

1 **Generalisable functional imaging classifiers of schizophrenia have multifunctionality as**
2 **trait, state, and staging biomarkers**

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1 **ABSTRACT (147 words)**

2 This study introduces a novel resting-state functional connectivity (rs-FC) magnetic resonance
3 imaging (MRI) biomarker for diagnosing schizophrenia spectrum disorder (SSD), developed
4 using customized machine learning on an anteroegradely and retrogradely harmonized dataset
5 from multiple sites, including 617 healthy controls and 116 patients with SSD. Unlike previous
6 rs-FC MRI biomarkers, this new biomarker demonstrated a high accuracy rate of 77.3% in an
7 independent validation cohort, including 404 healthy controls and 198 patients with SSD from
8 seven different sites, while overcoming across-scan variability. It specifically identifies SSD,
9 differentiating it from other psychiatric disorders. Our analysis identified 47 important FCs
10 significant in SSD classification, many of which are involved in the pathophysiology of SSD.
11 These FCs could be potential trait, state, and staging markers for effectively predicting
12 delusional tendencies, specific symptoms, and disease stages. This research underscores the
13 potential of rs-FC as a clinically applicable neural phenotype marker for SSD.

14

1 INTRODUCTION

2 Schizophrenia spectrum disorder (SSD) is a chronic mental disorder affecting approximately
3 1% of the global population, ranking among the top ten causes of disease burden. Individuals
4 with SSD experience symptoms such as hallucinations, delusions, and cognitive impairment¹.
5 SSD diagnosis is often subjective and relies on diagnostic criteria that categorise disorders
6 based solely on signs and symptoms. Similar to the case with other psychiatric disorders, there
7 are currently no widely applicable, objective biomarkers² that can distinguish individuals with
8 SSD from their healthy counterparts with minimal overlap.

9 Generating a classifier of SSD using resting-state functional connectivity (rs-FC)
10 magnetic resonance imaging (MRI) is an emerging research topic. However, the practical use
11 of these classifiers is hindered by several key challenges³⁻⁶. First, there is an accuracy problem.
12 In studies with a large sample size and external validation^{7,8}, classifier performance was below
13 biomarker requirements (approximately 80%). Accuracy typically decreases with increase in
14 sample size ($N > 200$) in whole-brain imaging biomarkers⁹. A machine learning algorithm
15 requiring many explanatory variables (FCs) needs more data to achieve accuracy and
16 generalisability; however, large training samples from multiple facilities might cause a large
17 site effect, deteriorating data quality¹⁰. Second, there is an issue with generalisability. Most
18 previous studies used only internal or limited external validation and lacked comprehensive
19 external validation from independent cohorts obtained in multiple sites not involved in the
20 initial research¹¹. Third, across-session variability poses a problem. The reliability of
21 resting-state functional (rs-f) MRI is questionable over repeated tests¹², which could affect the
22 stability of the biomarker. Lastly, there is a lack of specificity in potential biomarkers identified
23 in rs-FC MRI studies focusing on specific disorders. Overcoming these issues is crucial for
24 developing a truly effective biomarker for clinical use in SSD.

25 While disease trait markers, including diagnostic markers, assist early intervention,

1 other biomarkers are also essential: state markers for estimating dynamic changes in response
2 to treatment^{13,14} and staging markers for disease prevention or personalising interventions¹⁵.
3 These biomarkers have been developed separately in some studies^{16,17}, and other studies
4 assumed a shared background^{18–20}. In SSD research, few studies have succeeded in developing
5 state markers using neuroimaging, and for staging markers, there is no consensus on biological
6 staging models²¹.

7 Patient biological data may simultaneously encompass information on traits, states,
8 and disease stages. As machine learning exploits these features without making distinctions,
9 diagnostic markers developed using machine learning may also function as state and/or staging
10 markers. Previous rs-FC biomarker studies reported multiple aggregated FCs as
11 biomarkers^{22,23}; however, whether individual FCs can be used as one or more of these three
12 types of biomarkers remains unexplored. A previous study showed that FCs originally
13 identified as diagnostic markers dynamically changed after treatment²⁴; thus, they also
14 functioned as state markers. Nevertheless, thoroughly investigating individual FCs has not
15 been attempted, making it difficult to determine whether any FCs could benefit early disease
16 detection and intervention as a trait marker or treatment target selection and drug discovery as a
17 surrogate marker.

18 Our research focused on two key areas (**Fig. 1**). (I) Developing Clinically Applicable
19 SSD Classifiers. We aimed to develop a clinically viable rs-FC biomarker for SSD, addressing
20 the challenges mentioned above. This first involved using a large, harmonized dataset from
21 four institutions, harmonized both prospectively²⁵ under a national project (Strategic Research
22 Program for Brain Science (SRPBS) & Brain/Mind Beyond) and retrospectively using the
23 travelling subject method¹⁰. Second, we employed a customized sparse learning algorithm,
24 specifically, the least absolute shrinkage and selection operator (LASSO), similar to our
25 previous work on major depressive disorder (MDD)²⁶. This algorithm classified individuals

1 and identified key contributing FCs. Furthermore, we attempted to enhance the performance by
2 introducing a voting method²⁷. Third, we reduced across-imaging variability and improved
3 generalization to an independent validation cohort through spatial averaging of 100 classifiers,
4 each analysing tens of FCs. Finally, we tested the biomarker's disorder specificity on other
5 psychiatric disorders. In developing the biomarker, we identified the laterality effect on our
6 biomarker, an idea originating from the findings in FC patterns. (II) Exploring Trait / State /
7 Staging Markers of SSD. We aimed to investigate whether the diagnostic biomarker can be
8 used as state and staging markers, assuming shared underpinnings. We demonstrated this on
9 two levels: first, we assessed how much the important FCs (in the aggregate) had capability as
10 trait and/or state markers by predicting symptom severity of two clinical scales; second, we
11 investigated which individual FCs were related to any of the states or disease stages. The
12 findings of this study could help develop new approaches for improving the diagnosis and
13 treatment of various psychiatric disorders, not only SSD.

1 RESULTS

2 Datasets

3 We used two independent multi-disorder datasets: one for developing classifiers ('discovery
4 dataset') and the other for validating the classifiers ('validation dataset'). Participant
5 characteristics are presented in **Table 1** and **Supplementary Table S1**. Fifty-one participants
6 whose scrubbed rs-fMRI volumes exceeded +3 standard deviations (S.D.) were excluded from
7 all datasets. Therefore, 1,015 participants in the discovery dataset and 683 participants in the
8 validation dataset were included in further analyses. Through the scrubbing process, $9.2 \pm$
9 17.9% (mean \pm S.D.) of whole volumes per rs-fMRI scan were removed across all datasets.

10

11 Group difference of FCs and their reproducibility

12 First, we performed a mass univariate analysis of the difference in mean FC values between the
13 healthy control (HC) and SSD groups. **Supplementary Fig. 1** shows the t values obtained from
14 the discovery and validation datasets, where each plot corresponds to a single FC. By
15 comparing to the data with permuted diagnosis labels (5,000 permutations), the original t
16 values were significantly correlated between the two datasets ($r_{(71,631)} = 0.597$; 95% confidence
17 interval (CI) = [0.590, 0.605]; permutation test, $P < 0.05$, two-sided). Hence, the distribution of
18 the diagnostic effect across all FCs was sustained across the two mutually independent cohorts.

19

20 Performance of the LASSO classifiers

21 Through 10-fold cross-validation (CV) and averaging the subsample outputs, we obtained 100
22 classifiers. Within the discovery dataset, classification accuracy was 79.6%, with an area under
23 the receiver operating characteristic curve (AUC) of 0.876. Sensitivity, specificity, and
24 Matthews' correlation coefficient (MCC) were 81.5%, 79.2%, and 0.484, respectively. The
25 density curve of HCs and patients with SSD is shown in **Fig. 3a**, where a subject with a

1 predicted probability of >0.5 was classified as a patient and vice versa. The curves for each site
2 are shown in **Fig. 3b**. We found relatively high predictability for sites KUT and SWA, whereas
3 site UTO achieved somewhat lesser scores.

