

Diffusion trajectory of atypical morphological development in autism spectrum disorder

Corresponding Author: Professor Xujun Duan

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)

This study systematically investigated brain maturational dynamics in adolescents with autism. By integrating multi-modal analyses of structural morphometry and functional network topology, the authors reveal a previously unrecognized developmental transition in gray matter volumetric deviations—from early cortical overgrowth to delayed maturation trajectories—which represents a conceptual advance in understanding neurodevelopmental divergence in ASD. The application of Kullback-Leibler divergence to quantify maturational asynchrony, combined with network diffusion modeling, establishes a rigorous, biologically grounded analytical pipeline for decoding spatiotemporal brain reorganization. While these approaches are commendable for their methodological innovation, several concerns should be addressed to improve the study.

Q1: In 'Distribution deviation estimation based on Kullback-Leibler divergence estimation'. The top 10% threshold for identifying atypical regions is arbitrary. It would be valuable to discuss why this threshold was chosen and whether results are robust across different thresholds (e.g., top 5%, top 15%).

Q2: The inclusion of Energy Distance, Total Variation Distance, and Maximum Mean Discrepancy for validation is a strong point. However, details on how these comparisons were performed are lacking. A brief description would significantly strengthen the validation claims.

Q3: Why did the authors choose functional connectivity as the diffusion network instead of structural connectivity? This aspect has received relatively little discussion in this study.

Q4: In result, the description of early positive and later negative deviations in DEV is intriguing. However, quantitative measures (e.g., mean $\pm$ SD of DEV values across regions or groups) should be reported to support these statements more rigorously.

Q5: Only the results for the 13–15 group are shown in the main text. Please briefly summarize whether similar patterns were consistently observed across other age groups.

Q6: While the manuscript states that the findings "support the stability and reliability" of KL divergence, it would be helpful to briefly discuss why KL divergence may be theoretically preferred over the alternative metrics (ED, TVD, MMD) beyond empirical correlation results.

Q7: Several minor typographical errors should be corrected (e.g., "alaysis" should be "analysis"; "supplyment" should be "supplementary").

Q8: The should be further improved throughout the manuscript.

Q9: The methods are not clearly described. More details are needed. E.g., how to obtain the expected value of the PDF for a given brain region in the ASD and TDC groups?

Reviewer #2

(Remarks to the Author)

The authors analyzed data from 301 children and adolescents with Autism Spectrum Disorder (ASD) and 375 typically developing (TD) children and adolescents, including T1 and resting-state fMRI data from the Autism Brain Imaging Data Exchange (ABIDE), to quantify the deviation in gray matter development and examine how gray matter development is influenced by functional brain networks. Using the KL divergence for the deviation distribution (DEV) of the GM, they observed a development-dependent time course of DEV, such that a positive GMV deviation was found in specific brain regions for early adolescent ASD, which was followed by a negative shift in late adolescence. They further applied network diffusion modeling (NDM) and observed the effects of atypicality at the preceding stages on the subsequent abnormalities, which were constrained by the functional network architecture as defined by the resting-state fMRI data.

Overall, the authors reported significant findings regarding dynamic maturation changes in the ASD brain, utilizing advanced analytical techniques such as DEV and NDM analysis, as well as crossmodal data analysis. However, the reviewer has several concerns regarding the lack of essential details of the study.

The most significant concern is that details of the ABIDE data used for the analysis are missing. Because the ABIDE contains data from 18 sites, each of which collected data using different scanning parameters, sequences, and conditions, the authors should first provide details of the data for each of the 9 age ranges, from 8 to 18 years old. How many of them were from which data site? Because the developmental stage-dependent GMV alterations are the main finding of the study, it is critical to demonstrate that these findings are not contaminated by differences in the data site, each of which may have different proportions of data over the age range examined in this study. Furthermore, the ABIDE dataset contains highly heterogeneous data conditions, and sometimes one single site contains multiple scanning conditions. These differences in the data collection conditions should be clarified for each age bin.

Even more critically, did the authors use any data harmonization technique? - I could not find any descriptions regarding it. How was data from different sites combined? Without any harmonization procedures, such as ComBat, the effect of the data site might dominate the results. Some harmonization corrections are necessary for both structural and resting-state data.

Data quality control. I was able to locate the data quality control for the resting-state fMRI data, but I could not find any for the structural T1 image. The ABIDE contains many cases where structural images are messy even when the fMRI captions are acceptable.

Association between DEV and the functional connectome. The authors summed the DEV values of the neighbors of given nodes based on "directly connected neighbors" (lines 249 - 260). How did they determine whether nodes are "directly connected," given the multiple density levels of 10 - 50% (section 2.2.3)?

The analysis using a network-based diffusion is interesting, but I do not understand how the authors determine the time points of 100. Because the last time point of 100 is compared with the real data of the next developmental stage, I expect that the 100 time points will correspond to 2 years. However, it is unclear how one can see any correspondence between the time points in the and the lapse of time in the real world. It may not be appropriate to cut at an arbitrary time point while the alteration pattern continues to change.

Gradient analysis. The authors performed gradient analysis using a matrix of frequency of GM distribution alterations over the examined time windows. I see no explanations regarding how to interpret the x- and y-axes. Are they the first and second components? It resembles the plot from the Margulies PNAS 2016 paper, although the input data differs. Please explain more about how to interpret this result.

3.5 Robustness and replication analysis. They used data from a single site, NYU, for replication, but they do not provide any details about the selected data. Please give more details of how they calculated the single r value for "all primary analyses".

Minor points

Fig. 5 (C) What does it mean by " $r/p < 0.01$ "? The values in the table seem to indicate the r value, as far as I understand from R-squared.

line 513. What is "CMV"? Is this GMV?

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I have no further comments.

Reviewer #2

(Remarks to the Author)

The authors addressed all concerns adequately raised by the reviewer.

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Authors' response

Reviewer # 1

General comments:

This study systematically investigated brain maturational dynamics in adolescents with autism. By integrating multi-modal analyses of structural morphometry and functional network topology, the authors reveal a previously unrecognized developmental transition in gray matter volumetric deviations—from early cortical overgrowth to delayed maturation trajectories—which represents a conceptual advance in understanding neurodevelopmental divergence in ASD. The application of Kullback-Leibler divergence to quantify maturational asynchrony, combined with network diffusion modeling, establishes a rigorous, biologically grounded analytical pipeline for decoding spatiotemporal brain reorganization. While these approaches are commendable for their methodological innovation, several concerns should be addressed to improve the study.

Response:

We appreciate the positive evaluation and valuable comments from the reviewer. In accordance with the reviewer's comments, we have revised the manuscript thoroughly to address the concerns raised. A point-by-point response to each of the issues raised by the reviewer has been made. We believe that these revisions have substantially improved our manuscript.

Q1:

In 'Distribution deviation estimation based on Kullback-Leibler divergence estimation'. The top 10% threshold for identifying atypical regions is arbitrary. It would be valuable to discuss why this threshold was chosen and whether results are robust across different thresholds (e.g., top 5%, top 15%).

Response:

We sincerely thank the reviewer for this valuable comment. In the present study, we defined spatially atypical regions by selecting the top 10% of distribution deviation (DEV) values, in line with established conventions in neuroimaging research. The application of a 10%

threshold is a widely accepted approach for delineating salient neurobiological features, including the highest-ranking voxels in task-based activation maps (Lipkin et al., 2022), peak cortical vertex activations (Scheeringa et al., 2023), and the strongest functional connectivity edges (Knodt et al., 2023). This strategy facilitates consistency with prior work while enhancing interpretability and methodological rigor.

Nevertheless, we fully agree that the choice of threshold may influence spatial patterns. To address this concern and evaluate the robustness of our findings, we performed additional sensitivity analyses using alternative thresholds (top 5% and top 15%). As shown in the Figure S5 and Figure S6 (Supplementary, Figure S5 and S6), the extent of the identified regions varied slightly, but the core regions, predominantly localized in the frontal, parietal, and temporal lobes were consistently preserved. Furthermore, we observed developmental trajectories of DEV values across age groups remained stable under different thresholds. During early adolescence (before the 13–15 age group) (Supplementary Table S4 and S5), atypical regions in individuals with ASD exhibited positive values, whereas during late adolescence (after the 13–15 age group), the DEV became negative.

This pattern supports a non-linear developmental trajectory in ASD, characterized by early overgrowth followed by delayed or attenuated maturation. Importantly, these findings and the associated transition patterns remained robust across different thresholds. These additional analyses and corresponding data have been incorporated into the revised manuscript. In the revised manuscript:

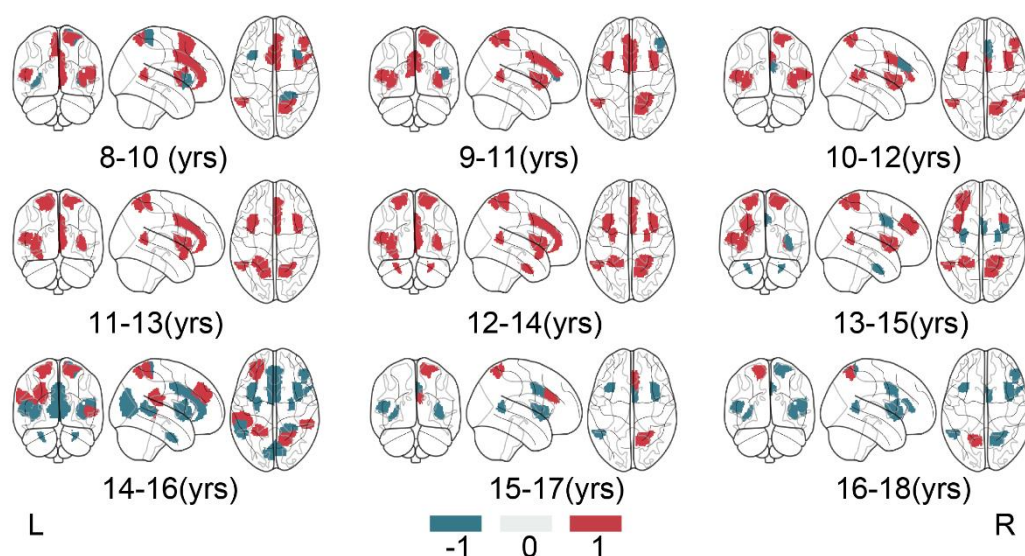


Fig. S5. Glass brain maps illustrating the values in the top 5% of spatial distribution deviation (DEV). Red areas indicate regions where DEV values are higher in the ASD group compared to TDCs, with the color bar label 1 representing the top 0–5% of positive deviations. Blue regions represent areas where DEV values are lower in the ASD group, with the label –1 corresponding to the top 0–5% of negative deviations. Label 0 denotes regions with DEV values outside these thresholds, darker colors indicate larger DEV scores. yrs refers to the years.

