the underlying additive effects β , however, the set of GWAS variants does not need to be the same as the set of the underlying variants (hence we use different subscripts i and j to distinguish between them, and assume that all M variants tested for association are present among the \bar{M} variants of the reference panel). Inference of GSA-MiXeR parameters is based on optimizing log-likelihood function $\log L(z_1, \dots, z_M | \theta) \rightarrow \max_{\theta}$ of observing a set of GWAS summary statistics

given model parameters, while controlling for the LD structure among variants and their allele frequencies. The log-likelihood computation is based on previously derived methodology^{16–18}, which established the following additive relationship between genetic effects (β_i) and GWAS z-scores (z_j), highlighting that z_j depends on β_i of all i -th variants tagged by j -th variant due to LD:

$$z_j = \delta_j + \epsilon_j = \sum_i \sqrt{N_j H_j} r_{ij} \beta_i + \epsilon_j , \quad (3)$$

where N_j is sample size at the j -th variant, r_{ij} is the LD allele correlation between i -th underlying and j -th GWAS variants, and ϵ_j is the normally distributed residual $\epsilon_j \sim N(0, \sigma_0^2)$ with variance σ_0^2 that accounts for potential inflation in GWAS summary statistics. Our two approaches for computing the log likelihood using “fast” and “full” models are described in the Supplementary Information. The “fast” model is based on the method of moments, approximating $p(z_j)$ is a way that preserves the second and fourth moments of the distribution, thus preserving variance and kurtosis; the third moment (skewness) is irrelevant because of symmetry of the $p(z_j)$ distribution. The “full” model performs more accurate sampling from the prior distribution $p(\beta_i)$ at the expense of longer computation times. Based on these methods of evaluating log-likelihood, we found an efficient computation procedure for computing the gradients $\frac{\partial L(z_1, \dots, z_M | \theta)}{\partial \pi_1}$ and $\frac{\partial L(z_1, \dots, z_M | \theta)}{\partial \sigma_i^2}$ in the context of “fast” and “full” models. Finally, from equation (1) one can compute the Jacobian matrixes $\left[\frac{\partial \sigma_i^2}{\partial \sigma_{\lambda, p}^2} \right]$, $\left[\frac{\partial \sigma_i^2}{\partial \sigma_{G, q}^2} \right]$, $\left[\frac{\partial \sigma_i^2}{\partial S} \right]$ and $\left[\frac{\partial \sigma_i^2}{\partial \ell} \right]$, thus allowing the computation of log-likelihood gradients with respect to the parameters of the final model: $\frac{\partial L(z_1, \dots, z_M | \theta)}{\partial \sigma_{\lambda, p}^2} = \sum_i \frac{\partial L(z_1, \dots, z_M | \theta)}{\partial \sigma_i^2} \frac{\partial \sigma_i^2}{\partial \sigma_{\lambda, p}^2}$, and similarly for all other parameters ($\sigma_{G, q}^2$, S , ℓ and π_1). This can be seen as an equivalent to error backpropagation in a neural network, with σ_i^2 representing the first layer of the network, and $\sigma_{\lambda, p}^2$, $\sigma_{G, q}^2$ representing the second layer. The total amount of computations needed to calculate the entire gradient vector (for π_1 and σ_i^2 across all variants) is similar to that needed

for computing the value of the likelihood function itself. This made it feasible to apply first-order optimization methods while avoiding computational burden despite adding tens of thousands of parameters to the model. For comparison, previous applications of the MiXeR model^{16–19} had a fixed number of 3, 5, 9, and 12 parameters, respectively, and were fitted with zero-order methods including Differential evolution⁵⁷, Nelder-Mead⁵⁸, and Brent's method⁵⁹.