4 To examine generalisability, we applied the classifiers to the validation dataset. The
5 classifiers distinguished patients with SSD from HCs with 77.3% accuracy and an AUC of
6 0.824, similar to the results from the discovery dataset (**Fig. 3c**). Sensitivity, specificity, and
7 MCC were 69.2%, 81.1%, and 0.490, respectively. A permutation test revealed that the AUC
8 and MCC were highly significant ($P<0.001$). The classifiers showed relatively high prediction
9 capability for sites KTT, COBRE, and TMU (**Fig. 3d**), whereas they showed lower but
10 acceptable capability for KUP. Notably, at JHU, the classifiers distinguished HCs correctly in
11 most cases (specificity: 83.6%) but not patients with SSD (sensitivity: 40.6%). The probability
12 density curve of SSD was closer to that of HC ($P=0.37$, two-sided binomial test), although the
13 distribution of the two curves was significantly different ($P=0.013$, two-sample
14 Kolmogorov-Smirnov test), suggesting that patients with early-stage SSD at JHU fell between
15 chronic-stage SSD and HC.

16

17 **Important FCs for predicting diagnosis**

18 We identified the important FCs that contributed more frequently to the prediction of LASSO
19 classifiers above the chance level. We permuted class labels in the discovery dataset, created
20 100 quasi-classifiers, and repeated the procedure 100 times to obtain a null distribution of the
21 maximal selection count by quasi-classifiers. At $P<0.05$ in the permutation test, the FCs
22 selected by 17 or more classifiers were deemed important. Ultimately, we identified 47
23 important FCs, as shown in **Fig. 4** and detailed in **Supplementary Table S2**

24 Furthermore, we investigated the detailed patterns of FC value differences between
25 HC and SSD groups and their reproducibility across the two datasets by plotting the mean

1 values for 47 FCs, facilitating a comparison between the datasets (**Fig. 6**). Notably, the
2 relationship between the mean FC values of the HC and SSD groups was maintained in the
3 validation dataset for 44 of 47 FCs.

4

5 **Performance of the voting classifiers**

6 Similar to the LASSO classifiers, we assessed the classification performance of the voting
7 classifiers in two steps using the same two datasets. **Supplementary Fig. 2a, b** displays the
8 probability density curve of the voting classifier's output through 10-fold CV in the discovery
9 dataset. Scores were lowest at UTO among the three sites that included HC participants and
10 those with SSD (as observed in the LASSO classifiers). However, all metrics except sensitivity
11 indicated higher scores in voting classifiers than those in the LASSO classifiers, narrowing the
12 performance gap between UTO and the other sites.

13 Independent validation for all sites in the validation dataset is presented in
14 **Supplementary Fig. 2c**. We conducted statistical tests to objectively compare the performance
15 of the voting classifiers with that of the LASSO classifiers. Sensitivity was significantly higher
16 for the voting classifiers than for the LASSO classifiers ($P < 0.001$, McNemar test, two-sided).
17 However, specificity showed no significant difference between groups ($P = 0.192$, McNemar
18 test, two-sided). The AUC of the voting classifiers was 0.841, which was not significantly
19 different from that of LASSO classifiers ($P = 0.11$, DeLong's test). At the individual site level
20 (**Supplementary Fig. 2d**), the performance (AUC, accuracy, sensitivity, specificity, and MCC)
21 was lower at JHU and KUP among the five sites that included HCs and patients with SSD for
22 LASSO and voting classifiers. Similar to UTO in the discovery dataset, JHU and KUP showed
23 relatively major improvements in performance, except for specificity, when considering the
24 minor shifts in performance indices seen at the other sites.

25

1 **Supplementary analyses on classifiers' characteristics**

2 LASSO and voting classifiers were built on subsamples in which the mean ages of the
3 HC and SSD groups were matched to mitigate the confounding effect of age. Furthermore, we
4 evaluated whether the classifiers' performance was influenced by confounding factors (see
5 **Supplementary Text S1 and Table S3**). Consequently, the performance of voting classifiers
6 could be partly influenced by age but not by other potential confounding factors. In contrast,
7 the LASSO classifiers were unaffected by any potential confounding variables.

8 We also investigated how the classification performance varied based on disease
9 severity in the validation dataset. Following a previous report²⁸, we identified subgroups with
10 different severities based on Positive and Negative Syndrome Scale (PANSS) total scores: mild
11 ($PANSS \leq 58$, $N=55$), moderate ($58 < PANSS \leq 75$, $N=36$), marked ($75 < PANSS \leq 95$, $N=20$),
12 severe ($95 < PANSS \leq 116$, $N=1$), and most severe ($116 < PANSS$, $N=0$). Except for the severe and
13 most severe subgroups, each of which contained only one or no participants, the sensitivity by
14 subgroup was highest in the marked subgroup, followed by the mild and moderate subgroups
15 (**Supplementary Table S4**). This order of performance was consistent for the LASSO and
16 voting classifiers, but the disparity among the subgroups decreased for the voting classifiers
17 compared with the LASSO, suggesting that voting classifiers can predict classes with less
18 imbalance across different disease severity levels.

19

20 **Important FCs and subanalysis on laterality**

21 Glasser's regions of interest (ROIs) included in the 47 important FCs were sorted by frequency
22 (**Supplementary Table S5**). By considering random combinations of two ROIs from the 379
23 ROIs, ROIs of three times or more were deemed "significantly frequent" ($P < 0.05$). Notably,
24 for the frequencies of these seven ROIs, there was a laterality imbalance between the left and
25 right hemispheres, except in the cerebellum. We assessed how this laterality might affect SSD

1 biomarkers in these ROIs by comparing the weighted linear sum (WLS) of the original FCs
2 that included the concerned ROI ($WLS_{original}$) with the WLS of the mirror FCs (WLS_{mirror})
3 (**Supplementary Text S2**). **Supplementary Fig. S3** displays the WLS for the original and
4 mirror FCs of the HC and SSD, respectively, for each of the seven ROIs. We found a significant
5 group difference for $WLS_{original}$ and WLS_{mirror} in five ROIs [two sample t -test,
6 Bonferroni-corrected $P < 0.05/21$]. To elucidate the specific differences by diagnosis originating
7 from laterality, we assessed the statistical significance of the difference between HC and SSD
8 on WLS difference ($WLS_{diff} = WLS_{original} - WLS_{mirror}$), and the group difference of this
9 index was significant in the putamen ($P = 2.19 \times 10^{-5}$) and the superior frontal language area, a
10 part of the supplementary motor area ($P = 1.06 \times 10^{-4}$) [two sample t -test, Bonferroni corrected
11 $P < 0.05/21$].

12 Furthermore, we investigated whether FC laterality correlated with anatomical
13 laterality. Previous studies have reported that volume laterality in subcortical brain regions is
14 altered in schizophrenia²⁹. Focusing on the same seven ROIs, we calculated the anatomical
15 laterality index (LI) of each ROI (**Supplementary Text S3** for method) and conducted a
16 correlation analysis with WLS_{diff} . We found no significant correlation between any of the
17 ROIs (**Supplementary Fig. S4**).

18

19 **Classifier specificity for SSD**

20 We explored the generalisability of the classifiers to other mental disorders by applying them to
21 data from participants with major depressive disorder (MDD), autism spectrum disorder
22 (ASD), and bipolar disorder (BP). If the output probability was > 0.5 , the participants were
23 assumed to have SSD-like characteristics. The discovery dataset included patients with MDD,
24 ASD, and BP, and the validation dataset only included patients with MDD. The LASSO
25 classifiers revealed that the patients with ASD and BP did not exhibit high- or low-SSD-like

1 characteristics. However, patients with MDD were less SSD-like ($P=2.24\times 10^{-7}$), resembling
2 the HCs (**Fig. 4, Supplementary Table S7**). Voting classifiers showed a similar pattern. These
3 results suggest that both classifiers exhibited high specificity for SSD.

4 In addition, we investigated whether each probability density curve was distributed
5 differently. Both the LASSO and voting classifiers revealed that the curve of each non-SSD
6 disorder had a significantly different distribution from those of HC and SSD in the discovery
7 and validation datasets (**Supplementary Table S8**). Furthermore, the curves for any two
8 non-SSD disorders showed no significant differences.

