Cell Genomics Transparent Peer Review Record

The shared genetic landscape of blood cell traits and risk of neurological and psychiatric disorders

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Summary

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Data freely available:	Yes
Code freely available:	Yes

Referees' reports, first round of review

Reviewer #1: In this study, Yang and colleagues investigate genetic correlation and potential causal relationships between blood cell traits and both neurological and psychiatric disorders. The study is largely performed with a high standard of statistical rigour and there are some interesting results that will be of interest to the journal readership. However, I have a few comments below that I would like to see implemented before I would recommend this paper for publication.

Major Comments

1. In terms of the analyses that used the rho-HESS local covariance estimates for causal inference, could the authors please elaborate on the rationale for using P < 1e-05 as the threshold for 'specific SNPs'. Many of the neurological traits are much less powered than the blood-cell traits, so this threshold is somewhat arbitrary, particularly if there is a trend towards this level of statistical significance in a region that will likely be reached with larger samples. Did the authors consider a more informative method to define 'specific SNPs' e.g., a Bayesian approach?

2. In a similar vein, can the authors elaborate on how they identified the "intersection" SNPs that were shared, was this based on trait-wise P values as well? Given the relationships identified in this section were not readily found using MR or LCV, I think these results should be presented more cautiously.

3. I don't agree with the authors statement that "causal relationships are unlikely in the absence of global (or local) genetic correlation.". This is particularly non-reflective for biochemical traits or gene expression that may have more concentrated genetic architectures, and IVs can be selected with well-characterised or cis-acting effects on a trait. Moreover, local correlation is calculated usually at the level of LD-blocks, and thus, likely misses more discrete signals, particularly if a LD-blocks fails to obtain local non-zero heritability for one of the traits. I would remove this statement or alter it to be more nuanced.

4. Have the authors considered performing gene-set enrichment analyses on the implicated shared genes to see what biological approaches may be implicated?

5. I don't agree with calling the LCV method "MR equivalent" as it does not produce an exposure to outcome causal estimate. The underlying hypothesis of LCV is related to directional dependency as indexed by the fourth co-moments (cokurtosis), rather than instrumental variables. I think it would be better to explicitly state this difference in text and brief explain what the posterior GCP is.

6. The authors should perform and report some sensitivity analyses for the MR results beyond just the MR methods with differing assumptions regarding IV validity. I recommend the following: testing whether the MR-Egger intercept is significantly non-zero to screen for confounding pleiotropy, testing heterogeneity between IVs, and a leave-one-out analysis with the MR estimate is iteratively recalculated upon holding out one of the IVs. If a leave-one-out analysis demonstrates outlier IVs, these should be investigated to see if they have biologically plausible effects on the blood cell trait.

Minor Comments

1. When reporting the genetic correlation estimates, I think it is a good idea to also report the standard error. Even though the P-value encapsulates this information statistically, it is easier for the reader to visualise precision when they see an estimate and its corresponding standard error.

2. Like the above, can the authors please report the 95% confidence intervals for all odds ratios. It would be useful to also define what the unit of the exposures are such that the effect size is better conceptualised, e.g are the blood cell properties in standard deviation units, or are they log transformed, etc. Please also report the posterior standard error and P value for the posterior mean GCP estimates where these are reported in text.

3. Were there genes implicated in the SMR analysis where the effect of expression on either trait was discordant than the direction of the overall genetic correlation or local covariance? For example, if the two traits were positively correlated, but increased expression of a gene is associated with an increase in one trait but a decrease in the other.

4. Discussion: It may be worth mentioning when comparing the results to Reay et al. explicitly that the schizophrenia GWAS in the Reay et al. study was the most recent schizophrenia GWAS, whereas this study used in the 2014 GWAS. Conversely, this study used a larger blood-cell GWAS than that study.

Reviewer #2: Yang and colleagues investigate the pleiotropy linking blood cell traits and neuropsychiatric disorders. This is surely an interesting aim and the authors applied a wide range of methods to investigate the genetic basis of the observed comorbidities. Although the methods are appropriate, I believe the authors missed to address a key point. Likely, factors such as smoking, drinking, educational attainment, and socioeconomic status play a key role in the genetic correlation and causal effects identified. Accordingly, I strongly recommend the authors apply methods based on multivariable statistics to understand whether the effects detected are independent of other variables correlated with the traits investigated. Additionally, the results are separately for each method used. This makes it hard to understand the consistency among the findings observed across methods. For example, it would be interesting to understand the intersection between the findings of the local genetic correlation and SMR analyses. Also, some of the statistics are mentioned in the main text, but the actual numbers are only reported in the supplemental material. I suggest providing key statistics also in the main text.

Authors' response to the first round of review

We thank each of the Reviewers' for taking the time to review our manuscript, and for their helpful and constructive feedback which has greatly improved our paper. We have endeavoured to carefully and thoroughly address each of their comments and suggestions, as detailed in our response below.

Reviewer 1

In this study, Yang and colleagues investigate genetic correlation and potential causal relationships between blood cell traits and both neurological and psychiatric disorders. The study is largely performed with a high standard of statistical rigour and there are some interesting results that will be of interest to the journal readership. However, I have a few comments below that I would like to see implemented before I would recommend this paper for publication.

We are grateful to the Reviewer for the positive feedback and valuable comments.

Major Comments:

1. In terms of the analyses that used the rho-HESS local covariance estimates for causal inference, could the authors please elaborate on the rationale for using P < 1e-05 as the threshold for 'specific SNPs'. Many of the neurological traits are much less powered than the blood-cell traits, so this threshold is somewhat arbitrary, particularly if there is a trend towards this level of statistical significance in a region that will likely be reached with larger samples. Did the authors consider a more informative method to define 'specific SNPs' e.g., a Bayesian approach?

We thank the Reviewer for raising this point. The rationale for using p-value < 1Å~10-5 as the threshold for trait-specific SNPs was that (as intimated by the Reviewer) some of the neuropsychiatric disorders (NPD) have relatively few genome-wide significant SNPs, meaning that estimates of local genetic correlations between blood cell traits (BCT) and NPDs in NPD-specific regions were imprecise when based on the conventional p-value < 5Å~10-8 threshold. We acknowledge that this is a limitation of our analysis, and that different p-value thresholds might result in different patterns of local genetic correlations between BCTs and NPDs, with the potential to influence inferred causal relationships. To

explore the impact of p-value threshold selection on our results, we performed a series of sensitivity analyses using four alternative p-value thresholds (i.e., 1Å~10-3, 1Å~10-4, 1Å~10-6, 5Å~10-8). Additionally, in response to the Reviewer's query, we conducted sensitivity analyses using the Bayesian SBayesR approach1 to define trait-specific SNPs.

