

Anterior-posterior systematic deficits of cortical thickness in early-onset schizophrenia

Corresponding Author: Professor Huafu 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)

The authors have tried to investigate system-level organization of coordinated structural changes in early onset schizophrenia (EOS) patients by applying a dimensional reduction approach to the structural covariance of cross-sectional cortical thickness data and found disrupted systematic organization of structural covariance patterns in EOS patients, which was supported by excessive distant connection profiles between two ends of the axis. The research question is relevant for understanding of pathophysiology of schizophrenia.

However, the following queries need to be addressed before the acceptance of manuscript for publication.

1. Provide ethical clearance number.
2. In case of subjects below 18 years of age, assent from the subject is required. Did the authors obtained assent for this study?

Otherwise, the manuscript is very well written and can be accepted for publication.

Reviewer #2

(Remarks to the Author)

This manuscript describes the results of a study that utilized cortical thickness to study structural covariance patterns in early onset schizophrenia (EOS). Using a young sample, the authors calculated multiple indices of structural connectivity and found that EOS patients exhibited a compressed covariance gradient along the anterior-posterior axis and increased structural covariance between distant cortical regions, indicating a contracted organizational axis of cortical thickness. Their findings suggest that early disruptions in cortical organization contribute to the pathology of EOS in support of a neurodevelopmental hypothesis of schizophrenia. In general, the article is well-written and utilizes sophisticated methodology that aligns with current conceptualizations of network integrity and neural development in a novel way. I believe it can make a strong contribution to the literature, however, there are some minor concerns below that should be addressed prior to consideration for publication:

Abstract:

- Good review of the article with relevant methodological and summary data
- Some brief inclusion of the clinical symptom findings may be helpful for indexing purposes (easier to find this study for those interested in this angle)

Introduction:

- Authors provide solid background on current conceptualizations of brain maturation, as well as metrics for evaluating it in terms of structural connectivity
- There is a relative de-emphasis on the network literature in EOS and the rationale for utilizing structural covariance, gradient measures, and physical distance. Indeed, most paragraphs in the Introduction end with only a brief sentence describing why to use each approach in EOS. Some additional context would be helpful; for example, what previous developmental neuroscience literature in EOS provides a strong rationale for the current study's approach?
- Some minor grammar issues throughout, e.g., pg. 4 line 126 should read "examined diagnosis and symptom effects"

Methods

- The cortical thickness findings from the groups at the end of the third paragraph seem more appropriate for the Results section
- Why align the EOS gradient maps with the TD ones versus a combined map? Provide more explicit reasons for this decision
- What was the rationale for dividing parcels into 10 equal-sized bins to examine symptom effects (as opposed to using other gradient scores or relative to the functional networks, etc.) – i.e., why is this the best way to study clinical symptoms in this sample?
- Why average the geodesic distance across hemispheres? Given known brain laterality differences in psychosis populations, it might be meaningful to not average (unless the authors were able to determine that the values in each hemisphere did not meaningfully differ from each other)
- There is some debate on whether to regress out the effects of mean global cortical thickness in regional-specific models. Have the authors considered what effect this had on their results and why; could they run the models without controlling for mean global cortical thickness to quantitatively determine this?

Results:

- The first mention of the HCP data is in the first paragraph – why was this used, what advantage does it have? This should be detailed in the Methods section.
- Pg. 9 lines 270-272 – if no parcels survived FDR correction, could not the “subtly decreased gradient scores” in frontal regions just as likely be spurious as meaningful? Might be best to remove this part, plus, Figure 2A seems to suggest there are just as many posterior (and cingulate) regions “subtly” involved too, so among them all, which are the most relevant (probably none)
- Pg. 9, lines 276-278 – why are group comparisons occurring with the G1 map when it is described in the paragraph above that the G2 axis was selected instead for comparison (pg. 8, lines 253-254)?
- Pg. 10, line 314 – minor grammar again “diagnosis effects”

Discussion:

- Pg. 13, lines 366-367 – the sentence about G1 and motor regions feels extraneous and not relevant to main story of the study, it reads a little awkward stuck in there without further explication/interpretation as well, might recommend removing
- Pg. 14, lines 408-409 – what would be an example of this compensatory mechanism, any insights from the literature?
- Pg. 15, line 456 – minor grammar issue, “...putamen infarct reportedly caused...”

Tables/Figures

- Many of the figures were difficult to see/interpret due to size and many small details

Replicability

- The authors are to be commended for following the rigorous practice of providing links to code and data to assist with replicability

Reviewer #3

(Remarks to the Author)

This study uses advanced image analysis methods on T1 scans with suboptimal resolution. It seems the authors clearly know what they are doing. The evidence corroborating their hypotheses is, generally, quite weak, I think. This is fine with me. However, it needs to be clearly reflected in the Discussion. In addition, the significant diagnostic differences should, if possible, be clarified with plots. If these graphics show considerable overlap between healthy and EOS individuals than this overlap should be discussed in the context of the methods used and EOS.

The authors state: "Our previous work using the same dataset found increased segregation of macroscale thalamocortical functional organization in EOS." That previous neuroimaging-EOS work (<https://pmc.ncbi.nlm.nih.gov/articles/PMC10483446/>) included 86 patients, the current one 95, why?

I've looked at this prior publication and the current manuscript. The authors provide no data on the time between scan-acquisition and diagnosis. Why not? When was the scan made? The previous work using the same dataset (<https://pmc.ncbi.nlm.nih.gov/articles/PMC10483446/>) states:

"Here, we contrasted resting-state fMRI in 86 antipsychotic-naïve first-episode early-onset schizophrenia (EOS) patients...", implying that patients were antipsychotic-naïve at scan-acquisition.

There are a few possible scenarios here. Perhaps the likely one is that scans were made at baseline assessment and after a minimum of 6 months (after scan-acquisition) the EOS diagnosis was confirmed. I suppose that anti-psychotic treatment started after scan-acquisition but months (?) before diagnosis. Is that correct?

They state that no patients had >12 months of diagnosis. That means, I think, that a patient could have a maximum of 18 months of active psychotic symptoms since baseline assessment. What I don't understand is why the authors mention this? What is, if any, the relationship between this statement and the rest of the sample description. If, in an alternative scenario, scan-acquisition was done at the time of diagnosis than the authors should explain the clinical follow-up really carefully b/c ethical concerns may be raised.

Please give a very clear description of the chronology of the inclusion and clinical follow-up process, including what (type of) treatment was given when and where. Mention disease duration. Also mention clearly when scan-acquisition took place in this whole process. If there was individual variation in the time between baseline assessment and scan-acquisition then provide quantitative indicators of this time/variation.

Global CT was calculated by averaging parcels? Are these parcels of similar size? Why not take a hemispheric mask and calculate the average directly over vertices (instead of an average of an average)? The mean difference between groups was 0.04 mm given a slice thickness of 1.2 mm. Is the software really able to detect such small sub-millimeter differences? Could the authors provide a plot showing the individual data-points by group? If there is huge overlap between cases and controls I deem it a good idea if the authors discuss the possible causes for the huge overlap between individuals with EOS and TD individuals. For example, what does this tell us about cortical thickness and EOS?

The authors state: "...frontal regions showed subtly decreased gradient scores in EOS patients relative to TD controls..". Why use uncorrected p-values? I think I didn't see a plot showing this subtle decrease in EOP vs TD. Can the authors add it? If there is huge overlap between cases and controls I deem it a good idea if the authors interpret this overlap and its consequences for the relationship between "gradient scores" and EOS.

I understand that comparisons were made between all bins, without a priori hypothesis. A few bins differed between groups, the majority didn't. A few were related to symptoms, the majority wasn't. I think the authors should be really carefully in their Discussion of these results and not generalize 'across bins', so to speak.

For the distances I have the same commentary as above, diagnostic differences are uncorrected or related to some bins but not others (why?). Taken together I think this means that considerable caution is warranted when discussing and interpreting the results.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)

The authors have done an excellent job addressing all of my concerns and revising the manuscript. I believe it has enhanced their work and have no further comment.

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Reviewers' Comments to Author (COMMSBIO-24-6662A):

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Reviewer #1

Remarks to the Author

The authors have tried to investigate system-level organization of coordinated structural changes in early onset schizophrenia (EOS) patients by applying a dimensional reduction approach to the structural covariance of cross-sectional cortical thickness data and found disrupted systematic organization of structural covariance patterns in EOS patients, which was supported by excessive distant connection profiles between two ends of the axis. The research question is relevant for understanding of pathophysiology of schizophrenia. However, the following queries need to be addressed before the acceptance of manuscript for publication.

We greatly appreciate the Reviewer for his/her appreciation of our work and the constructive suggestions. We have provided ethical clearance number and details about the recruitment of subjects in the revision.

Q1: Provide ethical clearance number.

Response:

We have provided ethical clearance number of the study in revised manuscript as below:

Page 6: *"This study was listed on the Chinese Clinical Trial Registry website (registration number: ChiCTR1900025838, <https://www.chictr.org.cn/showproj.html?proj=42160>), and was approved by the Ethics Committee of the First Hospital of Shanxi Medical University."*

Q2: In case of subjects below 18 years of age, assent from the subject is required. Did the authors obtained assent for this study?

Response:

Yes, for helping pediatric participants understand their condition and treatment, we offered and obtained informed assent from them (Diekema, Mercurio, & Adam, 2011), and have made sure that they have the right for appropriate decision making. In addition, informed consent was also obtained from their parents or legal guardians. We've clarified this point in the revision as below:

Page 6: *"Informed assent was obtained from all pediatric subjects for ensuring their knowledge of current condition and treatment, and involving them in appropriate*

decision-making tasks (Diekema, Mercurio, & Adam, 2011). In addition, informed consent was also obtained from their parents or legal guardians (Cotrim, Granja, Carvalho, Cotrim, & Martins, 2021).”

Bibliography in the Response to the Reviewer:

Diekema, D. S., Mercurio, M. R. & Adam, M. B. Clinical Ethics in Pediatrics: A Case-Based Textbook. (Cambridge University Press, 2011).

Cotrim, H., Granja, C., Carvalho, A. S., Cotrim, C. & Martins, R. Children's Understanding of Informed Assents in Research Studies. Healthcare 9, doi:10.3390/healthcare9070871 (2021).