9

10 **Prediction of clinical scale scores using important FCs**

11 Another objective of this study was to determine the extendibility of the trait marker to the state
12 or disease stage. In the first part of this investigation, we attempted to predict the scores of two
13 clinical scales with important FCs *in the aggregate* so we could aid clinical assessment and
14 estimate the extent to which our biomarker could function as a trait and/or state marker, using
15 the Peters et al. Delusions Inventory (PDI) and PANSS total scores. These scales have been
16 widely used to assess delusional thinking and psychotic syndrome³⁰. PDI may reflect trait³¹ as
17 well as state³², whereas the PANSS may reflect the overall symptom severity of psychosis at
18 that time point (state)²⁸.

19 Using a 10-fold CV, we performed linear regression on the discovery dataset and
20 predicted the scores of the test data for each model. Subsequently, the models were applied to
21 the validation dataset for prediction.

22 Regarding PDI total scores, the results of 10-fold CV in the discovery dataset were
23 acceptable ($r=0.231$, 95% CI=[0.00101, 0.438], $P=0.0492$, two-sided, mean absolute error
24 [MAE]=40.0). Weak correlations between the actual and predicted scores were observed in the
25 validation dataset ($r=0.331$, 95% CI=[0.0609, 0.556], $R^2=0.110$, $P=0.0177$, two-sided,

1 MAE=55.5) (**Fig. 5a**). A permutation test was conducted to objectively assess whether the
2 evaluation metrics were satisfactory. The correlation coefficients were significantly high
3 ($P_{\text{perm}}=0.01$) and MAE significantly low ($P_{\text{perm}}=0.018$).

4 Concerning PANSS total scores, the 10-fold CV in the discovery dataset resulted in no
5 correlation between actual and predicted scores ($r=-0.0065$, 95% CI=[-0.199, 0.186],
6 $P=0.948$, two-sided, MAE=17.5). The model did not predict scores in the validation dataset
7 either ($r=0.128$, 95% CI=[-0.0588, 0.306], $R^2=0.016$, $P=0.178$, two-sided, MAE=16.8) (**Fig.**
8 **5b**).

9 In summary, we predicted the PDI total score more efficiently than the PANSS total
10 score, suggesting that, collectively, the important FCs may function as trait and state markers.
11

12 **Individual FCs that were associated with PANSS factors**

13 In addition to the relationship between clinical scale scores and aggregated FC biomarkers, we
14 explored the roles of individual FCs. While individual FCs are expected to be associated with
15 certain traits, they might be linked to the state of SSD. Hence, we determined the association of
16 each important FC with the PANSS factors using linear regression models. In total, 17 FCs
17 were significantly associated with the PANSS factors. Using the three-factor model³³ of
18 PANSS, we identified significant associations in FCs #45 (R.TPOJ1 & R.Thalamus, with the
19 PANSS positive factor) and #8 (L.1 & R.3b, with the PANSS negative factor), regardless of the
20 score conversion method (**Table 2, Supplementary Table S9**). With the five-factor model³⁴ of
21 PANSS, we found that the significantly associated FCs, regardless of conversion method, were
22 FCs #18 (L.FOP1 & R.Putamen), #41 (R.6a & R.PoI1), #43 (R.FOP4 & R.Putamen) with the
23 excited factor, and FC #27 (R.RSC & R.SFL) with the depressed factor.

24 When the coefficient estimate was significant only for a state (not for the trait), such
25 FCs were assumed to be “pure” state markers. We found nine FCs (**Table 2**). Among these FCs,

1 FC #27 (R.RSC & R.SFL) was the only one consistently identified as a “pure” state marker,
2 regardless of conversion method.

3

4 **Individual FCs that were associated with disease stage**

5 Using a multiple regression model, we categorised important FCs into subgroups associated
6 with trait (diagnosis), the early stage, or the chronic stage of SSD. **Fig. 5c** presents the results
7 using the bootstrapping method (1,000 iterations). We assumed that an FC belonged to a certain
8 category with consistent regression results for over half (>500) of the iterations. Subsequently,
9 the important FCs were categorised most frequently as “trait and chronic stage” (61.7%),
10 followed by “trait and early stage” (6.4%), “trait only” (2.1%), and “trait, early, and chronic
11 stage” (2.1%). No FCs were categorised as “early stage only” or “chronic stage only,”
12 indicating that we did not identify any pure staging markers.

1 **DISCUSSION**

2 We developed a diagnostic rs-FC biomarker based on harmonised multicentre data and
3 validated it using large-sample independent data. LASSO and voting classifiers achieved high
4 performance, and we confirmed their generalisability through independent validation,
5 demonstrating minimal machine learning-induced inflation in diagnostic biomarker
6 performance. We also identified distinct properties of each type of classifier. By leveraging
7 logistic regression with LASSO, we extracted the important FCs involved in SSD classification
8 and depicted them within a constellation of large-scale networks. Our biomarker also
9 demonstrated high specificity for SSD across broader dimensionality, encompassing several
10 psychiatric disorders.

11 This study also aimed to maximise the potential of the rs-FC biomarker (originally
12 developed as a trait marker) to function as a state marker and a staging marker. We employed
13 two approaches, one of which involved predicting clinical scale scores based on the values of
14 important FCs. We successfully predicted delusion proneness in patients with SSD using
15 aggregated FCs. Multiple regression analysis focusing on individual FCs enabled us to
16 comprehensively elucidate the associations between individual FCs and state or disease stage.
17 This study revealed the state and staging of the biomarker characteristics of individual FCs
18 based on generalisable diagnostic biomarkers.

19 The accuracy, sensitivity, and specificity of the identified diagnostic marker were
20 approximately 80% for the discovery dataset. It demonstrated comparable performance in an
21 external validation with seven international cohorts; this presents a sharp contrast to previous
22 studies¹¹, including the report on a FC biomarker from our group²³. This achievement was
23 possible through bi-directional (prospective and retrospective) harmonisation, which enabled
24 the extraction of the disease factor itself, and with optimised machine learning method. Against
25 the concerns on session variability of rsfMRI, our previous study³⁵ revealed that test-retest

1 reliability was acceptable with the same methodology as demonstrated in this study.

2 The LASSO and voting classifiers exhibited distinct performance characteristics.
3 Voting classifiers demonstrated superiority in sensitivity and a more balanced profile over
4 LASSO classifiers, with the accuracy, sensitivity, and specificity falling within a narrow range
5 (LASSO: 69.2–81.1%, voting: 74.7–78.8%) in independent validation.

6 When we categorised patients with SSD into subgroups based on disease severity,
7 LASSO classifiers tended to identify patients with more severe symptoms correctly but not
8 those with milder symptoms; in contrast, voting classifiers identified patients with SSD more
9 evenly across severity subgroups. This observation suggests that voting classifiers capture the
10 SSD trait better, whereas LASSO classifiers are more influenced by the state.

11 We identified important FCs that significantly contributed more frequently to SSD
12 classification in LASSO classifiers. Notably, based on Anatomical Automatic Labelling (AAL),
13 the putamen, insula, thalamus, and cingulum were among the top ROIs most frequently found
14 in the important FCs (**Supplementary Table S5**). In schizophrenia research, these are
15 consistently implicated in grey matter volume reduction³⁶ or FC abnormality^{37,38}. Moreover,
16 these ROIs were associated with the cortico-striatal-thalamic-cortical loop and salience
17 network, both recognised for their pivotal roles in the psychopathology of SSD³⁹. In the context
18 of FC, these ROIs provide an intriguing discussion of SSD pathophysiology. Hypoconnectivity
19 between the putamen and anterior cingulate cortex (FC #13, #30, #37, #38) in the SSD group
20 aligns with a previous report⁴⁰, where greater ACC-putamen connectivity predicted better
21 treatment response. We also observed hypoconnectivity between the putamen and insula (FC
22 #17, #43), consistent with another report⁴¹ indicating hypoconnectivity between the dorsal
23 anterior insula and bilateral putamen in early-stage schizophrenia and high-risk clinical groups.
24 Conversely, in the SSD group, the thalamus exhibited increased connectivity with various
25 cortical ROIs (FC #11, #12, #24, #25, #35, #36, and #45), corresponding with a previous

1 report⁴² on thalamocortical connectivity in schizophrenia, implying disrupted information
2 filtering in SSD, consistent with the literature⁴³. Sensorimotor areas (precentral and postcentral
3 gyri, supplementary motor area) were also among the top ROIs (see below). Therefore, these
4 important FCs aptly reflected the neural correlates of schizophrenia and were considered trait
5 markers of SSD.

