Pharmacological enrichment of polygenic risk for precision medicine in complex disorders

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ABSTRACT

Individuals with complex disorders typically have a heritable burden of common variation that can be expressed as a polygenic risk score (PRS). While PRS has some predictive utility, it lacks the molecular specificity to be directly informative for clinical interventions. We therefore sought to develop a framework to quantify an individual's common variant enrichment in clinically actionable systems responsive to existing drugs. This was achieved with a metric designated the *pharmagenic enrichment score* (PES), which we demonstrate for individual SNP profiles in a cohort of cases with schizophrenia. A large proportion of these had elevated PES in one or more of eight clinically actionable gene-sets enriched with schizophrenia associated common variation. Notable candidates targeting these pathways included vitamins, insulin modulating agents, and protein kinase inhibitors with putative neuroprotective properties. Interestingly, elevated PES was also observed in individuals with otherwise low common variant burden. The biological saliency of PES profiles were observed directly through their impact on gene expression in a subset of the cohort with matched transcriptomic data, supporting our assertion that this framework can integrate an individual's common variant risk to inform personalised interventions, including drug repositioning, for complex disorders such as schizophrenia.

INTRODUCTION

A significant burden of disease is caused by complex traits including psychiatric and neurobehavioural disorders, inflammatory and autoimmune disorders, metabolic and cardiovascular disease, and cancer. Until relatively recently it was difficult to identify the heritable components of these traits, however, the emergence of well powered genome-wide association studies (GWAS) using large cohorts assembled by collaborative consortia are revealing important insights into their common variant architecture [1]. While collectively this information has been vital to map genes and pathways that are likely to be etiological factors, the small effect size of each variant, and their heterogeneity in the population make their relevance to individuals with the disorder highly variable, relatively specific, and fairly minor with respect to the total variant burden. They also present as relatively small targets for therapeutic intervention and may not attract the investment needed for pharmaceutical development. We therefore need mechanisms for using this vast amount of diverse genetic information to maximise its utility for therapeutic advances. This requires a personalised approach that can capture variant burden in affected individuals with respect to biological components that align with existing medications, and/or pathways of relevance to key pathophysiological processes, to provide sufficient support for the development of new interventions.

While approaches that summate the genomic risk burden in individuals, such as polygenic risk scoring (PRS), have demonstrated some predictive utility for complex traits (such as neuropsychiatric disorders [2; 3], diabetes [4; 5], cardiovascular disease [6; 7], and inflammatory disorders [8; 9]) their composition of heterogeneous risk factors lack the biological salience needed to design a precision treatment strategy. We, however, hypothesized that the biologically supervised enrichment of trait-associated common variants in clinically

actionable pathways would provide a means of pharmacologically annotating PRS in individuals. To test this proposal, we devised a statistical framework for scoring polygenic risk (at the multivariable level) in pathways relevant as therapeutic targets for complex traits. This quantitative approach, designated the *pharmagenic enrichment score* (PES), was developed to provide an indication of an individual's exposure to risk variants that are potentially treatable by existing pharmacological agents, including many that have never been considered or tested previously for the condition/disorder they are experiencing. By focusing on biological pathways with known drug targets, we endeavour to enhance the clinical utility of polygenic risk approaches by providing novel and specific opportunities to identify treatment targets and/or repurpose existing drugs. This application of genome-wide common variant genotyping should have particular relevance for the precision treatment of individuals that are resistant to currently indicated medications. In this study we outlined the PES approach and sought to exemplify its utility in individuals with the complex psychiatric condition, schizophrenia.

MATERIALS AND METHODS

Pipeline for the derivation of *pharmagenic enrichment scores*

The methodology developed for constructing *pharmagenic enrichment scores* (PES) is outlined in a schematic presented in Supplementary Figure 1. We exploit the results of gene-set association analysis to aggregate variants from GWAS into gene-sets which may be candidates for pharmacological intervention. These gene-sets were high quality canonical and hallmark pathways sourced from the molecular signatures database (MSigDB) [10]. Pathways were designated as clinically relevant if they contained at least one gene annotated to interact

100 with an approved pharmacological agent in the DrugCentral database as classified by the
101 Target Central Resource Database (TCRD, genes annotated as T_{Clin}) [11].
102
103 Firstly, this process tests the combined effect of variants at the gene level. We utilised an
104 omnibus *P* value test to achieve this, whereby a linear combination of variant-wise *P* values
105 in a gene is compared to a null distribution to derive a combined *P* value for that gene. This
106 was performed using the MAGMA package to account for linkage disequilibrium between
107 variants in the approximation of the null χ^2 distribution [12]. We mapped SNPs to protein
108 coding genes (hg19, NCBI) with the genic boundaries encompassing 5kb upstream and 1.5
109 kb downstream to capture variation in regulatory regions. Genes within the major
110 histocompatibility complex (MHC) on chromosome 6 were excluded from this study due to
111 the complexity of haplotypes in that region. Traditionally, the full breadth of variants
112 available in the summary statistics are utilised in these models. This, however, may miss
113 important aspects of the biological interpretation of the genomic signal. PRS derived from
114 large GWAS cohorts are tested at different P value thresholds (P_T) to exploit a model which
115 explains the most variance between the case and control groups (in a dichotomous construct).
116 For instance, a $P_T < 0.05$ means that only *P* values in the GWAS with a stronger association
117 (*P* value) than this threshold are included. A range of P_T have been suggested to be optimal
118 for PRS in several disorders depending on their genomic architecture [2; 7; 13]. Aggregating the
119 combined effects of variants in genes at different P_T may therefore capture the biological
120 complexity of the signal at varying degrees of polygenicity.
121
122 Once variants are aggregated in genes at varying significance thresholds (P_T), we conduct
123 gene-set association of the pre-defined druggable gene-sets with the trait of interest. This was
124 a competitive association test, which tests the null hypothesis of genes in the set being no

more strongly associated than all other genes. This model was implemented with the MAGMA package. Enriched pathways with a known drug target uncovered from this pipeline then form the basis of calculating the *pharmagenic enrichment score* (PES). To profile individuals for a PES, genes which compromise the candidate pathway are extracted and cumulative genomic risk calculated in an analogous fashion to PRS, assuming an additive model [2]. Each individual profiled thus has a genomic risk score within each clinically actionable pathway.