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Reviewer #2

Remarks to the Author

This manuscript describes the results of a study that utilized cortical thickness to study structural covariance patterns in early onset schizophrenia (EOS). Using a young sample, the authors calculated multiple indices of structural connectivity and found that EOS patients exhibited a compressed covariance gradient along the anterior-posterior axis and increased structural covariance between distant cortical regions, indicating a contracted organizational axis of cortical thickness. Their findings suggest that early disruptions in cortical organization contribute to the pathology of EOS in support of a neurodevelopmental hypothesis of schizophrenia. In general, the article is well-written and utilizes sophisticated methodology that aligns with current conceptualizations of network integrity and neural development in a novel way. I believe it can make a strong contribution to the literature, however, there are some minor concerns below that should be addressed prior to consideration for publication:

We greatly appreciate the Reviewer for his/her appreciation of our work and the constructive suggestions, which highly improve the quality of this work. We have provided more explicit rationale for our study design in the Introduction, and detailed methodological steps about gradient calculation and geodesic distance. We've modified all improper result descriptions. In addition, we have tested the effects of global cortical thickness and inter-hemispheric geodesic distance by re-calculating gradient maps without regressing global cortical thickness and covariance distance without averaging geodesic distance across hemispheres. We've re-plotted all figures with proper size in the revision.

Abstract:

Q1: Good review of the article with relevant methodological and summary data. Some brief inclusion of the clinical symptom findings may be helpful for indexing purposes (easier to find this study for those interested in this angle)

Response:

We've added clinical findings in the revised Abstract as below:

Page 2: *"Patients showed increased structural covariance between two ends of the anterior-posterior axis, with increased geodesic distance of covarying regions between two ends. Positive symptoms increased with the strengthening of structural covariance between two ends."*

Introduction:

Q2: Authors provide solid background on current conceptualizations of brain maturation, as well as metrics for evaluating it in terms of structural connectivity. There is a relative de-emphasis on the network literature in EOS and the rationale for utilizing structural covariance, gradient measures, and physical distance. Indeed, most paragraphs in the Introduction end with only a brief sentence describing why to use each approach in EOS. Some additional context would be helpful; for example, what previous developmental neuroscience literature in EOS provides a strong rationale for the current study's approach?

Response:

We thank the Reviewer for this valuable suggestion, and have added more previous developmental neuroscience literatures in EOS and emphasized the rationale for utilizing structural covariance, gradient measures, and physical distance in the revision as below:

Pages 3-5:

“An intuitive method for capturing coordinated changes in cortical thickness across the cortex is the “structural covariance” approach (Raznahan et al., 2011). By calculating the covariance of cross-sectional cortical thickness data, this approach measures the similarity of anatomical variations in the brain. The covariance pattern reflects the coordinated effects of specific micro- and mesoscopic factors, such as gene expression (Schmitt et al., 2009), synaptogenesis (Draganski et al., 2004), and laminar thickness (Saber et al., 2023). Moreover, the structural covarying pattern of childhood-adolescence reflects synchronized developmental changes in the cortex (A. Alexander-Bloch, Raznahan, Bullmore, & Giedd, 2013; Raznahan et al., 2011) and serves as a signature of coordinated developmental processes (A. Alexander-Bloch et al., 2013). For example, this structural covariance pattern has been shown to resemble intra-individual maturational coupling inferred from longitudinal data (Khundrakpam et al., 2019). More optimal topological organization of structural covariance network is associated with higher cognitive performance, implying its involvement in the development of intelligence (Khundrakpam et al., 2017). Altered covariance networks are related with a variety of mental health conditions in young patients with disorders such as depression (Vijayakumar et al., 2017) and anxiety symptoms (Rakesh, Elzeiny, Vijayakumar, & Whittle, 2023). Patients with schizophrenia have an aberrant pattern of fronto-temporal, fronto-parietal and fronto-thalamic covariations (Prasad et al., 2022a), and show a disruption of covariance network architecture (Prasad et al., 2022b). Atypical maturations of structural covariance have been shown in pediatric individuals at high risk for psychosis (Sandini et al., 2018). Yet, few EOS studies investigated

maturation processes of cortical thickness at a high resolution of structural covariance network (Zalesky et al., 2015).

Recently, brain maturation processes have been reported to occur in a system-like manner (Sydnor et al., 2021). Systematic patterns of brain organization have been described within a framework of “gradients” (Hilgetag & Goulas, 2020), that capture an orderly spatial progression of cortical features (Huntenburg, Bazin, & Margulies, 2018). For example, neuronal density changes systematically along spatially organized gradients (Economo, 1927). To characterize the systematic gradient pattern of cortical organization, a nonlinear dimensionality reduction technique, also called “diffusion embedding” has been proposed (Bernhardt, Smallwood, Keilholz, & Margulies, 2022; Margulies et al., 2016). By embedding cortical regions into a continuous gradient map according to the similarity of their structural covariance profiles, previous work has revealed a non-random spatial organization of structural coordination across the cortex (Kharabian Masouleh, Plachti, Hoffstaedter, Eickhoff, & Genon, 2020; Valk et al., 2020). In particular, the dominant structural gradient axis tends to distinguish posterior cortices from anterior cortices, reflecting the temporal sequence of neurogenesis (Valk et al., 2020). Generally, the posterior portion of the cortex has a greater number of neurons with a shorter cell cycle, while the anterior end has a smaller number with a longer cell cycle (Elston, 2000). Common disease effects across various psychiatric disorders have been found to follow a similar cortical thickness covariance gradient axis dissociating anterior and posterior regions (Hettwer et al., 2022). Accordingly, the anterior-posterior gradient axis of structural covariance shaped by neurodevelopmental mechanisms appears to be also affected by psychiatric conditions. Despite the potential interplay of development and illness effects on the gradient axis, it remains unknown whether the gradient patterns change in patients with EOS, and how the system-level disruptions relate to cortical thickness abnormalities.

The systematic pattern of brain organization is supported by many factors. Among them, physical distance along the cortical surface is an important determinant of how regions are connected and thus how the brain is organized (Ercsey-Ravasz et al., 2013). Indeed, a previous quantitative retrograde tracer analysis of macaque cortical networks suggests that physically close areas are more likely to be interconnected (Markov et al., 2011). In addition to physical distance, anatomical similarity between regions is also an important determinant (Barone, Batardiere, Knoblauch, & Kennedy, 2000; Saberi et al., 2023). For example, cortical regions with similar laminar thickness patterns have been reported to have higher structural and functional connectivity (Saberi et al., 2023). In fact, physically close regions tend to have similar microstructural profiles and high interregional connectivity (Barbas, 2015; M. M. Mesulam, 1998). A structural covariance network reflecting the similarity of anatomical

variations in the brain has shown a bias toward short-distance connections (A. Alexander-Bloch et al., 2013). Physical distance along the cortical surface has been suggested to be associated with system-level transitions of both microscale cortical cytoarchitectural covariance (Saber et al., 2023) and macroscale cortical thickness covariance (Valk et al., 2020). Together, physical distance, anatomical similarity, and brain connectivity seem to be intrinsically linked, and are essential to the formation of the systematic pattern of brain organization. Here we would test the relationship between geodesic distances, anatomical similarities and gradient patterns, and whether EOS affected it by examining geodesic distance between covarying regions in patients.”

Bibliography in the Response to the Reviewer:

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Zalesky, A., Pantelis, C., Cropley, V., Fornito, A., Cocchi, L., McAdams, H., . . . Gogtay, N. (2015). Delayed Development of Brain Connectivity in Adolescents With Schizophrenia and Their Unaffected Siblings. *JAMA Psychiatry*, 72(9), 900-908.

Q3: Some minor grammar issues throughout, e.g., pg. 4 line 126 should read “examined diagnosis and symptom effects”

Response:

We have checked and modified all these grammar errors about “diagnosis” in our revision as below:

Page 5: “...we examined diagnosis and symptom effects on structural covariance patterns ordered by the covariance gradient axis in TD controls.”

Page 8: “Next, we examined diagnosis effects by using a classical linear interaction model with diagnosis and cortical thickness as two factors”

Page 11: “We then tested for diagnosis effects on the reshaped structural covariance patterns.”

Page 14: “...two senior psychiatrists with more than 10 years of experience made the consensus diagnosis by interviewing both the child and parent(s).”

Page 23: “(A) Diagnosis effects on reshaped covariance.”

Methods:

Q4: The cortical thickness findings from the groups at the end of the third paragraph seem more appropriate for the Results section

Response:

We previously stated it in the Method part to explain the reason why we regressed out global cortical thickness. However, it is indeed more suitable for the Results section, and we’ve moved the findings to the first paragraph of the Result section as below:

Page 10: “Patients with EOS ($\text{mean} \pm \text{SD} = 2.58 \pm 0.45 \text{ mm}$) showed reduced global cortical thickness relative to TD controls ($\text{mean} \pm \text{SD} = 2.62 \pm 0.46 \text{ mm}$; $t = 2.41$, $p = 0.02$), consistent with previous findings (van Haren et al., 2011).”

Bibliography in the Response to the Reviewer:

van Haren, N. E., Schnack, H. G., Cahn, W., van den Heuvel, M. P., Lepage, C., Collins, L., . . . Kahn, R. S. (2011). Changes in cortical thickness during the course of illness in schizophrenia. *Arch Gen Psychiatry*, 68(9), 871-880.

Q5: Why align the EOS gradient maps with the TD ones versus a combined map? Provide more explicit reasons for this decision

Response:

In our study, we used the TD group as the normative population to show typical gradient patterns. To make the gradients comparable across individuals and eliminate the randomness of the direction of the gradients, we need to align individual gradient maps to a common gradient mask. Here we chose the TD ones as the gradient mask for removing the influence of disease on the gradient mask. We have provided explicit reasons for this step in our revision as below:

Page 7: *“To make the gradients comparable across individuals, we used a unique gradient mask to align two groups via Procrustes rotations. This mask was generated by the normative TD group for avoiding the influence of disease on gradient directions.”*

Q6: What was the rationale for dividing parcels into 10 equal-sized bins to examine symptom effects (as opposed to using other gradient scores or relative to the functional networks, etc.) – i.e., why is this the best way to study clinical symptoms in this sample?

Response:

Indeed, we could examine symptom effects at three scales including parcel-wise, network-wise, and bin-wise. We did not choose the parcel-wise scale for its high dimension. Besides, we examined symptom effects on structural covariance for interpreting the compressed anterior-posterior axis in EOS patients, so we need to align the structural covariance matrices to the gradient axis. Together, to reduce dimension and align to the gradient axis, we reorganized and averaged the structural covariance matrices according to the 10-binned normative gradient mask. We've added this explanation in the revision as below:

Page 8: *“To better visualize group differences along the gradient axis, we divided cortical parcels into 10 equal-sized bins according to their ranked gradient scores in TD controls, and performed paired t-tests on gradient scores of all parcels belonging to each bin (FDR corrected, $q_{FDR} < 0.05$).”*

Page 8: *“To reduce dimension and aligned to the gradient axis, we first divided cortical parcels into 10 equal-sized bins according to their ranked gradient scores in TD*

controls.”