These sensitivity analyses are summarized in the Figure R1 below (and in Figures S8-14 & Tables S19-25): each panel shows average local rg estimates (means and standard errors) for 319 BCT-NPD trait pairs from analyses performed using different p-value thresholds (total 15 plots for all pairwise comparisons of five p-value thresholds plus SBayesR). Local rg estimates are provided for each trait pair for "NPD-specific" regions (coloured in purple), "BCT-specific" regions (red), "Intersection" regions (blue) and "Neither" regions (green). These analyses clearly show that patterns of local genetic correlations between BCTs and NPDs in NPD-specific and "Intersection" regions are highly inconsistent, depending on the p-value threshold employed, with pairwise correlations between pvalue thresholds ranging from R = -0.03 to 0.52 and from -0.54 to 0.96, respectively. In contrast, local rg estimates in BCT-specific regions (R = 0.59 to 0.92) and "Neither" regions (R = 0.40 to 0.97) were moderately to highly consistent between analyses based on different p-value thresholds.

We wish to note that in revising this analysis in response to the Reviewer's comment, we realized that we had inadvertently failed to exclude the MHC region in our original analysis. Consequently, whereas we had previously reported two FDR-significant causal relationships (i.e., ALS-MONO# and ALS-MCH) based on a p-value threshold for defining trait-specific SNPs of 1Å~10-5, these associations no longer surpass correction for multiple testing. In our final analysis, no single BCTNPD pair showed a Bonferroni- or FDR significant pattern of local genetic correlations consistent with a putative causal relationship after excluding the MHC, irrespective of the p-value threshold used to select trait-associated SNPs and classify trait-related regions.

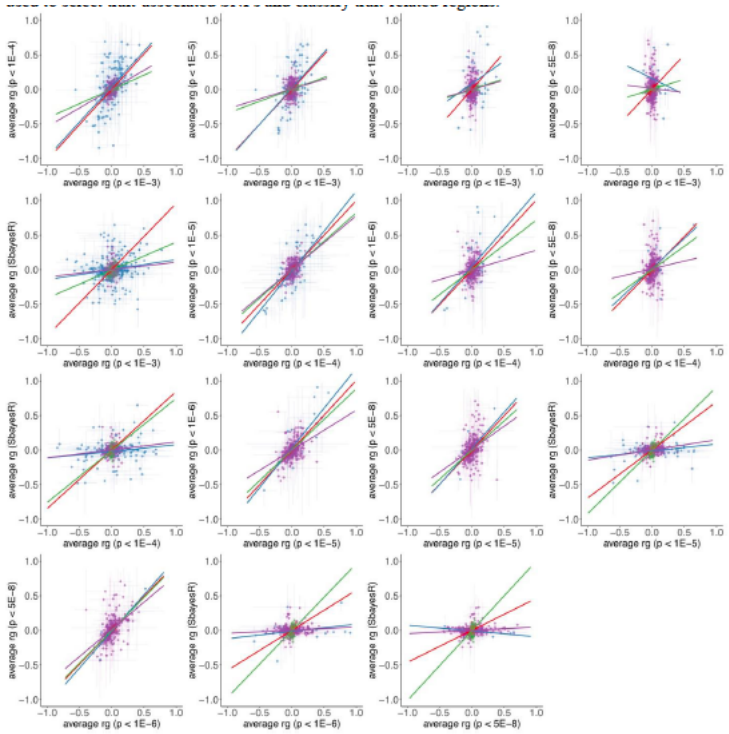

Figure R1. Comparison of average local genetic correlations between $N = 319$ BCTs and NPDs trait pairs from analyses performed using different p-value thresholds to define trait-specific regions. Dots in red, green, purple, and blue represent the estimated average local genetic correlations from the "BCT-specific" regions, "Neither" regions, "NPD-specific" regions, and "Intersection" regions, respectively, with estimated regression lines displayed in the corresponding colours. Error bars represent the standard errors of the estimated average local genetic correlations.

The sensitivity analyses described above are consistent with the Reviewer's perceptive point about the differential power of NPD and BCT GWAS, since we would expect higher levels of sampling variation – across different p-value thresholds – in the number of genomic regions harbouring NPD-specific SNPs, or both NPD-specific and BCT-specific SNPs (i.e., "Intersection" regions), relative to regions with BCT-specific SNPs or "Neither". These sensitivity analyses suggest that utilising patterns of local genetic correlations for causal inference has significant limitations in the presence of differential power between traits, which is the case in our study. For this reason, we have amended our manuscript to be substantially more cautious in relation to the use of the □-HESS results for causal inference. Importantly, these changes have minimal impact on the overall conclusions of our study.

In the Results, we have removed references to the previously reported FDR-significant pairs (ALSMONO#, ALS-MCH) and Figure 3 has been shifted from the main text to the supplementary (Figure S7). We have additionally added Figures S8-14 and Tables S19-25 to capture results of the sensitivity analyses across different p-value thresholds. The revised Results section now reads (page 6, line 30- 33):

"We identified no single BCT-NPD trait pair satisfying this criterion at Bonferroni ($p < 1.57$Å~10-4) or FDR ($p <$ ~1.50Å~10-4) correction for multiple testing, irrespective of the p-value cut-off (or SBayesR35) used to define trait-specific SNPs and genomic regions (Figure S8-14; Table S19-25)."

In the Discussion, we have removed the text (page 13) related to ALS-MONO# and ALS-MCH, along with the related text in the limitations (page 18), since we have de-emphasized the use of □-HESS for causal inference.