6 Reduced laterality in structure and function has been suggested in patients with
7 schizophrenia^{44,45}. Regarding the putamen and SFL ROIs, we observed significant group
8 differences in WLS_{diff} , which we interpreted as the effect of FC laterality on our LASSO
9 classifiers. Because our approach to measuring FC laterality was novel, a direct comparison of
10 these results with previous findings was not feasible. Nevertheless, we noted a convincing
11 agreement with a study⁴⁶ in which the authors reported meaningful laterality of target ROIs
12 when the bilateral putamen was set as the seed. Laterality in the superior frontal language area
13 (a part of the supplementary motor area) was decreased in the SSD group, consistent with
14 previous studies demonstrating diminished laterality in language-related areas in patients with
15 schizophrenia^{47,48}.

16 FC laterality and volume laterality were not correlated to any of the ROIs. A previous
17 report showed a correlation between functional laterality and grey matter volume laterality at
18 the whole-brain level; however, this was not specific to SSD⁴⁹. To our knowledge, no studies
19 have explored the relationship between FC laterality and volume laterality; thus, further
20 investigation is needed.

21 We observed that the LASSO and voting classifiers exhibited high specificity for SSD,
22 whereas other diagnoses (MDD, ASD, and BP) did not show specificity for HC or SSD.
23 Evidence indicates that these psychiatric disorders share several characteristics with SSD in the
24 alteration pattern of brain function⁵⁰⁻⁵². Moreover, these disorders have similar phenotypes
25 (e.g., cortical thickness) and genotypes⁵³. Thus, our biomarkers may, to some extent, reflect

1 neural changes common to psychiatric disorders⁵⁴.

2 The second objective of this study was to dissect the biomarker into a “trait marker”
3 and other components, such as a state marker or staging marker. Using aggregated important
4 FCs, we achieved an acceptable level of prediction for the PDI but not for the PANSS,
5 indicating that aggregated FCs were more strongly associated with traits. Predicting the
6 intensity of psychotic conditions is crucial for clinical decision-making. Few groups have
7 reported successful prediction of symptoms using FC. One study reported the prediction of the
8 PANSS total score improvement from baseline based on striatal resting-state markers with a
9 large sample size, but it lacked external validation¹⁸. Another group predicted PANSS positive
10 and negative scores that were weak to moderate correlations with the actual scores⁵⁵, benefiting
11 from individually defined ROIs; however, the sample size was small and lacked external
12 validation. In this study, the predicted PDI total score was significantly correlated to the actual
13 score in the discovery and validation datasets.

14 Although we successfully predicted scale scores with important FCs in the aggregate,
15 we still lack knowledge regarding which FC was specifically related to each state. Using linear
16 regression analysis, we identified state markers individually.

17 Using the three-factor model of the PANSS, we observed a significant association
18 between positive scores and FC #45 as well as negative scores and FC #8, irrespective of the
19 score conversion method. Evidence suggests that FC #45 significantly correlates with positive
20 symptoms⁵⁶. FC #8 also showed a significant association with the negative and disorganised
21 factors of the five-factor model, which could be linked to the finding that voxel-mirrored
22 homotopic connectivity of the precentral and postcentral gyri were negatively correlated with
23 the PANSS total score⁵⁷.

24 We discovered interesting overlaps of ROIs in the potential state marker FCs.
25 Concerning negative and depressed factors, sensorimotor areas, including the pre/postcentral

1 gyri and supplementary motor area, were noticeable. Specific functional alterations in these
2 ROIs related to neurological soft signs (NSSs), a core feature of SSD, have been reported⁵⁸.
3 Moreover, NSSs correlate with PANSS negative scores and depression scale scores⁵⁹. The
4 excited factor seems to involve the putamen, insula, Rolandic operculum, and middle cingulum,
5 regions likely to be associated with aggression, especially in studies on grey matter volume⁶⁰.
6 The Rolandic operculum and middle cingulum are related to aggression via disruption of the
7 cognitive control network⁶¹.

8 Although we did not discover any pure staging markers, our results provide a
9 compelling argument for “trait and early stage” markers exemplified by FCs #16, #20, and #23.
10 FCs #20 and #23 represented connections between regions around the superior temporal sulcus
11 (STS) or gyrus (STG). In groups with high risk for SSD⁶², the response to face recognition in
12 STS, as well as the connectivity between bilateral STS regions, was correlated with schizotypy
13 scores. A heightened response in carriers of a risk allele for SSD compared with non-carriers
14 was also reported. Hence, STS activity appeared to be associated with SSD traits. However, a
15 task-based fMRI study on working memory showed attenuated activity in the STG in patients
16 with early-stage psychosis compared with HCs⁶³. Moreover, the cortical thickness of the insula
17 (an ROI in FC #16) and the STS region are reduced in early-stage psychosis⁶⁴. This evidence
18 suggests that STS/STG alterations should also be markers of early-stage disease. The fact that
19 the probability curve for patients with early-stage psychosis at JHU lies in the middle between
20 the HC and SSD groups may also support this argument.

21 This study had several limitations. First, the participants with SSD in our study were
22 mostly medicated; hence, the applicability of our results to drug-naive patients cannot be
23 guaranteed. Second, we only confirmed that our classifiers distinguished patients with SSD
24 from healthy individuals not from individuals with other psychiatric disorders exhibiting
25 psychotic symptoms. Further studies are required to test the classifiers for these disorders to

1 maximise the clinical applicability. Third, in the analysis on laterality, only a small number of
2 regions were included in the important FCs, while the “lateralisation hypothesis” of psychosis
3 covers the entire brain⁶⁵. In future research, a broader scope is needed to verify FC laterality at
4 the whole-brain level across different disease stages and in other disorders.

5 In conclusion, we developed robust and clinically usable classifiers for SSD using a
6 combination of cutting-edge strategies. While constructing the classifiers, we identified FCs
7 that may play key roles in the pathophysiology of SSD. We also demonstrated that these
8 “important FCs” had multiple functions as SSD trait, state, or staging markers, which was
9 supported by their roles in predicting clinical scale scores and regression analyses that
10 identified individual FCs relevant to symptoms or disease stages. Our findings shed new light
11 on the early diagnosis of SSD as well as the selection of targets for neuromodulation.

12

13 **ONLINE METHODS**

14 **Participants**

15 The discovery dataset comprised data from four sites: Kyoto University Siemens TimTrio
16 scanner (KUT), Showa University (SWA), Centre of Innovation in Hiroshima University
17 (COI), and University of Tokyo (UTO). The dataset included 1,045 participants consisting of
18 617 HCs, 116 patients with SSD (which includes schizophrenia, schizoaffective disorder, and
19 delusional disorder), 148 patients with MDD, 125 patients with ASD, and 39 patients with BP
20 (**Table 1** and **Supplementary Table S1**).

21 The validation dataset comprised international cohorts, including the Japanese cohort
22 from Hiroshima Kajikawa Hospital (HKH), Hiroshima Rehabilitation Centre (HRC),
23 Hiroshima University Hospital (HUH), Kyoto University Siemens Trio scanner (KTT), Kyoto
24 University Siemens Prisma scanner (KUP). It also included the Taiwanese cohort (Taipei
25 Medical University, TMU) and the Centre of Biomedical Research Excellence (COBRE) open

1 dataset (http://fcon_1000.projects.nitrc.org/indi/retro/cobre.html), along with the Johns
2 Hopkins University cohort (United States), which included HCs and patients with early-stage
3 SSD. The total number of participants was 708 (405 HCs, 198 patients with SSD, and 105
4 patients with MDD; **Table 1** and **Supplementary Table S1**). There was no overlap of subjects
5 between the discovery and validation datasets.

6 Clinical scale scores: PANSS³³ and PDI 21-item version³¹, as well as information
7 about the dosage of antipsychotics, were available from a subset of participants in both datasets
8 (**Supplementary Table S10**).

9 This study was conducted in accordance with the recommendations of the review
10 boards of institutions affiliated with the principal investigators are affiliated—namely,
11 Hiroshima University, Kyoto University, Showa University, and University of Tokyo. The
12 majority of the material data in this study can be downloaded from the DecNef Project Brain
13 Data Repository⁶⁷ (<https://bicr-resource.atr.jp/srpbsopen/>).