Q7: Why average the geodesic distance across hemispheres? Given known brain laterality differences in psychosis populations, it might be meaningful to not average (unless the authors were able to determine that the values in each hemisphere did not meaningfully differ from each other)

Response:

We agree that brain laterality is meaningful in psychosis populations and it's improper to average geodesic distance across hemispheres. However, we did not average the geodesic distance for intra-hemispheric distance, but just averaged two hemispheres to represent the inter-hemispheric geodesic distance. Specifically, we separately calculated intra-hemispheric geodesic distance for the left hemisphere and the right hemisphere. Note that inter-hemispheric geodesic distances could not be directly calculated and were currently zero, we averaged intra-hemispheric geodesic distance across hemispheres to represent the inter-hemispheric geodesic distance.

Given the averaging step could also influence distance results, we re-calculated covariance distance without the second averaging step by using only intra-hemispheric geodesic distance. We found the replicated results were similar with our original results (below **Figure S3**), indicating little influence of the averaging step on covariance distance results. Furthermore, we've clarified our descriptions about averaging geodesic distance in the revision as below:

Page 8: *“Intra-hemispheric geodesic distances were separately calculated within the left hemisphere and the right hemisphere. Given inter-hemispheric geodesic distance could not be directly measured, they were represented by averaged intra-hemispheric geodesic distances across two hemispheres. We found this averaging step had little influence on following results (**Figure S3**).”*

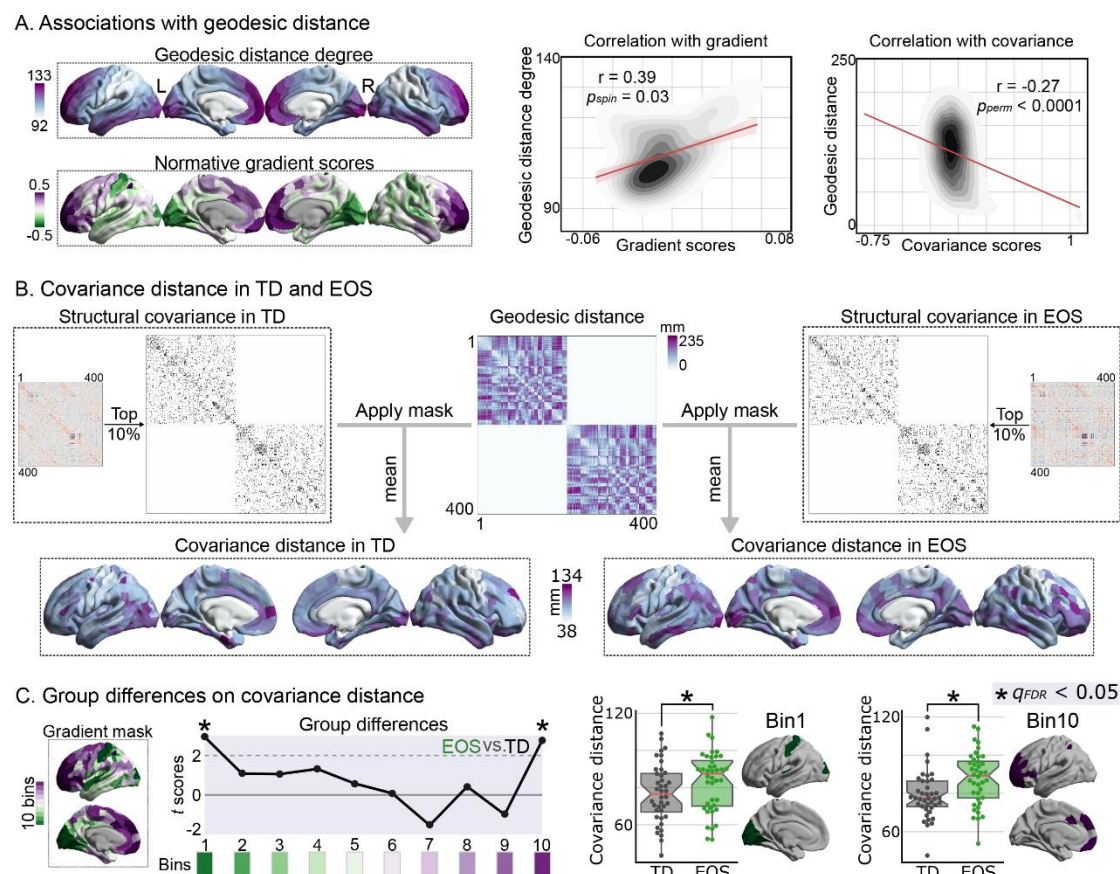


Figure S3. Replicated covariance distance using intra-hemisphere geodesic distance. Covariance distance was computed within the raw geodesic distance mask without averaging intra-hemispheric geodesic distances across two hemispheres.

Q8: There is some debate on whether to regress out the effects of mean global cortical thickness in regional-specific models. Have the authors considered what effect this had on their results and why; could they run the models without controlling for mean global cortical thickness to quantitatively determine this?

Response:

Indeed, there is always debate on the regression of global cortical thickness. Therefore, we've calculated both the covariance gradients with controlling global cortical thickness (our original results) and without controlling global cortical thickness (the replicated results). As shown in below **Figure S2**, we found the replicated gradient maps were closely related with the original gradients, and inferred that global cortical thickness had little effect on our gradient maps. We've clarified this point in our revision as below:

Page 7: "Additionally, we estimated covariance gradient maps without regressing global cortical thickness (**Figure S2**), and found similar gradient patterns as the original gradient maps (the first gradient: $r_{(\text{TD})} = 0.95$, $r_{(\text{EOS})} = 0.97$; the second gradient: $r_{(\text{TD})} =$

0.81, $r_{(EOS)} = 0.83$)."

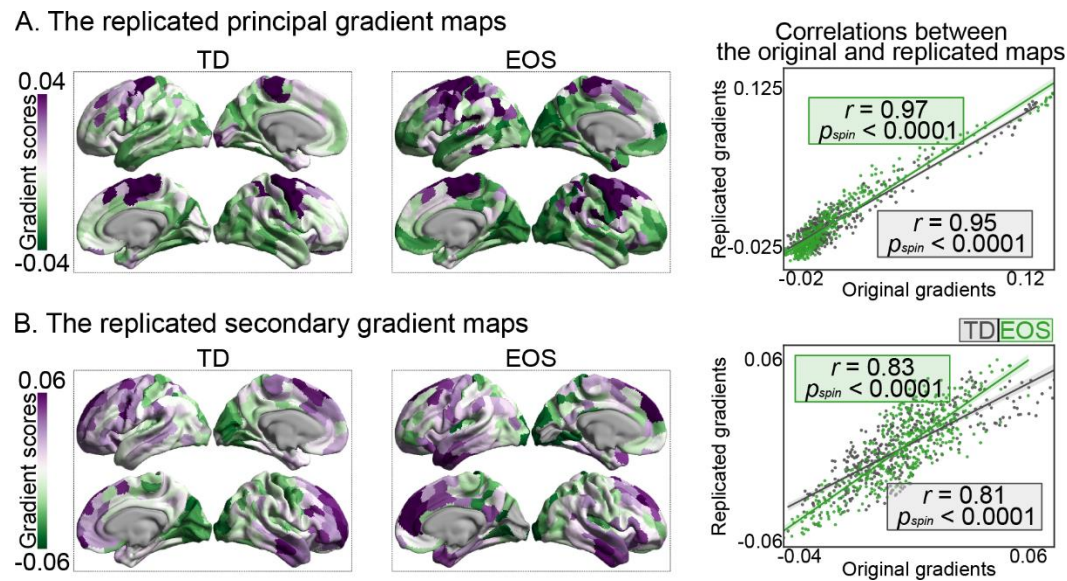


Figure S2. Replicated gradient maps without controlling global cortical thickness.

Results:

Q9: The first mention of the HCP data is in the first paragraph – why was this used, what advantage does it have? This should be detailed in the Methods section.

Response:

The Human Connectome Project (HCP) adult data is generally used to establish a reliable group-level structural covariance gradient map (Kharabian Masouleh, Plachti, Hoffstaedter, Eickhoff, & Genon, 2020; Valk et al., 2020). Consider that our gradients were based on pediatric subjects and might differ from that of adult subjects, we generated the gradient mask using the TD controls, and compared our normative gradient mask with the main gradient axis derived from HCP dataset. We've detailed the reason in the Methods section as below:

Page 7: "To make the gradients comparable across individuals, we used a unique gradient mask to align two groups via Procrustes rotations. This mask was generated by the normative TD group for avoiding the influence of disease on gradient directions. The gradient mask based on pediatric subjects was further compared with the well-established group-level cortical gradient map derived from the Human Connectome Project (HCP) adult data, which tends to distinguish posterior cortices from anterior cortices, reflecting the temporal sequence of neurogenesis (Valk et al., 2020)."

Bibliography in the Response to the Reviewer:

Kharabian Masouleh, S., Plachti, A., Hoffstaedter, F., Eickhoff, S., & Genon, S. (2020).

Characterizing the gradients of structural covariance in the human hippocampus. Neuroimage, 218, 116972. doi: 10.1016/j.neuroimage.2020.116972

Valk, S. L., Xu, T., Margulies, D. S., Masouleh, S. K., Paquola, C., Goulas, A., . . . Eickhoff, S. B. (2020). Shaping brain structure: Genetic and phylogenetic axes of macroscale organization of cortical thickness. Sci Adv, 6(39). doi: 10.1126/sciadv.abb3417

Q10: Pg. 9 lines 270-272 – if no parcels survived FDR correction, could not the “subtly decreased gradient scores” in frontal regions just as likely be spurious as meaningful? Might be best to remove this part, plus, Figure 2A seems to suggest there are just as many posterior (and cingulate) regions “subtly” involved too, so among them all, which are the most relevant (probably none)

Response:

Indeed, those uncorrected gradient differences might be spurious, and we agree with the Reviewer that it is not a good idea to use uncorrected results. We have deleted these descriptions about “subtly decreased gradient scores” in the Result part, and just showed the uncorrected group difference results as supplementary data (below **Figure S5**). In contrast, we added a plot showing FDR-corrected group comparisons of bins along the gradient axis (**Figure 2C**). We’ve modified corresponding part in the revision as below:

Page 10: “No parcels survived FDR correction for z score differences of gradient scores (**Figure S5**).”

Page 10: “We then divided cortical parcels into 10 equal-sized bins according to their ranked gradient scores in TD controls and tested group differences for each bin by using paired t-tests (**Figure 2C**). Patients showed increased gradient scores in the 3rd bin and decreased gradient scores in the 9th and 10th bins ($q_{FDR} < 0.05$), indicating a disrupted segregation for both the posterior and anterior parts.”