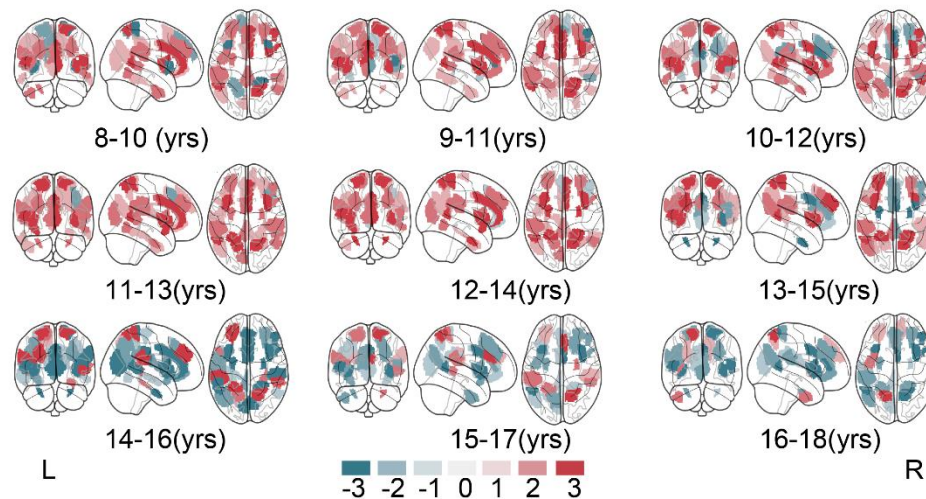


Fig. S6. Glass brain maps illustrating the values in the top 15% of spatial distribution deviation (DEV). Red areas indicate regions where DEV values are higher in the ASD group compared to TDCs (with color bar label 1 representing the top 11–15% of positive DEV values, and label 2 representing the top 6–10%, and label 3 representing the top 0–5%). Blue areas indicate regions where DEV values are lower in the ASD group (with color bar label 1 representing the top 11–15% of positive DEV values, and label 2 representing the top 6–10%, and label 3 representing the top 0–5%). Label 0 denotes regions with DEV values outside these thresholds, darker colors indicate larger DEV scores. yrs refers to the years.

Reference:

Lipkin B, Tuckute G, Affourtit J, Small H, Mineroff Z, Kean H, Jouravlev O, Rakocevic L, et al. (2022), Probabilistic atlas for the language network based on precision fMRI data from >800 individuals. *Sci Data* 9:529.

Scheeringa R, Bonnefond M, van Mourik T, Jensen O, Norris DG, Koopmans PJ (2023), Relating neural oscillations to laminar fMRI connectivity in visual cortex. *Cereb Cortex* 33:1537-1549.

Knodt AR, Elliott ML, Whitman ET, Winn A, Addae A, Ireland D, Poulton R, Ramrakha S, et al.

(2023), *Test–retest reliability and predictive utility of a macroscale principal functional connectivity gradient. Human brain mapping* 44:6399-6417.

Q2:

The inclusion of Energy Distance, Total Variation Distance, and Maximum Mean Discrepancy for validation is a strong point. However, details on how these comparisons were performed are lacking. A brief description would significantly strengthen the validation claims.

Response:

Thanks for the reviewer’s comment. To evaluate the robustness of KL divergence in capturing developmental distributional deviations, we incorporated three established distance metrics: Energy Distance (ED), Total Variation Distance (TVD), and Maximum Mean Discrepancy (MMD). Full methodological details are now provided in the supplementary [\(Supplementary Divergence Measures: ED, TVD, and MMD\)](#).

ED was computed following Székely and Rizzo ([Székely GJ and Rizzo ML, 2013](#)), quantifying distributional divergence between ASD and TDC groups through pairwise Euclidean distances for each brain region. The ED between the independent random variables $X \sim P$ and $Y \sim Q$ is defined as:

$$ED(X, Y) = 2E|X - Y| - E|X - X'| - E|Y - Y'|,$$

where $E|X| < \infty, E|Y| < \infty$, X' and Y' are independent copies of X and Y , respectively.

TVD referred to as statistical difference, is a fundamental metric used to quantify the discrepancy between two probability distributions. For two distributions P and Q over the sample space Ω , their TVD is defined as ([Feng W et al., 2024](#)):

$$\Delta TVD(P, Q) = \frac{1}{2} \sum_{x \in \Omega} |P(x) - Q(x)|,$$

it corresponds to the L_1 -norm of the difference between P and Q , linking to other statistical divergences including relative entropy.

MMD was estimated using a Gaussian radial basis function (RBF) kernel. Given samples $X = \{x_1, x_2, \dots, x_m\}$ denotes the samples from distribution P and $Y = \{y_1, y_2, \dots, y_m\}$

denotes the samples from distribution Q . The unbiased estimate of squared MMD (MMD^2) is (Jia X et al., 2018):

$$MMD^2(X, Y) = \frac{1}{m^2} \sum_{i,j=1}^m k(x_i, x_j) + \frac{1}{n^2} \sum_{i,j=1}^n k(y_i, y_j) - \frac{1}{mn} \sum_{i,j=1}^m k(x_i, y_j),$$

where $k(.,.)$ the Gaussian kernel with bandwidth chosen via the median heuristic (see [Supplementary Divergence Measures: ED, TVD, and MMD](#)).

Finally, we evaluated the concordance between KL divergence and alternative metrics by computing Pearson correlations based on atypicality frequency (defined as the number of developmental time windows in which each brain region was classified as atypical). As evidenced in [Supplementary Figure S8](#), the results demonstrated a significant correspondence between the atypical brain regions identified by KL divergence and those detected using alternative metrics. Specifically, regions identified via ED exhibited a spatial correlation of $r = 0.577$ ($p < 0.001$) with those derived from KL divergence, while spatial correlations for MMD and TVD were $r = 0.827$ ($p < 0.001$) and $r = 0.683$ ($p < 0.001$), respectively. These findings support the stability and reliability of KL divergence in characterizing atypical brain regions. In the revised manuscript:

Method: *“In the current study, to rigorously evaluate the reliability of KL divergence computation model, we implemented three established probability distribution distance metrics for comparative validation: Energy Distance (ED) [57], Total Variation Distance (TVD) [58], and Maximum Mean Discrepancy (MMD) [59] (see [Supplementary Divergence Measures: ED, TVD, and MMD](#)). Specifically, we calculated the spatial correlation (Pearson correlation) between their respective frequency matrices. Here, the frequency matrix was defined as a vector representing the number of developmental time windows in which each brain region was identified as atypical.”*

Reference:

Székely GJ, Rizzo ML (2013), *Energy statistics: A class of statistics based on distances*. *Journal of Statistical Planning and Inference* 143:1249-1272.

Feng W, Liu L, Liu T, *On deterministically approximating total variation distance*, *Proceedings*

of the 2024 Annual ACM-SIAM Symposium on Discrete Algorithms (SODA), SIAM, 2024, pp. 1766-1791.

Jia X, Zhao M, Di Y, Yang Q, Lee J (2018), Assessment of Data Suitability for Machine Prognosis Using Maximum Mean Discrepancy. *IEEE Transactions on Industrial Electronics* 65:5872-5881.

Q3:

Why did the authors choose functional connectivity as the diffusion network instead of structural connectivity? This aspect has received relatively little discussion in this study.

Response:

We sincerely thank the reviewer for the thoughtful comment. The use of functional connectivity (FC) as the diffusion network was primarily guided by the theoretical orientation of our study, which aims to examine how atypical neurodevelopmental patterns may propagate along intrinsic functional architectures. As demonstrated in Figure 3D, atypical brain regions (top 10% DEV values) showed spatial clustering along the principal functional gradient. Specifically, these regions were concentrated at the transmodal apex of Gradient 1 (As shown in Figure 3D of the manuscript), indicating a bias toward higher-order associative cortices, particularly in hubs of the Salience Network (SN), Default Mode Network (DMN), and Frontoparietal Network (FPN). This spatial pattern aligns with accumulating evidence implicating these higher-order functional networks as key of dysconnectivity in ASD. For example, studies have demonstrated that individuals with ASD exhibit functional abnormalities of brain regions in the DMN, including the prefrontal cortex, the insula, and the inferior frontal gyrus, which are involved in social cognition processing in both TDCs and ASD populations (Chen L et al., 2020). Attanasio M et al. suggested that early disruptions in SN connectivity may impair mechanisms for detecting salient social stimuli (e.g., human faces), potentially contributing to core ASD symptoms such as social communication deficits and atypical sensory processing (Attanasio M et al., 2024). Furthermore, functional connectivity within and between FPN and SN subnetworks is generally weaker in ASD, and these disruptions have been associated with co-occurring attentional and executive symptoms (Yerys BE et al., 2019). Importantly, we observed a topographical correspondence between regions of atypical

neurodevelopment and the intrinsic functional connectome, supporting our hypothesis that atypical developmental trajectories in ASD may be shaped or constrained by the architecture of large-scale functional brain networks.

