nature portfolio

Peer Review File

Edge-centric connectome-genetic markers of bridging factor to comorbidity between depression and anxiety

Corresponding Author: Professor Zhiyi Chen

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Chen and colleagues clearly presented sophisticated but intriguing findings regarding the connectome-transcriptomic biomarkers of a general bridging factor for comorbidity between depression and anxiety. As they recapitulated, this study proposes a novel conceptual framework theorizing the psychopathological mechanisms underlying the comorbidity of depression and anxiety by identifying a general structure that bridges symptoms, rather than focusing on hub or overlapping symptoms. This approach addresses ongoing theoretical challenges associated with both the common factor hypothesis and the network hypothesis in psychopathological systems.

Moreover, these structurally and systematically genetic-neuroimaging analyses were well conducted to substantiate neurobiological substrates of this conceptualized cb factor from the edge-centric CPM, eFC-based RSA and connectome-transcriptomic decoding models, powerfully enriching our understanding of neurobiological pathways of depression-anxiety comorbidity. Overall, this study sounds solid in both theory and methodology. Here are some questions that I do remain for improving its clarity and result robustness.

1) Theoretically specking, the main challenge the p factor theory is facing narrowed into cross-cultural or cross-cohort heterogeneity. I am very glad to see this eCPM could generalize prediction capability for cross-race validation, but concerned whether this cb factor varied from populations per se. For example, would these cb factor scores be differed from races (e.g., minority vs majority), genders (male vs female) or environment (e.g., post-covid-19 vs pre-covid-19)?

2) The edge-centric FC features indeed offer new and welcomed insights to understand neuropathological understructure of psychiatric comorbidity, but a robust body of studies have ever conducted to disentangle rFC changes of depression-anxiety comorbidity. At the introduction sec tion, such research progresses should be added.

3) There are some discrepancies between Results section and Methods section for the descriptions on subsampling. Line. 177 reported that the subsamples were derived from population-based demographics, but this sample split was conducted basing on the data collectors? Please clarify exactly and clearly how to set these samples in the CPM training, validation and testing.

4) For the results section, despite high SE values, the exact values should be added in the main texts to clarify relative differences of these bridging symptoms

5) Biological enrichment analyses in these canonical genomics datasets have extended comprehensions of genetic substrates of the cb factor from intermediate phenotype to microscale and cellular associations. However, rapid advances in single-cell spatial sequencing datasets (Annotation of Cell Types, ACT, http://xteam.xbio.top/ACT/; Single-cell genomics and regulatory networks dataset, 10.1126/science.adi5199) provided more multiscale and multidimensional insights into genetic biological annotations, particularly in the cell type enrichment. Such new findings could be added into this study enriching neurobiological knowledge of genetic processes of the cb factor.

Here are some minor typesetting and text advice
1) Line 490, “a total” should be changed to “a total of”
2) Line 517, removing “inflating false-positive rates.”

3) In the main texts, the “cb” was underlined in somewhere, without notes to indicate why did it.
In the SI, some texts are colored by light blue, withou

Reviewer #2

(Remarks to the Author)
Review Comments to NCOMMS-24-34272

The authors leveraged cutting-edge neuroimaging techniques, specifically edge-centric brain functional dynamics, to propose a new theoretical framework explaining the comorbidity between depression and anxiety (referred to as the “cb factor”) using large-scale samples and an independent longitudinal twin neuroimaging cohort. By establishing a novel connectome-based computational framework (eCPM), the authors demonstrated the significant predictive roles of edge functional connectivity (eFCs) for the cb factor, with strong validation of their generalizability. Furthermore, they identified the genetic neural substrates of the cb factor by demonstrating moderate heritability of eFCs through brain-behavior representation similarity. By decomposing eFC features into multiscale transcriptome-imaging architectures, the authors substantially enhanced the biological interpretability, elucidating the molecular and cellular associations of connectome-transcriptome interactions with the cb-factor phenotype. Overall, this study is well-conceptualized and well-written, providing both theoretical and subclinical insights into the understanding of depression-anxiety comorbidity. I have several minor comments regarding the writing structure and presentation of the results:

- For the theoretical establishment, despite disclosing the core pitfall of single-dimensional structure of psychopathology of comorbidity (i.e., p factor), more evidence should be added substantiating why network-theory framework could outperform this “general factor” system. That is to say, it could be clarified for the potential pitfalls of other multidimensional theoretical framework.

- Establishing a new theoretical conception should be highly welcomed and applauded, and authors stood for clear points on the cb factor conceptualization. Nonetheless, are there some specific hypotheses to neurobiological markers of this cb factor?

- Why the authors use the eFC matrices to identify the neural substrates for cb factors? Could it be more predictive than traditional functional connectivity methods? Please provide additional rationale.

- Line 133-134, please clarify what specific differences between the univariate correlation and network-between correlation.

- At the Result section, the Figure 2a illustrated the geospatial distribution of sampling population, but seemed to appear for some unclear labels. For instance, at the within-figure texts, it showed “30 minority races” for the whole sample, but “29 minority races” was reported in the main texts. Furthermore, why a few number of areas are given for their name, such as H.K., Macau?

- It is quite helpful to examine specificity of eCPM by regressing to total scores of depression and anxiety and p factor, respectively. I would recommend to move forward such specificity analysis to depression-anxiety distances (e.g., Euclidean space), which mirror the extent to which depression symptoms comorbid with anxiety symptoms (vice versa).

- Downstream analysis to the connectome-transcriptomic genetic markers of cb factor indeed added promising neurobiological knowledge to depression-anxiety comorbidity from existing normative maps (e.g., SEA), enriching fruitful insights into the biomarkers of this comorbidity. - However, the GSEA has been critiqued for plain cell type data on humans. Given that, I recommend authors to stretch such biological function decoding results by using PanglaoDB, a latest cell-type dataset from large-scale single cell sequencing methods.

- At the conclusion section, line 403-405, I feel confused for the dataset that used for connectome-transcriptomic analysis a bit. The eFC-cb-factor markers were captured in this independent twin sample? If in this case, why not use bivariate ACE model to examine rg. If it is not, please clarify this point.

- There are a few typos in the figure texts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The authors have addressed my previous comments, and I recommend publishing the paper.

Reviewer #2

(Remarks to the Author)
I have reviewed the response from the reviewers and am satisfied with their response. I have no further comments to add at this time.

August 20, 2024

RE: Decision on Nature Communications manuscript NCOMMS-24-34272

Dear esteemed reviewers,

We do appreciate you for taking valuable time to review our manuscript titled "**Edge-centric connectome-genetic markers of bridging factor to comorbidity between depression and anxiety**", and are very thankful to you for kindly sharing these quite helpful and insightful suggestions to improve the writing clarity and the neuroimaging-transcriptomic association robustness in this study. We highly valued and carefully read these comments, and thoroughly revised this manuscript by following your comments, one-by-one, particularly where we strengthened justifications of leveraging eFC over traditional nFC, deepened analyses to neurobiological decoding by cutting-edge single cell sequencing methods, and clarified methodological details in the subsampling.

We are very glad to see that this manuscript has been substantially improved by addressing all of these great points that both you shared. Again, we cannot thank you more enough for sharing these helpful comments, and sincerely hope this revised manuscript could be judged suitable for publication on *Nature Communications* now. Please see full details for what we have revised to address these comments in this **Author's Response Letter** underneath.

Many thanks and looking forward to hear from you for further suggestions (if any).