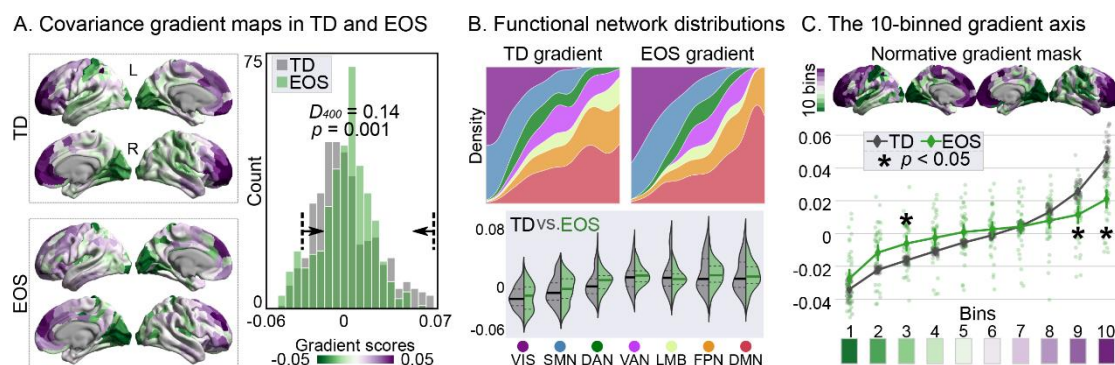


Figure 2. Structural covariance gradient patterns. (A) Covariance gradient maps in TD and EOS. In the right density map, green boxes represent gradient scores of EOS patients and grey

boxes represent TD controls, which suggested compressed gradient axis in EOS than TD (Kolmogorov-Smirnov test; $D_{400} = 0.13$, $p = 0.002$). **(B)** Network distributions. For each group, the continuous density maps of gradient scores were separately plotted for each network. Group differences between EOS and TD were tested by using paired t-tests [False discovery rate (FDR) corrections, $q_{FDR} < 0.05$]. VIS, visual network; SMN, sensorimotor network; DAN, dorsal attention network; VAN, ventral attention network; LMB, limbic network; FPN, frontoparietal network; DMN, default mode network. **(C)** The 10-binned gradient axis. We computed bin-level gradient scores according to the 10-binned normative gradient mask and tested group differences by using paired t-tests (FDR corrected, $p < 0.05$).

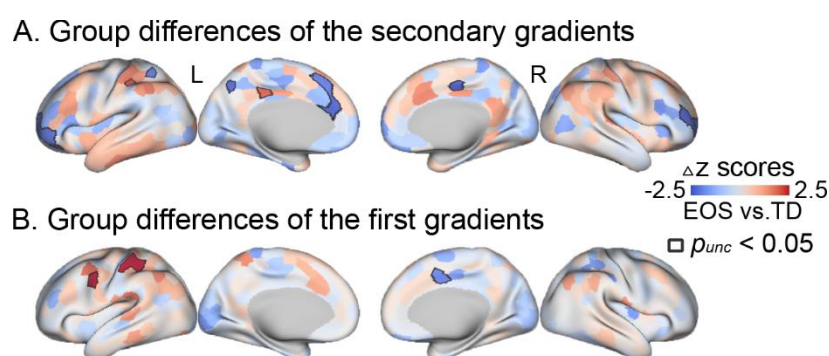


Figure S5. Parcel-wise gradient differences between EOS and TD. Brain maps show the group differences of z scores transformed from gradient scores (EOS vs. TD). Parcels showing uncorrected group differences ($p_{uncorrected} < 0.05$) are outlined in black. **(A)** Group differences of G2. The frontal regions showed subtly decreased gradient scores in EOS patients relative to TD controls. **(B)** Group differences of G1.

Q11: Pg. 9, lines 276-278 – why are group comparisons occurring with the G1 map when it is described in the paragraph above that the G2 axis was selected instead for comparison (pg. 8, lines 253-254)?

Response:

We also compared the first gradient (G1) maps between groups for offering more information of pediatric covariance gradient maps to readers. Indeed, our study focused on the second gradient (G2) map, thus the G1 results were less important and could mislead readers. We have moved the descriptions about G1 results to Supplementary materials as below:

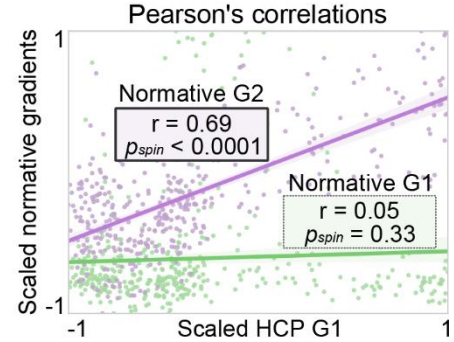
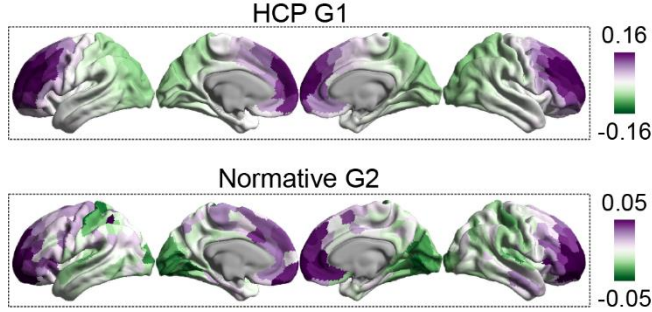
Page 2 in Supplement:

“The first gradient maps in TD and EOS

The first gradient component (G1) explained 20% of the variance in the TD group and 26% of the variance in the EOS group. The G1 axis distinguished motor regions from other cortical areas. However, the G1 map showed no correlation with either the human connectome project (HCP) G1 map ($r = 0.22$, $p_{spin} = 0.18$) or the HCP second gradient

(G2) map ($r = 0.20$, $p_{spin} = 0.28$) (**Figure S4**). Hence, we presented G2 results in our main document but the G1 findings here.”

A. Anterior-to-posterior gradient map



B. Superior-to-inferior gradient map

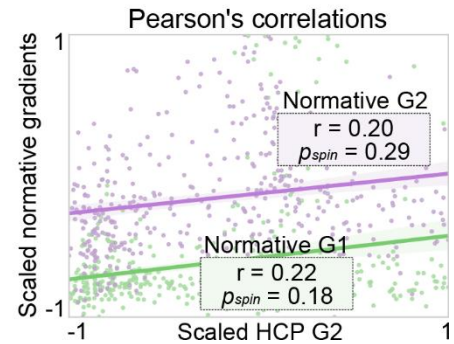
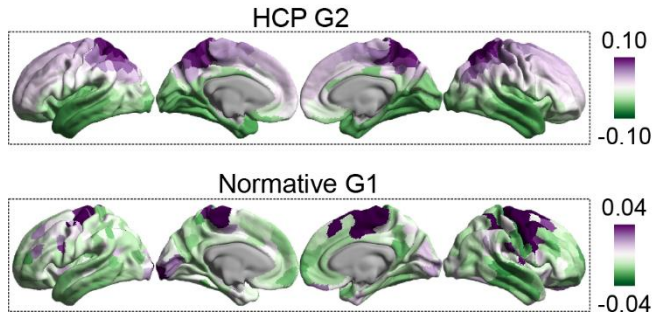


Figure S4. Relationships with gradient maps derived from human connectome project (HCP) data. (A) The first gradient (G1) map of HCP data showed an anterior-posterior transition, which was significantly correlated with the second gradient (G2) map of TD ($r = 0.69$, $p_{spin} < 0.0001$). (B) The HCP G2 map described a spatial arrangement from superior cortices to inferior cortices, while it showed no significant correlation with our gradient maps.

“Group comparisons of the G1 map suggested an extended axis in EOS patients compared to TD controls ($D_{400} = 0.19$, $p < 0.0001$) (**Figure S7A**). However, no parcel survived false discovery rate (FDR) correction for z score differences of gradient scores (**Figure S5B**). After pooling 400 cortical parcels into seven functional networks (**Figure S7B**), we found that patients showed decreased gradient scores in the visual network ($t = -4.2$, $q_{FDR} = 0.0006$) and the limbic network (LMB; $t = -3.28$, $q_{FDR} = 0.01$). After dividing cortical parcels into 10 equal-sized bins according to their ranked G1 scores in TD controls and testing group differences for each bin by using paired t-tests, we found that patients showed decreased gradient scores in the 9th bin ($q_{FDR} < 0.05$) (**Figure S7C**). In terms of covariance distance, we found the G1 map showed no correlation with geodesic distance (**Figure S8**).”

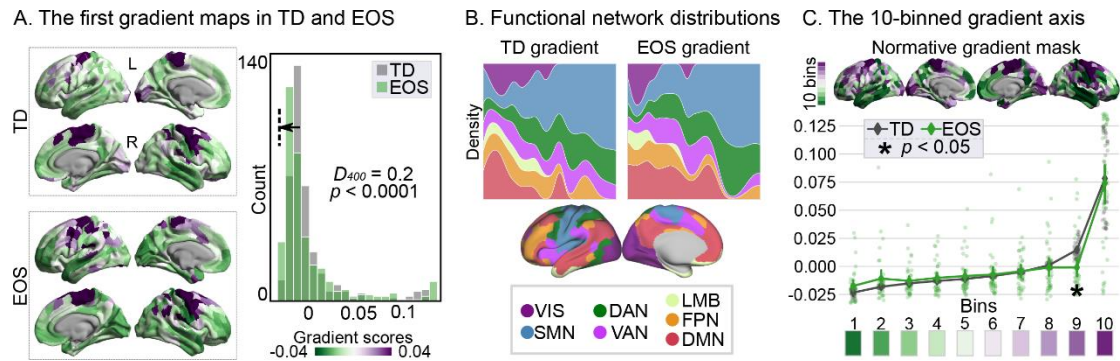


Figure S7. The first gradient of the structural covariance. VIS, visual network; SMN, sensorimotor network; DAN, dorsal attention network; VAN, ventral attention network; LMB, limbic network; FPN, frontoparietal network; DMN, default mode network.