Log-likelihood optimization

The log-likelihood function $\log L(z_1, \dots, z_M | \theta) = \sum_j w_j \log p(z_j | \theta)$ is weighted across variants using inverse LD score¹¹ to avoid over-counting evidence from large LD blocks, in a way that is similar to --w-ld option in sLDSC. The fit is first performed for the baseline model, using the Adam algorithm²⁰ with parameters $\beta_1 = 0.9$, $\beta_2 = 0.99$, $\epsilon = 10^{-8}$ in order to jointly fit parameters of the baseline GSA-MiXeR model. Optimization utilizes N=100 epochs, each of which passes over 22 batches (one batch per chromosome, re-shuffled in a random order for each epoch). The step-size parameter was gradually reduced from $\alpha = 0.064$ down to $\alpha = 0.0001$, reducing the value by a factor of 2 after every 10 epochs. Optimization of the full model constrains $\sigma_{A,0}^2$, $\sigma_{A,p}^2$, $\sigma_{G,0}^2$, σ_0^2 , S and ℓ parameters to their respective values from the baseline model. The optimization of the remaining parameters ($\sigma_{G,q}^2$ effect size variances for each gene) is initiated from the σ_G^2 value from the baseline model and is done separately for each chromosome using $\beta_1 = 0.1$ and $\beta_2 = 0.8$ in Adam algorithm. While GSA-MiXeR implementation allows fitting all parameters including π_1 and S from the data, the main results were obtained constraining the model to an infinitesimal model ($\pi_1 = 1$), and $S = -0.25$ parameter chosen in line with the LDAK recommendation¹⁴.

After fitting baseline and full GSA-MiXeR models, we apply Akaike Information Criterion (AIC) to identify genes and gene-sets that are improving the fit of the full model compared to the baseline model. More specifically, to assess whether in a certain genomic region (i.e. for a gene, or for a gene-set) our full model, θ_{full} , has a better likelihood than the baseline model, θ_{base} , we constructed a partial model, θ_{part} , which is equivalent to the full model θ_{full} except for the genomic region being tested, and in that region θ_{part} is equivalent to the baseline model θ_{base} ("equivalent" means that gene-level variance parameters σ_i^2 in θ_{part} are set to their respective values from either from full or baseline models). AIC was computed as $2 \log \left(\frac{L(\theta_{full})}{L(\theta_{part})} \right) - 2k$, where k represents the number of model parameters fitted for the genomic region being tested. In the case of non-overlapping genes, the k parameter equals to the number of genes in a gene-set being tested; in a more general case, this accounts for an overlap among adjacent genes occurring largely due to 10-kb up- and down-stream expansion.

SEs of fitted parameters were estimated from the observed Fisher's information matrix $-\Delta \Delta^T \log L|_{\theta^*}$ (the negative Hessian matrix of the log-likelihood function). For compound functions of multiple parameters, such as fold enrichment of heritability in a region, we sample N=100 realizations of the parameters vector θ from the posterior distribution $\theta \sim N(\theta^*, [-\Delta \Delta^T \log L|_{\theta^*}]^{-1})$, calculate the compound function (e.g., fold enrichment) for

each realization of θ , and report the standard deviations across realization. If the Hessian matrix was not positive definite, we use marginal errors of fitted parameters. The Fisher's information matrix was computed efficiently using sparse matrices (see Supplementary Information for further details). We validated SEs in simulations by comparing them to standard deviations of point estimates across simulation runs (Supplementary Table 3); for individual genes we also compared GSA-MiXeR SEs to SEs estimated by LDAK-GBAT, leading to satisfactory results (Supplementary Figure 9, Supplementary Table 9–10).

HRC and UKB references (LD structure)

We prepared two reference panels available for GSA-MiXeR analysis using Haplotype Reference Consortia (HRC) and UK Biobank (UKB) datasets.

Our HRC reference has 11,980,511 variants and 23,152 samples after basic quality control (QC) procedure. During sample QC, we select individuals with EUR ancestry (including Finnish population) defined from the first two principal components of 1000 genomes Phase3 data. Further, we prune related individuals using KING software (“king --unrelated -degree 2”). For variant QC, we use “plink --maf 0.001 --hwe 1e-10 --geno 0.1” parameters, and further exclude markers without RS IDs and excluding duplicated RS IDs.