Best and warm regards,

Shaozheng Qin

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Experimental Research Center for Medical and Psychological Science, School of Psychology, Third Military Medical University;

Contents

Response format: original comments in black color**, response in** blue color**; new texts added to the revised manuscript or supplement in blue color and bold**

Note: unpredictable changes to the line numbers may occur in converting manuscript file in the submission system, causing mismatch to the line numbers we provided in this ARL

Reviewer #1

Chen and colleagues clearly presented sophisticated but intriguing findings regarding the connectome-transcriptomic biomarkers of a general bridging factor for comorbidity between depression and anxiety. As they recapitulated, this study proposes a novel conceptual framework theorizing the psychopathological mechanisms underlying the comorbidity of depression and anxiety by identifying a general structure that bridges symptoms, rather than focusing on hub or overlapping symptoms. This approach addresses ongoing theoretical challenges associated with both the common factor hypothesis and the network hypothesis in psychopathological systems.

Response: We do appreciate you for this clear and accurate summary to our manuscript.

Moreover, these structurally and systematically genetic-neuroimaging analyses were well conducted to substantiate neurobiological substrates of this conceptualized cb factor from the edge-centric CPM, eFC-based RSA and connectome-transcriptomic decoding models, powerfully enriching our understanding of neurobiological pathways of depression-anxiety comorbidity. Overall, this study sounds solid in both theory and methodology. Here are some questions that I do remain for improving its clarity and result robustness.

Response: Many thanks to you for taking valuable time to kindly comment our manuscript, and put such positive evaluations. We do appreciate these helpful and constructive advice on strengthening the writing clarity and robustness of these findings. Please see details underneath for what we have done to address these crucial points that you raised, one-by-one. Please do let us know if there are further queries that we need to clarify.

1) Theoretically specking, the main challenge the p factor theory is facing narrowed into cross-cultural or cross-cohort heterogeneity. I am very glad to see this eCPM could generalize prediction capability for cross-race validation, but concerned whether this cb factor varied from populations per se. For example, would these cb factor scores be differed from races (e.g., minority vs majority), genders (male vs female) or environment (e.g., post-covid-19 vs pre-covid-19)?

Response: We greatly appreciate you for this insightful advice solidifying theoretical foundation of establishing this common bridging factor of comorbidity between depression and anxiety (*cb* factor). We fully concur with you for this strong point that one of the long-lasting challenges in such factorial theories is that the factor scores may vary from populations per se. As you kindly suggested, for the *cb* factor scores, we have estimated between-group differences on ethnicity (i.e., minority *vs.* majority), sex (i.e., males *vs.*

females) and environmental change (i.e., pre-pandemic *vs.* post-pandemic). As expected, by calculating Jeffreys-Zellner-Siow Bayesian factor (BF_{10}) evidence strengths (i.e., > 3 indicated strong evidence supporting between-group differences), we found weakly statistical evidences supporting between-group variations on ethnicity (BF_{10} = 0.2), sex (BF_{10} = 2.7) and pandemic periods (BF_{10} = 0.1), respectively, substantiating measure invariances of *cb* factor on populations per se. Thus, there were no significant across-population variations in the *cb* factor structure.

Again, many thanks to you for this very helpful comment, and we have added full results regarding between-population invariances into the Supplemental Results for evidencing the robustness of the *cb* factor structure across populations per se. Please see details underneath.

Supplemental Results Section (Page. 14, Supplementary Tab. S7)

"To ensure the measure invariance across populations, we have capitalized on the Jeffreys-Zellner-Siow Bayesian factor (BF_{10}) statistics with aprior Cauchy distribution for estimating between-group variations of the *cb* factor scores on sex (male vs. female), ethnicity (majority vs. minority) and pandemic periods (pre-pandemic vs. post-pandemic), respectively. Here, the strong posterior evidences to support between-group differences on populations were mathematically quantified as BF_{10} > 3. Results showed the weak Bayesian evidences to support significant between-group variations for *cb* factor scores, including sex (BF_{10} = 2.7), ethnicity (BF_{10} = 0.2) and pandemic periods (BF_{10} = 0.1), respectively, which demonstrated no prominent across-population variations in this conceptualized metric. Full results have been tabulated into the Tab S7.

Population variables	BF_{10}	95% Credible Interval	Median	Error, %
Sex	2.680	0.039 - 0.222	0.131	0.008
Ethnicity	0.163	-0.049-0.183	0.067	0.163
Pandemic periods	0.094	-0.095-0.178	0.041	0.207

Tab S7. Bayesian factor evidence strengths of population-based variances for the *cb* factor. BF_{10} indicated the strength of Bayesian evidence supporting alternative hypothesis than null hypothesis."

2) The edge-centric FC features indeed offer new and welcomed insights to understand neuropathological understructure of psychiatric comorbidity, but a robust body of studies have ever conducted to disentangle rFC changes of depression-anxiety comorbidity. At the introduction section, such research progresses should be added.

Response: We are wholeheartedly thankful to you for this helpful suggestion that adding knowledge of nFC-based neural substrates of depression-anxiety comorbidity could benefit

to clarify the research background of measuring the edge-centric FC architecture in the present study. Following this advice, we have added more literature evidences to introduce what nFC neural patterns exactly are for characterizing comorbidity between depression and anxiety. These modifications have been specified underneath:

Introduction Section (Page. 2, Line. 76-82)

"... It was well-documented that the brain connectome-based features provided robust neurobiological markers to characterize the biotype of depression/anxiety (even in comorbid conditions)[30-32], especially in comparing to regional change in specific region or plain neural circuits[33-35]. Specifically, the amygdala-modulated downstream rFC-connectomes (e.g., regions of limbic networks) were consistently captured as cross-disorder diagnostic markers for patients who comorbid with depression and anxiety [36,37]. Moreover, by synthesizing from numerous meta-analytic evidences, the rFC-wise abnormalities in the default mode network and frontoparietal network have been identified as domain-specific biomarkers predicting depression-anxiety comorbidity, showing decreased intra-connections in these brain networks when anxious and depressive symptoms co-occurred [38,39]. ..."

References in this response

Brandl, F., Weise, B., Mulej Bratec, S., Jassim, N., Hoffmann Ayala, D., Bertram, T., Ploner, M., & Sorg, C. (2022). Common and specific large-scale brain changes in major depressive disorder, anxiety disorders, and chronic pain: a transdiagnostic multimodal meta-analysis of structural and functional MRI studies. Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology, 47(5), 1071 – 1080. https://doi.org/10.1038/s41386-022-01271-y

Briley, P. M., Webster, L., Boutry, C., Cottam, W. J., Auer, D. P., Liddle, P. F., & Morriss, R. (2022). Resting-state functional connectivity correlates of anxiety co-morbidity in major depressive disorder. Neuroscience and biobehavioral reviews, 138, 104701. https://doi.org/10.1016/j.neubiorev.2022.104701

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3) There are some discrepancies between Results section and Methods section for the descriptions on subsampling. Line. 177 reported that the subsamples were derived from

population-based demographics, but this sample split was conducted basing on the data collectors? Please clarify exactly and clearly how to set these samples in the CPM training, validation and testing.

Response: We truly apologize for those vague descriptions that had confused you to understand the subsampling processes. There are no actual discrepancies to generate subsamples when describing in the Method and Result section. As we reported in the Methods section and Data availability statement, the whole sample (n = 2,020) was curated by a large-scale research consortium titled "GGBBP", with inclusions of multiple independent research teams (data collectors) in the same neuroimaging site. To keep independence of generating discovery, validation and generalization samples, we picked up 241, 240 and 244 participants from the original sample, respectively, for forming three subgroups (i.e., Discovery dataset, n = 241; Validation dataset n = 240; Generalization dataset, n = 244), because they are derived from three distinct research teams (data collectors).