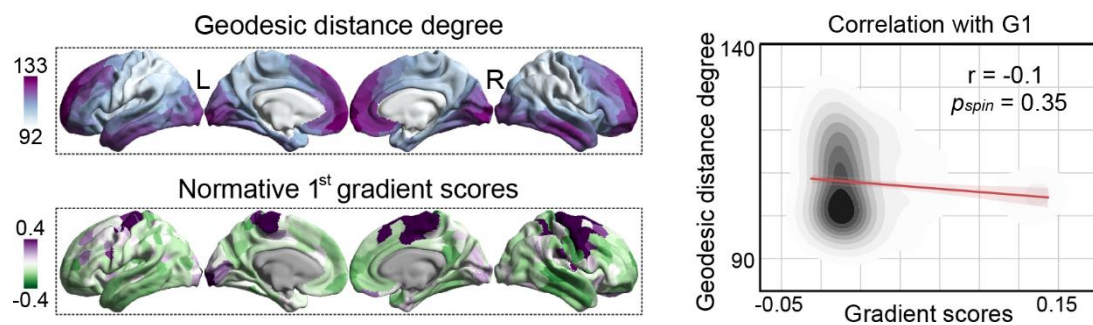


Figure S8. Association between geodesic distance degree and the first covariance gradient. No significant correlation was found between normative G1 map and node-wise geodesic distance degree map ($r = -0.1$, $p_{spin} = 0.35$).

Q12: Pg. 10, line 314 – minor grammar again “diagnosis effects”

Response:

We have corrected all these errors from “diagnose effects” to “diagnosis effects” in the revision.

Discussion:

Q13: Pg. 13, lines 366-367 – the sentence about G1 and motor regions feels extraneous and not relevant to main story of the study, it reads a little awkward stuck in there without further explication/interpretation as well, might recommend removing

Response:

We agreed that the sentence about G1 findings is extraneous and interrupted reader's train of thought. We have removed this sentence in our revised manuscript.

Q14: Pg. 14, lines 408-409 – what would be an example of this compensatory mechanism, any insights from the literature?

Response:

Here the compensatory mechanism indicated that patient's increased structural coupling was a downstream alteration of the brain during the pathological process of schizophrenia, to achieve similar activity patterns in biological networks as the generation population (Marder & Goaillard, 2006). We have added the explanation of compensatory mechanism in the revision as below:

Page 13: *"We found that the clinical severity of positive symptoms decreased with the strengthening of structural coupling between posterior and anterior regions, suggesting a compensatory neural mechanism in EOS. Particularly, patient's increased structural coupling was a downstream alteration of the brain during the pathological process of schizophrenia, to achieve similar activity patterns in biological networks as the generation population (Marder & Goaillard, 2006)."*

Bibliography in the Response to the Reviewer:

Marder, E., & Goaillard, J. M. (2006). Variability, compensation and homeostasis in neuron and network function. Nat Rev Neurosci, 7(7), 563-574.

Q15: Pg. 15, line 456 – minor grammar issue, "...putamen infarct reportedly caused..."

Response:

We have modified this grammar issue in our revised manuscript.

Tables/Figures

Q16: Many of the figures were difficult to see/interpret due to size and many small details

Response:

We have re-plotted all figures in our main document and adjusted their sizes to a proper level as below **Figures 1-4:**

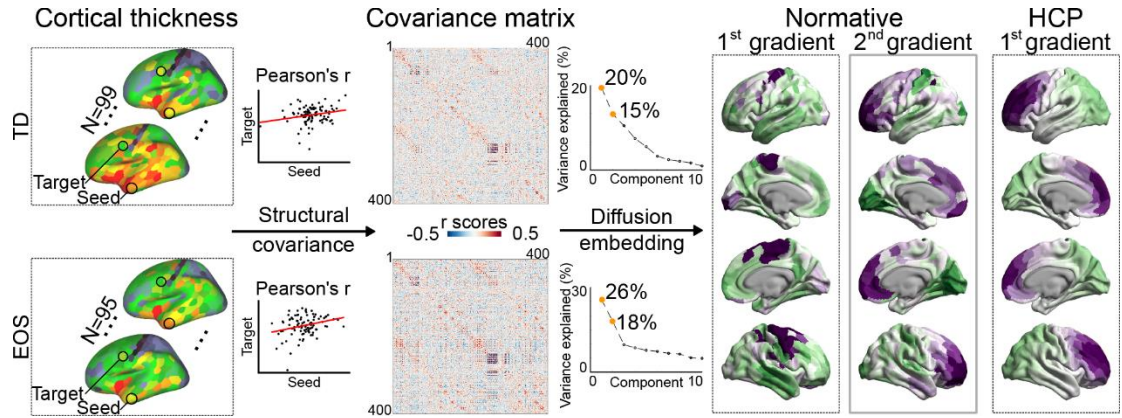


Figure 1. Method flowchart of the structural covariance gradient.

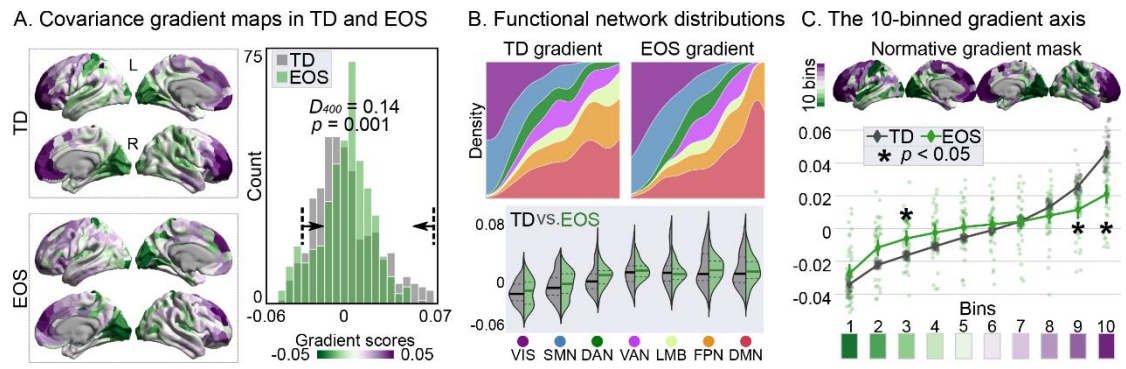


Figure 2. Structural covariance gradient patterns.

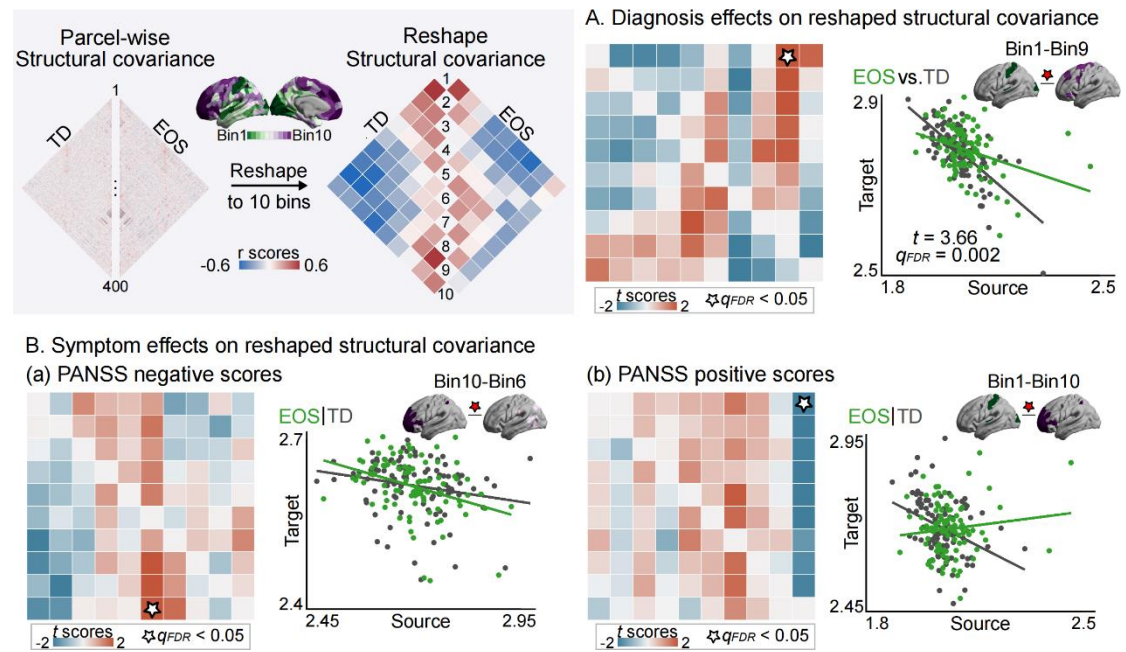


Figure 3. Disease effects on structural covariance patterns.

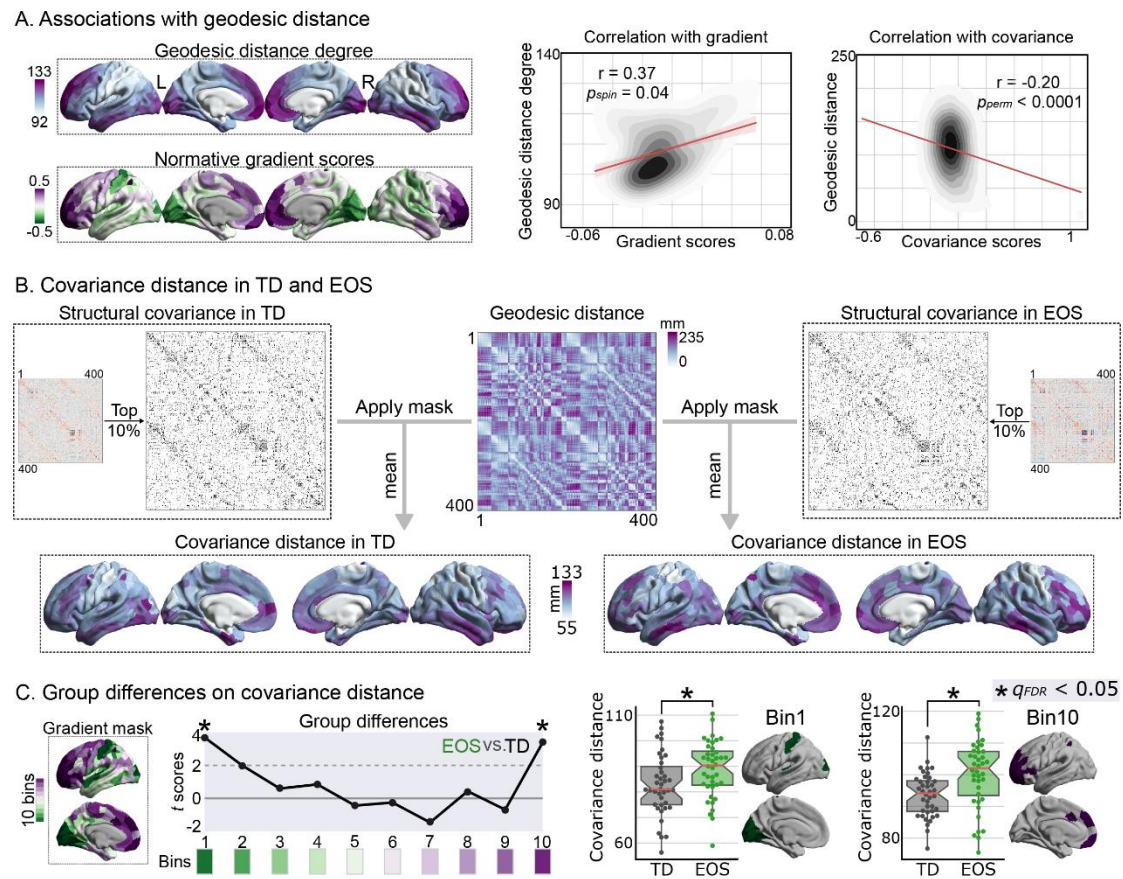


Figure 4. Covariance distance in TD and EOS.