Our UKB reference has 12,926,669 variants and 337,145 subjects, derived from the UK Biobank imputed v3 dataset. During sample QC, we select unrelated individuals with white British ancestry, remove sex chromosome aneuploidy, and exclude participants who withdrew their consent. Variant QC was applied as follows: “plink --maf 0.001 --hwe 1e-10 --geno 0.1”, in addition to filtering variants with imputation INFO score below 0.8 and excluding variants with duplicated RS IDs.

In both references, the calculation of allelic correlation (LD r^2) was done from hard calls, separately for each chromosome, with a window size of 10 MB, and using a minimum LD r^2 threshold of 0.01.

Functional categories and gene-sets

For functional categories, we used the set of 75 binary annotations designed for stratified LD score regression (baselineLD_v2.2).

To define gene boundaries, we used NCBI Entrez database, starting from a set of protein-coding (N=19,608 genes). After keeping only genes with known coordinates in the primary assembly of GRC37 human reference (annotation release 105.20201022), excluding genes on non-autosomes, and excluding genes without variants in MiXeR's UKB or HRC references, we obtained a final list of N=18,201 protein-coding genes. The boundaries of each gene were extended by 10 KB up- and downstream, both for the GSA-MiXeR and for the MAGMA analyses.

To define gene-sets, we used GO terms from MsigDB v7.5 (biological processes - c5.bp; cellular component - c5.cc; and molecular function - c5.mf; resulting in 10,402 gene-sets in total). This set was extended using the SynGO database (<https://syngoportal.org/>), which added 73 gene-sets after excluding those comprising 5 genes or less. Among 10,475

resulting gene-sets there were 19,366 unique genes present, of which 16,787 were present among the list of 18,201 genes available for analysis. To avoid reporting results for highly overlapping gene-sets, we pruned gene-sets that overlapped with a Dice coefficient of 0.8 or above, retaining smaller gene sets from each overlapping pair. This procedure excluded 1,771 gene-sets from the analysis, and the final list used in the analysis contained 8,704 gene-sets.

To validate whether gene-set enrichment was largely driven by a single gene, we performed additional leave-one-out analysis for all gene-sets with up to 25 genes, where enrichment was computed after removing one gene at a time and reporting the gene whose exclusion led to lowest enrichment.

GWAS Summary Statistics

Table 1 gives an overview of publicly available GWAS summary statistics used for this analysis. Summary statistics were converted to common format using cleansumstats pipeline. SNPs with MAF below 5% in the reference panel, strand-ambiguous SNPs, indels, and SNPs without an RS ID were excluded prior to the analysis. SNPs in the extended region around major histocompatibility complex (chr6:25–35 MB in GRCh37 genomic build) were also excluded due to long-range LD. In the replication analysis of schizophrenia, we used sub-study data to perform a variance-based meta-analysis on a randomly selected half of the cohorts. In our replication analysis of height, we performed GWAS on UK Biobank standing height (field 50), following the GWAS protocol from the Neale Lab. More specifically, we used PLINK for linear association analysis after standardizing height measures through an inverse rank-based normal transform done separately in males and in females, and controlling for age, sex, and the first twenty principal components of the genotype matrix.

Simulations

Simulations used a panel of $N=337,145$ subjects and $M=12,926,669$ variants from UK Biobank. For each run, we used PLINK to obtain GWAS summary statistics of a quantitative phenotype $y_k = \sum_i g_{ik}\beta_i + e_k$, where β_i were drawn from a specific model (depending on simulation scenario, as described below), and e_k residual was drawn from a normal distribution with zero mean and variance chosen in a way that sets heritability $h^2 = \text{var}(G) / \text{var}(y)$ to a predefined level ($h^2=0.1, 0.4$ or 0.7).

A subset of causal variants in simulations was selected by randomly pruning all reference variants at LD $r^2=0.1$ threshold so that causal variants were in weak LD with each other. This ensured that each genomic region had a well-defined heritability, as for a quantitative phenotype $y = \sum_i g_i\beta_i + \epsilon$ the variance of the genetic component under a fixed effects model is given by $\text{Var}(\sum_i g_i\beta_i) = \sum_i \beta_i^2 \text{Var}(g_i) + 2 \sum_i \sum_{j>i} \beta_i\beta_j \text{Cov}(g_i, g_j)$, where the second component is negligible if causal variants are not in LD with each other. The remaining component allows the definition of genetic variance in a specific region g as $\sum_{i \in g} \beta_i^2 \text{Var}(g_i)$.