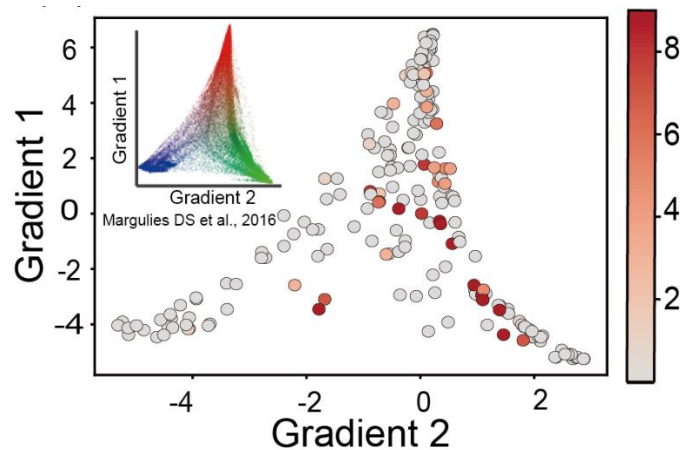


Figure 3D. Atypical regions projected onto the principal functional gradients of the cerebral cortex (Gradient 1 and Gradient 2; Margulies et al., 2016). The color bar indicating the number of times these abnormal regions appeared across the 9 age groups.

Reference:

Attanasio M, Mazza M, Le Donne I, Nigri A, Valenti M (2024), *Salience Network in Autism: preliminary results on functional connectivity analysis in resting state. European Archives of Psychiatry and Clinical Neuroscience.*

Chen L, Chen Y, Zheng H, Zhang B, Wang F, Fang J, Li Y, Chen Q, et al. (2020), *Changes in the topological organization of the default mode network in autism spectrum disorder. Brain Imaging and Behavior 15:1058-1067.*

Yerys BE, Tunç B, Satterthwaite TD, Antezana L, Mosner MG, Bertollo JR, Guy L, Schultz RT, et al. (2019), *Functional connectivity of frontoparietal and salience/ventral attention networks have independent associations with co-occurring attention-deficit/hyperactivity disorder symptoms in children with autism. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging 4:343-351.*

Q4:

In result, the description of early positive and later negative deviations in DEV is intriguing. However, quantitative measures (e.g., mean $\pm$ SD of DEV values across regions or groups)

should be reported to support these statements more rigorously.

Response:

We sincerely thank the reviewer for this helpful suggestion. We have now computed the mean $\pm$ standard deviation (SD) of DEV values in atypical brain regions for each age group, stratified by sliding age windows ([Supplementary Table S3](#)). These values provide a quantitative summary of the developmental shift in deviation patterns. Specifically, the mean DEV values were positive prior to the 13–15 age group and subsequently became negative, indicating a trajectory in which deviation values shift from early positive to later negative with increasing age. In addition, we also calculated the mean $\pm$ SD of DEV values within atypical regions defined by alternative thresholds (top 5% and top 15%) ([see Supplementary Tables S4 and S5](#)). These analyses revealed a consistent pattern: mean DEV values were positive in earlier age windows (before 13–15 age group) and turned negative in later stages, reinforcing the observed developmental transition. These analyses revealed a consistent pattern: mean DEV values were positive in earlier age windows and turned negative in later stages, reinforcing the observed developmental transition. These quantitative measures have been incorporated into the revised Results section to enhance the rigor, clarity, and interpretability of our findings. In the revised manuscript:

“As shown in Figure 2, the glass brain visualizations illustrate the top 10% of DEV regions for each age group, which were defined as atypical brain regions. These regions were primarily located in the frontal, parietal, and temporal lobes. During early adolescence (before the 13–15 age group), individuals with ASD exhibited positive values in DEV compared to TDCs (see Supplementary Table S3), suggesting that greater GMV density in ASD. Conversely, negative deviation emerged in later adolescence (after the 13–15 age group), indicating reduced GMV density in ASD compared to TDCs. This shift reflects a nonlinear developmental trajectory in ASD, characterized by early cortical overgrowth followed by delayed or attenuated maturation.

To evaluate the robustness of these findings, we repeated the analyses using different thresholds (top 5% and 15%). The spatial distribution of atypical regions remained largely consistent across thresholds. Although minor variations in extent were observed, the core regions, predominantly localized in the frontal, parietal, and temporal lobes were consistently preserved. Moreover, the developmental trajectories of DEV values across age groups remained stable under

different thresholds. During early adolescence (before the 13–15 age group), atypical regions in individuals with ASD exhibited positive deviations (Supplementary Table S4 and S5), whereas during late adolescence (after the 13–15 age group), the DEV values became negative.”

Table S3 mean and standard deviation of atypical regional (top 10%) DEV values

Age (yrs)	8-10	9-11	10-12	11-13	12-14	13-15	14-16	15-17	16-18
Mean	0.032	0.044	0.037	0.060	0.056	0.001	-0.051	-0.020	-0.034
SD	0.189	0.180	0.185	0.193	0.184	0.197	0.240	0.175	0.165

Note: SD refers to the standard; yrs refers to the years.

Table S4 mean and standard deviation of atypical regional (top 5%) DEV values

Age (yrs)	8-10	9-11	10-12	11-13	12-14	13-15	14-16	15-17	16-18
Mean	0.014	0.029	0.021	0.035	0.040	0.012	-0.039	-0.012	-0.020
SD	0.145	0.153	0.137	0.159	0.164	0.164	0.209	0.127	0.128

Note: SD refers to the standard; yrs refers to the years.

Table S5 mean and standard deviation of atypical regional (top 15%) DEV values

Age (yrs)	8-10	9-11	10-12	11-13	12-14	13-15	14-16	15-17	16-18
Mean	0.048	0.061	0.043	0.088	0.068	0.024	-0.064	-0.035	-0.036
SD	0.203	0.207	0.207	0.213	0.206	0.218	0.254	0.198	0.184

Note: SD refers to the standard; yrs refers to the years.

Q5:

Only the results for the 13–15 group are shown in the main text. Please briefly summarize whether similar patterns were consistently observed across other age groups.

Response:

Thanks for the reviewer’s comment. In the current study, we focused primarily on the 13–15 age group in the main text, as this developmental stage demonstrated a marked shift in the directionality of maturational deviation. However, we fully agree that presenting results across all age groups is important for completeness and transparency. Accordingly, we have now

included the full set of diffusion modeling results for all sliding age windows between 8 and 18 years in [Supplementary Figures S11](#). These results reveal that, the DEV scores of the seven core atypical regions demonstrate a dynamic age-related shift, gradually transitioning from positive to negative deviations, with negative DEV values emerging in late adolescence. In the revised Supplementary:

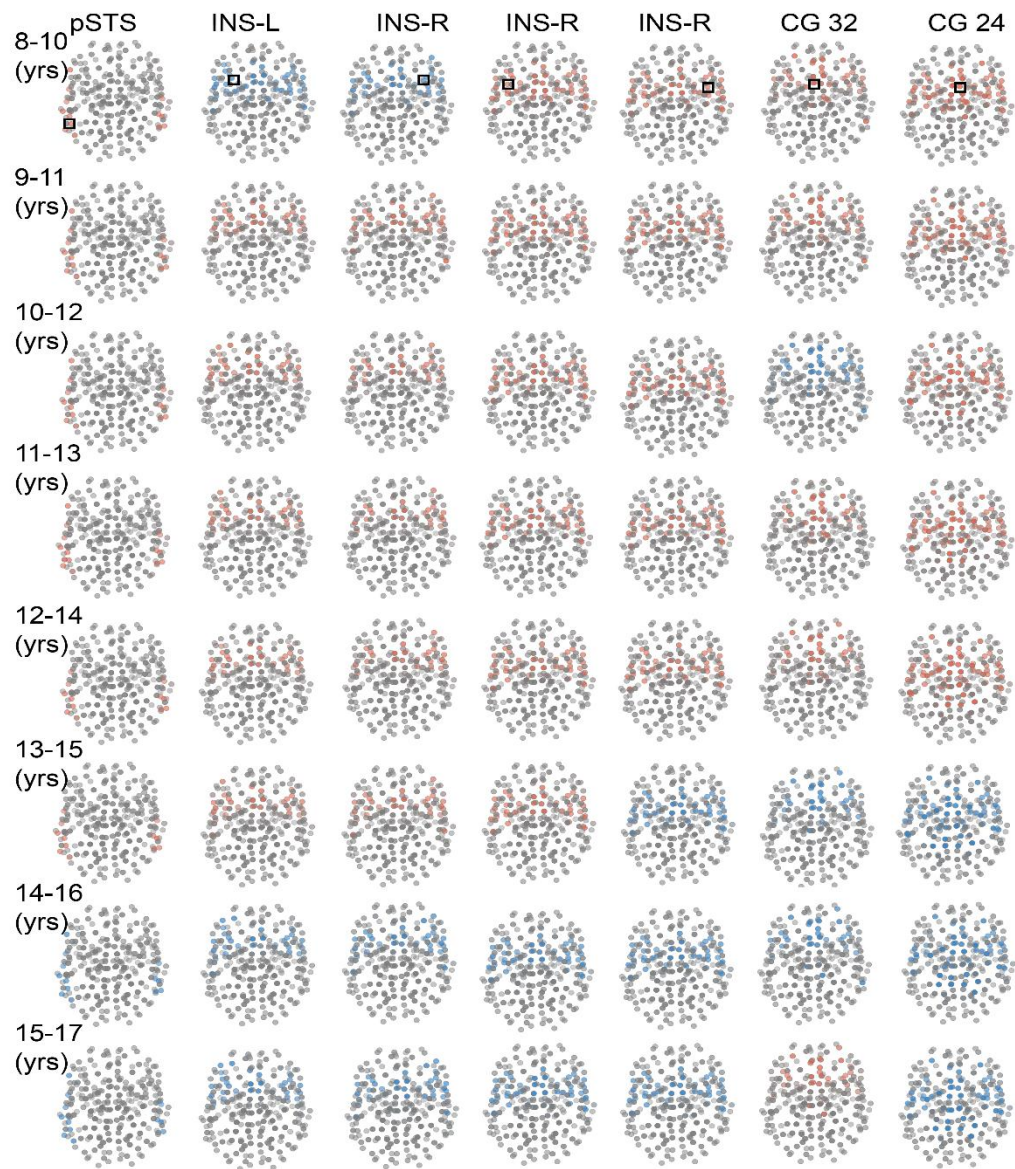


Fig. S11. Spatiotemporal diffusion patterns of seven representative nodes across all age group. Red and blue nodes denote regions with positive and negative DEV values, respectively. Nodes boxed in black indicate key regions referenced in seven representative nodes. yrs refers to the years.