Application of *pharmagenic enrichment score* to identify drug repurposing candidates for schizophrenia

To identify clinically actionable gene-sets and construct PES, we processed the 2014 psychiatric genomics consortium GWAS for the complex neuropsychiatric disorder schizophrenia with the pipeline described above [3]. Variants were selected based on their significance (*P* value) for inclusion in the at four different thresholds – all SNPs, $P < 0.5$, $P < 0.05$, and $P < 0.005$. Geneset association was conducted on 1012 MSigDB pathways with at least one druggable (T_{Clin}) gene. To capture a wide variety of pathways for a complex phenotype like schizophrenia, we used a nominal significance threshold of $P < 0.001$ to select candidate pathways for PES.

We annotated each of these candidate pathways for their drug interactions, tissue specificity, and phenotypic associations. Using WebGestalt, drugs which target a statistically significant number of genes in the PES gene-sets were identified after the application of multiple testing correction (FDR < 0.05) [14]. A minimum overlap of at least three targets overlapping the geneset for each pharmaceutical agent was also implemented. Drugs were also mapped to gene-sets using DGidb v3.02, with the top FDA approved drug per pathway was selected

based on the DGidb score of interaction confidence between a T_{Clin} gene and drug [15]. Further, the tissue specificity of expression for all genes from the eight pathways was investigated using GENE2FUNC application of FUMA [16]. Transcript expression in each of the 53 tissue types in the GTEx v7 dataset for the input pathway genes was tested for upregulation, downregulation and two-sided differential expression in comparison to the entire protein-coding genome. Enrichment of input genes for associated traits in GWAS catalogues was also tested in the FUMA framework.

Individual profiling of *pharmagenic enrichment scores* in a genotyped schizophrenia cohort

We sought to generate PES for the schizophrenia candidate pathways in a cohort of diagnosed schizophrenia cases and non-neuropsychiatric controls sourced from the Australian Schizophrenia Research Bank (ASRB) [17; 18]. Detailed descriptions of consent procedures along with inclusion and exclusion criteria for the ASRB have been extensively described elsewhere [17]. The Illumina Infinitium Human 610K (610-Quad) BeadChip platform was used to genotype genomic DNA extracted from peripheral blood mononucleocytes as per standard manufacturer protocols. Variant and individual level quality control, along with imputation using the 1000 genomes phase 3 European reference panel, has been outlined in detail for this cohort previously [18]. High quality autosomal sites with low missingness (< 2%) and an imputation score greater than 0.8 ($R^2 > 0.8$) were retained for analysis in this study. After the removal of individuals in the post-genotyping quality control, 425 schizophrenia cases and 251 controls were analysed in this study; cases were 67% male, whilst males comprised 44% of the control cohort (Supplementary Table 5). The use of these data was approved by the University of Newcastle Human Ethics Research Committee (HREC).

PES is calculated from SNPs mapped to genes which form the candidate pharmacologically actionable geneset. This comprises the following model (1) which sums the statistical effect size of each variant in the geneset multiplied the allele count (dosage) for said variant– for individual i, let $\widehat{\beta_j}$ denote the statistical effect size from the GWAS for each variant j in the candidate gene-set, multiplied by the dosage (G) of j in i .

$$PES_i = \sum_{j}^{M} \left(\widehat{\beta_j} \times G_{ij}\right) \qquad (1)$$

This was calculated for the individuals using the PRSice2 package [19]. For each PES, the P_T used to derive the score was selected based on which P_T the corresponding geneset was derived from the GWAS, when a geneset was associated at multiple P_T, the most significant was chosen. A genome-wide PRS (PRS_{Total}) was also constructed for this cohort, with the P_T which explained the most variance between cases and controls selected using Nagelkerke's R^2. Association for each of the scores with cases was conducted using binomial logistic regression, adjusted for sex and the first three principal components using R 3.4.4. *P* values were derived using the Wald test with and without the total PRS score at the optimum threshold as a covariate in the model.

Each PES was ranked for individuals within the ASRB cohort, with three metrics used to define a person with an 'elevated PES' score: the top percentile, decile, and quartile of the study population. The number of pathways which pass these thresholds were totalled for each individual and the association between these totals and schizophrenia assessed using the same model as for univariate PRS as described above. To investigate the relationship between PRS_{Total} and PES, genome wide PRS_{Total} and the count of PES in the top decile per individual were clustered using finite Gaussian mixture modelling (GMM) with the mclust package version 5.4 [20]. The optimal number of clusters was selected based on parametrisation of the

198 covariance matrix utilising the Bayesian Information Criterion (BIC), with the highest BIC
199 value used for selection of the number of clusters. Clusters were ellipsoidal, with the volume
200 of the ellipsoid, shape of the density contours, and orientation of the corresponding ellipsoid
201 also determined by the covariance matrix. We tested whether schizophrenia cases in each of
202 the four GMM derived clusters were overrepresented for carriers of a top percentile PES
203 using multinomial logistic regression with the nnet package (https://cran.r-
204 project.org/web/packages/nnet/index.html). The largest cluster, *Cluster 2*, was used as the
205 reference for the other clusters, with the model covaried for sex and prinicipal components as
206 above. After dividing the regression coefficients by their standard error to derive *z*, *P* values
207 were calculated using the Wald Test.
208
209 **Investigation of the effect of PES profiles on gene expression**
210 We sought to investigate the relationship between PES profiles and the expression of genes
211 which comprise their pathways in individuals with schizophrenia. A subset of schizophrenia
212 cases in this cohort (N = 75) had mRNA expression data available from a previous study [21].
213 These participants had a mean age of 42.21 (s.d. = 10.47), whilst the majority of the
214 subcohort was male (N_{Male} = 45, N_{Female} = 30). RNA extracted from peripheral blood
215 mononuclear cells was profiled using Illumina HT-12_V3 BeadChips and normalised as
216 described in Gardiner *et al.* [21]. Genes which comprise each PES pathway were extracted if
217 they were available on the array with normalised expression values which survived quality
218 control. The relationship between PES and the expression of each gene in that pathway as the
219 outcome was assessed using a linear model covaried for age, sex, and PRS_{Total} for
220 schizophrenia. These models were constructed in R version 3.4.4 using the *lm* function.
221 Multiple testing correction was applied to each PES model individually to account for the

number of genes tested in each pathway using Benjamini-Hochberg method via the *p.adjust* function in R.