14

15 **Data acquisition**

16 All data in the discovery dataset were collected using a unified protocol developed by SRPBS
17 (<https://bicr.atr.jp/decnefpro/>) Project in Japan²⁵. The MRI data comprised a T1-weighted
18 structural image, rs-fMRI acquired using an echo-planar imaging technique, and field map
19 images. The duration of rs-fMRI was 10 min. Participants were instructed to relax, stay awake,
20 fixate on the central crosshair mark, and not concentrate on specific things. The MRI data for
21 the validation dataset included a structural image and rs-fMRI similar to the discovery dataset;
22 however, some of the data lacked fieldmap images. Scanning parameters for the validation
23 dataset varied by site. The duration of rs-fMRI was approximately 4–6 min. Detailed imaging
24 parameters for both datasets are provided in **Supplementary Table S11**.

25

1 **Preprocessing**

2 The data were preprocessed according to a previous report²⁶. fMRIPrep version 20.1.11 was
3 used for data preprocessing⁶⁶. First, the first four volumes of the rs-fMRI scan were discarded
4 for T1 equilibration. The preprocessing steps were as follows: slice-timing correction,
5 realignment, coregistration, susceptibility-induced distortion correction using field maps,
6 segmentation of a T1-weighted structural image, normalisation to Montreal Neurological
7 Institute (MNI) space, and spatial smoothing with an isotropic Gaussian kernel of 6 mm
8 full-width at half-maximum. For the participants without field maps in the validation dataset,
9 we applied “fieldmap-less” distortion correction, which involves nonlinear registration of
10 fMRI to the same-subject T1-weighted image as the undistorted target, implemented in
11 fMRIPrep. Further details of the pipeline are available at
12 <http://fmriprep.readthedocs.io/en/latest/workflows.html>.

13

14 **Signal extraction**

15 We employed two approaches for fMRI signal extraction: (1) A surface-based approach
16 following the Human Connectome Project (HCP) pipelines. Using the ciftify toolbox version
17 2.3.2⁶⁷, we converted volume-based MRI data into data based on “greyordinate” (cortical grey
18 matter surface vertices and subcortical grey matter voxels). The value of a cortical vertex was
19 determined as the weighted mean of the voxel values in the cortical ribbon corresponding to the
20 vertex, similar to the procedure in the *fMRISurface* pipeline of HCP. (2) An approach based on
21 the ROI. For reliable surface-based brain parcellation, we adopted the ROIs from Glasser et
22 al.⁶⁸ (379 parcels in total, comprising 360 cortical parcels as surface ROIs and 19 subcortical
23 parcels as volume ROIs). Using these approaches, we extracted BOLD signal time courses
24 from 379 ROIs. To compare these ROIs with conventional annotations of brain areas and
25 intrinsic brain networks, we referred to the AAL and Ji et al.⁶⁹, respectively.

1

2 **Noise removal**

3 We used component-based noise correction (CompCor)⁷⁰, a module in fMRIPrep that performs
4 principal component analysis on the entire time series of the preprocessed rs-fMRI, to detect
5 physiological noise. Anatomical CompCor (aCompCor) was applied to the subcortical white
6 matter (WM) and cerebrospinal fluid (CSF) regions, and the top five principal components
7 were estimated as physiological noise, except for one participant who had only four
8 components. Accordingly, we regressed out these components together with six head-motion
9 parameters and averaged the signals over the entire brain.

10

11 **Temporal filtering**

12 We employed temporal bandpass filtering ranging from 0.01–0.08 Hz⁷¹ to the time series of
13 rs-fMRI to extract the low-frequency brain activity characterising resting state.

14

15 **Data scrubbing**

16 We removed volumes with considerable head motion based on framewise displacement (FD)⁷²,
17 which is one of the outputs of fMRIPrep. FD was calculated as the sum of the absolute
18 displacements in translation and rotation between two consecutive volumes. We removed
19 volumes with $FD > 0.5$ mm, as proposed in a previous study⁷². In addition, participants whose
20 scrubbed volume ratio exceeded the mean +3 S.D. were excluded.

21

22 **Calculation of the FC matrices**

23 We defined FC as the temporal correlation of rs-fMRI BOLD signals between two ROIs. We
24 calculated the Pearson's correlation coefficient of the preprocessed BOLD time series between
25 each pair of ROIs out of Glasser's 379 ROIs. Fisher's Z-transformed values of the correlation

1 coefficients constituted an FC matrix for each participant, where the total number of FC was

2 $\binom{379}{2} = 71,631$.

3

4 **Harmonisation of the site differences**

5 We harmonised the site effects in the discovery dataset using data from subjects who travelled
6 between sites (travelling subjects). Site effects were separately estimated as measurement bias
7 and sampling bias from the rs-fMRI data of these travelling subjects, each of whom underwent
8 scans at multiple sites¹⁰. We subtracted the estimated measurement bias to obtain harmonised
9 connectivity data (see **Supplementary Text S4**). For the validation dataset, we applied the
10 ComBat harmonisation method^{73–75}, which estimates the site effect as one because the scanners
11 in this dataset did not have travelling subject data. In the execution of ComBat harmonisation,
12 we inputted the diagnosis, PANSS total score, PDI total score, age, sex, handedness, and
13 duration of illness (for patients with SSD) as auxiliary variables to correct measurement bias.

14

15 **Group difference of FCs**

16 To delineate the cumulative effect of diagnosis on FCs, we conducted a mass univariate
17 analysis. We calculated the difference in mean FC values (Z-transformed) between the HC and
18 SSD groups for each FC and computed *t* statistic. To validate the reproducibility of the
19 diagnostic effect on FCs for both datasets, we performed a permutation test (5,000 times) using
20 *mult_comp_perm_corr* function in MATLAB (R2019a, MathWorks, USA) and assessed the
21 significance of the correlation of the *t* values mentioned above between the two datasets.

22

23 **Diagnostic classifiers for SSD**

24 Next, we constructed classifiers to differentiate patients with SSD from HCs using machine
25 learning based on 71,631 FC values as features. In subsequent sections, we used only the data

1 of HCs and patients with SSD, except for generalising the models to other disorders. Initially,
2 we created the classifiers using logistic regression with LASSO. The sparse method in LASSO
3 can prevent overfitting and simultaneously select important features (for the detailed
4 methodology, refer to **Supplementary Text S5**). Although we used LASSO to identify
5 important FCs for SSD classification, we applied a voting method to enhance the classification
6 performance of the LASSO classifiers.

7

8 **Building LASSO classifiers**

9 As illustrated in **Fig. 6a**, we implemented a nested 10-fold CV scheme. In the outer
10 loop, we divided the discovery dataset into ten folds. After leaving one fold as the test set, the
11 remaining nine folds were used as the training set. To minimise the bias arising from the
12 imbalance between the number of patients with SSD and HCs, we conducted subsampling with
13 undersampling simultaneously. We randomly sampled the same number ($N = 102$) of HCs and
14 SSDs from the training set, creating a subsample. During subsampling and undersampling, we
15 matched the mean ages of HCs and SSDs. We fit the logistic regression model to the subsample
16 while tuning a hyperparameter with an inner-loop 10-fold CV (for the detailed methodology,
17 see **Supplementary Text S5**). By repeating random subsampling and fitting the model ten
18 times, we obtained ten classifiers. We then predicted SSD probability for each participant in the
19 test set. By applying the ten classifiers built from a training set to a test set in each CV, we
20 obtained the probability values for the participant as the classifier outputs. When the mean
21 probability value was >0.5 , we considered the participant's class as SSD; otherwise, as HC.
22 Finally, by repeating the above procedure ten times in the outer loop, 100 classifiers were
23 obtained.

24 We evaluated the performance of the classifiers using the following indices: AUC,
25 accuracy, sensitivity, specificity, and MCC (for MCC, see **Supplementary Text S6** for details).

1

2 **Independent validation of LASSO classifiers**

3 To assess the generalisability of the obtained classifiers, we tested them on the validation
4 dataset. We applied the 100 classifiers to each participant in the validation dataset to compute
5 diagnostic probability values for each participant. We classified a participant as having SSD if
6 the average probability value was >0.5 .