We have also updated the STAR Methods, which now reads (page 24, line 18 to page 25, line 7):
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"We then followed the approach proposed by Shi et al.17 and classified the local genetic correlations into four groups for each pair of traits: (i) regions harbouring only NPD-specific SNPs, (ii) regions harbouring only BCT-specific SNPs, (iii) regions harbouring SNPs shared by both members of the BCT-NPD trait pair ("Intersection"), and (iv) other regions ("Neither"). We defined trait-specific SNPs (used to classify genomic regions into each of the four groups) as those with p < 1Å~10-5, and compared these results to four other p-value cut-offs (p < 5Å~10-8, p < 1Å~10-6, p < 1Å~10-4, and p < 1Å~10-3), as a means of accommodating differential GWAS power between BCTs and NPDs. We also performed a further sensitivity analysis using a Bayesian-based approach (SBayesR)35 to define trait-specific SNPs. SBayesR implements a Bayesian likelihood multiple regression procedure to refine the estimated effect sizes of trait-associated SNPs by updating the 'prior' SNP
effects of GWAS summary statistics to 'posterior' SNP effects. We used a sparse LD correlation matrix generated from Europeans in the UK Biobank as a reference for SBayesR. Genomic regions were excluded if the estimated local genetic correlation was missing (e.g., because the local estimated single-trait heritability was negative), less than -1 or greater than 1 (e.g., because at least one of the local estimated single-trait heritability estimates was close to zero). For each group that comprised □ 5 local genetic correlation estimates, we calculated the mean and standard error of the local genetic correlations within the group. A causal effect of BCT on NPD was suggested if the average local genetic correlation in regions harbouring BCT-specific SNPs was Bonferroni significantly non-zero (p < 1.57Å~10-4 for 319 pairs) and Bonferroni significantly different from that in "Intersection" regions, "Neither" regions, and regions harbouring NPD-specific SNPs (p < 1.57Å~10-4, based on a two-tailed Z-test). We used an equivalent strategy to identify patterns of local genetic correlations consistent with a potential causal effect of NPD on BCT."

2. In a similar vein, can the authors elaborate on how they identified the "intersection" SNPs that were shared, was this based on trait-wise P values as well? Given the relationships identified in this section were not readily found using MR or LCV, I think these results should be presented more cautiously.

We apologise for any lack of clarity in our description of the methods used in our □-HESS analyses. As suggested by the Reviewer, the "Intersection" SNPs were selected based on the trait-wise p-values. We have now clarified the definition of the four groups (including the "Intersection" group) in the □-HESS section of the STAR Methods (page 24, line 18-22). The revised section now reads:

"We then followed the approach proposed by Shi et al. and classified the local genetic correlations into four groups for each pair of traits: (i) regions harbouring only NPD-specific SNPs, (ii) regions harbouring only BCT-specific SNPs, (iii) regions harbouring SNPs shared by both members of the BCT-NPD trait pair ("Intersection"), and (iv) other regions ("Neither")."

We agree with the Reviewer that our □-HESS results should be presented more cautiously. As explained in our response to the Reviewer's previous point, we have de-emphasized these results, and re-drafted relevant sections of the Results and Discussion.

3. I don't agree with the authors statement that "causal relationships are unlikely in the absence of global (or local) genetic correlation". This is particularly non-reflective for biochemical traits or gene expression that may have more concentrated genetic architectures, and IVs can be selected with well-characterised or cis-acting effects on a trait. Moreover, local correlation is calculated usually at the level of LD-blocks, and thus, likely misses more discrete signals, particularly if a LD-blocks fails to obtain local non-zero heritability for one of the traits. I would remove this statement or alter it to be more nuanced.

We thank the Reviewer for this comment, with which we agree in principle. However, we note that all of the BCTs and NPDs analysed in our study show evidence for a highly polygenic architecture, as opposed to oligogenic architectures. For this reason, we believe that our rationale in focusing our analyses on trait pairs with evidence for a genetic correlation is defensible. We have nonetheless updated the relevant text in response to the Reviewer's concern (page 7, line 6-7):

"…, recognising that causal relationships between highly polygenic traits (such as the BCTs and NPDs included in our study) are more likely in the presence of a global genetic correlation."

4. Have the authors considered performing gene-set enrichment analyses on the implicated shared genes to see what biological approaches may be implicated?

We are grateful to the Reviewer for this suggestion, and agree that gene set enrichment analysis (GSEA) is worthwhile exploring, given the potential to identify shared biological processes underlying pairs of BCTs and NPDs. We conducted GSEA for sets of candidate genes identified using SMR, whose expression levels were Bonferroni-significant for both members of specific BCTNPD trait pairs. To maintain power and sensitivity, we focused on candidate gene sets comprising five or more genes, resulting in analysis of 11 gene sets, including those for four MS-BCT pairs and seven SCZ-BCT pairs (Table S50). We performed GSEA using the ShinyGO tool2 based on the Gene Ontology (GO) annotation resource, comprising a hierarchy of biological processes, cellular components and molecular functions. We identified 31 pathways containing ≥ 2 gene set members at an FDR < 0.05, including N = 5 for MS-EO#, N = 2 for MS-RDW, N = 5 for SCZ-LYMPH#, N = 12 for SCZ-MONO%, N = 3 for SCZ-NEUT%, N = 1 for SCZ-RET# and N = 3 for SCZ-RET% (Table S51). However, whereas the minimum gene set enrichment for these pathway terms was > 5-fold, no single gene set had a proportion of pathway-specific genes > 0.05. For this reason, we have interpreted the results with caution and note that larger studies will be needed for robust inference of biological pathways shared by specific pairs of BCTs and NPDs. We have added the following text to the revised manuscript:

Results (page 11, line 26 to page 12, line 2):
"Lastly, we conducted gene set enrichment analysis (GSEA) using ShinyGO41 to identify biological pathways shared between specific pairs of BCTs and NPDs. We focused on 11 trait pairs with ≥ 5 shared genes identified using SMR applied to blood-based or brain-based cis-eQTLs (Table S50). We identified 31 pathways containing ≥ 2 gene set members at an FDR < 0.05, including N = 5 for MS-EO#, N = 2 for MS-RDW, N = 5 for SCZ-LYMPH#, N = 12 for SCZ-MONO%, N = 3 for SCZ-NEUT%, N = 1 for SCZ-RET# and N = 3 for SCZ-RET% (Table S51). However, whereas the minimum gene set enrichment for these pathway terms was > 5-fold, the highest proportion of pathway-specific genes was only 0.04, for tau protein binding in SCZ and MONO% (2 of 45 pathway genes, 338-fold enrichment, FDR p-value = 4.68Å~10-4)."