RESULTS

Clinically actionable pathways enriched with common variant risk in schizophrenia

Schizophrenia is a typical complex trait disorder with a prevalence around 0.7% and heritability in the region of 80% [22; 23]. A substantial proportion of this heritability was accounted for in the 2014 psychiatric genomics consortium (PGC) mega GWAS, which identified over 100 common variant loci at rigorous genome-wide significance level [3], making the disorder a suitable candidate to test the implementation of the PES framework. Using the complete summary statistics, we identified eight clinically actionable gene-sets (at the different P_T) containing known drug targets (Table 1). The most significantly associated of these was the *HIF-2 pathway* ($P = 3.12 \times 10^{-5}$, $\beta = 0.435$, $SE = 0.109$, $P_T < 0.005$), which is comprised of genes in the hypoxia inducible factor 2 (HIF-2) alpha transcription factor network. *One carbon pool by folate* was the second most significant pathway with a putative drug interaction ($P = 1.4 \times 10^{-4}$, $\beta = 0.433$, $SE = 0.119$, $P_T < 0.05$). Two gene-sets were related to the function of the neurotransmitters GABA and Acetylcholine, whilst other signalling pathways represented were *NOS1* (Nitric Oxide Synthase I), *Hedgehog* signalling and the semaphorin related *CRMP (Collapsin Response Mediator) proteins in Sema3A signalling* pathway. In addition, the geneset *Regulation of Insulin Secretion* passed the threshold for inclusion.

The genes which constitute these eight pathways had upregulated expression in the brain relative to the rest of the protein coding genome, with the anterior cingulate cortex the most highly enriched region after multiple testing correction, $P_{Adj} = 6.45 \times 10^{-13}$. Conversely, they

were downregulated ($P_{Adj} < 0.05$) in several peripheral tissues including the stomach and skin (Supplementary Fig. 2). These genes were also overrepresented in the GWAS catalogue for traits relevant to psychiatry including schizophrenia, post-traumatic stress disorder, nicotine dependence and cognitive performance ($P_{Adj} < 0.05$, Supplementary Table 1).

Pathway	*P* threshold (P_T)	*P*
NOS1 pathway	All SNPs	6.3×10^{-4}
Regulation of insulin secretion	$P < 0.5$	3.9×10^{-4}
CRMPs in Sema3A signalling	$P < 0.5$	9.4×10^{-4}
GABA synthesis, release, reuptake and degradation	$P < 0.5$	5.8×10^{-4}
One carbon pool by folate	$P < 0.05$	1.4×10^{-4}
Hedgehog signalling	$P < 0.05$	1.9×10^{-4}
HIF-2 pathway	$P < 0.005$	3.1×10^{-5}
Acetylcholine binding and downstream events	$P < 0.005$	3.8×10^{-4}

Table 1. Pathways enriched with common variation associated with schizophrenia with putative clinical actionability. Pathways with putative clinical actionability by virtue of having targets for existing drugs with potential for repurposing. Enrichment *P* values refer gene-set association aggregated SNPs associated with schizophrenia in the PGC GWAS.

The eight gene-sets prioritised by our pipeline are indicative of a diverse range of drug classes. We sought to investigate a selection of candidate pharmacological agents which may be utilised for each PES input pathway. Firstly, we extracted the genes classified in the TCRD as T_{Clin} from each of the gene-sets and matched them to their known drug-interactions using the drug gene interaction database (DGidb v3.02, Supplementary Table 2). The top FDA approved drug per pathway was selected based on the DGidb score of interaction confidence between a T_{Clin} gene and drug. After annotation via the anatomical chemical

(ATC) classification system two candidate drugs were anti-neoplastic and immunomodulating agents (ATC = L), two were classified as nervous system (ATC = N), whilst the remaining encompassed one of the following: blood and blood forming organs (ATC = B), musculoskeletal system (ATC = M), sensory organs (ATC = S) and alimentary tract and metabolism (ATC = A). Clinical trials for schizophrenia, either completed or in the recruiting phase, were registered for three of these compounds – glycine, varenicline and exenatide.

Drugs which target a statistically significant number of genes in each pathway were derived using over-representation analysis in WebGestalt [14]. Of the eight gene-sets tested, six had a significant drug enrichment with a minimum overlap of three genes after multiple testing correction (Table 3, Supplementary Table 3). Nervous system drugs were the most common ATC category (level 1) across all the input pathways. Some interesting repurposing candidates with previous clinical trials in the disorder included the psychostimulant Atomoxiene [24], the α4β2 nicotinic acetylcholine receptor subtype partial agonist Varenicline [25], acetylcysteine (*N*-acetylcysteine) - a precursor to the antioxidant glutathione [26-28], ascorbic acid (Vitamin C) [29; 30], vitamin E[30], and memantine [31; 32]. Whilst the results of these trials were mixed, targeting such interventions to specific individuals based on genomic risk is yet to be investigated.

Pathway	Top Drug*	Overlap#	ATC Code
NOS1	Milnacipran	5	Other antidepressants
GABA	Temazepam	14	Benzodiazepine derivatives
Insulin	Clodronic acid	3	Bisphosphonates
HIF-2	Ascorbic acid (Vitamin C)	3	Ascorbic acid (Vitamin C)
Acetylcholine	Nicotine	11	Drugs used in nicotine dependence
Folate	Tetrahydrofolic acid	11	Folic acid and derivatives

Table 2. The top enriched drug target for each *pharmagenic enrichment score* pathway with at least three interacting genes after multiple testing correction. Most significantly associated drug after multiple testing correction was selected, when corrected *P* values were equal, the drug with the highest geneset overlap was selected. Overlap refers to the number of genes targeted by the drug in the candidate pathway.