After selecting the set of causal variants, their effect sizes β_i were selected to ensure pre-defined fold enrichment for a selected set of genes. We consider two regions in a

genome: G (representing the set of all genes) and $g \subset G$, representing the subset of $N=1, 3, 10, 30$, or 100 enriched genes. Let H_G and H_g be total heterozygosity for variants in G and g , respectively. To generate a trait with pre-defined fold enrichments, $f(g)$, we draw β_i from a normal distribution $N\left(0, h^2 \frac{f(g)}{H_G}\right)$ for variants in enriched gene-set g , and from $N\left(0, h^2 \frac{H_G - f(g)H_g}{H_G(H_G - H_g)}\right)$ for the remaining variants in $G \setminus g$. As shown in Supplementary Information, this translates to a true fold enrichment equal to the pre-selected $f(g)$ value.

We chose to keep the sample size constant throughout all simulations as the power of GWAS depends on z-scores, which in turn depend on the product of the sample size and heritability (as long as polygenicity is kept constant). Thus, our simulations using QCed UKB genotypes ($N=337,145$ subjects) and $h^2=0.1$ yield results that are expected to be equivalent to simulations with $N=1,000,000$ for a trait with SNP heritability of $h^2=0.03$, or to $N=100,000$ for a trait with $h^2=0.3$. Polygenicity in the “base” scenario is defined to match the polygenicity of schizophrenia, with its estimated 30,000 causal variants¹⁷ (one of the highest estimates across complex traits).

Statistics and Reproducibility

Statistical methods for all analyses, including estimation of GSA-MiXeR and LDK-GBAT standard errors, MAGMA gene-set enrichment p-values, and the design of simulation experiments are detailed in the corresponding Methods section. In simulations, n is the number of independent experiments. No statistical method was used to predetermine the sample size. No data were excluded from the analyses. We use previously collected datasets; thus, the experiments were not randomized, and the investigators were not blinded to allocation during experiments and outcome assessment. All codes to replicate the analysis are available as part of code availability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Funding was provided by the Research Council of Norway (RCN) #334920 - K.O.; Norwegian Health Association #22731 - S.B.; European Union's Horizon 2020 (EU H2020) under the Marie Skłodowska-Curie Actions (Scientia fellowship) #801133 - N.P.; RCN #223273, #273291, #300309, #324252, #324499, #326813, South-Eastern Norway Regional Health Authority (HSØ) #2022073, The Kristian Gerhard Jebsen Stiftelsen #SKGJ-MED-021, EU H2020 grant #847776 (CoMorMent) and #964874 (RealMent), EEA and Norway grant #EEA-RO-NO-2018-0573, American National Institutes of Health (NIH) grant 5R01MH124839-02 (PGC4) - O.A.A.; NIH grants U24DA041123; R01AG076838; U24DA055330, OT2 HL161847 - A.M.D. This research has been conducted using the UK Biobank Resource under Application Number 27412. This work used the Extreme Science and Engineering Discovery Environment (XSEDE), including ExpansE and OASIS resources provided by San Diego Supercomputer Center at UC San Diego through allocation IBN200001. This work also used the TSD (Tjeneste for Sensitive Data) facilities, owned by the University of Oslo, operated and developed by the TSD service group at the University of Oslo, IT-Department (USIT, tsd-drift@usit.uio.no), using resources provided by UNINETT Sigma2 - the National Infrastructure for High Performance Computing and Data Storage in Norway. The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.