Q6:
While the manuscript states that the findings "support the stability and reliability" of KL

divergence, it would be helpful to briefly discuss why KL divergence may be theoretically preferred over the alternative metrics (ED, TVD, MMD) beyond empirical correlation results.

Response:

Thanks for the reviewer's insightful comment. In the current study, we adopted KL divergence in combination with expected value estimates to assess distributional deviations in GMV across development. We selected KL divergence as the primary metric is grounded in its widespread application in neuroimaging for quantifying differences between probabilistic representations of brain morphology. Numerous prior studies have demonstrated the biological interpretability and empirical reliability of KL divergence ([Kullback S and Leibler RA, 1951](#); [Sebenius I et al., 2022](#); [Wang H et al., 2016](#)). For example, Wang and colleagues further validated the effectiveness of this method in constructing morphological similarity networks ([Wang H et al., 2016](#)). Furthermore, Sebenius et al. utilized symmetric KL divergence to derive a structural similarity metric that exhibited strongly alignment with known cortical symmetry, underscoring its biological interpretability ([Sebenius I et al., 2022](#)). Together with its strong theoretical foundation in information theory, these findings establish KL divergence as a biologically meaningful and theoretically grounded tool for modeling developmental deviations in GMV.

While KL divergence was selected as the primary metric in the current study, we additionally incorporated (ED, TVD, and MMD) as complementary measures to validate the robustness of our findings. Importantly, this was not intended to suggest that KL divergence is categorically superior, but rather to ensure that the observed deviation patterns were consistent across theoretically distinct metrics. Specifically, ED emphasizes sample-wise Euclidean distances, TVD quantifies the maximum difference in probabilities across distributions, and MMD assesses divergence within a reproducing kernel Hilbert space. The strong regional and spatial concordance among these metrics supports the stability and generalizability of our KL-based findings ([Supplementary Figure S8](#)). We have now expanded the Discussion section to clarify both the theoretical rationale for prioritizing KL divergence and the complementary roles of alternative distance measures in supporting the robustness of our results. In the revised manuscript:

" In the current study, we assessed the stability and robustness of the KL divergence computation model by introducing three widely used alternative measures of distributional distance: ED, TVD, and MMD. These metrics were chosen for their complementary theoretical properties and relevance in statistical comparison of probability distributions. Specifically, ED captures sample-wise differences based on pairwise Euclidean distances [56], TVD quantifies the maximum difference in probability mass between two distributions [99], and MMD assesses discrepancies between distributions in a reproducing kernel Hilbert space using a kernel-based framework [100]. By calculating the spatial correlation patterns between the three alternative metrics and KL divergence, we observed a high degree of spatial and statistical concordance in the overall atypicality maps. This strongly supports the robustness of KL divergence in detecting meaningful group-level distributional deviations and reinforces its reliability in identifying atypical morphological patterns in ASD, independent of the specific choice of distance metric."

Reference:

Kullback S, Leibler RA (1951), On information and sufficiency. *The annals of mathematical statistics* 22:79-86.

Sebenius I, Seidlitz J, Warrier V, Bethlehem RA, Alexander-Bloch A, Mallard TT, Garcia RR, Bullmore ET, et al. (2022), Mind networks: Robust estimation of structural similarity from brain mri. *bioRxiv:2022.2010. 2012.511922*.

Wang H, Jin X, Zhang Y, Wang J (2016), Single-subject morphological brain networks: connectivity mapping, topological characterization and test-retest reliability. *Brain Behav* 6:e00448.

Q7:

Several minor typographical errors should be corrected (e.g., "alaysis" should be "analysis"; "supplyment" should be "supplementary").

Response:

We thank the reviewer for pointing out this error and apologize for the mistake. We have carefully reviewed the manuscript and corrected all identified error. This correction has been incorporated into the revised manuscript as follows:

"Gradient analysis";

“More information about NDM can be found in supplementary”;

Q8:

The language should be further improved throughout the manuscript.

Response:

Thanks for the reviewer’s valuable suggestion. We have carefully revised the manuscript to improve the clarity, flow, and overall linguistic quality throughout the text. To ensure a high standard of academic writing, the revised text has been carefully reviewed and edited with input from colleagues with expertise in scientific English. We believe these improvements have significantly enhanced the readability, precision, and professional tone of the manuscript.

Q9:

The methods are not clearly described. More details are needed. E.g., how to obtain the expected value of the PDF for a given brain region in the ASD and TDC groups?

Response:

Thanks for reviewer’s suggestion. We apologize for the lack of clarity in the initial description. In the revised manuscript, we have carefully revised and clarified the relevant parts of the Methods section. Specifically, we now provide explicit descriptions of how the expected value of the probability density function (PDF) is calculated for each brain region in both ASD and TDC groups. The corresponding mathematical formulation and computational procedures have been added to the [Supplementary Material \(Figure S2\)](#). In addition, we have revised and redrawn our analytic workflow diagram. These updates will enhance the transparency of our group-wise regional deviation quantification. Below is the revised content of the supplementary material Supplementary S2:

supplementary material: *“Fig. S2. shows three randomly generated probability density functions (PDFs) with the same standard deviation: PDF 1, PDF 2, and PDF 3. PDF 2 is represented by a solid line, while PDF 1 and PDF 3 are shown as dashed lines, with PDF 1 positioned to the left of PDF 2 and PDF 3 to the right. As illustrated, the Kullback–Leibler (KL) divergence between the dashed PDFs (PDF 1 and PDF 3) and PDF 2 yields only positive values regardless of the direction of shift, due to its inherent asymmetry and non-negativity. Therefore,*

KL divergence alone cannot determine whether a distribution is shifted to the left or right relative to the reference distribution.

To address this limitation, we incorporate the concept of the mathematical expectation, defined for a continuous probability distribution $f(x)$ as:

$$E[x] = \int_{-\infty}^{\infty} xf(x)dx$$

We compute the expected values of both the dashed PDFs and PDF 2. By taking the difference between them ($E[PDF_{dashed}] - E[PDF_2]$), we derive a directional indicator. A negative value indicates that the dashed PDF is shifted to the left of the reference PDF 2, while a positive value indicates a shift to the right.”

Methods: Distribution deviation estimation based on Kullback-Leibler divergence estimation : “In the current study, to rigorously evaluate the reliability of KL divergence computation model, we implemented three established probability distribution distance metrics for comparative validation: Energy Distance (ED) [58] , Total Variation Distance (TVD) [59], and Maximum Mean Discrepancy (MMD) [60] (see Supplementary Divergence Measures: ED, TVD, and MMD). Specifically, we calculated the spatial correlation (Pearson correlation) between their respective frequency matrices. Here, the frequency matrix was defined as a vector representing the number of developmental time windows in which each brain region was identified as atypical.”

Methods: Gradient analysis: “To investigate the functional distribution patterns of atypical developmental brain regions, we performed a gradient analysis. Specifically, we constructed a matrix capturing the frequency with which each brain region was identified as atypical across developmental time windows. We then mapped these regions into the functional gradient space, using a standardized cortical gradient provided by Margulies et al [65], as implemented in the neuromaps toolbox [66]. This reference gradient reflects the principal axis of functional organization across the cortex, enabling spatial interpretation of atypicality patterns in relation to large-scale cortical hierarchies. We retained the first two gradient dimensions for visualization and interpretation, as they captured the most prominent axes of functional

differentiation. which allows standardized gradient mapping across brain parcellations.”

Methods: Statistics and Reproducibility: *“To validate the robustness and reproducibility of our findings, we conducted several complementary analyses. First, we repeated all primary analyses using alternative brain parcellation schemes consisting of 200 and 400 cortical regions, and confirmed the consistency of results. Second, to assess the generalizability of our results, we repeated the primary analyses in two subsamples: (i) a single-site cohort from New York University (NYU), which offers one of the largest and most balanced datasets in ABIDE (ASD = 55, TDC = 62). Full demographic details for the NYU is provided in Supplementary Table S2, and (ii) a male-only subsample to control for potential sex-related effects. Third, to quantify the reproducibility of the spatial patterns of atypical development, we focused on the frequency maps of atypical regions across developmental time windows. For each group, we computed a 246×1 vector representing the frequency with which each brain region was identified as atypical. We then calculated the Pearson correlation coefficient (r) between the frequency maps derived from each subsample and those from the full dataset. Finally, to evaluate the influence of site-related variability and the role of ComBat harmonization in our findings, we performed a set of control analyses using GMV data without applying ComBat correction.”*