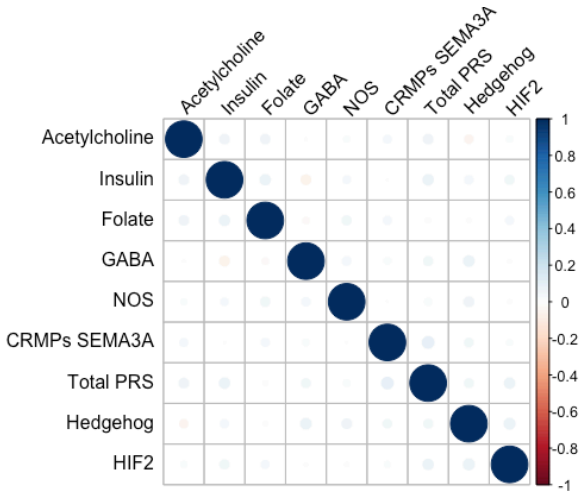
Individual profiling of pharmagenic enrichment scores in a schizophrenia cohort

We profiled PES in a cohort of schizophrenia patients and screened healthy controls [17] and identified members of the cohort with relatively high PES in clinically actionable gene-sets. Firstly, we examined individuals in the top percentile of the ASRB cohort for each PES, to explore the phenotypic characteristics of an elevated risk score with high confidence. There were 55 individuals with a top percentile PES, as one schizophrenia case had elevated PES in both the *One Carbon Pool by Folate* and the *GABA synthesis, release, reuptake and degradation* pathways. From this subset, the majority were schizophrenia patients (N=38), however, there was no significant association between top percentile status and diagnosis (z = 0.975, P = 0.33). We investigated clinical characteristics obtained for ASRB participants to prioritise top percentile PES carriers who may benefit most from a personalised treatment regime. Three variables were selected as a proxy of a more clinically challenging presentation of the disorder: clozapine prescription (as a surrogate for treatment resistance), a

global assessment of functioning (GAF) score < 50, and an adolescent onset of the disorder before the age of 18 [33; 34]. Interestingly, of the 38 schizophrenia cases with an elevated PES, 71% of this subset meet at least one of these criteria (N =27): clozapine prescription (N = 9), GAF < 50 (N = 12), onset age < 18 (N = 9).

In addition, two less stringent partitions of elevated PES were implemented, specifically, a decile and quartile cut-off for PES in the entire cohort was used to triage patients at elevated risk of dysfunction in that pathway. The highest number of PES in the top decile or quartile respectively for an individual was six (Supplementary Fig. 3). An increasing number of PES in both the top quartile (OR = 1.1493 [95% CI: 1.016 – 1.303], $P = 0.0287$) and decile (OR = 1.207 [95% CI: 1.013 – 1.447], $P = 0.0384$) was associated with schizophrenia. However, this signal was not significant after adjustment for PRS_{Total}. As visualised with kernel density estimation in figure 1b-c, there is evidence of skew towards high PRS_{Total} for those individuals with at least four top quartile or decile PES categories. Whilst the aim of this study was not to find association with cases for this cohort, two PES were nominally associated with schizophrenia in the ASRB - *Regulation of Insulin Secretion* ($z = 2.262$, $P = 0.0237$) and the *Acetylcholine Binding and Downstream Events* pathways ($z = 2.167$, $P = 0.0303$). However, significance was diminished when covaried for total schizophrenia PRS (PRS_{Total}, $P > 0.05$).

a



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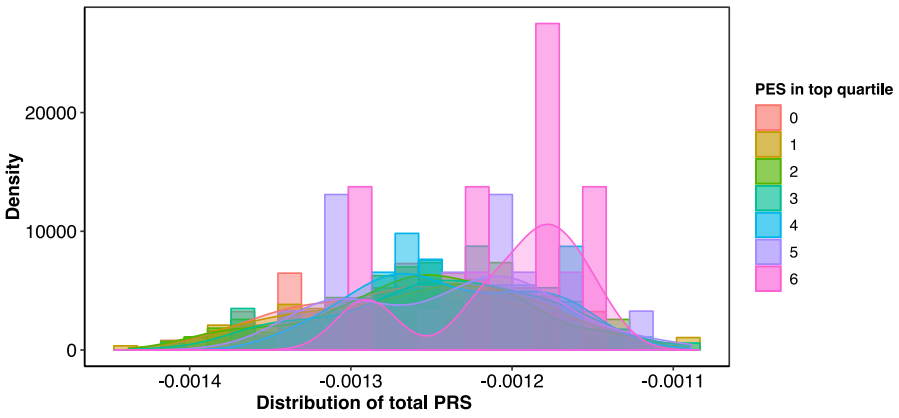

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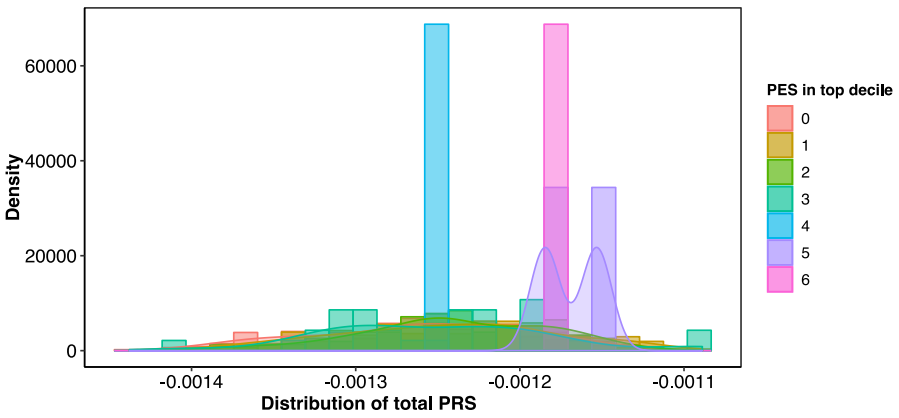

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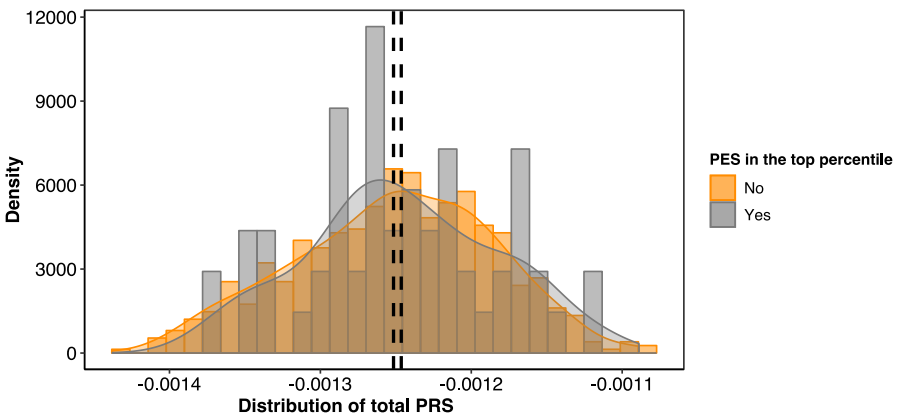

Fig 1. Relationship between genome wide schizophrenia PRS and *pharmagenic enrichment scores* in the ASRB cohort. (**a**) Pairwise univariate correlation between each of the PES and total PRS. Scale represents strength of relationship in the positive or negative direction. Kernel density estimation of distribution of total PRS amongst individuals with multiple PES in the top quartile (**b**) or decile (**c**). Scale refers to the number of PES over the threshold in an individual, that is, a score of six represents an individual with six PES categories in the top quartile or decile of the ASRB cohort. (**d**) Distribution of total PRS between ASRB participants with at least one PES in the top percentile of the cohort (grey) or without (orange). Black dashed line represents the mean PRS_{Total} for the cohort with a top percentile PES (right) and without (left).