Replicability

Q17: The authors are to be commended for following the rigorous practice of providing links to code and data to assist with replicability

Response:

We thank the Reviewer again for his/her patience in reviewing our manuscript. These comments help us so much, and highly improved our work to be a well-organized and detailed study with discreet result descriptions and discussions. We sincerely hope our open code and data could assist with replicability and the development of future works.

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Reviewer #3

Remarks to the Author

This study uses advanced image analysis methods on T1 scans with suboptimal resolution. It seems the authors clearly know what they are doing. The evidence corroborating their hypotheses is, generally, quite weak, I think. This is fine with me. However, it needs to be clearly reflected in the Discussion. In addition, the significant diagnostic differences should, if possible, be clarified with plots. If these graphics show considerable overlap between healthy and EOS individuals than this overlap should be discussed in the context of the methods used and EOS.

We greatly appreciate the Reviewer for his/her appreciation of our work and the constructive suggestions, which highly improve the quality of this work. We have detailed the chronology of subject inclusion and clinical follow-up process. Moreover, we've reorganized our results and plots to provide clearer overview of evidence supporting our hypotheses. We've further discussed the influence of cortical thickness overlap between patients and controls in our results in the revision.

Q1: The authors state: "Our previous work using the same dataset found increased segregation of macroscale thalamocortical functional organization in EOS." That previous neuroimaging-EOS work (<https://pmc.ncbi.nlm.nih.gov/articles/PMC10483446/>) included 86 patients, the current one 95, why?

Response:

Indeed, these two studies used the same dataset, i.e., a total of 199 subjects with 99 patients with early-onset schizophrenia (EOS) and 100 typically developing (TD) controls. However, our previous work used both T1-weighted MRI data and resting-state functional MRI data. As we stated (Fan et al., 2023):

"Four patients and two controls were excluded from the study because of incomplete scanning data, nine patients and six controls due to excessive head motion [mean frame-wise displacement, (FD) > 0.2 mm or outliers > 50%], and one control due to poor quality of intrasubject brain registration. Ultimately, 86 EOS patients and 91 demographically-matched TD controls were included in the analysis."

In the current study, only T1-weighted MRI data was used to estimate cortical thickness. Therefore, here four patients were excluded due to incomplete scanning and one control due to poor quality of cortical parcellation, resulting in a final sample including 95 EOS patients and 99 demographically-matched TD controls.

Altogether, it is the difference between included imaging modalities and preprocessed steps that makes the final sample sizes different. We have clarified our

descriptions in the revision as below:

Page 2: *“Here, we investigated whether pathological alterations of schizophrenia originate from an early disruption of cortical organization by using T1-weighted structural MRI data from 95 patients with antipsychotic-naïve first-episode early-onset schizophrenia (EOS) and 99 typically developing (TD) controls (recruited sample of 99 EOS and 100 TD).”*

Bibliography in the Response to the Reviewer:

Fan, Y. S., Xu, Y., Bayrak, S., Shine, J. M., Wan, B., Li, H., . . . Chen, H. (2023). Macroscale Thalamic Functional Organization Disturbances and Underlying Core Cytoarchitecture in Early-Onset Schizophrenia. *Schizophr Bull*, 49(5), 1375-1386.

Q2: I've looked at this prior publication and the current manuscript. The authors provide no data on the time between scan-acquisition and diagnosis. Why not? When was the scan made? The previous work using the same dataset (<https://pmc.ncbi.nlm.nih.gov/articles/PMC10483446/>) states: "Here, we contrasted resting-state fMRI in 86 antipsychotic-naïve first-episode early-onset schizophrenia (EOS) patients...", implying that patients were antipsychotic-naïve at scan-acquisition. There are a few possible scenarios here. Perhaps the likely one is that scans were made at baseline assessment and after a minimum of 6 months (after scan-acquisition) the EOS diagnosis was confirmed. I suppose that anti-psychotic treatment started after scan-acquisition but months (?) before diagnosis. Is that correct?

Response:

Yes, MRI scans were made at baseline assessment, and then the diagnosis of EOS was re-confirmed after a minimum of 6 months (after scan-acquisition). However, only imaging data of patients that passed the second confirmation were used in our study. Some patients with severe active symptoms were treated by antipsychotic medications right after scan-acquisition with their agreement. Although the medication time was before the second confirmation, it is more important to make sure patients get the treatment in time. We have detailed the scanning, diagnosis, and treatment time in the revision as below:

Page 6: *“Clinical and imaging data of patients were collected right after baseline diagnosis of schizophrenia to ensure that all data were drug-naïve. Next, individualized aggressive medication treatment was implemented under their agreement, in majority of cases with Clozapine effectively managing symptoms at the lowest possible dose.”*

Q3: They state that no patients had >12 months of diagnosis. That means, I think, that a patient could have a maximum of 18 months of active psychotic symptoms since baseline assessment. What I don't understand is why the authors mention this? What is, if any, the relationship between this statement and the rest of the sample description. If, in an alternative scenario, scan-acquisition was done at the time of diagnosis than the authors should explain the clinical follow-up really carefully b/c ethical concerns may be raised.

Response:

Actually, here "Patients were also excluded if they had the disease for > 1 year." refers to exclude patients for a duration of active psychotic symptoms > 12 months, not "no patients had >12 months of diagnosis". We apologize for our misleading description and have corrected this in the revision.

We mentioned "no longer than 1 year duration of psychotic symptoms" for making sure that patients are first-episode. Of course, "no longer than 1 year duration of symptoms" is not a standard criterion for the first episode period of schizophrenia. Usually, the first episode was defined by less than five years duration of psychotic symptoms. In contrast, some definitions of the chronic stage were patients with major negative symptoms and decline. Thus, here we claimed that patients with less than 12 months illness duration were not at their chronic stage of schizophrenia, and were still at their first episode period.

Page 6: *"All patients were at their first episode period with exhibiting at least one type of positive or negative symptoms for 1-12 months."*

Q4: Please give a very clear description of the chronology of the inclusion and clinical follow-up process, including what (type of) treatment was given when and where. Mention disease duration. Also mention clearly when scan-acquisition took place in this whole process. If there was individual variation in the time between baseline assessment and scan-acquisition then provide quantitative indicators of this time/variation.

Response:

We thank the Reviewer for this suggestion. The chronology of patient recruitment and clinical follow-up process was as follow:

1) Baseline assessment: Once a pediatric patient came to the hospital, the diagnostic progress was made by two psychiatrists via interviewing both the child and parent(s), including ruling out other mental health conditions (such as schizoaffective disorder, depressive or bipolar disorder) that may have similar psychotic features, and making the diagnosis of schizophrenia according to the Structured Clinical Interview

for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

2) Data collection: Right after baseline assessment, clinical and MRI scanning data of all patients were collected in the hospital. Those patients exhibiting at least one positive or negative symptom longer than 12 months were excluded in our study for making sure that patients are at their first-episode period.

3) Treatment: Once the baseline diagnosis was affirmed, individualized aggressive medication treatment started with the agreement of both patients and their legal guardians. Although the time of treatment is before the second confirmation, timely treatment for patients is more important. The treatment includes medication treatment, family education, and individual counseling. Most of the antipsychotics used in pediatric patients are the same as those used for adults with schizophrenia, in majority of cases with Clozapine. The type and dose of medication depend on individuals, with a goal of effectively managing symptoms at the lowest possible dose. However, we did not access detailed medication information from the hospital.

4) Re-confirmation: After 6-month follow-up, the diagnosis of schizophrenia was re-confirmed. Only clinical and MRI scanning data of those re-confirmed patients were used in our study.

We have clarified corresponding information in the revision as below:

Page 6: *“Ninety-nine drug-naïve first-episode EOS patients and 100 TD controls were recruited from the First Hospital of Shanxi Medical University, Taiyuan, China. The diagnosis of schizophrenia was made according to the Structured Clinical Interview for Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, and confirmed by two senior psychiatrists after at least 6-month follow-up. All patients were at their first episode period with exhibiting at least one type of positive or negative symptoms for 1-12 months. The psychiatric symptomatology of 71 patients was assessed using the Positive and Negative Syndrome Scale (PANSS). Clinical and imaging data of patients were collected right after baseline diagnosis of schizophrenia to ensure that all data were drug-naïve. Next, individualized aggressive medication treatment was implemented under their agreement, in majority of cases with Clozapine effectively managing symptoms at the lowest possible dose. Both TD controls and their first-degree relatives had no prior history of schizophrenia and were confirmed by the Structured Clinical Interview for DSM-IV Non-Patient Edition. Exclusion criteria for all subjects included i) age over 18 years; ii) history of neurological MRI abnormalities; iii) substance abuse; and iv) any electronic or metal implants; v) co-morbid Axis-I or Axis-II disease.”*

Q5: Global CT was calculated by averaging parcels? Are these parcels of similar size? Why not take a hemispheric mask and calculate the average directly over vertices (instead of an average of an average)? The mean difference between groups was 0.04 mm given a slice thickness of 1.2 mm. Is the software really able to detect such small sub-millimeter differences? Could the authors provide a plot showing the individual data-points by group? If there is huge overlap between cases and controls I deem it a good idea if the authors discuss the possible causes for the huge overlap between individuals with EOS and TD individuals. For example, what does this tell us about cortical thickness and EOS?

Response:

Indeed, we calculated global cortical thickness by averaging parcels, and these parcels were not the same size, thus potentially influencing the result of global cortical thickness. We've re-calculated global cortical thickness across the cortex by averaging all vertices within the hemispheric mask. However, as shown in below **Figure S1B**, we found global cortical thicknesses calculated by these two averaging strategies were almost the same, indicating little effect of the averaging strategy. Anyway, we changed the original parcel-wise averaging method to the vertex-level averaging strategy in our revision. We found similar covariance gradient results with global cortical thickness covariates averaged across vertices.