Discussion (page 17, line 23-32):
"The application of gene set enrichment analysis to Bonferroni significant genes identified through SMR identified multiple FDR-significant biological pathways that were shared by specific pairs of BCTs and NPDs. The top-ranked pathway was tau protein binding for SCZ-MONO%, which is notable given that aberrantly phosphorylated tau protein has been associated with risk for SCZ72, and there is evidence that monocyte-derived macrophages play an important role in phagocytosing extracellular oligomeric tau protein73. Although this observation has potential therapeutic implications, an important caveat is that the proportion of pathway-specific genes was small (< 5%). This was also the case for other FDR-significant pathways identified by GSEA, which suggests that larger studies will be needed for robust inference of biological pathways shared by specific pairs of BCTs and NPDs."

STAR Methods (page 29, line 22-30):

"Gene set enrichment analysis (GSEA)
Finally, we performed GSEA to identify biological pathways shared by specific pairs of BCTs and NPDs. We used the ShinyGO tool41 based on the Gene Ontology (GO) annotation resource, comprising a hierarchy of biological processes, cellular components and molecular functions. To maintain power and sensitivity, we applied ShinyGO to candidate gene sets comprising five or more genes whose expression levels were Bonferroni-significant for both members of specific pairs of BCTs and NPDs, based on SMR analysis of blood-based and/or brain-based cis-eQTLs (Table S50). We defined significantly enriched pathways as those with ≥ 2 pathway-specific genes and an FDR < 5%."

5. I don't agree with calling the LCV method "MR equivalent" as it does not produce an exposure to outcome causal estimate. The underlying hypothesis of LCV is related to directional dependency as indexed by the fourth co-moments (cokurtosis), rather than instrumental variables. I think it would be better to explicitly state this difference in text and brief explain what the posterior GCP is.

We apologise for failing to make this concept clearer in our original submission. We no longer refer to LCV as an "MR-equivalent method", and we have clarified the definition of 'LCV' and 'GCP' in the relevant section of the Methods. The revised text now reads (page 26, line 20-26):

"Furthermore, we inferred putative causal relationships between BCTs and NPDs using the LCV (latent causal variable) model24. The LCV method assumes that the genetic correlation between two traits is mediated by a latent variable, which has a causal effect on each trait and can be quantified by estimating the GCP (genetic causality proportion) using the mixed fourth moments of marginal SNP effect sizes for each trait. GCP estimates vary between 0 (no causal relationship) and 1 (fully causal relationship), with higher values implying a stronger partially causal relationship."

6. The authors should perform and report some sensitivity analyses for the MR results beyond just the MR methods with differing assumptions regarding IV validity. I recommend the following: testing whether the MR-Egger intercept is significantly non-zero to screen for confounding pleiotropy, testing heterogeneity between IVs, and a leave-one-out analysis with the MR estimate is iteratively recalculated upon holding out one of the IVs. If a leave-one-out analysis demonstrates outlier IVs, these should be investigated to see if they have biologically plausible effects on the blood cell trait.

We thank the Reviewer for these constructive suggestions, each of which we have now actioned for the two trait pairs with consistent evidence for a causal relationship (i.e., stroke and PCT and PD and PDW).

First, we examined the MR-Egger intercepts and found no evidence for a non-zero estimate in either of the (bidirectional) analyses (see Table R1 and Table S29), suggesting an absence of uncorrelated pleiotropy on these two putative causal relationships.

Table R1. Estimates of the intercept term in the MR-Egger regression

Exposure – Outcome	MR-Egger Intercept	SE	P-value
PCT – Stroke	-0.0018	0.0008	0.04
Stroke – PCT	-0.0069	0.0142	0.63
PDW – PD	-0.0017	0.0011	0.12
PD – PDW	0.0018	0.0016	0.27

Second, we performed leave-one-out analyses using each of the four two-sample MR methods. In all instances, there was no evidence that a single instrumental SNP was responsible for the inference of a causal relationship for either PCT-stroke or PDW-PD. These results are now provided in Table S30-33, and can be clearly seen in the Figure R2 (Figure S15), which shows the distribution of beta values

from leave-one-out analyses for each trait-pair and MR method.

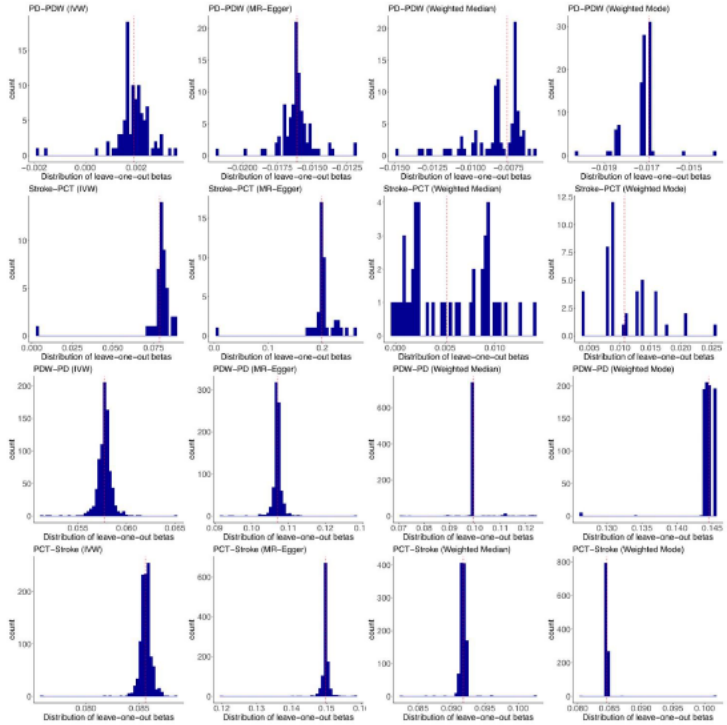

Figure R2. Histograms for estimated beta values from leave-one-out analyses assessing the impact of each instrumental SNP on the putative causal relationships between PCT-stroke and PDW-PD, using IVW, MR-Egger, weighted median, and weighted mode model, respectively. Plots are also shown for the reverse analyses (i.e., stroke-PCT and PD-PDW). Red dashed lines represent the estimated beta values from the original analyses based on all instrumental SNPs.