7 We also conducted a statistical analysis of the classification performance for
8 independent validation using a permutation test. We created 100 quasi-classifiers using the
9 same procedure as for building genuine classifiers, with the participants' classes permuted in
10 the discovery dataset. We predicted the diagnosis in the same manner as mentioned above
11 using the quasi-classifiers on the validation dataset for each permutation. By repeating random
12 permutations 500 times and obtaining null distributions of the AUC and MCC, we evaluated
13 the statistical significance of the true classifiers.

14

15 **Identifying important FCs for predicting diagnosis**

16 We investigated which FCs were utilised to predict the diagnosis in each classifier by
17 identifying the nonzero coefficients in LASSO. We counted the number of classifiers (out of
18 100) that selected each FC as an explanatory variable. We performed a permutation test to
19 identify the most informative FCs from 71,631 FCs. Each time we randomly permuted the
20 class labels of participants in the discovery dataset, we built 100 quasi-classifiers using 10-fold
21 CV with 10-time subsampling, following the methodology described in the previous section
22 (see "**Building LASSO classifiers**" section). We determined the maximum counts for which
23 each FC was selected as a predictive explanatory variable using 100 quasi-classifiers for each
24 permutation. This procedure was repeated 100 times, resulting in a null distribution of 100
25 values for the maximum selection count. An FC was considered significantly informative

1 ('important FC') when the selection count in the genuine 100 classifiers exceeded the threshold
2 that corresponded to $P < 0.05$ in the null distribution.

3

4 **Voting classifiers**

5 In addition to logistic regression with LASSO, we further explored the diagnostic biomarker's
6 potential by combining multiple machine-learning methods. We incorporated support vector
7 machine (SVM)⁷⁶, random forest (RF)⁷⁷, light gradient boosting machine (LGBM)⁷⁸, and
8 multi-layer perceptron (MLP)⁷⁹ as representative algorithms. The machine-learning packages
9 for each algorithm were LightGBM version 3.2.1 for LGBM, Keras version 2.6.0 for MLP, and
10 scikit-learn version 0.24.1 for SVM and RF. For each algorithm, we conducted CV,
11 subsampling with undersampling, and training on the discovery dataset following the same
12 procedure used to build the LASSO classifiers. Hyperparameters were tuned using Optuna
13 version 2.9.1 in 10-fold CV within the inner loop of the nested CV. We constructed 10
14 classifiers by repeating the subsampling and training of the model 10 times. In each fold of the
15 outer-loop CV, we predicted the class of each participant in a test set using these 10 classifiers
16 (for LASSO, LGBM, and MLP, the default output was in the form of a probability value.
17 Therefore, when the probability value was > 0.5 , the predicted class was 'SSD' and vice versa).
18 Similar to voting, we counted the number of times the participant was predicted to have an SSD
19 by ten classifiers for each of the five machine learning algorithms (maximal count: 10
20 classifiers \times 5 algorithms = 50). When the count exceeded 25, the participant was considered to
21 have SSD. Using 10-time subsampling and 10-fold CV for each of the five algorithms, we
22 obtained 500 classifiers. Next, we predicted the diagnostic class in the validation dataset using
23 each of these classifiers, and the final prediction by voting was determined as the class
24 predicted by the majority of the classifiers (> 250).

25 To statistically compare the classification capability of the voting classifiers with that

1 of the LASSO classifiers, we used the R programmes Compbdt and pROC. Using Compbdt⁸⁰,
2 we compared the two classifiers based on their sensitivity and specificity using the McNemar
3 test. We used pROC to conduct DeLong's test on the AUC. The significance level for all tests
4 was set at $P < 0.05$.

5

6 **Generalisation of the classifiers to other psychiatric disorders**

7 Regarding the LASSO classifiers, we applied 100 classifiers to all the patients with MDD,
8 ASD, and BP from discovery and validation datasets. If the output probability was >0.5 , the
9 participants were assumed to have SSD-like characteristics. Regarding the voting classifiers,
10 we applied 500 classifiers to the same patients, and the participants were assumed to be
11 SSD-like if over half (>250) of the classifiers predicted as such. The discovery dataset included
12 patients with MDD, ASD, and BP, whereas the validation dataset only included patients with
13 MDD. The outputs of the classifiers for HC and SSD were used for the comparison with MDD,
14 ASD, and BP. Regarding HC and SSD, the output for test data in the 10-fold CV was used in
15 the discovery dataset, and the output of 100 classifiers was used in the validation dataset.

16 To evaluate if the classifying results of patients with any of the other disorders (MDD,
17 ASD, BP) had similarity to HC, SSD, or neither, we conducted a two-sided binomial test (the
18 significance level: $P < 0.05$). We also checked whether each probability density curve was
19 distributed differently using the two-sample Kolmogorov-Smirnov test. We conducted this test
20 for every combination of HC, SSD, MDD, ASD, and BP; thus, ten combinations were obtained
21 in total. The level of significance was $P < 0.05/10 = 0.005$ [Bonferroni-corrected].

22

23 **Prediction of clinical scores using important FCs**

24 We predicted the scores on the clinical scales (PDI and PANSS total scores) using important
25 FCs as explanatory variables. We applied a nested 10-fold CV scheme to the discovery dataset

1 (only SSD participants with available target scale scores) to build prediction models
2 (**Supplementary Fig. 5a**). In the outer loop, we divided the discovery dataset into 10 folds.
3 After leaving one fold as the test set, we used the remaining nine folds as the training set. We
4 fitted the linear regression model to the training set (the *LinearRegression* module in
5 scikit-learn version 0.24.1). In the inner loop, we searched for the most suitable number of
6 important FCs to be used as features to avoid overfitting (for the detailed methodology, see
7 **Supplementary Text S7 & Supplementary Fig. 5b**). We predicted the score for each
8 participant in the test set using a linear regression model with the best number of features for
9 the fold. Using 10-fold CV, we obtained 10 models with different numbers of features. For the
10 validation dataset, we averaged the outputs from the ten models to create the final predicted
11 score (**Supplementary Fig. 5c**). Notably, at any step of this analysis, the predicted value was
12 adjusted within the range of the scale score (if the prediction was lower than the lowest possible
13 value, it was adjusted to the lowest possible value and vice versa).

14 We conducted a permutation test to assess the evaluation metrics statistically.
15 Specifically, we permuted clinical labels (diagnosis and symptom scores) against FC values,
16 created quasi-models following the same procedure as the genuine models, and repeated these
17 steps 500 times to obtain a null distribution of the evaluation metrics. Differences were
18 considered statistically significant if each evaluation metric for the genuine models was better
19 than the cut-off value of $P=0.05$ of the null distribution.

20

21 **Separately identifying FCs associated with the state**

22 In the second part of the analysis of state and staging markers, we focused on individual FCs
23 from the perspective of their association with various factors of state and disease stages. To
24 identify the FCs associated with state (i.e., symptom scale scores), we conducted multiple
25 regression analyses on each of the important FCs. We chose PANSS as the state index because

1 it changes dynamically with treatment and has been used as a representative scale for the state
2 of schizophrenia. All HC participants and patients with SSD having data on PANSS scores
3 across the discovery and validation datasets were included in the analysis. We examined two
4 models of the PANSS: the original three-factor model³³ composed of positive, negative, and
5 general pathological factors, and the five-factor model³⁴ composed of positive, negative,
6 disorganised, excited, and depressed factors. Because the general pathological factor was not
7 categorised as a specific symptom dimension, we used only positive and negative factors as
8 explanatory variables for the three-factor model.

9 Due to the exploratory nature of this analysis, we converted the PANSS scores into
10 explanatory variables in two ways: one by min-max normalisation and the other by binary
11 dummy variables. The formula to fit was the same for both methods:

$$12 \quad FC = \beta_0 + \beta_1 x_t + \sum_{j=1}^{n_{factor}} (\beta_{j+1} x_{s_j}) + e \quad (\text{Eq. 1})$$

13 where x_t represents the trait or diagnosis (HC: 0; SSD: 1), x_{s_j} represents the existence of
14 symptoms for the j -th factor of the PANSS, and e represents a random error.