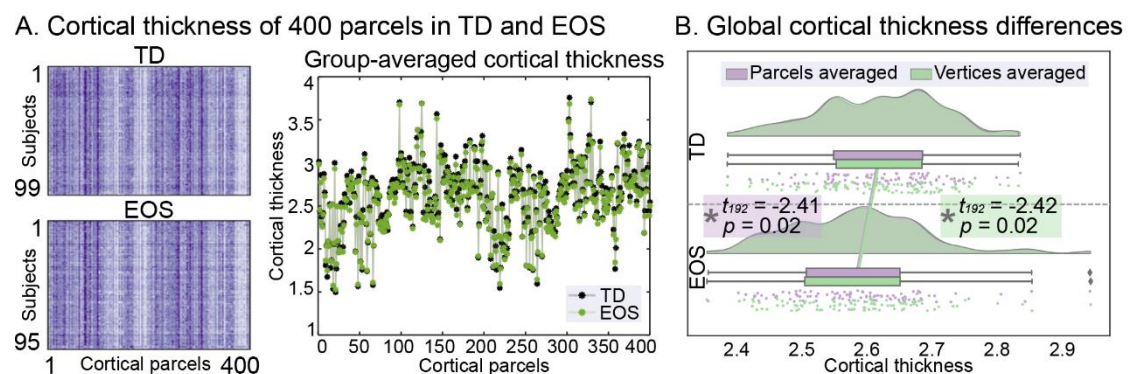


Figure S1. Parcel-wise and vertex-wise cortical thickness in typically developing (TD) and early-onset schizophrenia (EOS). (A) Cortical thickness of 400 parcels. For each parcel, group-averaged cortical thickness could be separately obtained for TD (colored in dark) and EOS (colored in green). (B) Global cortical thickness differences between different averaging strategies and groups. For each subject, global cortical thickness of parcel-wise averaging strategy (colored in purple) was similar with that using vertex-wise averaging strategy (colored in green). Global cortical thickness for both strategies showed significant group differences between TD and EOS.

Yes, the software was able to detect the sub-millimeter differences of cortical thickness. The thickness of a MRI slice is not directly link to obtained cortical thickness, and doesn't need to be thinner than cortical thickness of the cortex. Conceptually, slice thickness refers to the thickness of individual image slices acquired during the scanning process, measuring the depth of the section being imaged. However, the cortical thickness estimation was not simply based on the estimation of one imaging slice. In detail, we applied Freesurfer toolbox on all MRI slices to extract vertex-wise cortical thickness, including normalization, segmentation, and surface reconstruction. After surface reconstruction by tessellating the filled hemisphere with triangles and identifying vertices with the places where the points of the triangles meet, vertex-wise cortical thickness could be defined as the distance between the pial surface and the white matter surface. All values of cortical thickness could be accurate to four decimal places. Hence, our mean difference of global cortical thickness between groups was 0.04 mm, smaller than the slice thickness of 1.2 mm. Of course, increased slice thickness could result in a lower spatial resolution and may lose fine details. Generally, the ideal slice thickness for Freesurfer to estimate cortical thickness is 1 mm, and a thickness of up to 1.5 mm is also acceptable. On the contrary, too thinner slice thickness could also increase signal-to-noise ratio of the image. Altogether, the software is able to detect this sub-millimeter differences of cortical thickness.

Individual data points of global cortical thickness for TD and EOS are shown in **Figure S1B**. We found patients showed significant decreased global cortical thickness by using both parcel-wise averaging strategy ($t = -2.41$, $p = 0.02$) and vertex-wise strategy ($t = -2.42$, $p = 0.02$). However, as shown in **Figure S1A**, there were indeed some overlap data points of cortical parcels between the two groups, indicating that though on average there are differences, single cortical parcel of patients cannot be identified based on cortical thickness difference alone. These regional overlap between controls and patients might contribute to the findings of gradient overlap, i.e., no significant group difference in parcel-wise gradient scores. We have discussed their relationships in our revision as below:

Page 7: *“For each subject, global cortical thickness was calculated by averaging all vertices within the hemispheric mask and was then compared between controls and patients (Figure S1).”*

Page 13: *“However, group differences of gradient scores could not be observed at the parcel level. We inferred that the parcel-wise overlap of cortical thickness between patients and controls (Figure S1A) might be responsible for this negative finding. Despite the parcel-wise overlap, patients with EOS exhibited systematic disruptions of cortical thickness, underscoring the critical need for future investigation from a system-*

level perspective.”

Q6: The authors state: "...frontal regions showed subtly decreased gradient scores in EOS patients relative to TD controls...". Why use uncorrected p-values? I think I didn't see a plot showing this subtle decrease in EOP vs TD. Can the authors add it? If there is huge overlap between cases and controls I deem it a good idea if the authors interpret this overlap and its consequences for the relationship between "gradient scores" and EOS.

Response:

Indeed, it is not a good idea to use uncorrected p -values. In our original group comparisons of gradient scores, we found no parcel survived FDR correction and then reported uncorrected group differences. Patients' uncorrected reductions of gradient scores were mainly observed in frontal parcels (**Figure S5A**). However, as also mentioned by **the Reviewer 2** in **Q10**, these "subtly decreased gradient scores in frontal parcels" could just likely be spurious. Therefore, we have deleted these descriptions and provided these uncorrected group differences as supplementary data (**Figure S5**).

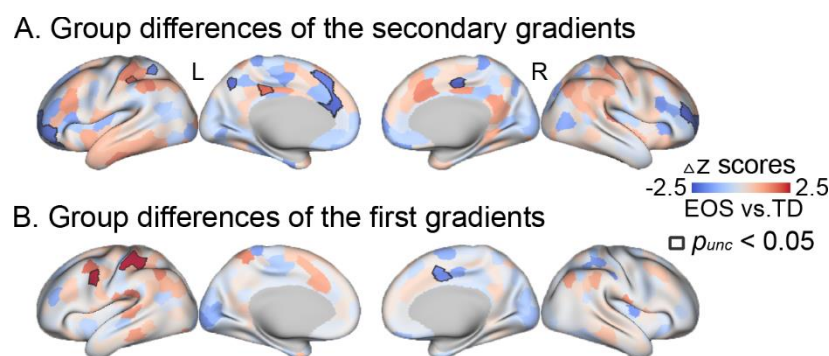


Figure S5. Parcel-wise gradient differences between EOS and TD. Brain maps show the group differences of z scores transformed from gradient scores (EOS vs. TD). Parcels showing uncorrected group differences ($p_{\text{uncorrected}} < 0.05$) are outlined in black. **(A)** Group differences of G2. The frontal regions showed subtly decreased gradient scores in EOS patients relative to TD controls. **(B)** Group differences of G1.

In the revised **Figure 2C**, we added a plot showing FDR-corrected group comparisons of the bins along the normative gradient axis. The 9th and 10th bins, which were mainly comprised of frontal areas, showed significant group differences in gradient scores. Furthermore, the relationships between parcel-wise gradient overlap and cortical thickness overlap have been discussed as abovementioned. We've modified corresponding Result part in the revision as below:

Page 10: “No parcels survived FDR correction for z score differences of gradient scores (**Figure S5**).”

Page 10: “We then divided cortical parcels into 10 equal-sized bins according to their ranked gradient scores in TD controls and tested group differences for each bin by using paired t-tests (**Figure 2C**). Patients showed increased gradient scores in the 3rd bin and decreased gradient scores in the 9th and 10th bins ($q_{FDR} < 0.05$), indicating a disrupted segregation for both the posterior and anterior parts.”

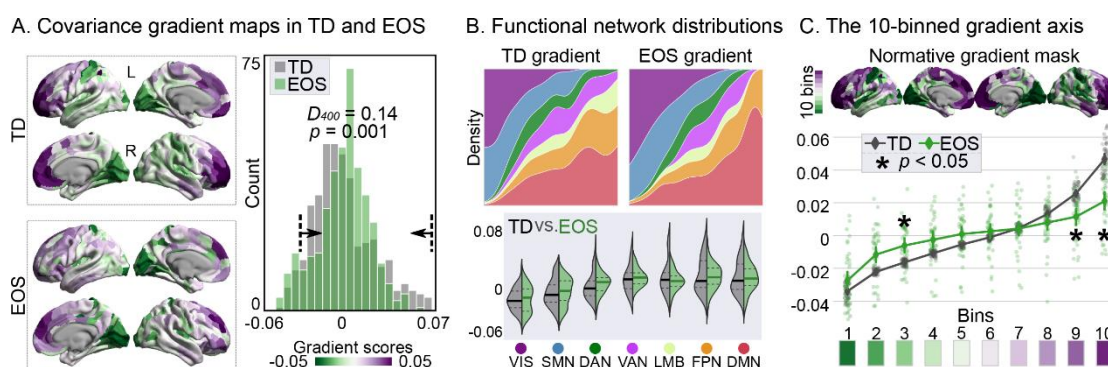


Figure 2. Structural covariance gradient patterns. (A) Covariance gradient maps in TD and EOS. In the right density map, green boxes represent gradient scores of EOS patients and grey boxes represent TD controls, which suggested compressed gradient axis in EOS than TD (Kolmogorov-Smirnov test; $D_{400} = 0.13$, $p = 0.002$). **(B)** Network distributions. For each group, the continuous density maps of gradient scores were separately plotted for each network. Group differences between EOS and TD were tested by using paired t-tests [False discovery rate (FDR) corrections, $q_{FDR} < 0.05$]. VIS, visual network; SMN, sensorimotor network; DAN, dorsal attention network; VAN, ventral attention network; LMB, limbic network; FPN, frontoparietal network; DMN, default mode network. **(C)** The 10-binned gradient axis. We computed bin-level gradient scores according to the 10-binned normative gradient mask and tested group differences by using paired t-tests (FDR corrected, $p < 0.05$).

Q7: I understand that comparisons were made between all bins, without a priori hypothesis. A few bins differed between groups, the majority didn't. A few were related to symptoms, the majority wasn't. I think the authors should be really carefully in their Discussion of these results and not generalize 'across bins', so to speak.

Response:

Indeed, a few bins differed between groups, but the majority didn't. For example, patients showed increased gradient scores in the 3rd bin, and decreased gradients in the 9th and 10th bins (See above **Figure 2C**). Actually, it was exactly this small part showing group differences that explained the compressed gradient axis in patients. To be specific, the 9th and 10th bins, i.e., the positive end in the anterior cortex showed

decreased gradient scores in patients, indicating less segregation of the anterior part along the gradient axis.