In contrast to the results described above, we did observe substantial heterogeneity among instrumental SNPs (quantified using Cochran's Q and I2) for both IVW and MR-Egger (see Table R2 and Table S29).

Table R2. Heterogeneity statistics for IVW and MR-Egger regression with and without removal of pleiotropic SNPs identified by GSMR

Exposure – Outcome	Model	All instrumental SNPs			Pleiotropic SNPs (identified by GSMR) removed		
		Q	I^2	P-value (Q)	Q	I^2	P-value (Q)
PCT – Stroke	IVW	1551	0.31	1.45×10^{-20}	856	0	1
	MR-Egger	1545	0.31	2.62×10^{-20}	855	0	1
Stroke – PCT	IVW	3292	0.99	0	40	0.22	0.14
	MR-Egger	3274	0.99	0	38	0.21	0.15
PDW – PD	IVW	1543	0.48	3.58×10^{-49}	733	0.03	0.29
	MR-Egger	1538	0.48	7.76×10^{-49}	729	0.02	0.32
PD – PDW	IVW	607	0.85	4.51×10^{-76}	109	0.27	0.02
	MR-Egger	599	0.84	4.88×10^{-75}	106	0.26	0.02

In principle this may be cause for concern, but there was no evidence for heterogeneity of SNP effects (i.e. non-significant Q and I2) after removing pleiotropic SNPs identified by GSMR (see Table R3 and Table S29), and estimated causal relationships for PCT-stroke and PDW-PD based on the filtered sub-set of instrumental SNPs were highly consistent with our original analyses (see Table below and Table S29). This was also the case for the other two-sample MR methods (i.e., Weighted Median, Weighted Mode).

Table R3. Estimated causal effects using two-sample MR models before and after the removal of pleiotropic SNPs identified by GSMR

Exposure – Outcome	Model	Original results		Results after the removal of pleiotropic SNPs identified by GSMR	
		Beta (SE)	P-value	Beta (SE)	P-value
PCT – Stroke	IVW	0.09 (0.0172)	6.32×10^{-7}	0.07 (0.0150)	4.38×10^{-6}
	MR-Egger	0.15 (0.0349)	1.96×10^{-5}	0.10 (0.0308)	2.06×10^{-3}
	Weighted Median	0.09 (0.0258)	5.28×10^{-4}	0.08 (0.0260)	2.42×10^{-3}
	Weighted Mode	0.08 (0.0425)	0.049	0.09 (0.0423)	0.041
Stroke – PCT	IVW	0.08 (0.0596)	0.19	0.02 (0.0092)	0.07
	MR-Egger	0.20 (0.2572)	0.44	-0.02 (0.0352)	0.61
	Weighted Median	0.01 (0.0122)	0.68	0.02 (0.0117)	0.13
	Weighted Mode	0.01 (0.0213)	0.64	-0.01 (0.0266)	0.79
PDW – PD	IVW	0.06 (0.0211)	6.13×10^{-3}	0.05 (0.0161)	4.85×10^{-3}
	MR-Egger	0.11 (0.0382)	5.23×10^{-3}	0.10 (0.0290)	1.03×10^{-3}
	Weighted Median	0.10 (0.0289)	7.08×10^{-4}	0.07 (0.0296)	0.01
	Weighted Mode	0.14 (0.0410)	1.87×10^{-4}	0.14 (0.0384)	3.47×10^{-4}
PD – PDW	IVW	0.00 (0.0075)	0.80	-0.01 (0.0037)	0.08
	MR-Egger	-0.02 (0.0180)	0.37	-0.02 (0.0094)	0.04
	Weighted Median	-0.01 (0.0050)	0.12	-0.02 (0.0050)	1.91×10^{-3}
	Weighted Mode	-0.02 (0.0077)	0.02	-0.02 (0.0084)	0.01

Considering the results from all three MR sensitivity analyses together, we are confident that the evidence supporting causality of PCT on risk for stroke and PDW on risk for PD is robust. These results are now available in the revised Figure S15 and Table S29-33. We have also updated the relevant sections throughout the revised manuscript, which now read:
Results (page 8, line 15-28):

"To further evaluate the reliability of the inferred causal effects of PCT on stroke and PDW on PD, we performed several additional sensitivity analyses. We first checked the MR-Egger intercept terms in each analysis, confirming there was no evidence for non-zero estimates, and thus no indication that the MR-Egger causal estimates were confounded by pleiotropy (Table S29). Second, we performed leave-one-out analyses for each trait pair using each of the four two-sample MR methods (inverse variance weighting [IVW], MREgger, weighted median [WMe], weighted mode [WMo]). In all instances, there was no evidence that any single instrumental SNP was responsible for the inference of a causal relationship for either PCT-stroke or PDW-PD (Figure S15, Tables S30-33). Third, we checked for heterogeneity of instrumental SNP effects in the IVW and MR-Egger analyses, and although there was evidence for heterogeneity, after removing pleiotropic SNPs identified by generalised summary-data-based Mendelian randomisation (GSMR), the causal estimates from IVW and MR-Egger for PCT-stroke and PDW-PD remained significant and highly consistent with the original estimates (Table S29)."

STAR Methods (page 27, line 8-15):
"To evaluate the reliability of our MR results, we implemented several additional sensitivity analyses to evaluate the validity of instrumental SNPs for pairs of traits with consistent evidence for a causal relationship across all MR models103. The sensitivity analyses included: (i) checking whether the intercept term in MREgger regression is significantly different from zero; (ii) checking for heterogeneity among instrumental SNPs using Cochran's Q and I2; and (iii) performing leave-one-out analyses using each of the four two-sample MR models to evaluate if single instrumental SNPs may be responsible for the inferred causal relationship(s). MR results satisfying all three sensitivity analyses were considered robust."

Minor Comments:
1. When reporting the genetic correlation estimates, I think it is a good idea to also report the standard error. Even though the P-value encapsulates this information statistically, it is easier for the reader to visualise precision when they see an estimate and its corresponding standard error.

We thank the Reviewer for this suggestion. We agree and have added the standard error for each genetic correlation estimate throughout the revised manuscript and supplementary notes.