15 *Conversion 1. Min-max normalisation of the scores.*

16 The value x_{s_j} was determined using the following formula:

$$17 \quad x_{s_j} = \frac{m_j - \min(m_j)}{\max(m_j) - \min(m_j)} \quad (\text{Eq. 2})$$

18 where m_j represents the participant's average score in the j -th factor of the PANSS, and
19 $\min(m_j)$ (or $\max(m_j)$) represents the minimum (or maximum) average score of the j -th
20 PANSS factor across all patients with SSD with available PANSS subscore data.

21 *Conversion 2. Binarising the scores.*

$$22 \quad x_{s_j} = \begin{cases} 0 & \text{if } m_j \leq 2 \\ 1 & \text{if } m_j > 2 \end{cases} \quad (\text{Eq. 3})$$

23 The value x_{s_j} was determined as in Eq.3 since three points or more is considered pathological

1 in PANSS rating.

2 All s_j for HC participants were assumed to be zero. In the regression analysis for
3 each FC, the FC was regarded as a potential state marker when any of the coefficient estimates
4 for PANSS factor ($\beta_{(j+1)}, j = 1, 2, \dots, n_{\text{factor}}$) was non-zero at a significance level $P < 0.05$ with
5 a one-sample t -test. Of note, we reported FCs as being significant only when the state's
6 coefficient concerned ($\beta_{(j+1)}$) was of the same sign as w of the FC in terms of consistency
7 with the underlying LASSO classifiers.

8

9 **Identifying FCs associated with the disease stage**

10 Lastly, we constructed another multiple regression model to identify FCs associated with the
11 disease stage of SSD, which could be referred to as 'staging' markers. Following a pre-existing
12 definition^{81,82}, we divided the SSD group into an early-stage psychosis subgroup (duration of
13 illness (DOI) of less than 5 years) and a chronic-stage subgroup (DOI of 5 years or longer). We
14 formulated a regression model similar to that described in the previous section.

$$15 \quad FC = \beta_0 + \beta_1 x_t + \beta_2 x_e + \beta_3 x_c + \beta_4 x_a + e \quad (\text{Eq. 4})$$

$$16 \quad \text{such that } x_e = \begin{cases} 1 & \text{if DOI} < 5 \text{ yrs.} \\ 0 & \text{if DOI} \geq 5 \text{ yrs.} \end{cases}, \quad x_c = \begin{cases} 0 & \text{if DOI} < 5 \text{ yrs.} \\ 1 & \text{if DOI} \geq 5 \text{ yrs.} \end{cases} \quad (\text{Eq. 5})$$

17 where x_e represents the early stage of SSD, x_c represents the chronic stage, and x_t and e are
18 defined in the same way as in Eq. 1. The value x_a , the age of the participant, was introduced as
19 a covariate because the DOI was supposed to correlate with age. All HC participants and
20 patients with SSD with DOI data across the discovery and validation datasets were included in
21 the analysis. Using this model, we categorised important FCs into six groups based on which
22 trait, early/chronic stage, was significantly associated: (1) trait only, (2) early stage only, (3)
23 chronic stage only, (4) trait and early stage, (5) trait and chronic stage, and (6) all three. In terms
24 of consistency with our classifiers and within the coefficients, every coefficient estimate

1 concerned must be of the same sign as w .; statistical significance was determined at the level P
2 < 0.05 , with a one-sample t -test for each coefficient estimate. Considering the relative
3 instability of the model fit due to singularity, we adopted the bootstrap method (1,000
4 iterations) to count the number of times a certain FC was sorted into each category.

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17

18

19 **Author contributions:**

20 T.K., A.Y., M.K., and H.T. designed the study. T.K., Y.Y., Y.K., N.O, K.K., M-C.H., A.S.,
21 T.M., J.M. and H.T. recruited participants for the study and collected their clinical and
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25

1 **Competing interests:**

2 MK is an inventor of patents owned by the Advanced Telecommunications Research Institute
3 International related to the present work (PCT/JP2014/061544 [WO2014178323] and
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- 25

1 **Table 1. Demographics of the participants (HC and patients with SSD)**

Site	Abbreviation	HC			SSD			Total (HC & SSD)			
		Number	M/F	Age	Number	M/F	Age	Number	M/F	Age	
Discovery dataset											
Kyoto University (TimTrio)	KUT	223	127/96	33.4±13.2	61	32/29	40.7±13.2	284	159/125	35.1±13.2	
Showa University	SWA	101	86/15	31.4±7.9	19	15/4	42.7±8.4	120	101/19	30.7±9.5	
Centre of Innovation, Hiroshima University	COI	124	46/78	51.9±13.4	-	-	-	124	46/78	51.9±13.4	
University of Tokyo	UTO	169	77/92	35.7±17.5	36	24/12	23.3±10.3	205	101/104	34.9±16.5	
Summary		617	336/281	36.9±16.0	116	71/45	38.5±11.4	733	407/326	37.2±15.3	
Validation dataset											
Kyoto University (Trio)	KTT	72	44/28	28.7±9.4	48	23/25	37.8±9.4	120	67/53	32.4±10.4	
Kyoto University (Prisma)	KUP	11	7/4	35.0±8.5	18	11/7	40.2±12.9	29	18/11	38.2±11.6	
Hiroshima University Hospital	HUH	66	29/37	34.6±13.0	-	-	-	66	29/37	34.6±13.0	
Hiroshima Kajikawa Hospital	HKH	29	12/17	45.4±9.5	-	-	-	29	12/17	45.4±9.5	
Hiroshima Research Centre	HRC	49	13/36	41.7±11.7	-	-	-	49	13/36	41.7±11.7	
COBRE	COB	73	50/23	35.7±11.6	67	57/10	37.4±13.8	140	107/33	36.6±12.7	

Taipei Medical University	TMU	29	26/3	31.4±5.0		32	23/9	35.3±6.1		61	49/12	33.4±5.9
Johns Hopkins University	JHU	75	37/38	24.4±4.1		33	23/10	22.5±4.7		108	60/48	23.8±4.4
Summary		404	218/186	32.3±11.6		198	137/61	34.9±11.9		602	355/247	33.8±11.7

- 1
- 2 The distributions of age and sex were not significantly different between the HC and SSD groups in the discovery dataset ($P > 0.05$). In the
- 3 validation dataset, the age distribution was not significantly different ($P > 0.05$); however, the sex distribution was significantly different ($P < 0.05$).
- 4 HC: healthy control, SSD: schizophrenia spectrum disorder.

1 **Table 2. Individual FCs significantly associated with PANSS factorial scores**

Score conversion	Three factor model		Five factor model				
	Positive	Negative	Positive	Negative	Disorganised	Excited	Depressed
1. Min-max transformation	#41: R.6a & R.Pol1 #45: R.TPOJ1 & R.Thalamus	#1: L.3b & L.1 #8: L.1 & R.3b	#5: L.5L & R.Cereb #42: R.i6-8 & R.PeEc #45: R.TPOJ1 & R.Thalamus			#17: L.MI & R.Putamen #18: L.FOP1 & R.Putamen #41: R.6a & R.Pol1 #43: R.FOP4 & R.Putamen	#27: R.RSC & R.SFL #28: R.POS2 & R.SFL
2. Binarisation	#33: R.6ma & R.Cereb #45: R.TPOJ1 & R.Thalamus	#2: L.POS2 & R.SFL #8: L.1 & R.3b #28: R.POS2 & R.SFL	#3: L.PCV & L.POS1	#2: L.POS2 & R.SFL #8: L.1 & R.3b	#8: L.1 & R.3b	#14: L.IFSa & R.AVI #18: L.FOP1 & R.Putamen #38: R.p32pr & R.Putamen #41: R.6a & R.Pol1 #43: R.FOP4 & R.Putamen #44: R.FOP1 & R.Putamen	#27: R.RSC & R.SFL

2 Using multiple regression analyses, the FCs significantly associated with each PANSS factor were identified. When the coefficient for the
 3 factor was positive, the FC is coloured red, and when it was negative, the FC is coloured blue. “Pure state markers” (the case in which the
 4 explanatory variable of the PANSS factor was solely associated with the FC) are highlighted in bold letters. The ROIs described here are from

- 1 Glasser's⁶⁸ parcellation. FC: functional connectivity, PANSS: Positive and Negative Syndrome Scale, ROI: region of interest.