Relationship between pathway-based annotation and genome wide polygenic risk for schizophrenia

We sought to define the relationship between PRS_{Total} and PES in further detail. Pairwise correlation between each of the scores demonstrated no significant univariate relationship between any PES or with PRS_{Total} (Fig. 1a). In addition, top percentile PES individuals were not enriched with PRS_{Total} in comparison to the rest of the cohort: $z = 0.819$, $P = 0.413$ (Fig. 1d). This presented a clinically significant subset of schizophrenia cases with a less polygenic phenotype, that is, low PRS_{Total} relative to the schizophrenia cohort but high heritable risk in one or more pathways. Analysis of the bottom quartile of PRS_{Total} in the ASRB schizophrenia cohort revealed cases (N=10) with top percentile PES but depleted PRS_{Total}. The pathways encompassed in these individuals were: *Acetylcholine* (N=3), *Hedgehog signalling* (N=2), *CRMPs in Sema3A* (N=2), *GABA* (N=1), *HIF-2* (N=1) and *Insulin secretion* (N=1). This information may be of great clinical value as these cases have less marked common variant burden genome-wide, but localised risk in a geneset. Furthermore, three of these patients

were prescribed clozapine (surrogate for treatment resistance), a further three had low global functioning (GAF < 50), along with two adolescent onset cases – potentially highlighting a heightened need for precision intervention in these individuals.

To investigate the relationship between low polygenic load and elevated PES, genome wide PRS and the PES category count in the top decile per individual were clustered using finite Gaussian mixture modelling (GMM). The optimal number of clusters was selected based on parametrisation of the covariance matrix utilising the Bayesian Information Criterion (BIC), with the highest BIC value used for selection of the number of clusters (Fig. 2a). Four clusters were derived from the data (*BIC* = 13871.21, VEV: variable volume, equal shape, variable orientation) (Fig. 2b). Clusters were ellipsoidal, with the volume of the ellipsoid, shape of the density contours, and orientation of the corresponding ellipsoid also determined by the covariance matrix. The first two clusters were comprised of schizophrenia patients with at least one PES in the top decile of the ASRB cohort, with *Cluster 1* having low PRS_{Total} relative to *Cluster 2*. The third and fourth clusters had no elevated PES but *Cluster 3* represents patients with greater polygenic load, that is PRS_{Total}, than the *Cluster 4*. The distribution of PRS_{Total} in *Cluster 1* reinforces the concept that a subset of schizophrenia patients with lower polygenic risk may have concentrated elevation in one or more specific biological systems. Analogous to its univariate relationship with PRS_{Total} individuals with extreme PES in the top percentile of the ARSB cohort were not enriched in any of the GMM clusters relative to the largest cluster, *Cluster 2* (*Cluster 2* vs *Cluster 1*: $P = 0.668$; *Cluster 2* vs *Cluster 3*: $P = 0.492$; *Cluster 2* vs *Cluster 4*: $P = 0.977$).