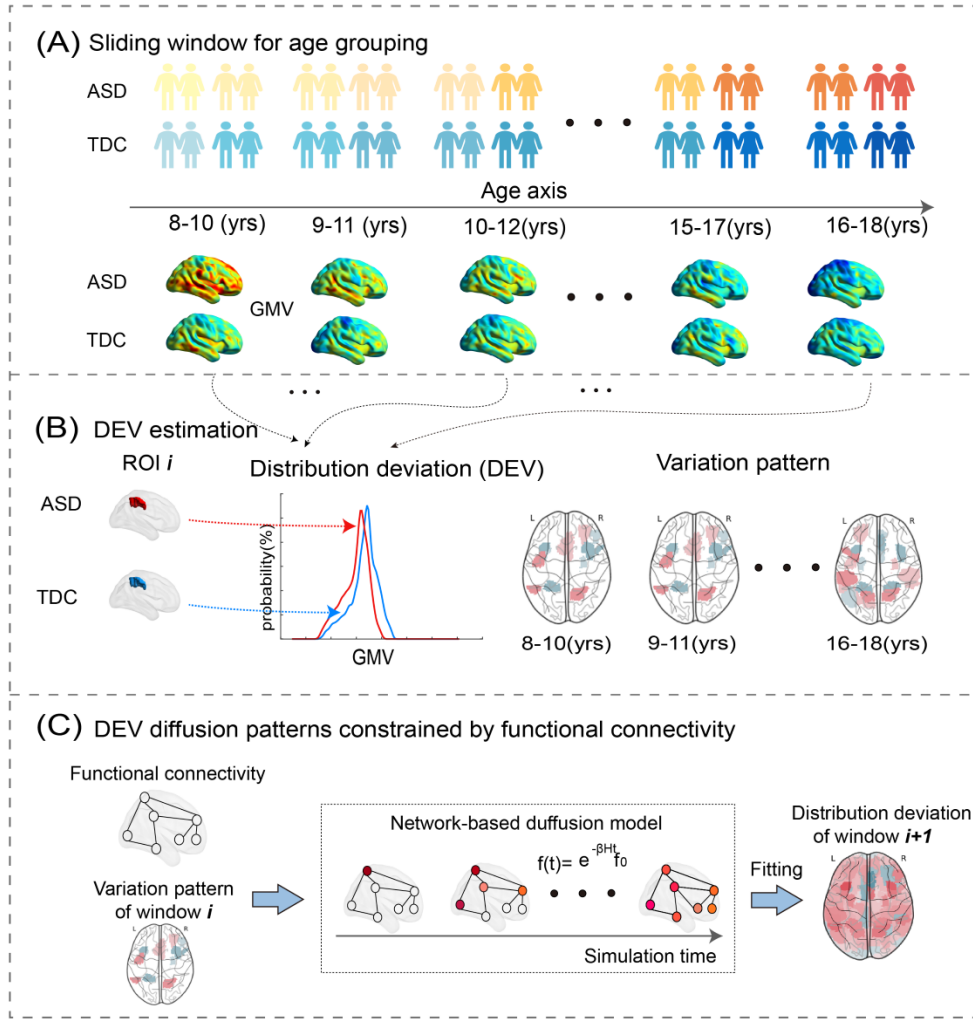


Fig. 1. Flow chart illustrating data analysis pipeline. (A) Sliding window approach for age grouping: Participants from the ASD and TDC groups were divided into overlapping age bins, each spanning 2 years with a 1-year overlap. For each age group, regional gray matter volume (GMV) maps were extracted separately for ASD and TDC individuals. (B) Estimation of distribution deviation (DEV): For each region of interest (ROI), the probability density functions (PDFs) of GMV values were estimated for both ASD and TDC groups, and using the Kullback–Leibler (KL) divergence and expected values to quantified DEV between the two distributions. The top 10% of regions with the highest DEV values were identified as atypical regions for each age group, forming age-specific variation maps. (C) Functional connectivity-constrained DEV diffusion modeling: To examine whether observed developmental changes in atypicality patterns are shaped by intrinsic brain connectivity, a network-based diffusion model (NDM) was applied. Using functional connectivity (FC) networks, atypical regions from a given age window served as seed inputs to simulate the spread of atypicality over time. The model fitting was performed by comparing simulated maps to observed DEV patterns in the subsequent age window. yrs refers to the year.

Reviewer # 2General comments:

The authors analyzed data from 301 children and adolescents with Autism Spectrum Disorder (ASD) and 375 typically developing (TD) children and adolescents, including T1 and resting-state fMRI data from the Autism Brain Imaging Data Exchange (ABIDE), to quantify the deviation in gray matter development and examine how gray matter development is influenced by functional brain networks. Using the KL divergence for the deviation distribution (DEV) of the GMV, they observed a development-dependent time course of DEV, such that a positive GMV deviation was found in specific brain regions for early adolescent ASD, which was followed by a negative shift in late adolescence. They further applied network diffusion modeling (NDM) and observed the effects of atypicality at the preceding stages on the subsequent abnormalities, which were constrained by the functional network architecture as defined by the resting-state fMRI data.

Overall, the authors reported significant findings regarding dynamic maturation changes in the ASD brain, utilizing advanced analytical techniques such as DEV and NDM analysis, as well as cross modal data analysis. However, the reviewer has several concerns regarding the lack of essential details of the study.

Response:

We sincerely thank the reviewer for the thoughtful and constructive feedback. We fully agree with the reviewer that additional methodological details are essential to improve the clarity, transparency, and reproducibility of the study. In response, we have thoroughly revised the manuscript to address these concerns. We believe the manuscript is much improved and hope it is now suitable for publication.

Q1:

The most significant concern is that details of the ABIDE data used for the analysis are missing. Because the ABIDE contains data from 18 sites, each of which collected data using different scanning parameters, sequences, and conditions, the authors should first provide details of the data for each of the 9 age ranges, from 8 to 18 years old. How many of them

were from which data site? Because the developmental stage-dependent GMV alterations are the main finding of the study, it is critical to demonstrate that these findings are not contaminated by differences in the data site, each of which may have different proportions of data over the age range examined in this study. Furthermore, the ABIDE dataset contains highly heterogeneous data conditions, and sometimes one single site contains multiple scanning conditions. These differences in the data collection conditions should be clarified for each age bin.

Response:

We sincerely thank the reviewer for this important comment. In selecting participants from the ABIDE dataset, our primary goal was to include as many participants as possible who fell within the developmental period of interest. We restricted the age range to 8–18 years based on the following considerations:

1) Data quality control: All participants underwent rigorous quality assessment procedures. These included visual inspection of raw and preprocessed structural MRI scans, exclusion of participants with motion artifacts or incomplete imaging data, and the use of standardized image preprocessing pipelines;

2) Sample size stability: Outside this range (i.e., <8 or >18 years), most sites had fewer than 20 participants per group, limiting statistical power and the reliability of normative modeling;

3) Balanced group representation: Within the 8–18-year window, each one-year age interval in both the ASD and TDC groups included at least 20 participants, ensuring robust age-specific comparisons;

4) Demographic matching: ASD and TDC groups were matched in terms of age, sex, and handedness. In the revised manuscript, we have provided detailed breakdowns of the number of participants by scanning site and age (8–18 years), for both the ASD and TDC groups. This information is presented in Table S1 ([Supplementary Table S1](#)). These tables clearly outline the age distribution across sites, enabling assessment of potential site-age confounds.

We acknowledge that, after stratification by age, several sites contributed relatively few participants to certain age range. This reflects an inherent limitation of the ABIDE dataset, in

which the distribution of ages and scanning protocols is uneven both across and within sites. To address potential concerns regarding site variability across age groups, we provide the following clarifications and methodological considerations: 1) The heterogeneity in age distribution across sites is a known limitation of the ABIDE dataset, which was aggregated retrospectively across multiple centers with varying recruitment strategies and scanning protocol; 2) To address this, we applied ComBat harmonization to the structural imaging data, including age, sex, and diagnosis as biological covariates. This approach effectively corrected for site-related variance while preserving meaningful developmental and diagnostic effects. Moreover, our analytic framework did not rely on traditional group-level case-control comparisons between ASD and TDC participants. Instead, we modeled normative developmental trajectories and quantified individual-level deviations, thereby reducing susceptibility to site-driven confound.

We also thank the reviewer for drawing attention to the potential influence of ComBat harmonization on our findings. We acknowledge that due to a production oversight, Figure 5B in the original manuscript mistakenly presented results derived from non-ComBat-corrected data, while it was described as reflecting harmonized outputs. We sincerely apologize for this error and have now corrected it in the revised version of the manuscript.

To ensure full transparency and allow direct comparison, the corresponding results based on non-harmonized data have been included in the Supplementary Materials as [Supplementary Figure S10](#). To further assess the impact of ComBat harmonization, we conducted additional robustness analyses using GMV data without applying ComBat correction. As shown in [Supplementary Figure S10A](#), the atypicality maps derived from harmonized and non-harmonized data were highly spatially correlated ($r = 0.860$, $p < 0.001$), indicating that the harmonization procedure did not materially affect the identification of atypical regions. Furthermore, [Supplementary Figure S10B](#) shows that atypicality brain regions from one developmental window significantly predicted DEV scores in the subsequent window ($p < 0.001$), suggesting that the developmental trajectories identified in our analysis are stable and not driven by site-specific artifacts. Taken together, these results further support the reliability of our conclusions and demonstrate that our approach captures meaningful patterns of neurodevelopmental atypicality that are not confounded by site-related variability. In the

revised manuscript:

Methods: “To address inter-site variability inherent in the multi-site ABIDE dataset, we applied ComBat harmonization to the regional GMV estimates. This method corrects for site-specific effects while preserving biologically meaningful variation. In line with recommendations from prior studies [47], we included age, sex, and diagnostic group as biological covariates in the ComBat model to ensure that developmental and group-related effects were retained following harmonization.”