2. Like the above, can the authors please report the 95% confidence intervals for all odds ratios. It

would be useful to also define what the unit of the exposures are such that the effect size is better conceptualised, e.g are the blood cell properties in standard deviation units, or are they log transformed, etc. Please also report the posterior standard error and P value for the posterior mean GCP estimates where these are reported in text.

We thank the Reviewer for this helpful suggestion. We have now added 95% confidence intervals for all MR odds ratios, and posterior standard errors and p-values for all LCV GCP estimates throughout the revised manuscript and supplementary notes. We have also updated both the Methods (page 26, line 1-5) and Results (page 7, line 1-4) to clarify the interpretation of effect sizes in the MR analyses.

Results (page 7, line 7-10):
"We identified three Bonferroni-significant (p < 3.57Å~10-4, i.e., 0.05 70×2) causal relationships (increased LYMPH# on risk for MS: odds ratio [ORCAUSE] = 1.20 [i.e., a 1.2-fold increase in risk for MS for each standard deviation increase in LYMPH#], 95% CI = 1.11-1.31, p = 3.94Å~10-5; …".

STAR Methods (page 27, line 2-6):
"The interpretation of the OR for "BCT → NPD" is that, as an example, if the estimated OR is 1.2, the NPD risk is increased by 1.2-fold for each standard deviation increase in the BCT. Similarly, the interpretation of the OR for "NPD → BCT" at 1.2 (as an example) is that the level of BCT is increased by 1.2-fold per standard deviation increase in NPD liability."

3. Were there genes implicated in the SMR analysis where the effect of expression on either trait was discordant than the direction of the overall genetic correlation or local covariance? For example, if the two traits were positively correlated, but increased expression of a gene is associated with an increase in one trait but a decrease in the other.

We thank the Reviewer for this interesting question. We did identify a small number of genes where the effect of expression on the two respective BCT and NPD traits was discordant in comparison to their genome-wide genetic correlation. However, in all cases, the effect of expression of these genes on the two traits was consistent with the local genetic correlation. For example, down-regulation of the BACH2 gene was associated with increased MS risk and reduced LYMPH%, and whereas the genetic correlation between MS and LYMPH% is positive (rg = 0.04, se = 0.02, p = 3.13Å~10-2), their local genetic correlation in the relevant genomic region (chromosome 6: 89973052–91843196) was estimated to be -0.79 (se = 0.28; p = 5.81Å~10-3). A related comment was made by Reviewer #2 (see below), in response to which we made the following changes to the Results:

Page 11, line 6-11:
"In addition, for all 12 genes that were Bonferroni-significant for both members of specific BCT-NPD trait pairs across the three tiers of SMR analyses, the effect of expression on the two traits was concordant with their local genetic correlations (N = 13; Table S43). Additionally, a high proportion (10/13) of these local genetic correlations surpassed a 5% nominal significance threshold, indicating a high degree of consistency between SMR and □-HESS in relation to shared genetic risk factors."

Page 11, line 22-24:
"In each case, the expression effects of these genes on specific pairs of BCTs and NPDs were consistent with their respective local genetic correlations (Table S44)."

4. Discussion: It may be worth mentioning when comparing the results to Reay et al. explicitly that the schizophrenia GWAS in the Reay et al. study was the most recent schizophrenia GWAS, whereas this study used in the 2014 GWAS. Conversely, this study used a larger blood-cell GWAS than that study.

We thank the Reviewer for this suggestion. In addressing this comment, we realised that we had inadvertently referenced the 2014 PGC SCZ GWAS when in fact the dataset used in our analyses was a pre-publication release (Ntotal = 123,575; Ncase = 51,900, Ncontrol = 71,675;) of the complete PGC3 SCZ GWAS (Ntotal = 130,644; Ncase = 53,386, Ncontrol = 77,258). We have now updated the SCZ GWAS citation from PGC SCZ2 (2014) to PGC SCZ3 (2022). Given that the sample size of the SCZ GWAS used in our study is highly similar to the full PGC3 SCZ GWAS used by Reay et al., we believe that any resulting difference in inference is likely to be negligible. Nonetheless, we have now amended the Discussion to acknowledge this possibility (page 13, line 16-23):

"Interestingly, we failed to replicate some previously reported positive genetic correlations between attention deficit hyperactivity disorder (ADHD)-RET%, MDD-WBC and SCZ-LYMPH#6, potentially due to the utilisation of different GWAS summary statistics for some psychiatric disorders (e.g., a subset of the European-based PGC3 SCZ GWAS [N = 123,575] used in this study versus complete PGC3 SCZ GWAS [N = 130,644] in Reay et al.) and BCTs (i.e., our BCT GWAS were obtained from Vuckovic et al.43 whereas the previous study used the publicly-available blood-based biomarker GWAS from http://www.nealelab.is/uk-biobank), …".

Reviewer 2

Yang and colleagues investigate the pleiotropy linking blood cell traits and neuropsychiatric disorders. This is surely an interesting aim and the authors applied a wide range of methods to investigate the genetic basis of the observed comorbidities.

We thank the Reviewer for taking the time to review our manuscript and for acknowledging the relevance of our contribution to the field.

1. Although the methods are appropriate, I believe the authors missed to address a key point. Likely, factors such as smoking, drinking, educational attainment, and socioeconomic status play a key role in the genetic correlation and causal effects identified. Accordingly, I strongly recommend the authors apply methods based on multivariable statistics to understand whether the effects detected are independent of other variables correlated with the traits investigated.

We are grateful to the Reviewer for this insightful suggestion. We agree that it is important to investigate if the genetic correlations and putative causal relationships reported in our study are influenced by common exposures associated with risk for disease, including smoking, drinking, educational attainment and socioeconomic status.

To address the Reviewer's comment, we focused on exploring the influences of each risk factor on the four BCT-NPD trait pairs with Bonferroni-corrected or FDR significant HDL-based genetic correlations (i.e., MS and LYMPH#, MS and WBC, SCZ and MONO%, migraine and PLT#) and the two pairs of traits with putative causal relationships (i.e., PCT and stroke, PDW and PD). We first estimated the genetic correlations between each of these focal BCTs and NPDs and each risk factor using HDL. We identified moderate and statistically significant genetic correlations between some pairs of risk factors and BCTs or NPDs (see Figure R3 below), suggesting there is potential for specific risk factors to influence the genetic relationship between these BCT-NPD trait pairs.