Data availability

The datasets analyzed in this study are available for download from the following URLs: Schizophrenia GWAS⁶⁰ from PGC, <https://pgc.unc.edu/for-researchers/download-results/>; Molecular Signatures Database v7.5, <https://www.gsea-msigdb.org/gsea/msigdb/>; Synaptic Gene Ontologies 20210225 release, <https://syngoportal.org/>; functional categories (baselineLD_v2.2), <https://alkesgroup.broadinstitute.org/LDSCORE/>; 1000 Genomes Phase3 data, <http://ftp.1000genomes.ebi.ac.uk/vol1/ftp/release/20130502/>. UK Biobank, <https://ams.ukbiobank.ac.uk/ams/>; HRC Release 1.1, <https://ega-archive.org/datasets/EGAD00001002729>; TOP cohort, <https://app.cristin.no/projects/show.jsf?id=2505365>, and country-level GWAS summary statistics for PGC SCZ, <https://pgc.unc.edu/for-researchers/data-access-committee/data-access-information/>, have controlled data access. These data are not publicly available due to national data privacy regulations as they contain information that could compromise research participant privacy and/or consent. Statistical Source Data is provided for Fig. 1–4. Minimum dataset⁶¹ of this study is made publicly available, and contains reference data formatted to GSA-MiXeR format, including definitions of genes, gene-sets, functional categories, and sharable non-sensitive data derived from UK Biobank and HRC references.

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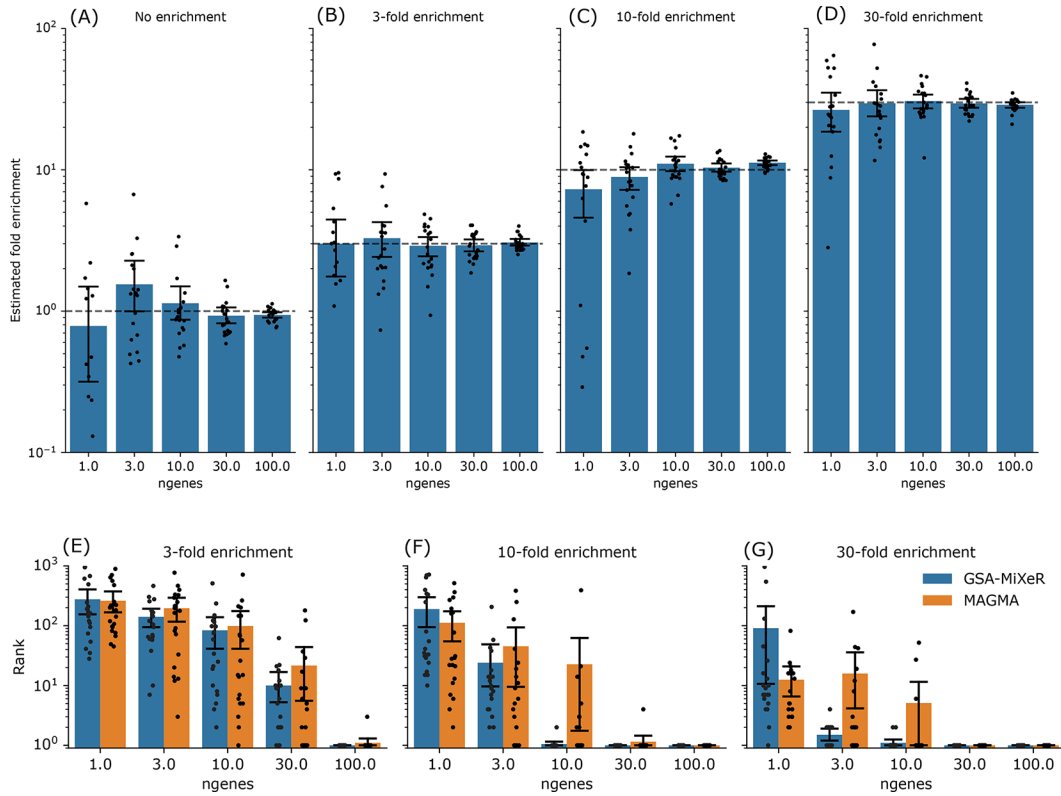