Table S1 Information of site

Site\age(yrs)	8	9	10	11	12	13	14	15	16	17	Total
<i>YALE</i> (ASD/TDC)	1/1	1/2	0/2	3/0	2/1	3/2	2/2	0/2	4/1	0/1	16/14
<i>USM</i>	--	0/1	--	1/0	1/1	0/2	0/1	2/0	3/1	2/1	9/7
<i>UM</i>	0/1	4/2	1/4	2/5	4/1	7/3	5/2	4/8	6/3	1/12	34/41
<i>UCLA</i>	1/1	3/4	5/1	4/6	3/6	4/6	5/5	2/3	3/0	3/1	33/33
<i>TRINITY</i>	--	--	--	--	0/2	3/2	1/4	3/3	0/1	1/1	8/13
<i>STANFORD</i>	4/2	2/3	1/1	3/1	1/1	--	--	--	--	--	11/8
<i>SDSU</i>	0/1	3/2	5/1	1/2	2/3	4/3	4/5	4/3	5/6	7/3	35/29
<i>PITT</i>	--	--	--	1/0	1/3	1/0	2/1	0/2	2/0	0/1	7/7
<i>OLIN</i>	--	--	--	--	2/0	--	1/1	1/1	1/1	1/1	6/4
<i>NYU</i>	12/11	7/6	10/9	8/11	5/6	3/3	5/5	3/4	1/6	1/1	55/62
<i>LEUVEN</i>	--	--	--	--	2/4	2/0	6/3	1/3	0/4	1/0	12/14
<i>KKI</i>	6/11	1/24	5/20	3/13	1/8	--	--	--	--	--	16/76
<i>CALTECH</i>	--	--	--	--	0/1	--	--	--	--	1/1	1/2
<i>UCD</i>	--	--	--	--	1/1	3/1	2/1	1/2	3/2	3/2	13/9
<i>TCD</i>	--	--	2/0	--	0/2	0/2	1/1	0/3	0/1	0/1	3/10
<i>SU</i>	1/0	3/4	4/2	3/3	5/4	0/1	--	--	--	--	16/14
<i>IU</i>	--	--	--	--	--	--	--	--	--	2/0	2/0
<i>GU</i>	1/9	3/4	6/8	6/5	5/4	3/2	--	--	--	--	24/32
<i>Total</i>	26/37	27/52	39/48	35/46	35/48	33/27	34/31	21/34	28/26	23/26	301/375

Note: yrs refers to the years.

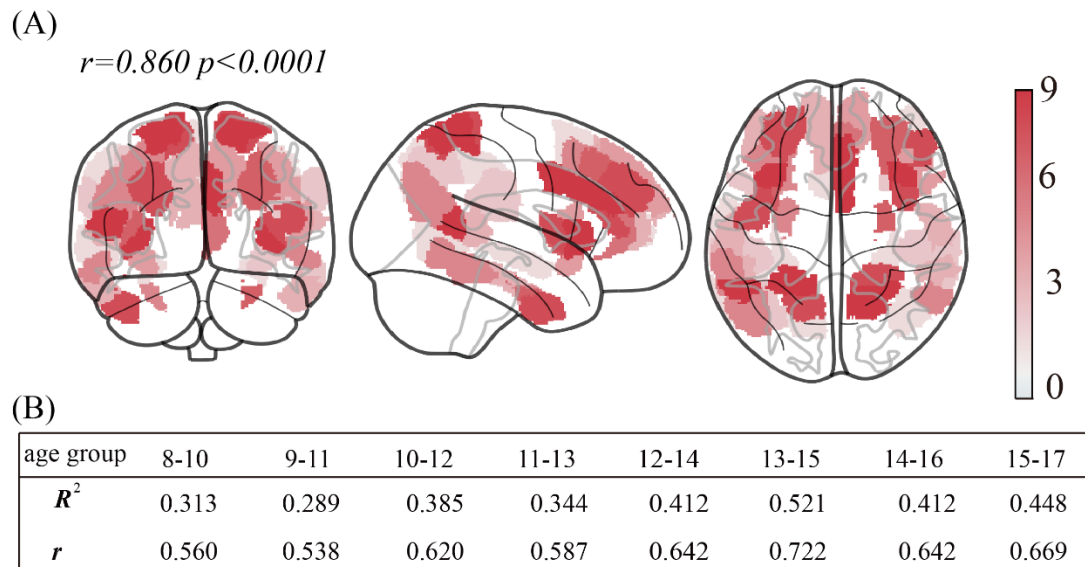


Fig. S10. Validation of Model Robustness Without ComBat Harmonization. (A) The glass brain map shows the atypical brain regions identified without applying ComBat correction, which exhibited a spatial correlation of $r = 0.860$ ($p < 0.001$) with the atypical regions derived from ComBat-corrected data. The color bar represents the frequency with which each region was identified as atypical across age groups. (B) The summary table derived from non-ComBat-corrected data displays the model fitting performance of the NDM between adjacent developmental age groups. Each row presents the explanatory power (R^2) and r between predicted and actual DEV scores from one age group to the next (Permutation test $p < 0.001$);

Q2:

Even more critically, did the authors use any data harmonization technique? - I could not find any descriptions regarding it. How was data from different sites combined? Without any harmonization procedures, such as ComBat, the effect of the data site might dominate the results. Some harmonization corrections are necessary for both structural and resting-state data.

Response:

We apologize for the lack of clarity in the original manuscript regarding harmonization procedures. For structural MRI data, we applied ComBat harmonization to the regional GMV estimates. This method effectively corrects for site-specific effects while preserving biologically meaningful variation. In line with recommendations from prior multi-site

neuroimaging studies (Bayer JM et al., 2022; Campo F et al., 2023), we included age, sex, and diagnostic group (ASD vs. TDC) as biological covariates in the ComBat model to retain relevant developmental and clinical signals. The full details of this harmonization procedure are now explicitly described in the revised Methods section.

Regarding resting-state functional connectivity (FC) data, ComBat harmonization was not applied to the FC data used in the network diffusion modeling analysis. This decision was based on both the nature of our analytic framework. Specifically, in our diffusion modeling analysis, we first averaged individual resting-state FC matrices within each age group, generating group-level FC matrices. These were then used to simulate the spatial diffusion of atypicality across the functional network architecture. The purpose of this analysis was not to compare ASD and TDC groups, but to examine whether the distribution of structural atypicality could be predicted by age-specific intrinsic functional network organization. Because the analysis was performed entirely on group-averaged FC matrices, and not on individual-level connectivity data, the potential influence of site-related variance was inherently minimized. The averaging procedure itself reduces noise introduced by individual, site-level heterogeneity, and the analysis was purely exploratory in modeling the spatial correspondence between FC and atypical structural patterns.

To further confirm the robustness of our findings, we conducted validation analyses using two homogeneous sub-cohorts: a single-site sample from NYU (ASD = 55, TDC = 62, the largest homogeneous subsample in ABIDE), and (2) an all-male sample (ASD = 252, TDC = 312). Critically, core findings replicated across both validation samples (Supplementary Figure S9), supporting the robustness of the findings and reducing the likelihood that they were influenced by site-related variability in FC data. In the revised manuscript:

“ To address inter-site variability inherent in the multi-site ABIDE dataset, we applied ComBat harmonization to the regional GMV estimates. This method corrects for site-specific effects while preserving biologically meaningful variation. In line with recommendations from prior studies [47], we included age, sex, and diagnostic group as biological covariates in the ComBat model to ensure that developmental and group-related effects were retained following harmonization.”

Limitation: *“Despite the valuable insights provided by this study, several limitations*

should be acknowledged. First, although our diffusion modeling approach revealed that atypical structural development may be constrained by functional network architecture, the analysis was limited to functional connectivity (FC). This choice was based on both the spatial correspondence of maturational deviations with known functional gradients and the limited availability of high-quality diffusion MRI data in the ABIDE cohort, which prevented robust structural connectivity (SC) reconstruction. Future work integrating SC-based network diffusion models will be important for validating and extending the current results. Second, the multi-site nature of the ABIDE dataset introduces considerable heterogeneity in data acquisition protocols, scanner types, and demographic distributions across sites and age groups. Although we used ComBat harmonization to reduce site-related variance and included biological covariates to preserve meaningful effects, we acknowledge that residual confounds factors may still exist, especially in underrepresented age groups from certain sites. Finally, the study primarily focuses on structural brain development and functional connectivity, and future research could explore how other neurobiological factors, such as genetic or environmental influences, contribute to atypical brain development in ASD.”

Reference:

Bayer JM, Dinga R, Kia SM, Kottaram AR, Wolfers T, Lv J, Zalesky A, Schmaal L, et al. (2022), Accommodating site variation in neuroimaging data using normative and hierarchical Bayesian models. NeuroImage 264:119699.

Campo F, Retico A, Calderoni S, Oliva P (2023), Multi-Site MRI data harmonization with an adversarial learning approach: implementation to the study of brain connectivity in autism spectrum disorders. Applied Sciences 13:6486.

Q3:

Data quality control. I was able to locate the data quality control for the resting-state fMRI data, but I could not find any for the structural T1 image. The ABIDE contains many cases where structural images are messy even when the fMRI captions are acceptable.

Response:

We apologize for our unclear description. In the revised Methods section, we have now provided a detailed account of the preprocessing and quality assurance steps applied to the structural MRI data. Specifically, all T1 images were visually inspected by three experienced raters for motion artifacts and preprocessing errors. Image quality was rated on a 5-point scale (1 = poor, 5 = excellent), and subjects with an average score ≤ 2 were excluded from further analysis. This step ensured robust skull stripping, tissue segmentation, and normalization quality. Subsequently, we used the Computational Anatomy Toolbox (CAT12) with SPM12, following a standardized and widely accepted pipeline. This included global intensity correction, segmentation, DARTEL registration, and spatial smoothing. To ensure data quality, we implemented multi-level quality control combining both automated and manual procedures. Automated quality metrics provided by CAT12, such as noise-to-contrast ratio, inhomogeneity, and image resolution—were used to flag potentially problematic images. In the revised manuscript:

“All T1-weighted images were visually evaluated for motion artifacts by three experienced raters. Image quality was scored on a 5-point scale (1 = poor, 5 = excellent), and participants with an average score ≤ 2 were excluded from further analysis. The MRI data processing was performed with Computational Anatomy Toolbox, CAT12 ([http:// www. neuro.uni- jena. de/ cat/](http://www.neuro.uni-jena.de/cat/)) and Statistical Parametric Mapping 12 ([https:// www. fil. ion. ucl. ac. uk/ spm/ softw are/ spm12/](https://www.fil.ion.ucl.ac.uk/spm/software/spm12/)) [43], using standard pipeline: (1) global intensity correction; (2) modulation and segmentation of the images into gray matter, white matter and cerebrospinal fluid (CSF) after blood vessel correction and skull stripping [44]; (3) registration to standard space using diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) algorithm; (4) thorough quality control of the structural images, comprising both automated image quality metrics provided by CAT12 (including noise-to-contrast ratio, inhomogeneity; and (5) co-registration to Montreal Neurological Institute (MNI) space [45], and spatial smoothing with a 6-mm FWHM kernel. All procedures were carried out following the guidelines provided in the Cat12 software manual [46].”