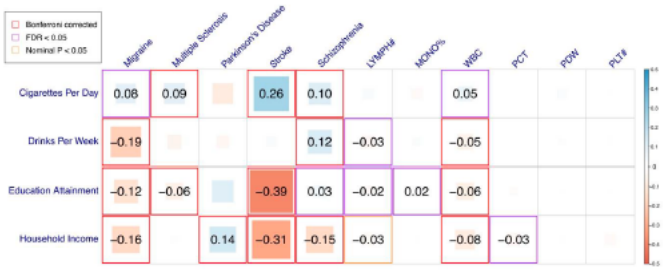

Figure R3. Estimated genetic correlations between risk factors (cigarettes per day, drinks per week, education attainment, household income) and focal BCTs and NPDs using the HDL method. Estimated genetic correlations are displayed for all trait pairs with HDL p-value < 0.05. Genetic correlations surpassing Bonferroni or FDR corrections are highlighted in red and purple, respectively.

Next, we used multi-trait-based conditional & joint association analysis (mtCOJO) to condition GWAS summary data of specific BCTs and NPDs on the GWAS summary data of each risk factor (i.e., cigarettes per day3, drinks per week3, educational attainment4, and household income5) separately. We used genotype data from unrelated Europeans in the UK Biobank as a reference. We then re-estimated the genetic correlations for each BCT-NPD trait pair by applying HDL to the conditioned GWAS summary statistics. These analyses revealed that genetic correlations based on the conditioned traits were highly consistent with the estimates from our original (unadjusted) analyses (see Figure below & Figure S5). This suggests that although there is evidence (see above) for genetic overlap between these risk factors and some of the focal BCTs and NPDs, they have negligible effects on the estimated genetic correlations between the six focal trait-pairs.

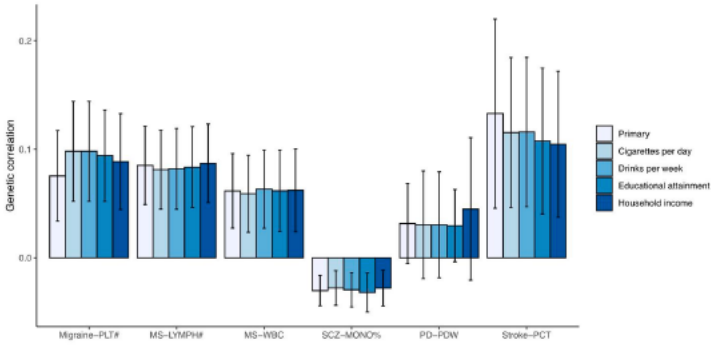

Figure R4. Summary of genetic correlations for specific BCT–NPD pairs estimated using HDL, comparing estimates from our primary analyses to those obtained after conditioning GWAS summary data of specific BCTs and NPDs on the GWAS of each of four risk factors (i.e., cigarettes per day, drinks per week, educational attainment, and household income). BCT–NPD pairs were included if they had a Bonferroni- or FDR-significant genetic correlation (i.e., MS and LYMPH#, MS and WBC, SCZ and MONO%, migraine and PLT#) or a putative causal relationship (i.e., stroke and PCT, PD and PDW). Error bars represent the 95% CIs for the estimated genetic correlations.

We then repeated each of the Mendelian randomization analyses (i.e., CAUSE, GSMR, IVW, MREgger, Weighted Median [WMe], Weighted Mode [WMo]) and LCV using the conditioned GWAS summary statistics for PCT and stroke, and PDW and PD generated by mtCOJO. These conditional MR (and LCV) analyses were highly consistent with the results from our primary analyses, irrespective of the analysis method employed and risk factor conditioned on (see Figure R5, Figure S16 & Table S34).

Finally, we applied the multivariable MR (MVMR) analysis method to PCT-stroke and PDW-PD, adjusting for potential pleiotropic effects of all four risk factors concurrently (see Table R4 & Table S35). Again, these analyses were highly consistent with the causal inference from our primary analyses, supporting unidirectional causal effects of PCT on stroke and PDW on PD.

These results, in combination with the conditional MR (and LCV) and HDL results strongly suggest that our conclusions are robust, and that both genetic correlations and causal effects reported in our study are independent of cigarettes per day, drinks per week, educational attainment and household income.

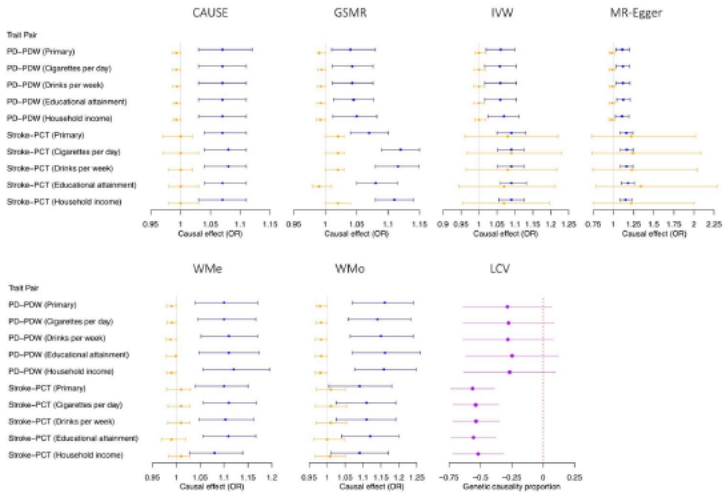

Figure R5. Summary of the putative causal relationships between PD and PDW as well as between stroke and PCT after adjusting for the effects of cigarettes per day, drinks per week, educational attainment, and household income, respectively. Results coloured in blue represent the estimated causal effect of BCTs on NPDs, while results coloured in orange represent the estimated causal effect of NPDs on BCTs. Error bars for MR methods represent 95% CIs and those for LCV-based GCP point estimates represent standard errors. For LCV, a negative GCP indicates a causal effect of BCT on NPD, and *vice versa*.

Table R4. Summary of MVMR results after adjusting both exposure and outcome traits for cigarettes per day, drinks per week, educational attainment and household income conjointly.