Fig. 1. Selected results from simulations a-d, comparison of GSA-MiXeR fold enrichment estimates versus ground truth; e-g, accuracy of sorting gene-sets according to the MAGMA enrichment p-values versus GSA-MiXeR fold enrichment. The simulations cover four levels of enrichment: 1 - null enrichment (a), 3-fold (b, e), 10-fold (c, f), and 30-fold (d, g); and vary in terms of the size of the enriched gene-set: 1, 3, 10, 30, and 100 genes (shown on the horizontal axis); ground truth is shown by the horizontal dashed line. Panels e-g show the position of the truly enriched gene-set among 1000 randomly generated gene-sets in simulations (expected rank is 1, lower values are better). In all simulations, the trait was simulated with the “base” scenario of genetic architecture (causal variants uniformly distributed across protein-coding genes), and total heritability of 0.4. Data are presented as mean values, with error bars showing standard deviation of the model estimate across twenty simulation runs. Individual simulation runs are shown as dot points. Additional simulations are presented in Supplementary Fig. 1–2.

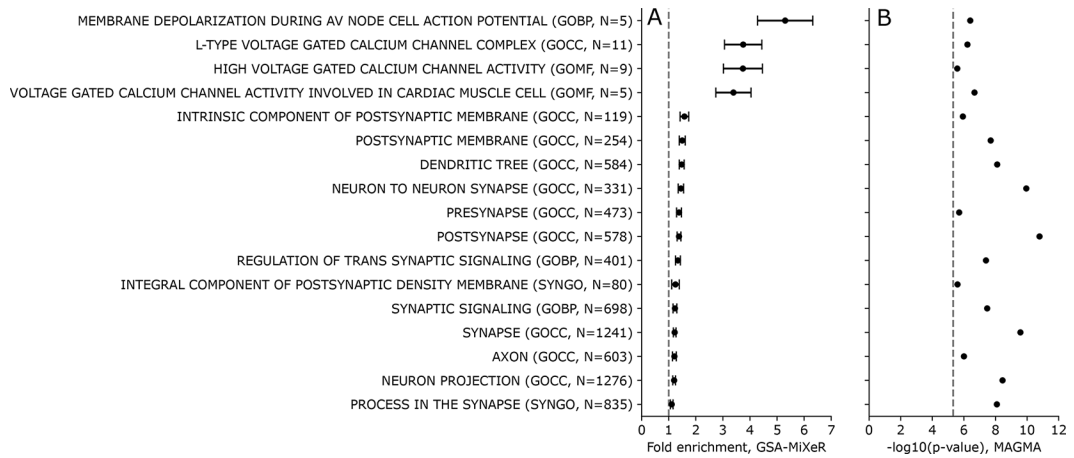


Fig. 2. Main GSA-MiXeR results for schizophrenia a-b, gene-sets implicated by MAGMA analysis, ordered by GSA-MiXeR fold enrichment. (a) GSA-MiXeR fold enrichment estimates, with error bars showing hessian-based standard error of the model estimates; vertical dashed line indicates no enrichment. (b) \log_{10} of MAGMA's gene-set enrichment p-value resulting from a test on the regression coefficient β , testing the null hypothesis $H_0: \beta = 0$ against the one-sided alternative $\beta > 0$; vertical dashed line indicates significance threshold after Bonferroni correction for multiple comparisons across $N=10475$ gene-sets. The number of genes per gene-set is indicated in parenthesis after gene-set name. GOMF – Gene Ontology Molecular Function; GOBP – Gene Ontology Biological Processes; GOCC – Gene Ontology Cellular Component; SYNGO – Synaptic Gene Ontologies.