Q4:

Association between DEV and the functional connectome. The authors summed the DEV values of the neighbors of given nodes based on “directly connected neighbors” (lines 249 - 260). How did they determine whether nodes are “directly connected,” given the multiple density levels of 10 - 50% (section 2.2.3)?

Response:

Thanks for the reviewer’s comment. In the current study, “directly connected” nodes were defined based on a binary FC matrix thresholded at a 10% sparsity level, consistent with prior studies (Knodt AR et al., 2023; Vos de Wael R et al., 2020). Specifically, the top 10% of strongest pairwise correlation values in the FC matrix were retained and binarized such that edges representing the highest 10% of connectivity strengths were assigned a value of 1 (indicating a direct connection), while all remaining edges were set to 0. To ensure that our findings were not driven by the specific choice of this threshold, we repeated all analyses across a range of sparsity levels (10% to 50%). The results remained consistent across thresholds, supporting the robustness of the observed associations between DEV and neighboring nodes. These results are presented in [Supplementary Table S6](#), which summarize the association strength between regional DEV and neighborhood DEV at each sparsity level.

Table S6 Correlation coefficients and corresponding p-values for different neighboring windows across various percentages.

sparsity levels		Age group (yrs)							
		8-10~9-11	9-10~10-12	10-12~11-13	11-13~12-14	12-14~13-15	13-15~14-16	14-16~15-17	15-17~16-18
10%	<i>r</i>	0.560	0.538	0.623	0.587	0.642	0.722	0.642	0.669
	<i>p</i>	<i>all<0.001</i>							
20%	<i>r</i>	0.697	0.623	0.663	0.627	0.699	0.580	0.694	0.587
	<i>p</i>	<i>all<0.001</i>							
30%	<i>r</i>	0.668	0.659	0.665	0.619	0.670	0.564	0.703	0.565
	<i>p</i>	<i>all<0.001</i>							
40%	<i>r</i>	0.670	0.663	0.496	0.602	0.651	0.577	0.709	0.573
	<i>p</i>	<0.001	<0.001	0.041	<0.001	<0.001	<0.001	<0.001	<0.001

50%	<i>r</i>	0.663	0.676	0.470	0.526	0.5820	0.064	0.684	0.579
	<i>p</i>	<0.001	<0.001	<0.064	=0.001	<0.001	<0.001	<0.001	<0.001

Notes: p represents the p-value obtained from the permutation test; yrs refers to the years.

Reference:

Knodt AR, Elliott ML, Whitman ET, Winn A, Addae A, Ireland D, Poulton R, Ramrakha S, et al. (2023), Test–retest reliability and predictive utility of a macroscale principal functional connectivity gradient. *Human brain mapping* 44:6399-6417.

Vos de Wael R, Benkarim O, Paquola C, Lariviere S, Royer J, Tavakol S, Xu T, Hong S-J, et al. (2020), BrainSpace: a toolbox for the analysis of macroscale gradients in neuroimaging and connectomics datasets. *Communications biology* 3:103.

Q5:

The analysis using a network-based diffusion model is interesting, but I do not understand how the authors determine the time points of 100. Because the last time point of 100 is compared with the real data of the next developmental stage, I expect that the 100 time points will correspond to 2 years. However, it is unclear how one can see any correspondence between the time points in the model and the lapse of time in the real world. It may not be appropriate to cut at an arbitrary time point while the alteration pattern continues to change.

Response:

We thank the reviewer for this important comment. We apologize for the unclear description in the original manuscript. In the context of the network diffusion model (NDM), the “time points” do not correspond to real-world chronological time, such as years. Instead, they represent iterations or steps in the simulation of heat diffusion over the network topology, and are thus unitless in terms of biological time.

In the current study, we selected $t = 0$ to 100 as the representative diffusion window because the model reaches a stabilized state at this stage while yielding maximal correlation between simulated patterns and empirical deviation patterns observed in subsequent age

groups, thereby achieving optimal diffusion modeling. This practice is consistent with previous studies employing network diffusion approaches (Chopra S et al., 2023; Raj A et al., 2012). As shown in **Figure S1C** of the supplementary, the optimal t value corresponding to the maximum correlation coefficient (r) was identified within the range of $t = 0$ to $t = 100$. All derivations and implementation details of the NDM model are provided in the [Supplementary Materials: Development of a Network Diffusion Model](#).

NDM model performance

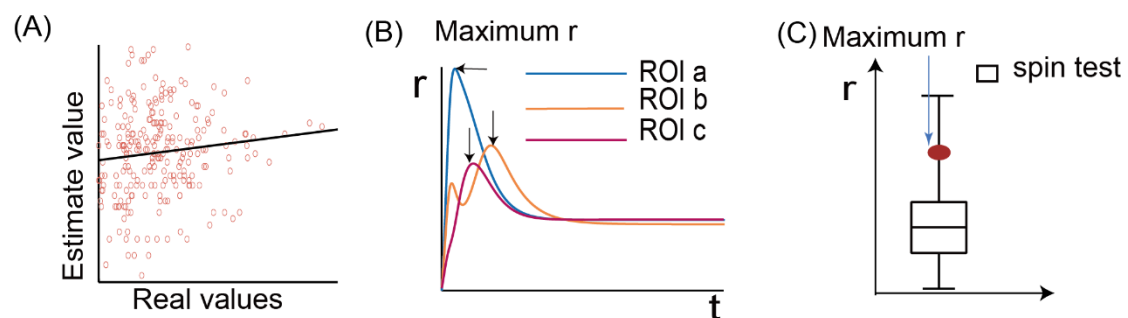


Fig.S1. Evaluation of network diffusion model (NDM) performance. (A): The scatter plot shows the relationship between observed and estimated distribution deviation (DEV) values across brain regions, with the strength of association quantified using Pearson correlation (r). (B): For each seed region (e.g., ROI a, b, c), the correlation (r) between observed and simulated DEV values was computed across diffusion time points. The maximum r value was extracted to characterize the optimal model fit. (C): The observed maximum r value (red dot) was compared against a null distribution of maximum r values generated from spatially permuted (spin test) surrogates. Statistical significance was determined by assessing whether the observed value exceeded the 95th percentile of the null distribution.

Reference:

Chopra S, Segal A, Oldham S, Holmes A, Sabaroedin K, Orchard ER, Francey SM, O'Donoghue B, et al. (2023), Network-Based Spreading of Gray Matter Changes Across Different Stages of Psychosis. *JAMA Psychiatry* 80:1246-1257.

Raj A, Kuceyeski A, Weiner M (2012), A network diffusion model of disease progression in dementia. *Neuron* 73:1204-1215.

Q6:

Gradient analysis. The authors performed gradient analysis using a matrix of frequency of GM distribution alterations over the examined time windows. I see no explanations

regarding how to interpret the x- and y-axes. Are they the first and second components? It resembles the plot from the Margulies PNAS 2016 paper, although the input data differs. Please explain more about how to interpret this result.

Response:

Thanks for the reviewer's comment. We apologize for the unclear description. In our gradient analysis, we constructed a matrix representing the frequency with which each brain region exhibited atypical developmental patterns. Specifically, occurrences within the top 10% of DEV values across developmental time windows. These regions were then projected into a standardized functional gradient space derived from the principal functional gradient reported by Margulies et al ([Margulies DS et al., 2016](#)). This gradient captures a macroscale axis of cortical organization from unimodal (sensorimotor) to transmodal (default-mode) areas and was implemented using the neuromaps toolbox ([Markello RD et al., 2022](#)). By mapping atypical regions onto this reference gradient, we were able to examine whether developmental structural asynchrony preferentially aligned along known functional hierarchies of the cortex. We have now clarified the interpretation of the x- and y-axes in the revised Results and Methods sections.

Methods: "To investigate the functional distribution patterns of atypical developmental brain regions, we performed a gradient-based analysis. Specifically, we constructed a matrix capturing the frequency with which each brain region was identified as atypical across developmental time windows. We then mapped these regions into the functional gradient space, using a standardized cortical gradient provided by the Margulies et al [64], as implemented in the neuromaps toolbox [65]. This reference gradient reflects the principal axis of functional organization across the cortex, enabling spatial interpretation of atypicality patterns in relation to large-scale cortical hierarchies. We retained the first two gradient dimensions for visualization and interpretation, as they captured the most prominent axes of functional differentiation."