To strictly examine the generalizability of this eCPM prediction model, we additionally screened remaining participants from the whole sample for generating three independent generalization samples, which are highly heterogeneous compared to the training sample: the generalization sample 1 included 133 participants who derived from 29 ethnic minorities in the Chinese population; the generalization sample 2 contained 237 participants who all came from ethnic majority in the Chinese population; the generalization sample 3 enrolled 219 participants who were scanned after the covid-19 pandemic but were without infection history. All the participants are completely independent to be modeled in training, validation and generalization.

To clarify this subsampling strategy, we have added more descriptions in the Results section, and offered full details at the Methods section. Please see below for more details.

Results Section (Page. 5, Line. 201-210)

"... Here, to guard against data leakage risks[61], we used external validations to evaluate prediction performances of this model, rather in-sample k-fold cross-validation method. We split original sample into three independent subsamples for model training (one discovery sample, N = 241) and performance evaluation (one external validation sample (N = 240) and one external generalization sample (N = 244)), because these three subsamples were independently curated from three distinct research teams (see Methods). Based on the population characteristics (e.g., ethnic groups, covid-19 exposure), the remaining participants in the original sample were grouped into three independent generalization samples to rigorously examine model generalizability (see Methods)."

Methods Section (Page. 12-13, Line. 523-534)

"... In the present analysis, these participants in the original sample were divided into six

groups beforehand for model training, validation and generalizations. A total of 724 participants in the original sample were grouped into three independent samples as they were independently recruited from three distinct teams in this data project, including discovery sample 1 (N = 241, used for training this model), validation sample 2 (N = 240, used for validating prediction performance of this trained model) and generalization sample 3 (N = 244, used for testing generalizability of this trained model). For rigorously examining generalizability of this eCPM, we generated three independent samples from remaining participants in the original sample, which were highly heterogeneous compared to discovery sample that used to train this model (generalization sample 4, N = 133, derived from 29 ethnic minorities in the Chinese population; generalization sample 5, N = 237, derived from ethnic majority; generalization sample 6, N = 219, scanned after the covid-19 pandemic)."

4) For the results section, despite high SE values, the exact values should be added in the main texts to clarify relative differences of these bridging symptoms.

Response: Many thanks to you for coming up with this great advice strengthening results clarity. Following this helpful suggestion, the exact values of SE have been added in the main texts, which are perceived very helpful for readers understanding relative changes of SE on each bridging symptom. Please see specific modifications underneath:

Results Section (Page. 4, Line. 166-172)

"... To address the discrepancies arising from these varying metrics, we calculated the normalized Shannon's entropy ($SE_{normalized}$, Supplemental Methods 6), which quantified the likelihood of each bridging symptoms being identified as "bridge node" across these metrics. This analysis identified 12 bridging symptoms with significantly high SE values (all SE > 0.8; Supplemental Results 2, Tab. S9), such as "exhaustion" ($SE_{normalized}$ = 1.0), "meaningless life" ($SE_{normalized}$ = 1.0), "depressive feeling" ($SE_{normalized}$ = 0.98) and "psychomotor agitation" ($SE_{normalized}$ = 0.94) (Fig. 3d)."

5) Biological enrichment analyses in these canonical genomics datasets have extended comprehensions of genetic substrates of the cb factor from intermediate phenotype to microscale and cellular associations. However, rapid advances in single-cell spatial sequencing datasets (Annotation of Cell Types, ACT, http://xteam.xbio.top/ACT/; Single-cell genomics and regulatory networks dataset, 10.1126/science.adi5199) provided more multiscale and multidimensional insights into genetic biological annotations, particularly in the cell type enrichment. Such new findings could be added into this study enriching neurobiological knowledge of genetic processes of the cb factor.

Response: We truly express our gratitude to you for recommending us to stretch biological enrichment analyses by using these cutting-edge single-cell spatial sequencing methods. We found that the Annotation of Cell Types (ACT) could substantially enrich comprehension of cell-type enrichment, particularly in offering well-organized hierarchy map of enriched cell

types, mapping relationship between canonical markers and differently up-regulated genes, estimating the prevalence of canonical markers, and providing their visualization in integrative multiple organ scRNA-seq expression data of human.

Though we have indeed utilized this ACT model to analyze cell-type enrichment (species: Human; Tissue: All) as you suggested, we have found no more enriched annotations outside GSEA that we have previously identified in the original manuscript. This indicated that the cell-type enrichment of these the-*cb*-factor-specific gene sets may be all annotated. Results of this analysis could be found by referring this jobID (20240803193418N582BTWHD3KFQN; (http://xteam.xbio.top/ACT/ResultAction.action?jobID=20240803193418N582BTWHD3KFQ). We have offered all the details of reproducing these ACT analyses underneath in case you have interests of verifying or reproducing them:

Job Information
JobID: 20240803193418N582BTWHD3KFQN
Species: Human
Tissue: PanTissue,Adipose tissue,Adipose tissue of abdominal region,Subcutaneous adipose tissue,Adrenal gland,Bladder organ,Blood,Blood plasma,Blood vessel,Aorta,Artery,Coronary artery,Bone marrow,Brain,Forebrain,Telencephalon,Cerebral cortex,Breast,Bronchus,Calcareous tooth,Cartilage element,Intervertebral disk,Cartilage tissue,Cortex,Embryo,Fetal gonad,Esophagus,Esophagus mucosa,Eye,Cornea,Retina,Fallopian tube,Gut wall,Crypt of Lieberkuhn,Mucosa of small intestine,Mucosa of stomach,Epithelium of stomach,Gastric gland,Heart,Right cardiac atrium,Hindlimb stylopod,Intestine,Large intestine,Colon,Small intestine,Knee,Larynx,Liver,Lung,Alveolar system,Lymph node,Lymphoid tissue,Mammary gland,Manus,Mouth,Jaw region,Minor salivary gland,Mouth mucosa,Saliva-secreting gland,Major salivary gland,Tongue,Muscle organ,Nose,Nasal cavity mucosa,Nasal cavity epithelium,Ovary,Pancreas,Paranasal sinus,Penis,Skin of prepuce of penis,Peritoneum,Pes,Placenta,Skin of body,Nail,Spleen,Stomach,Tendon,Thymus,Trachea,Uterus,Endometrium,Uterine cervix,Vertebral column,Vertebra
GSEA: FALSE

As for the single-cell spatial sequencing datasets that you kindly indicated, we have accessed this very valuable databank titled "PsychEncode" that curated from 388 adult DLPFC samples (http://brainscope.psychencode.org/). This databank incorporates single-cell-resolution multi-omic data, including snRNA-Seq, snATAC-Seq, snMultiome, and genotype data. Unfortunately, this databank has not yet included phenotype of anxiety or anxiety-depression comorbidity, thus making this enrichment analysis not practically applicable and feasible. Another hurdle to preclude our additional analysis from this dataset is that it has narrowed single cell sequencing and omics data into the DLPFC alone, rather the whole-brain entity that we analyzed in the present study. Though we indeed found this state-of-the-art multi-omic databank very valuable, it may be not suitable and applicable in the present study.

Again, many thanks to you for offering these fancy and helpful venues to enhance our understanding of neurobiological processes of genetic markers of the *cb* factor. Despite not applicable in the present study, we do believe they would benefit for our studies in the future.

Here are some minor typesetting and text advice

1) Line 490, "a total" should be changed to "a total of"

Response: Many thanks to this writing advice. We have changed it.

2) Line 517, removing "inflating false-positive rates."

Response: We have removed it as you kindly suggested.