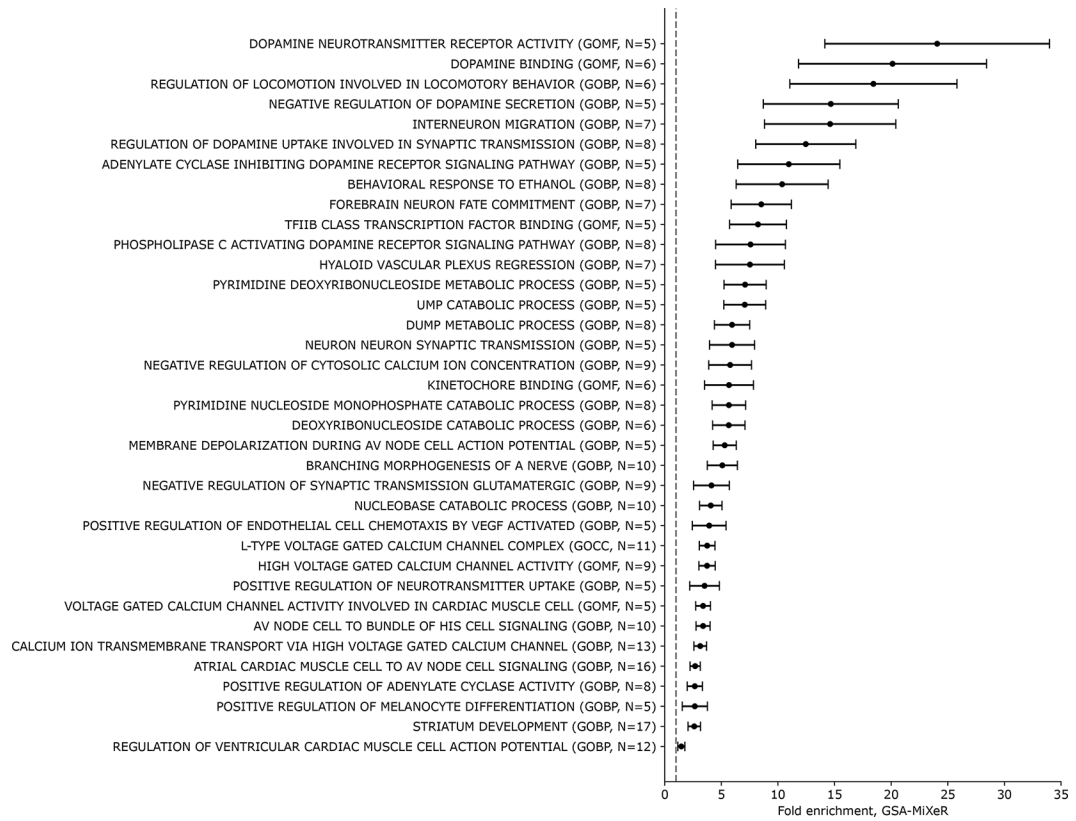


Fig. 3. Exploratory GSA-MiXeR results for schizophrenia GSA-MiXeR fold enrichment estimates for gene-sets with a positive AIC (Akaike information criterion) value. Positive AIC indicates a better fit of the GSA-MiXeR full model compared to the baseline model for each of the implicated gene-sets. Error bars show hessian-based standard error of the model estimates. The number of genes per gene-set is indicated in parentheses after each gene-set name. GOMF – Gene Ontology Molecular Function; GOBP – Gene Ontology Biological Processes; GOCC – Gene Ontology Cellular Component.

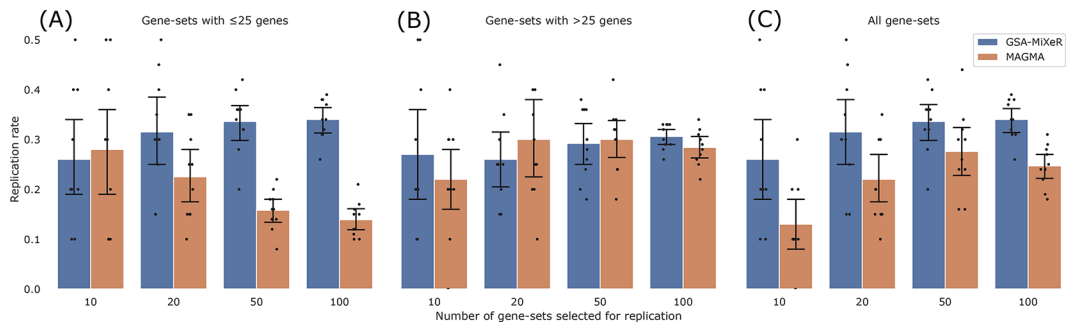


Fig. 4.