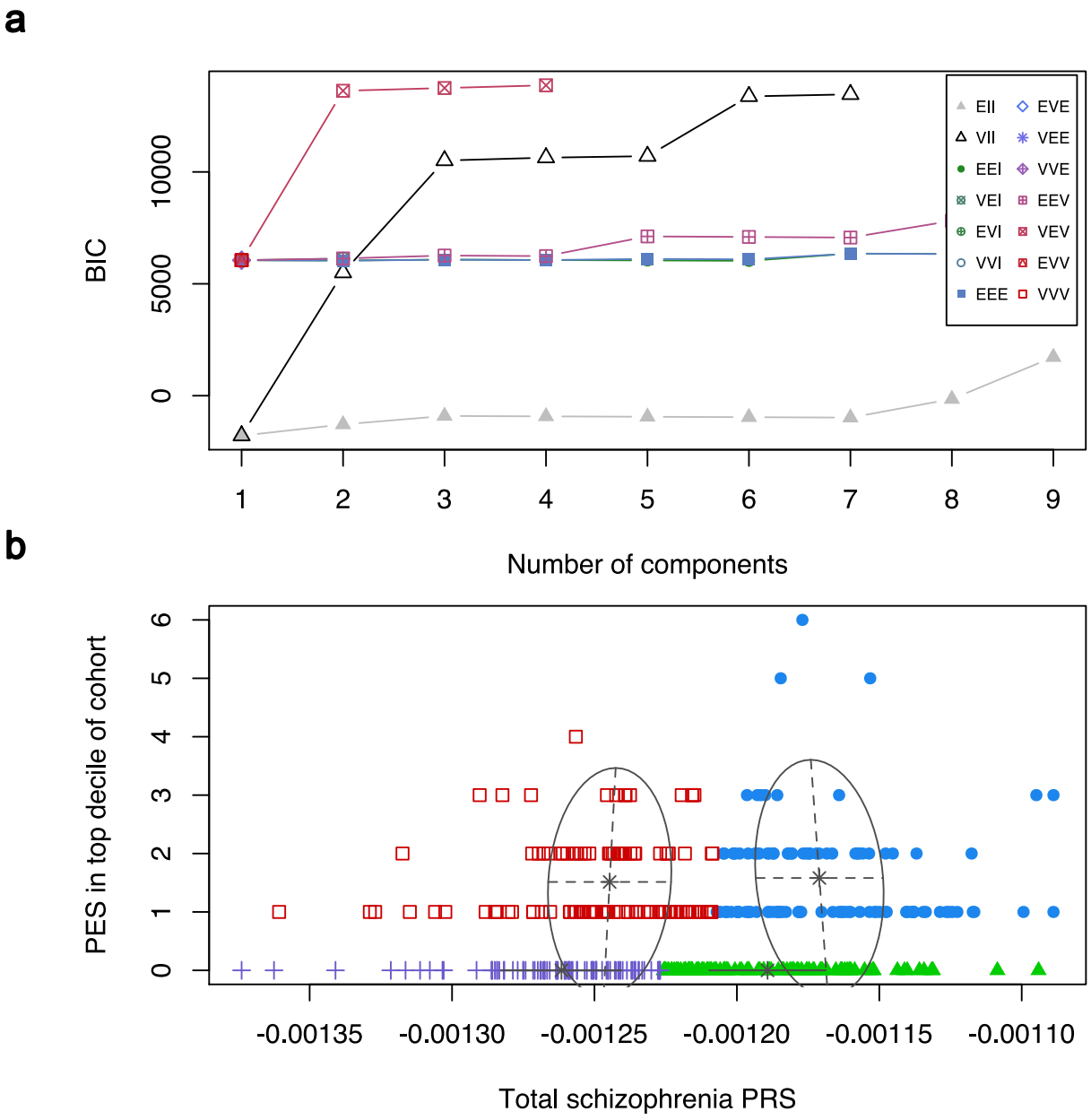

Fig 2. Clustering of genome wide PRS and the individual count of elevated *pharmagenic enrichment score* using finite Gaussian mixture modelling. (**a**) Selection of the number of clusters using the Bayesian information criterion (BIC). The scale represents the fourteen different Gaussian models (see Supplementary Table 5 for definitions) tested for parametrisation of the within-group covariance matrix. (**b**) Derived clusters of genome wide schizophrenia PRS (Total PRS) and the number of PES in the top decile of the ASRB cohort

per individual. Red boxes = *Cluster 1*, blue circles = *Cluster 2*, green triangles = *Cluster* 3, purple crosses = *Cluster 4*.

PES profiles impact the expression of genes within the candidate pathways

Each PES profile was tested for association with the peripheral blood expression of genes within their respective pathways for a subset of schizophrenia cases with expression data available (Fig. 3, Supplementary Table 6). After covariation for sex, age, and PRS_{Total}, the *NOS1* PES was associated with downregulated expression of the calcineurin subunit gene *PPP3CC* (t = -3.08, P = 2.9 x 10^{-3}, q = 0.05). This was followed by the *regulation of insulin secretion* PES, which was associated with decreased expression of the syntaxin gene *STX1A* (t = -3.5, P = 8.1 x 10^{-4}, q = 0.055); and the *One carbon pool by folate gene* PES, which was associated with downregulation of serine hydroxymethyltransferase 2 (*SHMT2*; t = -2.94, P = 4.5 x 10^{-3}, q = 0.072). Excluding these three genes (q < 0.1), there were eleven others with a nominally significant (Raw P < 0.05) relationship with a PES, with all PES profiles except *CRMPs in Sema3A signalling* having at least one such nominally significant model.

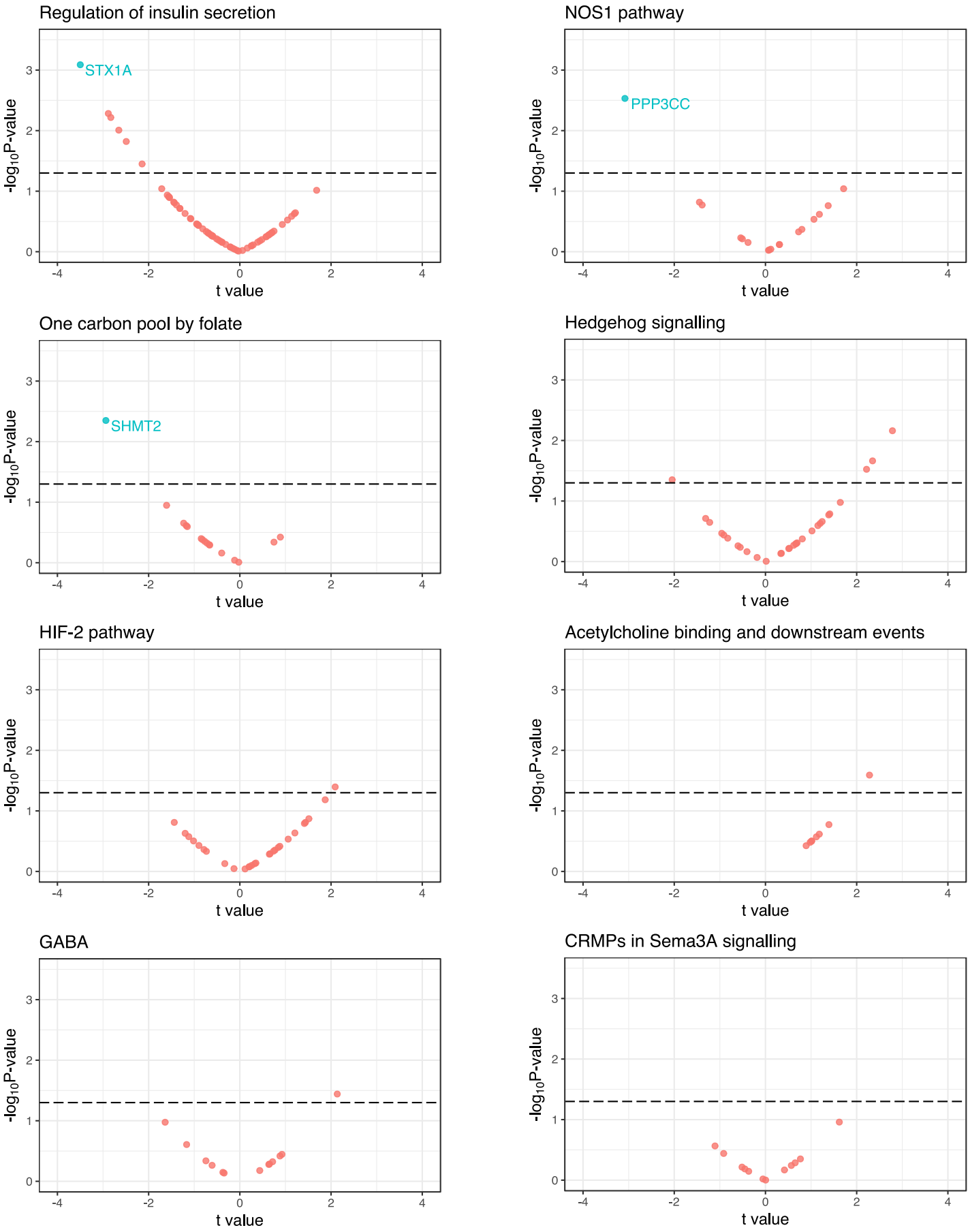

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Figure 3. The relationship between PES profiles and the expression of pathway genes in peripheral blood mononuclear cells. Plot for each PES of the results of a model which investigated the association of PES with the expression of genes which compromise the PES pathway. The *t* values on the x-axes were derived from the regression model for each PES-gene pair ($t = \widehat{\beta} / \widehat{SE}$), with the -$\log_{10}$ *P* value of association on the y-axes. The dotted line is indicative of uncorrected $P < 0.05$, genes highlighted blue were significant after correcting for the number of genes in the set with a liberal false discovery rate cut-off ($q < 0.1$).