3) In the main texts, the "cb" was underlined in somewhere, without notes to indicate why did it. In the SI, some texts are colored by light blue, withou

Response: We are sorry to remain these redundant underlines and change markers in the main texts and SI, when formatting the manuscript from last round of review & revision. They had been removed.

Reviewer #2

The authors leveraged cutting-edge neuroimaging techniques, specifically edge-centric brain functional dynamics, to propose a new theoretical framework explaining the comorbidity between depression and anxiety (referred to as the "cb factor") using large-scale samples and an independent longitudinal twin neuroimaging cohort. By establishing a novel connectome-based computational framework (eCPM), the authors demonstrated the significant predictive roles of edge functional connectivity (eFCs) for the cb factor, with strong validation of their generalizability. Furthermore, they identified the genetic neural substrates of the cb factor by demonstrating moderate heritability of eFCs through brain-behavior representation similarity. By decomposing eFC features into multiscale transcriptome-imaging architectures, the authors substantially enhanced the biological interpretability, elucidating the molecular and cellular associations of connectome-transcriptome interactions with the cb-factor phenotype. Overall, this study is well-conceptualized and well-written, providing both theoretical and subclinical insights into the understanding of depression-anxiety comorbidity. I have several minor comments regarding the writing structure and presentation of the results:

Response: We are genuinely grateful to you for reviewing our manuscript, and put these compendious sum-ups, as well particularly thank you for such kind words and positive evaluations on the novelty and quality of the present manuscript. We have carefully studied, and further addressed all of these concerns that you kindly raised, one-by-one, especially in justifications of constructing edge-centric FC connectome and in the clarification of presenting figures. Please see the point-by-point response underneath.

- For the theoretical establishment, despite disclosing the core pitfall of single-dimensional structure of psychopathology of comorbidity (i.e., p factor), more evidence should be added substantiating why network-theory framework could outperform this "general factor" system. That is to say, it could be clarified for the potential pitfalls of other multidimensional theoretical framework.

Response: We do appreciate you for proposing this quite valuable suggestion to strengthen theoretical understructure of establishing single-factorial *cb* factor in re-conceptualizing depression-anxiety comorbidity. As you exactly commented, we have provided robust evidences justifying the merits of leveraging network theory in the transdiagnostics over traditional symptom-centered framework, but remained weaknesses on clarifying why the *cb*-factor structure surpassed multidimensional alternatives. Following this helpful suggestion, we have added more theoretical evidences in the Introduction section to clarify the main pitfalls of using multidimensional framework to interpret psychiatric comorbidity, particularly from the Hierarchical Taxonomy of Psychopathology (HiTOP) system and Research Domain Criteria (RDoC) framework. Please see specific contexts for what we have added in the Introduction section to address this point underneath:

Introduction Section (Page. 1-2, Line. 46-66)

"... Therefore, the sole *p* factor showed unstable structure in explaining comorbidity with highly heterogeneous symptoms, which indicated alternative factors to derive co-concurrences of psychiatric disorders[23,24].

Several multi-factorial nosological theories had ever been established to understand symptomatology structures of psychiatric comorbidities, particularly in the Hierarchical Taxonomy of Psychopathology (HiTOP) and Research Domain Criteria (RDoC) frameworks. The HiTOP proposed a multidimensional diagnostic system embedded into a hierarchical framework, with combinations of subfactors (e.g., internalizing/external problems) to constitute high-order factors for diagnosing "comorbidity"[26,26]. However, compared to the single-factor nosological structure, this theory was consistently challenged for poor clinical practicability and especially discrepancies in the neurobiological interpretations[27,28]. To consolidate theoretical foundation of multidimensional structure, another nosological system enriched by neurobiological architectures, that was the RDoC, had been established[29]. Despite prominent merits on neurobiological interpretability, it was still questioned for poor theoretical constructs, given its "Reductionism" assumptions[30,31]. Therefore, to address these issues, synthesizing these heterogeneous bridging symptoms into one-factor structure might be one promising pathway and theoretical framework to understand the common neuropsychopathological mechanism to comorbidities[11,32-34]. By combining "common cause theory" to "comorbidity hypothesis", we aimed to establish a common bridging component to understand the "bridging factor" in the depression-anxiety comorbidity (referred as the "*cb* factor") (Fig. 1)."

References in this response

Bedwell, J. S., Spencer, C. C., & O'donnell, J. P. (2018). Promise and challenges with the research domain criteria framework. Adult Psychopathology and Diagnosis, Eighth Edition, 33-44.

Conway, C. C., Kotov, R., Krueger, R. F., & Caspi, A. (2023). Translating the hierarchical taxonomy of psychopathology (HiTOP) from potential to practice: Ten research questions. The American psychologist, 78(7), 873–885. https://doi.org/10.1037/amp0001046

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Forbes, M. K., Ringwald, W. R., Allen, T., Cicero, D. C., Clark, L. A., DeYoung, C. G., Eaton, N., Kotov, R., Krueger, R. F., Latzman, R. D., Martin, E. A., Naragon-Gainey, K., Ruggero, C. J., Waldman, I. D., Brandes, C., Fried, E. I., Goghari, V. M., Hankin, B., Sperry, S., Stanton, K., … Wright, A. G. C. (2024). Principles and procedures for revising the hierarchical taxonomy of psychopathology. Journal of psychopathology and clinical science, 133(1), 4–19. https://doi.org/10.1037/abn0000886

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Kotov, R., Krueger, R. F., & Watson, D. (2018). A paradigm shift in psychiatric classification: the Hierarchical Taxonomy Of Psychopathology (HiTOP). World psychiatry : official journal of the World Psychiatric Association (WPA), 17(1), 24–25. https://doi.org/10.1002/wps.20478

Kotov, R., Krueger, R. F., Watson, D., Achenbach, T. M., Althoff, R. R., Bagby, R. M., Brown, T. A., Carpenter, W. T., Caspi, A., Clark, L. A., Eaton, N. R., Forbes, M. K., Forbush, K. T., Goldberg, D., Hasin, D., Hyman, S. E., Ivanova, M. Y., Lynam, D. R., Markon, K., Miller, J. D., … Zimmerman, M. (2017). The Hierarchical Taxonomy of Psychopathology (HiTOP): A dimensional alternative to traditional nosologies. Journal of abnormal psychology, 126(4), 454–477. https://doi.org/10.1037/abn0000258

- Establishing a new theoretical conception should be highly welcomed and applauded, and authors stood for clear points on the cb factor conceptualization. Nonetheless, are there some specific hypotheses to neurobiological markers of this cb factor?

Response: We do thank you for raising this practical and helpful comment on improving clarity to our research questions. As you exactly supposed, the present study is established by theory-driven research framework, and thus contains specific research hypotheses when designing these analyses. To shorten the paper length, these descriptions for research hypotheses had been removed from the main texts in the last round of review & revision. In this revision, we have added specific hypotheses in the Introduction section. Please see full details as follow:

Introduction Section (Page. 3, Line. 116-128)

"**... Here, we collected the symptoms of depression and anxiety by self-reported questionnaires, and identified the *cb* factor using factor analysis model (see Methods). We hypothesized that the single-factor structure could be optimum in modeling these heterogeneous bridging symptoms. To probe neurobiological substrates of this conceptualized *cb* factor, we developed an eFC-connectome-based predictive model (eCPM) to examine whether the whole-brain eFC could reliably predict the *cb* factor (Fig. 2b). From what has been mentioned above, we speculated that the eFC could be robust biomarkers of this conceptualized *cb* factor. Once confirming this prediction, we employed a multivariate representation similarity analysis (RSA) to delineate what specific eFC markers characterize the *cb* factor, particularly in limbic, frontoparietal and default mode networks (Fig. 2c). Given the genetic influences on the brain connectome, we finally extended to capture the eFC-genetic signatures of the *cb* factor by recruiting an independent twin cohort and incorporating extensive neurocognitive and biological datasets (Fig. 2d).**"

- Why the authors use the eFC matrices to identify the neural substrates for cb factors? Could it be more predictive than traditional functional connectivity methods? Please provide additional

rationale.