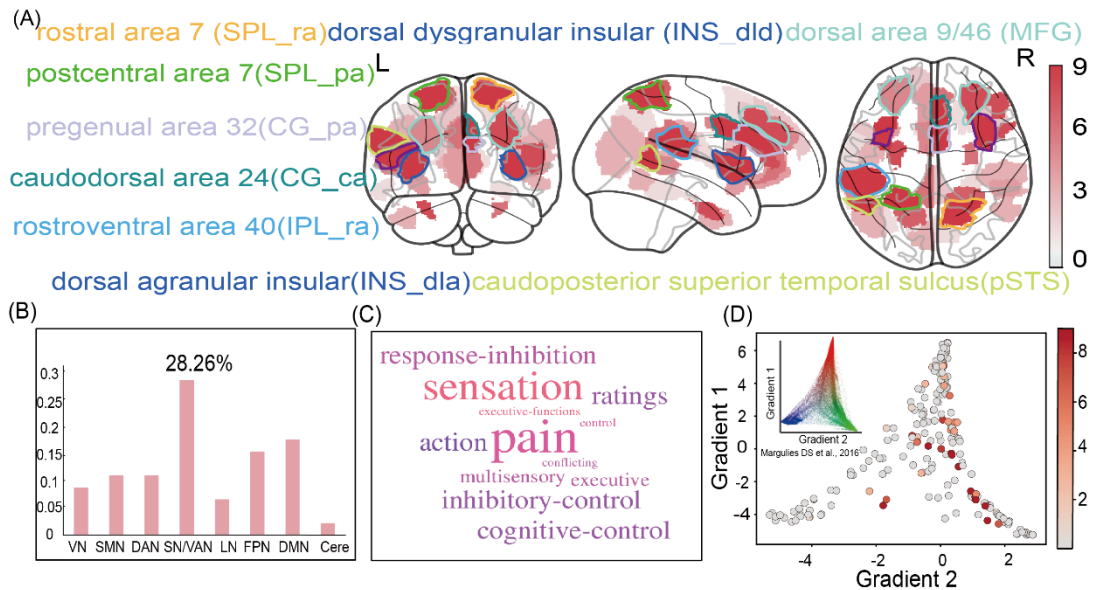


Fig. 3. Spatial distribution and functional profiling of atypical brain regions in ASD. (A) A glass brain map displaying the spatial distribution of the top 10% most atypical brain regions, aggregated across all age groups. The color bar represents the frequency with which each region was identified as atypical across age groups. (B) Proportion of atypical regions assigned to each functional network. The bar plot shows the proportion of atypical regions located in each network. (C) Potential psychological processes associated with atypical regions, decoded using Neurosynth. (D) Atypical regions projected onto the principal functional gradients of the cerebral cortex (Gradient 1 and Gradient 2; Margulies et al., 2016). The color bar indicating the number of times these abnormal regions appeared across the 9 age groups.

Reference:

Margulies DS, Ghosh SS, Goulas A, Falkiewicz M, Huntenburg JM, Langs G, Bezgin G, Eickhoff SB, et al. (2016), Situating the default-mode network along a principal gradient of macroscale cortical organization. *Proc Natl Acad Sci U S A* 113:12574-12579.

Markello RD, Hansen JY, Liu Z-Q, Bazinet V, Shafiei G, Suárez LE, Blöstein N, Seidlitz J, et al. (2022), Neuromaps: structural and functional interpretation of brain maps. *Nature Methods* 19:1472-1479.

Q7:

3.5 Robustness and replication analysis. They used data from a single site, NYU, for replication, but they do not provide any details about the selected data. Please give more details of how they calculated the single r value for “all primary analyses”.

Response:

Thanks for the reviewer's comment. For the replication analysis, we selected participants from the New York University (NYU) site in the ABIDE database, which contributed the largest single-site sample. The NYU subset included 55 ASD participants (11.35 ± 2.45 years) and 62 TDC participants (11.92 ± 2.68 years) after quality control. The demographic details of this subset are provided in Table S2 ([Supplementary Table S2](#)), and the same preprocessing pipeline was applied to ensure methodological consistency.

To evaluate the robustness and reproducibility of our primary findings, we focused on the spatial frequency map of atypical regions—specifically, a 246×1 vector for each age group indicating how frequently each brain region was identified as atypical across all developmental time windows. We then calculated the Pearson correlation coefficient (r) between the frequency map obtained from the NYU subset and those from the full multi-site dataset. This correlation reflects the overall spatial similarity in atypical region distributions across the two samples, thereby serving as a compact summary of replication fidelity across all primary analyses. The full replication results, including correlation values and visualizations, are presented in [Supplementary Figure S9](#).

Method: *"To validate the robustness and reproducibility of our findings, we conducted several complementary analyses. First, we repeated all primary analyses using alternative brain parcellation schemes consisting of 200 and 400 cortical regions, and confirmed the consistency of results. Second, to assess the generalizability of our results, we repeated the primary analyses in two subsamples: (i) a single-site cohort from New York University (NYU), which offers one of the largest and most balanced datasets in ABIDE (ASD = 55, TDC = 62). Full demographic details for the NYU is provided in Supplementary Table S2, and (ii) a male-only subsample to control for potential sex-related effects. Third, to quantify the reproducibility of the spatial patterns of atypical development, we focused on the frequency maps of atypical regions across developmental time windows. For each group, we computed a 246×1 vector representing the frequency with which each brain region was identified as atypical. We then calculated the Pearson correlation coefficient (r) between the frequency maps derived from each subsample and those from the full dataset. Finally, to evaluate the influence of site-related variability and the role of ComBat harmonization in our findings, we performed a set of control analyses using GMV data without applying ComBat*

correction."

Table S2 NYU subject demographics

	ASD (55)	TDCs (62)	<i>p</i>
Age	11.35±2.45	11.92±2.68	0.23 ^a
Sex(male/female)	49/6	54/8	0.74 ^b
Handedness(Right/left/mixed/)	43/8/4	58/4/0	0.02 ^b *
FIQ	106.45±18.18	114.24±15.44	0.01 ^a *

Mean and standard deviation are given for each continues measure; yrs refers to the years.

a The *P* value was obtained by a 2-sample *t*-test, 2-tailed

b The *P* value was obtained by the Pearson's chi-square test

* Significant at $p < 0.05$

Q8:

Fig. 5 (C) What does it mean by " $r/p < 0.01$ "? The values in the table seem to indicate the *r* value, as far as I understand from R-squared.

Response:

We thank the reviewer for pointing out this confusion. The label " $r/p < 0.01$ " in Figure 5C was indeed misleading. What we intended to convey was that the reported Pearson correlation coefficients (*r*) were statistically significant after correction, with $p < 0.001$. We have now corrected this mislabeling in the figure and clarified the legend accordingly in the revised manuscript.

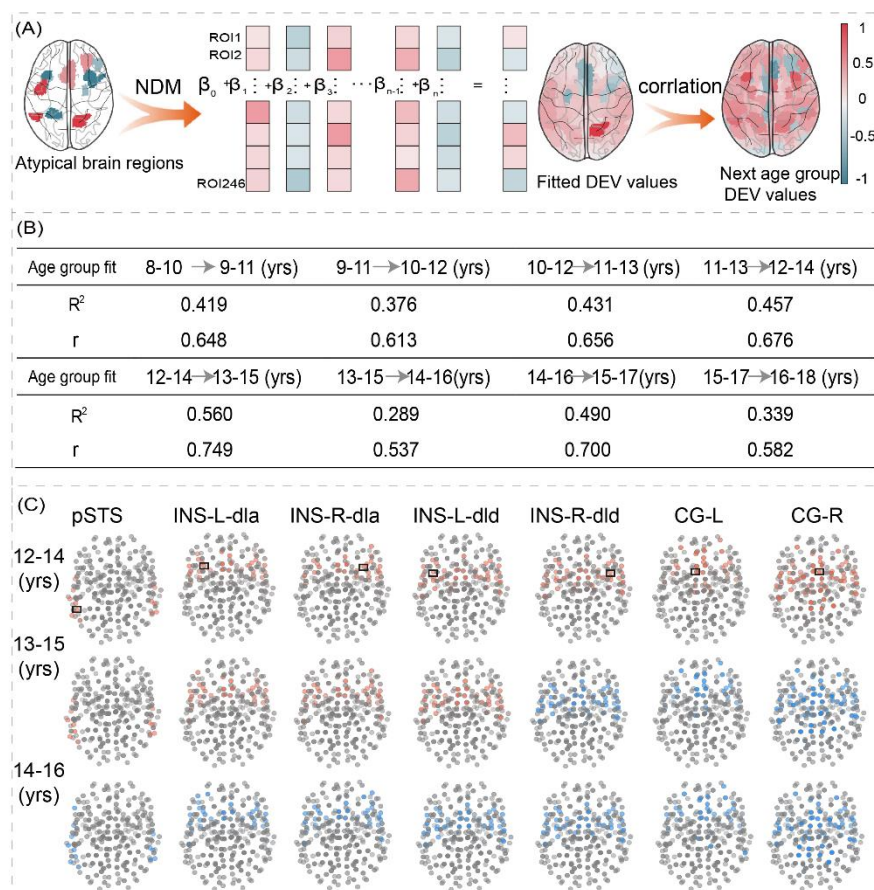


Fig.5. Network Diffusion Modeling (NDM) of atypical brain regions in ASD across age groups. (A) Schematic illustration of the NDM framework applied to atypical brain regions identified at each age stage. For a given age window, n atypical regions were used as source nodes in the NDM. Each source region generated a 246-dimensional diffusion map, and these n diffusion maps were subsequently used to estimate the fitted DEV scores. Pearson correlation analysis was conducted between the predicted DEV scores and the observed DEV scores from the subsequent age group. The color bar denotes both the intensity and polarity of the DEV values. (B) Summary table showing the performance of NDM-based model fitting between adjacent age groups. Each row presents the explanatory power (R^2) and r between predicted and actual DEV scores from one age group to the next (Permutation test $p < 0.001$); (C) Spatiotemporal diffusion patterns of seven representative nodes across three consecutive developmental windows. Red and blue nodes denote regions with positive and negative DEV values, respectively. Nodes boxed in black indicate key regions referenced in 7 representative nodes, yrs refers to the years.

Q9:

line 513. What is "CMV"? Is this GMV?

Response:

Thanks for the reviewer pointing out this error. We apologize for the mistake. The term “CMV” on line 513 was a typographical mistake and should have been “GMV” (gray matter volume). We have carefully reviewed the entire manuscript to identify and correct similar inconsistencies and have fixed this error in the revised version to ensure clarity and accuracy throughout.

“The results of current study reveal a significant relationship between the DEV of GMV and the functional network architecture in individuals with ASD.”