DISCUSSION

The drug development pipeline continues to be prohibitively expensive and time consuming in the translation of novel compounds for clinical practice [35; 36]. Repositioning of previously approved drugs for other human health conditions can be a more readily achievable action, particularly for rare disorders where a causal factor can be identified. However, in complex disorders, such as schizophrenia, this approach is hindered by the complexity of the pathophysiology and heterogeneity of genomic risk, along with inter-individual variability in illness onset and clinical course [37; 38]. Annotation of the individually-relevant (personalised) genetic components associated with complex syndromes, for the purpose of delineating clinically meaningful biological systems, will both better target existing treatments and reveal new opportunities for drug repurposing (Fig. 4a). In this study, we developed a novel method for capturing common variant risk in biological networks with known drug interactions – *pharmagenic enrichment scores* (PES) – to facilitate precision treatment design relevant to individuals with a particular set of risk variants. A distinct advantage of our PES approach is that it can capture latent enrichment of polygenic signal in pathways relevant to pharmaceutical actions, among individuals whose overall trait PRS is low relative to others with a shared phenotype. Even in cases where polygenic burden is high, genome-wide PRS

(as a biologically unannotated instrument) does not necessarily provide insight into pathways suitable for pharmacological intervention in individuals. Our approach for selection of putative drug targets exemplified in schizophrenia GWAS has revealed potential targets for drug repurposing with substantial clinical utility.

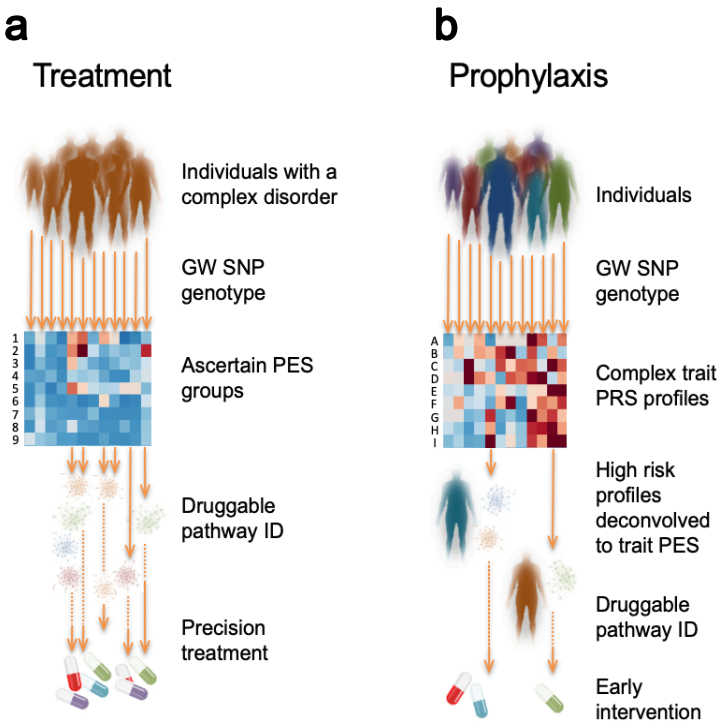
Aggregation of common variation from schizophrenia GWAS into biological pathways with known drug interactions revealed a diverse array of systems relevant to eight distinct PES categories. These candidate pathways displayed common variant enrichment at a range of P_T, indicative of the degree of polygenicity, ranging from using all SNPs as input, to a significance threshold below 0.005 ($P_T < 0.005$). While two of these pathways included GABAergic and cholinergic neurotransmission, both of which are intuitive candidates that have been extensively implicated in schizophrenia with associated drugs already in common practice for neuropsychiatric disorders [39; 40], many others were more surprising. The most significantly associated gene-set pertained to the HIF-2 transcription factor network, an important mediator in response to decreases in available cellular oxygen. This has clear significance for biological mechanisms involved in psychiatric disorders, for example in dopaminergic signalling [41]. Enrichment of ascorbic acid (vitamin C) targets in this pathway is notable from a therapeutic perspective because of its antioxidant capabilities, along with preliminary evidence for its efficacy as an adjuvant in the treatment of the disorder [29; 30]. The interaction between HIF-2 signalling and NOS1 signalling, another candidate pathway with pharmagenic enrichment in schizophrenia, is supported by previous evidence of redox dysfunction in the disorder [42; 43]. The activity of glutamate receptors in the NOS1 system suggests that psycholeptics and psychoanaleptics are likely to modulate this pathway. We also observed common variant enrichment in two developmental pathways that can be pharmacologically modulated: CRMPs in semaphorin 3a signalling and Hedgehog signalling.

503 The former is able to interact with the tyrosine kinase inhibitor Dasatinib, which is postulated
504 to have neuroprotective properties [44]. Enrichment in these actionable pathways is consistent
505 with longstanding hypotheses of deficits in neurodevelopment contributing to the aetiology of
506 schizophrenia [45]. There is also evidence for aetiological overlap between schizophrenia and
507 diabetes beyond what is attributable to metabolic effects of antipsychotic treatment, which
508 supports our identification of an insulin related pathway as a candidate PES [46-48].
509
510 The breadth of drugs which target these pathways used to construct PES suggests that
511 individual level treatment formulation can become highly specific depending on which
512 systems genomic risk is localised. This would include the stratification of individuals for
513 precision treatment with compounds previously tested on undifferentiated schizophrenia
514 cohorts, including, N-acetylcysteine, vitamin C, Atomoxiene, and Varenicline which were
515 identified using PES in this study [24-26; 29]. Repurposing drugs for individuals informed by
516 their genetic liability may assist in the reduction of response heterogeneity, which hinders the
517 implementation of novel treatments in very complex phenotypes like schizophrenia. We
518 suggest that the individuals with PES in the top percentile of any pathway, particularly those
519 with low genome wide PRS, present as the most tractable candidates for this approach;
520 whereas the clinical significance of particular sets of common variant burden would be
521 missed by an unannotated genome wide association indexed by total PRS alone.
522
523 In order to better understand how PES profiles could be leveraged for treatment, the effect of
524 sequence variation which comprises the PES needs to be investigated. We outlined an
525 example of this approach in this study by testing the effect of PES on the expression of genes
526 which comprise each pathway in schizophrenia. Several associations between PES and
527 mRNA expression were observed after correcting for the number of genes within the tested