Response: We fully appreciated you for pointing out this crucial and insightful question with respect to justification of establishing edge-centric FC over the traditional nodal FC. In present study, two solidly technical merits on the edge-centric FC have driven us to leverage it. One of the noteworthy technical superiorities is that the eFC indeed surpasses prediction performance than traditional FC methods, particularly in the robustness. Several empirical evidences converged into the line that the eFC outperformed nFC on the differential identifiability for individual idiosyncrasies (Faskowitz et al., 2020; Jo et al., 2021). As shown in the **Figure. R1a**, the individual differential identifiability in the eFC-based prediction models consistently outperformed in the nFC-based ones, particularly in extending scanning time points. Moreover, by using multi-dimensional scaling (MDS) algorithm, the significantly higher distance was observed in eFC-based model than do in nFC ones, showing superior identifiability/robustness from eFC features over nFC ones **(Figure. R1b)**. Compared to stable idiosyncrasies (e.g., personality traits, neurological diseases with high homogeneity), the psychiatric disorders, and even in complicated comorbidity, were highly heterogeneous across populations, along with various biotype and etiologies (Romero et al., 2022; Segal et al., 2023; Wendit et al., 2020). Thus, leveraging the eFC features to capture biomarkers of the *cb* factor, which maximally decomposed heterogeneous bridging symptoms between depression and anxiety, could substantially benefit to improve the robustness of such brain-symptom predictions.

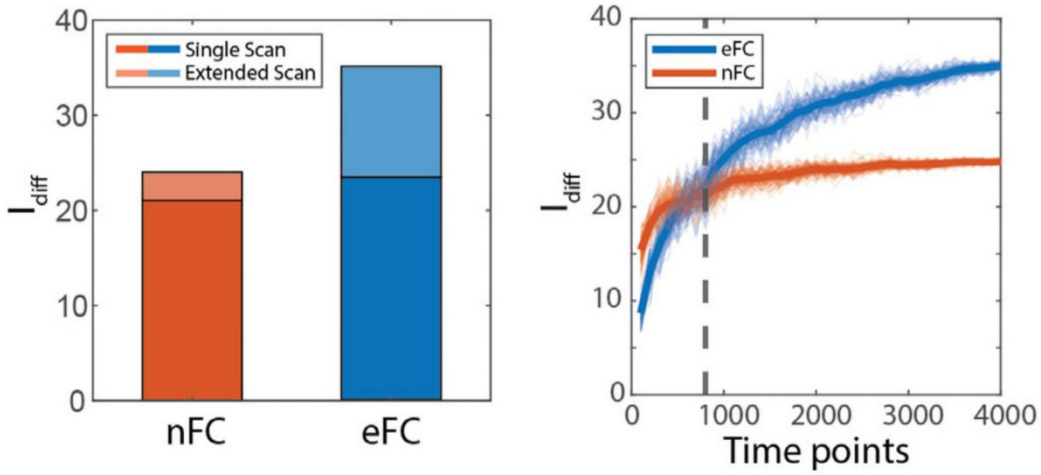

Figure R1a. Subject identification of eFC and nFC across scanning length (reprinted from Jo et al., 2021, original authors and publishers reserve permission and rights).

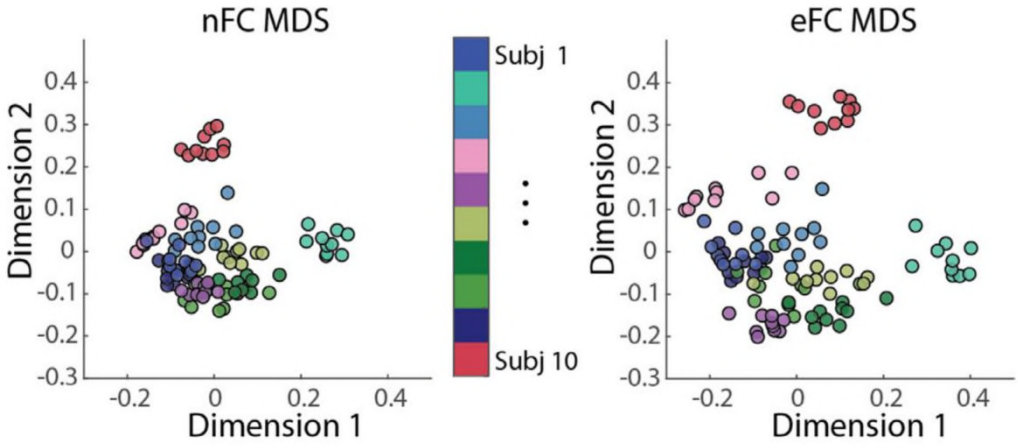

Figure R1b. Multi-dimensional scaling plots to individuals from nFC and eFC features (reprinted from Jo et al., 2021, original authors and publishers reserve permission and rights).

Another technical merit propelling this decision to use eFC features is that the eFC features provide a promising venue to capture time-varying fluctuations in functional connectivity, which powerfully increased temporal resolution to understand high-level architecture of system-level organization in the brain (Betzel et al., 2023; Novelli & Razi, 2022). It has long been challenged on traditional nFC method that the nFC oversimplified the brain organization as a low-level point-to-point (between-region) communication system (Betzel et al., 2023; Faskowitz et al., 2020). By estimating eFC to show high-resolution temporal architectures of brain organizations, this concern could be well addressed as it identified the similarity across time-point cofluctuations, such as the functional dynamics of "line-to-line" (between-FC) connections (Faskowitz et al., 2020) **(Figure. R2)**. In other words, in the present study, we capitalized on the eFC features not only to improve robustness of brain-symptom phenotyping and prediction, but also to yield unique insights into high-order brain connectome-based architectures of the depression-anxiety comorbidity.