Replication analysis for schizophrenia a-c, replication analysis for schizophrenia using sub-studies from Psychiatric Genomic Consortia, using (a) gene-sets with up to 25 genes; (b) gene-sets with more than 25 genes, and (c) all gene-sets. Replication rate is computed for GSA-MiXeR and MAGMA analyses using two-fold cross-validation, after applying both methods to two independent and equally sized GWAS sub-samples. After ranking gene-sets according to fold enrichment of heritability (GSA-MiXeR) or enrichment p-value (MAGMA) obtained in the discovery sample, the replication rate (shown on the vertical axis) was then defined as the fraction of gene-sets that remain within top N gene-sets ($N=10, 20, 50$, and 100 , shown on the horizontal axis) in the replication half of the dataset. Data are presented as mean values, with error bars showing standard deviation of the replication rate across ten simulation runs. Individual simulation runs are shown as dot points.

Table 1.

Summary of main and exploratory GSA-MiXeR results for schizophrenia and 20 other diverse human traits and disorders.

Traits	Sample size	Main results			Exploratory results		
		Count of gene-sets	Fold enrichment average (RMSE)	Average size of gene-sets (#genes)	Count of gene-sets	Fold enrichment average (RMSE)	Average size of gene-sets (#genes)
Alcohol consumption ²²	112117	0			1	21.12 (8.96)	7.0
Alzheimer's disease ³³	86531/676386	13	10.30 (4.13)	65.5	51	9.36 (4.56)	11.1
Body mass index ³⁵	795640	16	1.28 (0.11)	900.8	41	4.86 (1.81)	9.0
Chronic kidney disease ³⁴	41395/439303	0			5	6.42 (3.03)	7.8
Chronotype ³²	345148	1	1.47 (0.16)	427.0	3	11.45 (5.49)	6.7
Cognition ²⁹	269867	1	3.49 (0.91)	5.0	6	3.54 (1.64)	6.8
Crohns disease ²³	12194/34915	77	2.59 (0.63)	513.6	52	15.93 (4.63)	9.1
Educational attainment ²⁸	765283	10	1.32 (0.07)	549.7	34	2.85 (0.80)	7.8
Fasting glucose ²¹	281416	9	3.94 (1.00)	195.7	71	6.87 (2.46)	8.8
Fasting insulin ²¹	281416	0			6	7.17 (4.63)	6.2
Glycated hemoglobin ²¹	281416	3	5.35 (1.76)	460.7	57	8.41 (2.73)	10.9
Heart failure ³⁰	47309/930014	0			1	31.27 (11.55)	5.0
Height ³²	385748	40	2.26 (0.33)	636.1	405	4.69 (1.52)	17.5
Hospitalized covid ³¹	32519/2062805	0			1	18.56 (6.92)	5.0
Migraine ²⁵	48975/540381	0			8	7.39 (3.08)	7.8
Schizophrenia ⁸	53386/77258	17	1.97 (0.39)	441.4	36	7.28 (3.41)	7.8
Sleep ³²	384225	2	6.55 (1.96)	11.5	9	9.03 (3.31)	9.7
Stroke ²⁷	110182/1503898	0			3	14.82 (6.42)	7.0
Systolic Blood Pressure ²⁴	745820	25	1.82 (0.44)	706.8	202	4.70 (1.75)	14.5
Type 2 diabetes ²⁶	74124/824006	7	3.89 (0.73)	320.3	161	7.99 (2.75)	13.8
Ulcerative colitis ²³	12366/34915	33	2.06 (1.01)	353.8	20	11.98 (4.36)	7.2

These main results are based on gene-sets selected through MAGMA analysis, annotated with fold enrichment estimates computed by GSA-MiXeR. Exploratory results are based on GSA-MiXeR analysis, implicating gene-sets with positive AIC (Akaike Information Criterion). Sample size indicates the total number of participants in GWAS for quantitative traits and the number of cases and controls for binary traits, respectively; RMSE, root mean square error.