528 set. These effects may arise due to direct *cis*-acting loci within the PES and/or the
529 downstream biologic effects of variation which effect genes with interrelated functionality.
530 This was exemplified by the *Regulation of insulin secretion* PES, which was negatively
531 correlated with *STX1A* expression, a syntaxin postulated to play a role in insulin homeostasis.
532 Interestingly, *STX1A* has been shown to be positively correlated with glucose stimulated
533 insulin secretion, suggesting downregulation conferred by the PES may have an important
534 effect through this biological pathway[49; 50]. Similarly, downregulation of the calcineurin
535 subunit gene *PPP3CC* was associated with the *NOS1 pathway* PES. Previous research
536 suggests there is a bidirectional relationship between nitric oxide signalling and calcineurin,
537 where the calcineurin subunit is both regulated by redox products and able to induce nitric
538 oxide synthesis [51; 52]. Several other genes had suggestive association with PES profiles which
539 may be established with a larger cohort. We suspect that this approach to validation would be
540 particularly informative for schizophrenia in genotyped expression cohorts from brain tissue.
541
542 While we expect that in most circumstances the aggregate of variation constituting high PES
543 represent pathology of the target pathways, a current limitation of this methodology is that it
544 does not integrate the direction of effect. While this may be possible as more functional
545 annotations become available, this would present an immensely complex paradigm to predict
546 *in silico* due to the vast array of factors which influence the penetrance of genomic risk. We
547 believe that an analysis of the effect of PES profiles on gene expression in larger cohorts will
548 be an important future direction of this work. The impact of candidate molecules could also
549 be modelled in patient derived cell lines to, firstly, establish the extent of dysregulation
550 conferred by elevated PES and, secondly, investigate the interaction with the compounds
551 implicated. The current analysis also used very stringent criteria for drug pathway gene
552 inclusion and there are likely to be many more genes and pathways that may be implicated in

future ontologies and other investigator curated gene-sets. In a recent example of this we observed an enrichment of retinoid gene variation in schizophrenia [18].

New GWAS summary statistics are emerging daily on ever larger samples and these too will further enrich the substrate for PES determination and increase its clinical utility. Despite the aforementioned challenges, we believe that this methodology provides a useful framework to better utilise the breadth of available GWAS data for personalised treatment formulations. Particularly, as there remains a largely unmet need to translate polygenic risk for complex disorders into tractable treatment outcomes for affected individuals. Whilst we have demonstrated here the potential utility in schizophrenia, there is clearly scope to adapt this analytical approach to other complex disorders with summary statistics from well-powered GWAS. This methodology may also be applicable to prophylactic intervention for individuals at high risk for a complex phenotype (Fig. 4b). This could be implemented conservatively with lifestyle or dietary measures implicated by clusters of enrichment captured within the PES framework. For example, in schizophrenia we identified multiple actionable pathways quantified by PES that are modulated by vitamins, which represent a relatively uncomplicated intervention for individuals at high genetic risk for this disorder.



578 **Fig. 4. Implementation of *pharmagenic enrichment score* (PES) in precision treatment**
579 **and prophylaxis of complex disorders.** (**a**) Using the PES framework individuals with a
580 complex disorder provide DNA for common variant SNP genotyping, which is used to
581 ascertain individuals with a high PES. The PES groups (heatmap rows 1-9, with enrichment
582 denoted by darker colour) intrinsically identify precision treatment options tailored to the
583 individual's biological enrichment (of pathways with known drug targets) for polygenic risk
584 in that pathway. (**b**) A more advanced implementation of PES could be achieved for
585 prophylactic intervention for individuals in the population at very high polygenic risk for a
586 variety of complex traits with clinical actionability (heatmap rows A-I, with high PRS
587 denoted by darker colour). Conservative prophylactic measures in this context may account
588 for environmental risk exposure and focus on lifestyle interventions, such as diet and
589 exercise, rather than pharmaceutical treatments that may not be justified without symptom
590 presentation because of their side effect and/or cost.
591
592 **SUPPLEMENTAL DATA**
593 **Supplementary Fig 1.** Methodology for identifying pharmacologically-relevant pathways
594 enriched with GWAS risk variants.
595 **Supplementary Fig 2.** Tissue specific expression of genes contained within candidate PES
596 pathways derived from schizophrenia GWAS
597 **Supplementary Fig. 3.** Distribution of schizophrenia and healthy control patients with
598 multiple elevated *pharmagenic enrichment scores*.
599 **Supplementary Table 1.** Overrepresentation of genes within candidate PES pathways in the
600 GWAS catalogue traits with relevance to psychiatry after multiple testing correction.
601 **Supplementary Table 2.** Highest confidence drug interaction between of a member of each
602 pathway enriched with common polygenic risk for schizophrenia.

603 **Supplementary Table 3.** Enriched drug targets for each *pharmagenic enrichment score* with
604 at least three interacting genes after multiple testing correction (FDR < 0.05).
605 **Supplementary Table 4**. Geometric characteristics of the Gaussian models used for
606 parameterisations of the within-group covariance matrix.
607 **Supplementary Table 5.** Characteristics of the ASRB cohort analysed using the PES
608 methodology.
609 **Supplementary Table 6.** Effect of PES on gene expression for genes which comprise each
610 of the candidate pathways.
611

612 **DECLERATIONS OF INTERESTS**

613 The authors declare no conflicting financial interests

614 **DATA AVAILABILITY**

615 Schizophrenia GWAS data is available from the Psychiatric Genomics Consortium
616 (https://www.med.unc.edu/pgc/results-and-downloads). SNP array data used in this study is
617 available upon application to the Australian Schizophrenia Research Bank, URL:
618 https://www.neura.edu.au/discovery-portal/asrb/. Command line arguments for the
619 bioinformatics tools in this study are available upon reasonable request to the authors.
620

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