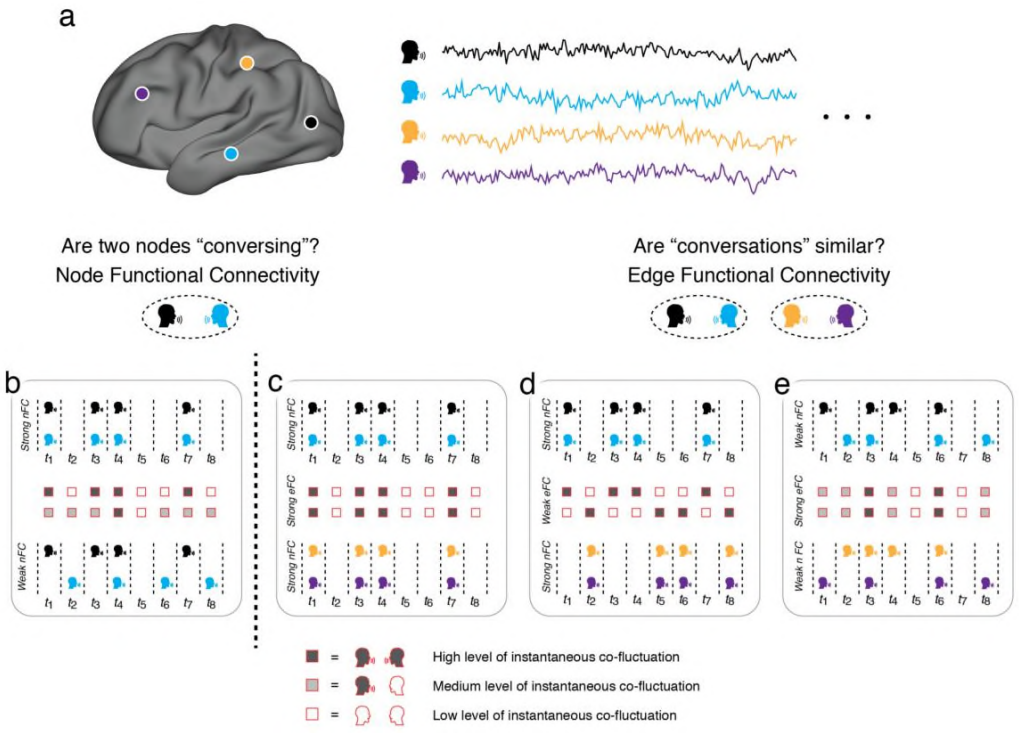

Figure R2. An intuitive schematic of eFC (reprinted from Faskowitz et al., 2020, original authors and publisher reserve permission and rights)

To clarify the rationale of using eFC, we have added more knowledge (we mentioned above) into the Introduction section of the revised manuscript. Please see specific modification underneath:

Introduction Section (Page. 2, Line. 84-92)

"... The eFC not only surpassed traditional rFC in robustness of phenotyping and in the differential identifiability to individual idiosyncrasies[36,37], but also showed superior performance in characterizing intrinsic neural patterns of neuropsychiatric disorders and neurological diseases[38-41]. More importantly, compared to rFC, the eFC shifted constructions of brain connectome from between-regions spontaneous synchronization to instantaneous co-fluctuations, thus yielding unique insights into brain high-resolution temporal FC architectures[42-43]. Therefore, in the present study, we intended to identify the eFC markers associated with the *cb* factor to probe its neurobiological substrates."

References in this response

Betzel, R. F., Faskowitz, J., & Sporns, O. (2023). Living on the edge: network neuroscience beyond nodes. Trends in cognitive sciences, 27(11), 1068–1084.

Dai, W., Qiu, E., Lin, X., Zhang, S., Zhang, M., Han, X., Jia, Z., Su, H., Bian, X., Zang, X., Li, M., Zhang, Q., Ran, Y., Gong, Z., Wang, X., Wang, R., Tian, L., Dong, Z., & Yu, S. (2023). Abnormal Thalamo-Cortical Interactions in Overlapping Communities of Migraine: An Edge Functional Connectivity Study. Annals of neurology, 94(6), 1168–1181.

Faskowitz, J., Esfahlani, F. Z., Jo, Y., Sporns, O., & Betzel, R. F. (2020). Edge-centric functional network representations of human cerebral cortex reveal overlapping system-level architecture. Nature neuroscience, 23(12), 1644–1654.

Fan, Y., Wang, R., Yi, C., Zhou, L., & Wu, Y. (2023). Hierarchical overlapping modular structure in the human cerebral cortex improves individual identification. iScience, 26(5), 106575.

Jo, Y., Zamani Esfahlani, F., Faskowitz, J., Chumin, E. J., Sporns, O., & Betzel, R. F. (2021). The diversity and multiplexity of edge communities within and between brain systems. Cell reports, 37(7), 110032.

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Romero, C., Werme, J., Jansen, P. R., Gelernter, J., Stein, M. B., Levey, D., ... & Van der Sluis, S. (2022). Exploring the genetic overlap between twelve psychiatric disorders. Nature genetics, 54(12), 1795-1802.

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Wang, W., Du, R., Wang, Z., Luo, X., Zhao, H., Luan, P., ... & Liu, S. (2023). Edge-centric functional network reveals new spatiotemporal biomarkers of early mild cognitive impairment. Brain-X, 1(3), e35.

Wendt, F. R., Pathak, G. A., Tylee, D. S., Goswami, A., & Polimanti, R. (2020). Heterogeneity and polygenicity in psychiatric disorders: a genome-wide perspective. Chronic Stress, 4, 2470547020924844.

- Line 133-134, please clarify what specific differences between the univariate correlation and network-between correlation.

Response: Many thanks to you for putting this helpful comment. This univariate correlation tested the simple linear association between two variables (herein referred as total scores of depression and anxiety, respectively). To further probe into the multivariate correlation between symptom-centered networks of depression and anxiety, we used the Mantel test for modeling association of these two inter-subject across-symptom correlation matrices (Smouse, Long, & Sokal, 1986; Somers & Jackson, 2022). Compared to simple linear Pearson correlation, the network-wise correlation estimated the spatial similarity between two positive-definite symmetric correlation matrices, thus directly examining multivariate association in network-wise data structures. By doing so, we not only revealed comorbidity between depression and anxiety by the correlation between total scores, but also uncovered the mutual association between across-symptoms structures of depression and anxiety. Following this helpful advice, we have added brief descriptions in the Results section to differentiate univariate correlation and multivariate correlation.

Results Section (Page. 4, Line. 157-161)

"We found the significant correlations for symptoms between depression and anxiety (*r* = .71, p < .001, univariate Pearson's correlation of total scores; *r* = .40, p < .001, multivariate Mantel's correlation of inter-subject across-symptom correlation networks; Supplemental Methods 4, Fig. 3b), thereby demonstrating the presence of comorbid conditions within this subclinical population. ..."

References in this response

Smouse, P. E., Long, J. C., & Sokal, R. R. (1986). Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Systematic zoology, 35(4), 627-632.

Somers, K. M., & Jackson, D. A. (2022). Putting the Mantel test back together again. Ecology, 103(10), e3780.

- At the Result section, the Figure 2a illustrated the geospatial distribution of sampling population, but seemed to appear for some unclear labels. For instance, at the within-figure texts, it showed "30 minority races" for the whole sample, but "29 minority races" was reported in the main texts. Furthermore, why a few number of areas are given for their name, such as H.K., Macau?

Response: We are quite sorry to remain such typos in the Figure 2a. The "29 minority races" is correct, and we have rectified this error in the Figure 2a. Furthermore, these labels of giving full name of these locations indicated participants who either overrepresented from these provinces/cities (Top 10%) or were underrepresented from these ones (Bottom 10%). To obviate confusions, we have removed these labels in the Figure 2a. Please see the revised Figure 2a underneath:

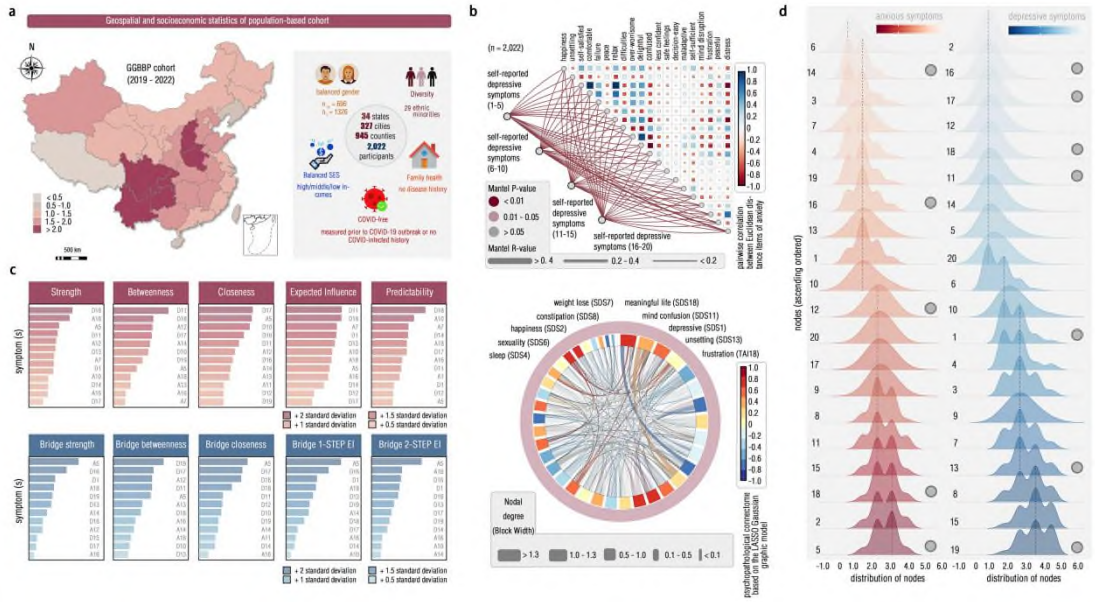

Figure Legend: Sociodemographic characteristics and Gaussian Graphic Model (GGM) of depression-anxiety inter-symptom network. a, The geospatial and socioeconomic statistics of this subclinical sample (GGBBP sample recruited from 2019 to 2022) demonstrate the geographic diversity. The scale indicated the number of included subjects after Log transformation. Icons in this panel were generated from the open-access web-based software (ICONFINDER, https://www.iconfinder.com/). The "male gender" icon by Anna Litviniuk, titled "Avatar, male, man icon", used under Free for commercial use license, available at https://www.iconfinder.com/icons/403019/download/png/512. The "female gender" icon by Anna Litviniuk, titled "Avatar, user, woman icon", used under Free for commercial use license, available at https://www.iconfinder.com/icons/403023/download/png/512. The "diversity" icon by Dumitriu Robert, titled "Guy, individual, man icon", used CC-BY 3.0 license, available at https://www.iconfinder.com/icons/3289573/download/png/512. This icon is modified by changing colors and replicating in the figure, as permitted by this license. The "socioeconomic status" icon by Pongsakorn Tan, titled "Banking, business, cash icon", used under Free for commercial use license, available at https://www.iconfinder.com/icons/4288564/download/png/512. The "family health" icon

by Paomedia, titled "House icon", used CC-BY 3.0 license, available at https://www.iconfinder.com/icons/299061/download/png/512. The "COVID-free" icon by Omeneko , titled "Corona, coronavirus, positive icon", used CC-BY 3.0 license, available at https://www.iconfinder.com/icons/6217233/download/png/512. This geographic map, along with this compass label, was produced by the open-access software titled "EasyShu (3.61)" (https://www.yuque.com/easyshu/). **b,** Mantel's test plot was illustrated here (p < .001, one-sided Mantel's test, uncorrected), and each point into the lower triangle indicated the mean values of corresponding items. **c,** We illustrated the centrality of each symptom (item) from network model by descending order, with the "D" for indicating "depressive symptom" and with the number of this label for indicating the item in this questionnaire (EI = Expected Influence). **d,** This showed density with Gaussian kernel function for each symptom by descending order, with each circuit (gray) for indicating the high integrative centrality. Source data are provided as a Source Data file.

- It is quite helpful to examine specificity of eCPM by regressing to total scores of depression and anxiety and p factor, respectively. I would recommend to move forward such specificity analysis to depression-anxiety distances (e.g., Euclidean space), which mirror the extent to which depression symptoms comorbid with anxiety symptoms (vice versa).

Response: Many thanks to you for offering this very helpful and practical advice to strengthen the specificity of this trained eCPM model. As you exactly proposed, we have ever examined the generalization performance of this trained eCPM model for the total scores of depression, anxiety and even p factor, respectively. Results showed that, this eCPM that trained by the eFC features, did not outperform prediction to all of them, including depression scores, anxiety scores, total scores of combining depression and anxiety, and p factor scores, which demonstrated the high model specificity **(Figure. R3-4)**. These findings have been sorted and presented in the main text at the Extended Data Fig. 1.

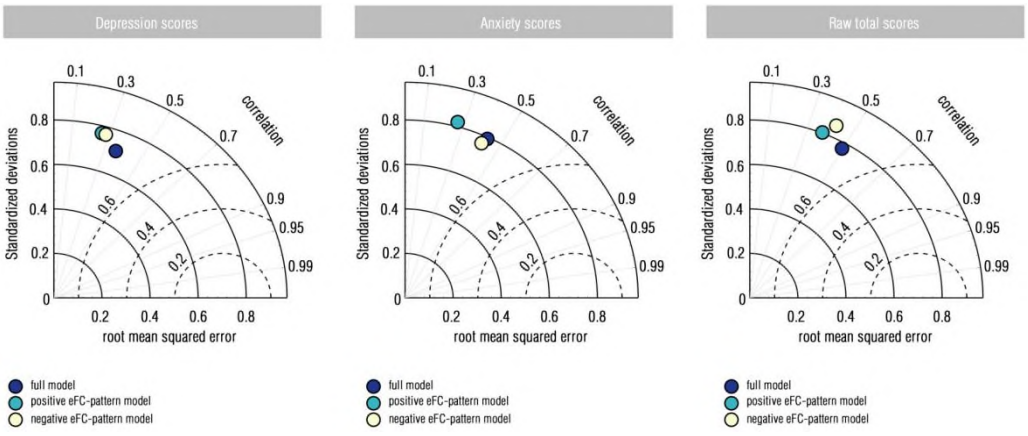

Figure. R3 Model performance for the trained eCPM on single-disorder symptoms. By testing this trained eCPM for the single-disorder symptoms (raw total scores), we found the decreased predictability of this model for these single symptoms, irrespective of training from positive (positive eFC-pattern model), negative (negative eFC-pattern model) or the combined eFCs (full model).

As for the depression-anxiety Euclidean distances, we do appreciate you for sharing this intriguing and promising advice. When validating prediction performance of this trained eCPM to the within-subject Euclidean distances between depression and anxiety, we found the significantly poorer goodness-of-fitness compared to others (e.g., p factor), these findings that further indicated high specificity of this eCPM **(Figure R4)**. Taken together, many thanks to you for this practical and helpful comment favoring to solidify specificity of the eCPM in the present study.

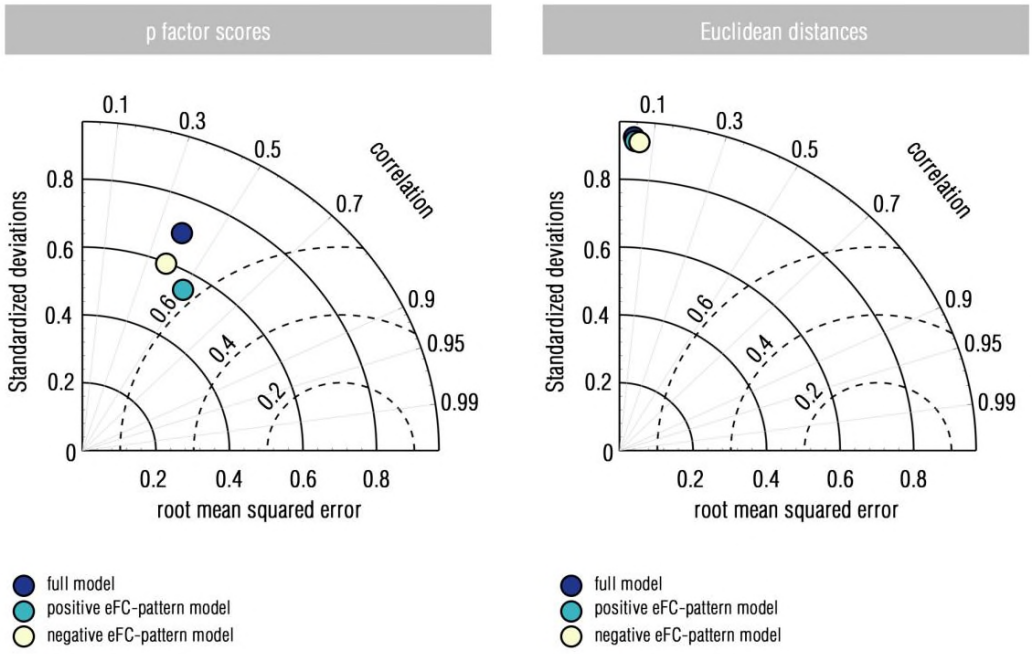

Figure. R4 Model performance for the trained eCPM on p factor and Euclidean distances between depression and anxiety.