In terms of structural covariance comparisons, they were performed to investigate underlying basis of the compressed anterior-posterior gradient axis. We found that patients had increased covariance values between the 1st and 9th bins (**Figure 3A**). This increased covarying between the anterior and posterior ends could partly contribute to reduced segregation of the anterior part. Additionally, we found that increased covariance values between the 1st and 10th bins were related with less positive symptoms in patients (**Figure 3B**). This finding suggested that increased covarying between the anterior and posterior ends was related with positive symptoms.

In sum, these group comparison findings could not generalize 'across bins', while it is reasonable that only a few bins along the gradient axis showed group differences, e.g., a compressed gradient axis might have decreased absolute value of two end but not in the middle. We have interpreted the meaning of group-differed bins along the axis in the revision as below:

Pages 10-11: *“To further investigate the compressed anterior-posterior axis in EOS patients, we reorganized and averaged the structural covariance matrices according to the 10-binned normative gradient mask (**Figure 3**). Along the normative gradient axis, close ranks of two bins reflected similar structural covarying profiles. As expected, the further apart two bins were along the gradient axis, the lower the covariance value. We then tested for diagnosis effects on the reshaped structural covariance patterns. We found that patients had increased covariance values between the 1st and 9th bins ($t = 3.66$, $q_{FDR} = 0.002$), i.e., less negative covariance values in EOS compared to TD (**Figure 3A**). Here the increased covariation between the anterior and posterior ends partly explained abovementioned disruptions of gradient segregation. Additionally, we found more severe positive symptoms (PANSS-P scores) were related with reduced covariance between the 1st and 10th bins ($t = -3.01$, $q_{FDR} = 0.009$) (**Figure 3B**). More severe negative symptoms were related with increased covariance between the 10th and 6th bins ($t = 2.71$, $q_{FDR} = 0.02$).”*

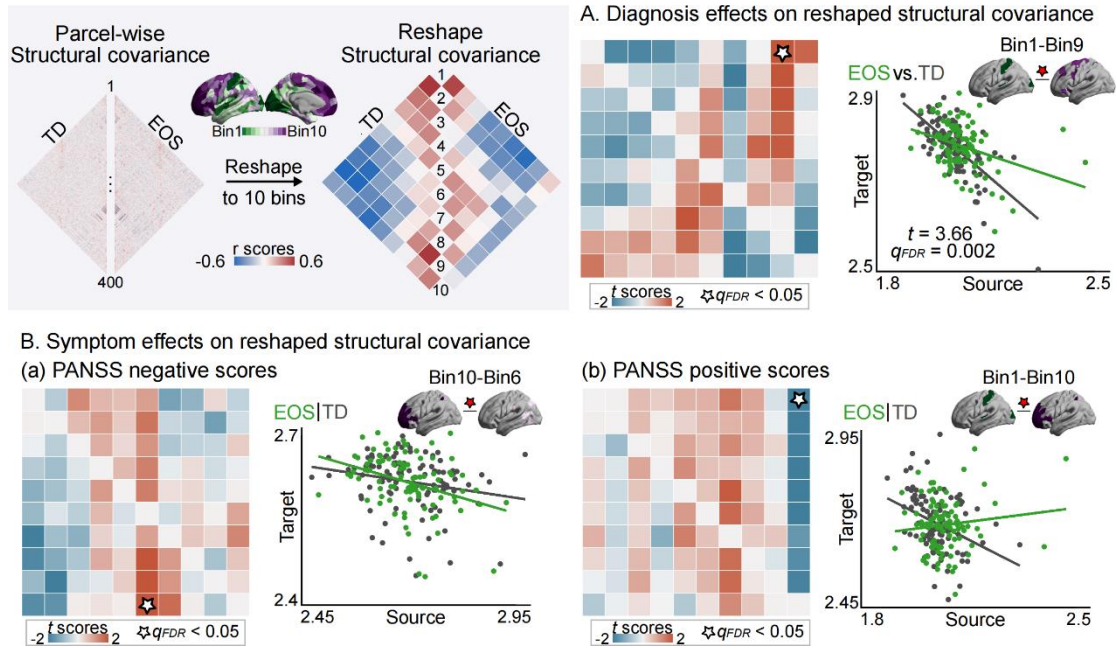


Figure 3. Disease effects on structural covariance patterns. Covariance matrices were reshaped into 10×10 matrices according to a 10-binned mask generated from the anterior-posterior gradient of TD controls. **(A)** Diagnosis effects on reshaped covariance. The diagnosis effect was examined by using a classic interaction linear model with group and cortical thickness as factors (FDR corrected, $p < 0.05$). Green dots and lines represent EOS patients and grey dots and lines represent TD controls. **(B)** Symptom effects on reshaped covariance. We used N scores of the Positive and Negative Syndrome Scale (PANSS) to quantify the severity of negative symptoms, and PANSS-P scores for positive symptoms. In the EOS group, symptom effects were examined by the interaction linear model with PANSS-P/N scores and cortical thickness as factors (FDR corrected, $p < 0.05$).

Q8: For the distances I have the same commentary as above, diagnostic differences are uncorrected or related to some bins but not others (why?). Taken together I think this means that considerable caution is warranted when discussing and interpreting the results.

Response:

We have removed the uncorrected diagnostic differences results of covariance distance in the revised **Figure 4**. Indeed, covariance distance differences were related to some bins but not others. To better illustrate this point, we plotted a line graph in **Figure 4C**. Each dot represents a bin along the gradient axis, the value of which was calculated by paired t -tests between patients and controls. Patients showed increased covariance distances in both ends of the gradient axis, but decreased covariance distances in the middle of the axis. Here a high covariance distance score of a region indicates a pattern dominated by remote connectivity, and a low score indicates local connectivity. Therefore, patients showed increased remote connectivity of two ends

along the gradient axis, potentially contributing to their compressed gradient map.

Page 11: “After resampling the covariance distance maps along the gradient axis (Figure 4C), we found that patients showed increased covariance distances in both ends of the gradient axis, but decreased covariance distances in the middle of the axis. Specifically, patients with EOS showed statistically increased covariance distances in the 1st bin ($t = 3.69$, $q_{FDR} = 0.007$) and the 10th bin ($t = 3.40$, $q_{FDR} = 0.008$) compared to TD controls.”

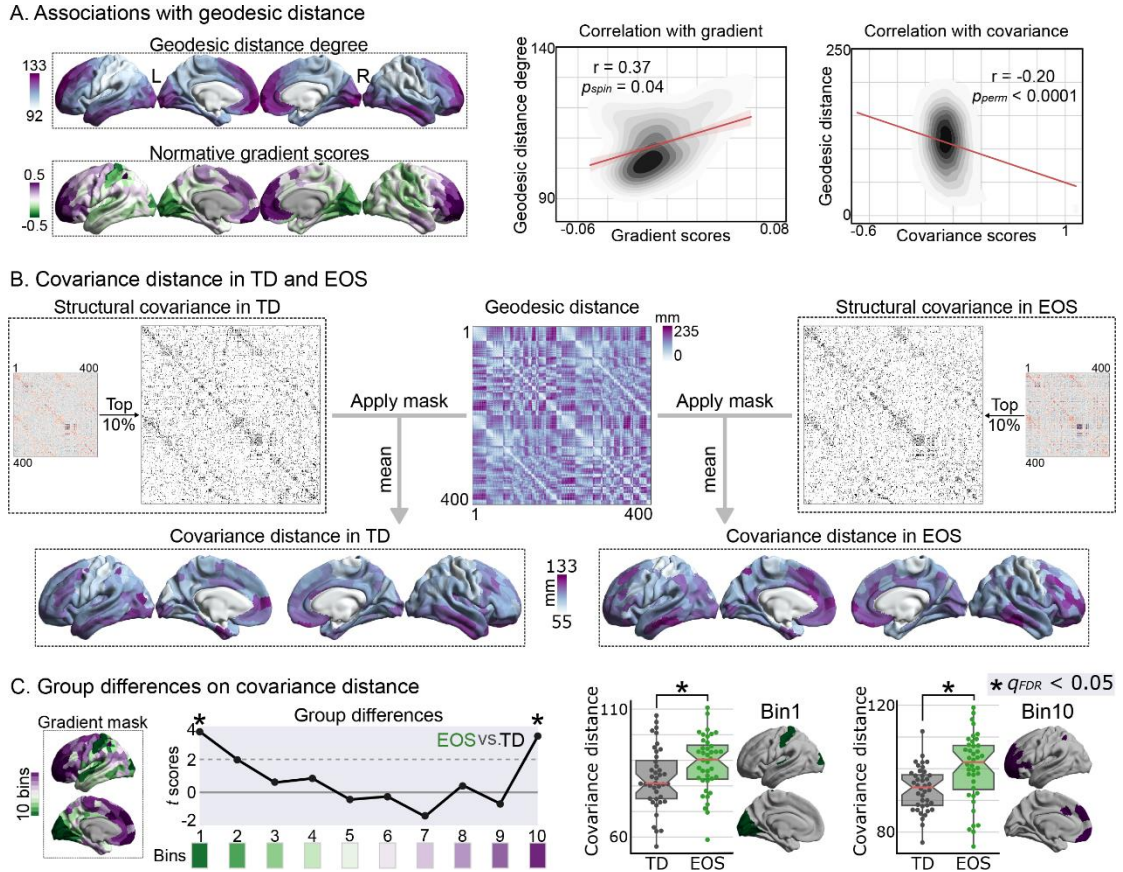


Figure 4. Covariance distance in TD and EOS. (A) Correlations between geodesic distances and covariance gradients and covariance matrices. The node-wise geodesic distance degree was calculated by averaging the geodesic distance for each node. The spin test was used to control for spatial autocorrelations ($p_{spin} < 0.05$, 10,000 times) (A. F. Alexander-Bloch et al., 2018). The edge-wise geodesic distance was then correlated with the structural covariance matrix, and the statistical significance was assessed using permutation tests ($p_{perm} < 0.05$, 10,000 times). (B) Covariance distance calculation. For each group, we column-wise thresholded the structural covariance matrix at 90%, and then binarized it as a mask. Covariance distance was then calculated using the averaging geodesic distance profiles of a seed region multiplied by the mask. (C) Effects of disease on covariance distances. In the left line graph, one dot represents a bin along the normative gradient axis, showing t values evaluated by paired t -tests (* represents $q_{FDR} < 0.05$). In the right box-and-whisker plots, the red horizontal lines in the box indicate the mean of each group, and the lower and upper

horizontal lines indicate the lower and upper quartiles, respectively. Green boxes represent covariance distances of EOS patients and grey boxes represent TD controls.

Bibliography in the Response to the Reviewer:

Alexander-Bloch, A. F., Shou, H., Liu, S., Satterthwaite, T. D., Glahn, D. C., Shinohara, R. T., . . . Raznahan, A. (2018). On testing for spatial correspondence between maps of human brain structure and function. *Neuroimage*, 178, 540-551.