Exposure – Outcome	No. SNP	OR	95% CI	P-value
PCT – Stroke	1027	1.09	1.05-1.13	3.79×10^{-7}
Stroke – PCT	40	1.12	0.98-1.27	0.09
PDW – PD	798	1.05	1.01-1.10	0.01
PD – PDW	87	1.00	0.98-1.02	0.97

We have incorporated these new results into relevant sections of the revised manuscript, and added Figure S5 & S16 and Tables S5, S34 and S35. These changes have greatly strengthened the manuscript, and we thank the Reviewer again for bringing this issue to our attention.

Results:

Page 5, line 10-16:

"We then re-estimated each of these four genetic correlations after using multi-trait-based conditional & joint analysis (mtCOJO)23 to condition each trait on each of four potential confounding factors, including cigarettes per day30, drinks per week30, educational attainment31, and household income32. The rg estimates from these conditional HDL analyses were highly consistent with our original estimates, suggesting that these confounding factors have negligible effects on the shared genetics underlying the focal pairs of BCTs and NPDs (Figure S5 & Table S5)."

Page 8, line 30 to page 9, line 7:

"As a final sensitivity analysis, we explored if the causal effects of PCT on stroke and PDW on PD were influenced by common environmental factors associated with disease risk, including smoking30, alcohol consumption30, educational attainment31 and socioeconomic status32. We conditioned GWAS statistics for PCT, stroke, PDW and PD on each potential cofounder using mtCOJO and then repeated each of the MR analyses (i.e., CAUSE, GSMR, IVW, MR-Egger, WMe, WMo) and LCV using the conditioned GWAS summary statistics (see Methods). These conditional MR (and LCV) analyses were all highly

consistent with our primary results (Figure S16, Tables S34). We also applied multivariable MR (MVMR)36 analysis to PCT-stroke and PDW-PD, adjusting for potential pleiotropic effects of all four confounders concurrently. Again, these analyses were highly consistent with the causal inference from our primary analyses (Tables S35), further supporting unidirectional causal effects of PCT on stroke and PDW on PD."

STAR Methods:
Page 23, line 28 to page 24, line 6:
"Investigating potential confounding factors mediating the shared genetics underlying pairs of traits with significant genetic correlations For BCT-NPD trait pairs with Bonferroni-corrected or FDR significant genome-wide rg (from HDL or LDSC), we investigated whether their shared genetics were driven by potential confounding factors, including smoking, drinking, educational attainment and socioeconomic status, that have been reported to be commonly associated with BCTs7,95,96 and NPDs97-100. We investigated the roles of these potential confounding factors using a conditional approach. First, we utilised mtCOJO23 to condition GWAS summary data of BCTs and NPDs on the GWAS of cigarettes per day30, drinks per week30, educational attainment31, and household income32, respectively. We used genotype data from unrelated Europeans in the UK Biobank as a reference. We then re-estimated the genetic correlations between specific pairs of BCTs and NPDs on the basis of their conditional GWAS summary statistics, using HDL."

Page 27, line 17-25:
"Finally, we investigated the contribution of four potential confounding factors (i.e., cigarettes per day30, drinks per week30, educational attainment31, household income32) on estimates of inferred causality between specific pairs of BCTs and NPDs, using two approaches. First, we re-estimated the causal effects for each pair of traits with consistent evidence for a causal relationship by applying the same MR methods (and LCV) to their conditional GWAS summary statistics, generated using mtCOJO. Second, we applied MVMR36 analysis to each BCT-NPD trait pair with consistent evidence for a causal relationship. The MVMR method is capable of estimating causal effects between an exposure and outcome whilst adjusting for the potential pleiotropic effects of multiple outcome-related confounding factors concurrently."

2. Additionally, the results are separately for each method used. This makes it hard to understand the consistency among the findings observed across methods. For example, it would be interesting to understand the intersection between the findings of the local genetic correlation and SMR analyses.

We thank the Reviewer for this suggestion. We have updated Figure 1 to provide a better description of the key analyses in our study, and how they are related. We have also added additional text to the Results to provide further explanation of the intersection between the SMR findings and □-HESS results:

Page 11, line 6-11:
"In addition, for all 12 genes that were Bonferroni-significant for both members of specific BCT-NPD trait pairs across the three tiers of SMR analyses, the effect of expression on the two traits was concordant with their local genetic correlations (N = 13; Table S43). Additionally, a high proportion (10/13) of these local genetic correlations surpassed a 5% nominal significance threshold, indicating a high degree of consistency between SMR and □-HESS in relation to shared genetic risk factors."

Page 11, line 22-24:
"In each case, the expression effects of these genes on specific pairs of BCTs and NPDs were consistent with their respective local genetic correlations (Table S44)."

3. Also, some of the statistics are mentioned in the main text, but the actual numbers are only reported

in the supplemental material. I suggest providing key statistics also in the main text.

We thank the Reviewer for this suggestion, which has helped us to more clearly articulate our findings. As noted in our response to Reviewer #1, we have provided all key statistics (including point estimates, standard errors, p-values, 95% CIs) for all BCT-NPD trait pairs with significant genetic correlations and/or causal relationships throughout the manuscript. We have also updated Figure 5 (originally Figure 6) summarising the SMR analyses, to include all significant genes associated with both members of BCT-NPD trait pairs, including those with significant genome-wide genetic correlations and in regions of significant local genetic correlation for trait pairs with negligible genome-wide genetic correlations.

References

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2. Ge, S.X., Jung, D. & Yao, R. ShinyGO: a graphical gene-set enrichment tool for animals and plants. Bioinformatics 36, 2628-2629 (2020).
3. Liu, M. et al. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. Nat Genet 51, 237-244 (2019).
4. Okbay, A. et al. Polygenic prediction of educational attainment within and between families from genome-wide association analyses in 3 million individuals. Nat Genet 54, 437-449 (2022).
5. Hill, W.D. et al. Genome-wide analysis identifies molecular systems and 149 genetic loci associated with income. Nat Commun 10, 5741 (2019).

Referees' report, second round of review

Reviewer #1: I thank the authors for taking the time to thoroughly respond to my feedback and comments. As a reviewer, it is much appreciated when feedback is taken onboard and implemented so clearly. I'm happy now to recommend this paper be accepted for publication.

Reviewer #2: The authors fully addressed my previous concerns.

Authors' response to the second round of review

We thank both Reviewers for taking the time to review our manuscript, and for their valuable and constructive feedback which has greatly improved our paper.