- Downstream analysis to the connectome-transcriptomic genetic markers of cb factor indeed added promising neurobiological knowledge to depression-anxiety comorbidity from existing normative maps (e.g., SEA), enriching fruitful insights into the biomarkers of this comorbidity. - However, the GSEA has been critiqued for plain cell type data on humans. Given that, I recommend authors to stretch such biological function decoding results by using PanglaoDB, a latest cell-type dataset from large-scale single cell sequencing methods.

Response: As we replied to the Reviewer #1 above, we truly welcome and are glad to use such new and large-scale single cell sequencing methods to enrich neurobiological annotations in the downstream analyses of imaging-transcriptomic markers, and we thus appreciate you for recommending this new and cutting-edge tool very much.

We have followed your kind suggestion to stretch genetic annotations methods from GSEA to PanglaoDB with 1368 scRNA-seq cell-type samples (https://panglaodb.se/). Given the restriction for the gene set size (as this method required), for the-*cb*-factor-specific genes,

we have decoded them for annotating cell-type clusters that are overexpressed (enriched) in the humans, by narrowing into top 5 genes of the PLS1+, PLS1-, PLS2+, PLS2-, respectively. Results demonstrated the cell-type enrichment converging on Basal cells and Germ cells, which indeed extended our understanding of neurobiological functions of these genes **(Table R1-4)**.

Gene	Description	Type	No. Samples	No. cell clusters
MORC4	MORC family CW-type zinc finger 4	protein-coding gene	11	19
EID4EBP1	eukaryotic translation initiation factor 4E binding protein 1	protein-coding gene	82	280
CMTM3	CKLF like MARVEL transmembrane domain containing 3	protein-coding gene	23	42
TMC8	transmembrane channel like 8	protein-coding gene	1	1
ZNF438	zinc finger protein 438	protein-coding gene	7	19

Table R1. The annotation results of top 5 genes from PLS1+ at the Panglao dataset.

Gene	Description	Type	No. Samples	No. cell clusters
UGCG	-	-	-	-
LDHB	lactate dehydrogenase B	protein-coding gene	154	1059
PPM1A	protein phosphatase, Mg2+/Mn2+ dependent 1A	protein-coding gene	33	104
JKAMP	JNK1/MAPK8 associated membrane protein	protein-coding gene	23	69
CORO2A	coronin 2A	protein-coding gene	6	13

Table R2. The annotation results of top 5 genes from PLS1- at the Panglao dataset. "-" indicated

no records in the Panglao dataset to date.

Gene	Description	Type	No. Samples	No. cell clusters
SCRID	scribbled planar cell polarity protein	protein-coding gene	6	7
HDAC7	histone deacetylase 7	protein-coding gene	31	44
ANKS3	ankyrin repeat and sterile alpha motif domain containing 3	protein-coding gene	2	2
CCDC57	coiled-coil domain containing 57	protein-coding gene	37	96
FTCD	formimidoyltransferase cyclodeaminase	protein-coding gene	21	72

Table R3. The annotation results of top 5 genes from PLS2+ at the Panglao dataset.

Gene	Description	Type	No. Samples	No. cell clusters
INPP4A	inositol polyphosphate-4-phosphatase type I A	protein-coding gene	1	1
RMND1	required for meiotic nuclear division 1 homolog	protein-coding gene	17	25
EIF2B2	eukaryotic translation initiation factor 2B subunit beta	protein-coding gene	33	81
SIK2	salt inducible kinase 2	protein-coding gene	1	2
CORO2A	coronin 2A	protein-coding gene	11	32

Table R4. The annotation results of top 5 genes from PLS2- at the Panglao dataset.

Nonetheless, once we use the Boolean logic to probe into the "Gene set" co-expression enrichment (rather individual top 5 genes as we did above), no clusters of cell types were enriched to reached statistical significance. This Panglao dataset indeed enriched the biological knowledge of genes from their fruitful cell-types samples, but these findings (we obtained above) were decoded by each single gene in the PLS component, thus annotating single-gene cell-type expressions. In the present study, the main goal of imaging-transcriptomic analysis is to capture gene sets correlating to the *cb* factor, rather to univariate correlations between single gene and this conceptualized phenotype (i.e., *cb* factor). Therefore, despite merits, these findings are not directly relevant to the

cb-factor-specific genetic markers, but annotated cell type functions for each gene per se. Again, many thanks to you for sharing this valuable and user-friendly tool for enriching understanding of cell-type expressions of these genes, but we do not include them into the revised manuscript, given weak relevance to the present study.

- At the conclusion section, line 403-405, I feel confused for the dataset that used for connectome-transcriptomic analysis a bit. The eFC-cb-factor markers were captured in this independent twin sample? If in this case, why not use bivariate ACE model to examine rg. If it is not, please clarify this point.

Response: We are quite sorry to confuse you as we used vague descriptions here. In the independent twin cohort sample, we built upon the univariate ACE model to estimate the heritability of eFC features that were already identified to be biomarkers of the *cb* factor by the IS-RSA analyses from the main sample. In this independent twin cohort sample, participants have not yet measured for depression and anxiety by SDS and STAI-T, which thus made the within-sample bivariate ACE analyses to directly examine r_g not applicable in the present study. That is to say, the eFC-*cb*-factor neuroimaging-transcriptomic markers are still probed in the main sample. To clarify this point, we have modified these statements in the Conclusion section. Please see the specific revision as follow:

Conclusion Section (Page. 10, Line. 429-437)

"In conclusion, we established a common bridging factor (*cb* factor) to characterize the general structure of these heterogeneous bridging symptoms in the depression-anxiety comorbidity. By adopting the eCPM and RSA models, we identified neural markers that underpinned this *cb* factor, showing the crucial roles of eFC connectomes within attention and frontoparietal networks to this comorbidity. In an independent twin cohort sample, we revealed the moderate heritability of these *cb*-factor-specific eFC connectomes. Thus, by aligning to other normative genetic and neurobiological datasets, we identified specific connectome-transcriptional genetic signatures of the *cb* factor, which further disentangled complex associations of the *cb* factor to vasculature and cerebellar developments. ..."

- There are a few typos in the figure texts.

Response: Many thanks to for this kind reminder. We have checked texts in figures, one-by-one, and further rectified these typos.

REVIEWER #1

The authors have addressed my previous comments, and I recommend publishing the paper.

Response: We do appreciate you for sharing these helpful suggestions, and thank you for satisfy our revisions.

REVIEWER #2

I have reviewed the response from the reviewers and am satisfied with their response. I have no further comments to add at this time.

Response: Many thanks to you for taking valuable time to re-review our manuscript, and truly thank you for satisfying our revision and response.