1 **FIGURE LEGENDS**

2 **Figure 1. Outline of the study**

3 This study was composed of two parts. **(I)** Constructing SSD Classifier: Using the
4 discovery dataset, we processed rs-fMRI images into an FC matrix for each participant,
5 which was then inputted into machine learning (LASSO) to build SSD classifiers. We
6 obtained the classification performance through 10-fold CV and examined its external
7 generalisability using the validation dataset. “Important FCs” were those that made the
8 highest contribution to the classification. We conducted further analyses on the
9 applicability of another machine learning method (voting classifiers) to other mental
10 disorders and on FC laterality. **(II)** Exploring Trait / State / Staging markers of SSD:
11 We investigated the different types of biomarkers inherent in important FCs. First, we
12 attempted to predict clinical scale scores using aggregated FCs. Second, we searched
13 for individual FCs associated with the state and/or disease stage. CV: cross-validation,
14 SSD: schizophrenia spectrum disorder, LASSO: least absolute shrinkage and selection
15 operator, rs-fMRI: resting state functional magnetic resonance imaging, FC:
16 functional connectivity.

17

18 **Figure 2. Probability density curves based on LASSO classifiers**

19 The abscissa represents the predicted probability of SSD as an output of the
20 classifiers. If a participant’s probability was over 0.5 (vertical dashed line), the
21 participant was classified as SSD, otherwise classified as HC. The ordinate indicates
22 the density at a certain probability. **(a)** Results for all the sites combined in the
23 discovery dataset. **(b)** Results for individual sites in the discovery dataset. COI has
24 only an HC curve since it has no patients with SSD. **(c)** Results for all the sites
25 combined in the validation dataset. **(d)** Results for individual sites in the validation
26 dataset. Since three sites in Hiroshima (HKH, HRC, and HUH) did not have any
27 patients with SSD, these sites have a curve for HCs only. HC: healthy control, SSD:
28 schizophrenia spectrum disorder, AUC: area under the curve, MCC: Matthews’
29 correlation coefficient, COI: Center of Innovation in Hiroshima University, KUT:
30 Kyoto University TimTrio, SWA: Showa University, UTO: University of Tokyo,
31 LASSO: least absolute shrinkage and selection operator, HKH: Hiroshima Kajikawa
32 Hospital, HRC: Hiroshima Rehabilitation Centre, HUH: Hiroshima University
33 Hospital, KTT: Kyoto University Trio, KUP: Kyoto University Prisma, TMU: Taipei
34 Medical University, JHU: Johns Hopkins University, COBRE: Centre of Biomedical
35 Research Excellence.

36

37 **Figure 3. Important FCs ($P < 0.05$) in diagnosis prediction by LASSO classifiers**

38 **(a)** Each node on the inner circle corresponds to an ROI. The line width of the FC
39 shows how many times it was selected by the classifiers, and the line colour denotes
40 the direction to which it contributes to the logistic regression model (red means that the
41 higher the FC value is, the more likely the classifier’s output is to be SSD; blue means
42 a lower FC value for a higher likelihood of SSD). **(b)** The 47 important FCs were
43 projected onto glass brains. The colours of the ROIs in relation to each intrinsic brain

1 network and the red/blue line colours correspond to those in (a). (c) The mean FC
2 values (Z-score, on the ordinate) of the 47 important FCs for the HC and SSD groups
3 are shown as a bar plot for the discovery and validation datasets. Error bars represent
4 standard error. The FC numbers on the abscissa correspond to those in (a) and
5 **Supplementary Table S2**. FC: functional connectivity, HC, healthy control; SSD:
6 schizophrenia spectrum disorder, ROI: region of interest, LASSO: least absolute
7 shrinkage and selection operator.

8

9 **Figure 4. Probability density curves for HC, SSD, and other disorders reveal**
10 **classifier specificity for SSD**

11 (a) Results for the LASSO classifiers. (b) Results for the voting classifiers. HC:
12 healthy control, SSD: schizophrenia spectrum disorder, MDD: major depressive
13 disorder, ASD: autism spectrum disorder, BP: bipolar disorder, LASSO: least absolute
14 shrinkage and selection operator.

15

16 **Figure 5. Trait, state, and staging marker analyses.**

17 (a, b) Prediction results of PDI total and PANSS total scores. The relationship between
18 the predicted scores (on the ordinate) and actual scores (on the abscissa) for the
19 validation dataset is shown in a scatter plot. The grey translucent band represents the
20 95% confidence interval of the regression line. (a) PDI total scores. (b) PANSS total
21 score. (c) Individual FCs associated with disease stage. This heat map shows the
22 results of the bootstrap method, where the number in the colour bar indicates the
23 number of times the FC was sorted into a category out of 1,000 iterations. n.s.: not
24 significant ($P \geq 0.05$ for all three explanatory variables or any of the coefficients' signs
25 were inconsistent with the average weight in LASSO classifiers). MAE: mean
26 absolute error. PDI: Peters et al. Delusion Inventory, PANSS: Positive and Negative
27 Syndrome Scale. FC: functional connectivity, LASSO: least absolute shrinkage and
28 selection operator.

29

30 **Figure 6. Scheme of the procedures for building classifiers**

31 (a) Workflow chart of the nested 10-fold CV applied to the discovery dataset. By
32 repeatedly subsampling and undersampling from a training set ten times, we obtained
33 ten subsets for each CV fold. Ultimately, 100 classifiers were obtained. (b) The
34 resulting 100 classifiers were applied to the validation dataset. The average value of
35 the classifier output was the final output. CV: cross-validation, HC: healthy control,
36 SSD: schizophrenia spectrum disorder.

I. Developing Clinically Applicable SSD Classifiers

Concerns about rsfMRI biomarkers

1. Inadequate accuracy
2. Insufficient generalisability
3. Across-scan variability
4. Lack of disease specificity

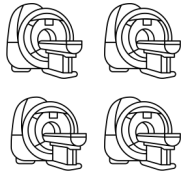
Aim: To overcome these by the methodology below

II. Exploring Trait / State / Staging markers of SSD

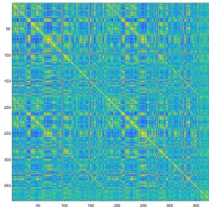
- Trait** Diagnosis, delusional tendency etc.
- State** Symptom (subject to change)
- Stage** e.g. early, chronic

Discovery dataset

(4 sites)

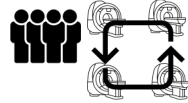


- Preprocessing
- Calculate FCs



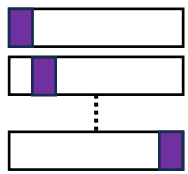
FC matrix

Unified imaging protocol (prospective harmo.)



Retrospective harmo. (travelling subjects)

Nested 10-fold CV + subsampling



Customised machine learning



SSD classifiers

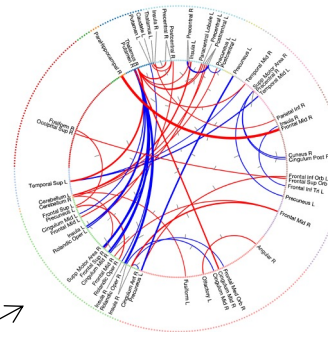
Additional analyses

- Voting classifiers
- Laterality in FCs

Validation dataset (7 sites)



Other psychiatric disorders

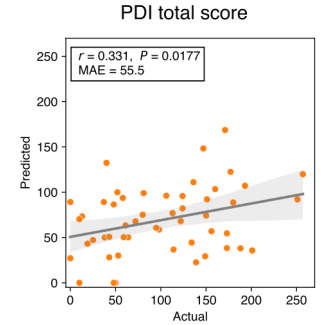


Important FCs

Aggregated

FC → trait, state

Scale score prediction



Individual

trait, state, staging → FC

Catalog of each marker type

State markers

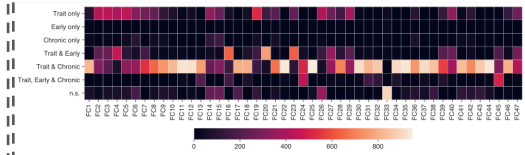
Three factor model

Positive #33, #41, #45
Negative #1, #2, #8, #28

Five factor model

Positive #3, #5, #42, #45
Negative #2, #8
Disorganised #8
Excited #14, #17, #18, #38, #41, #43, #44
Depressed #27, #28

Staging markers

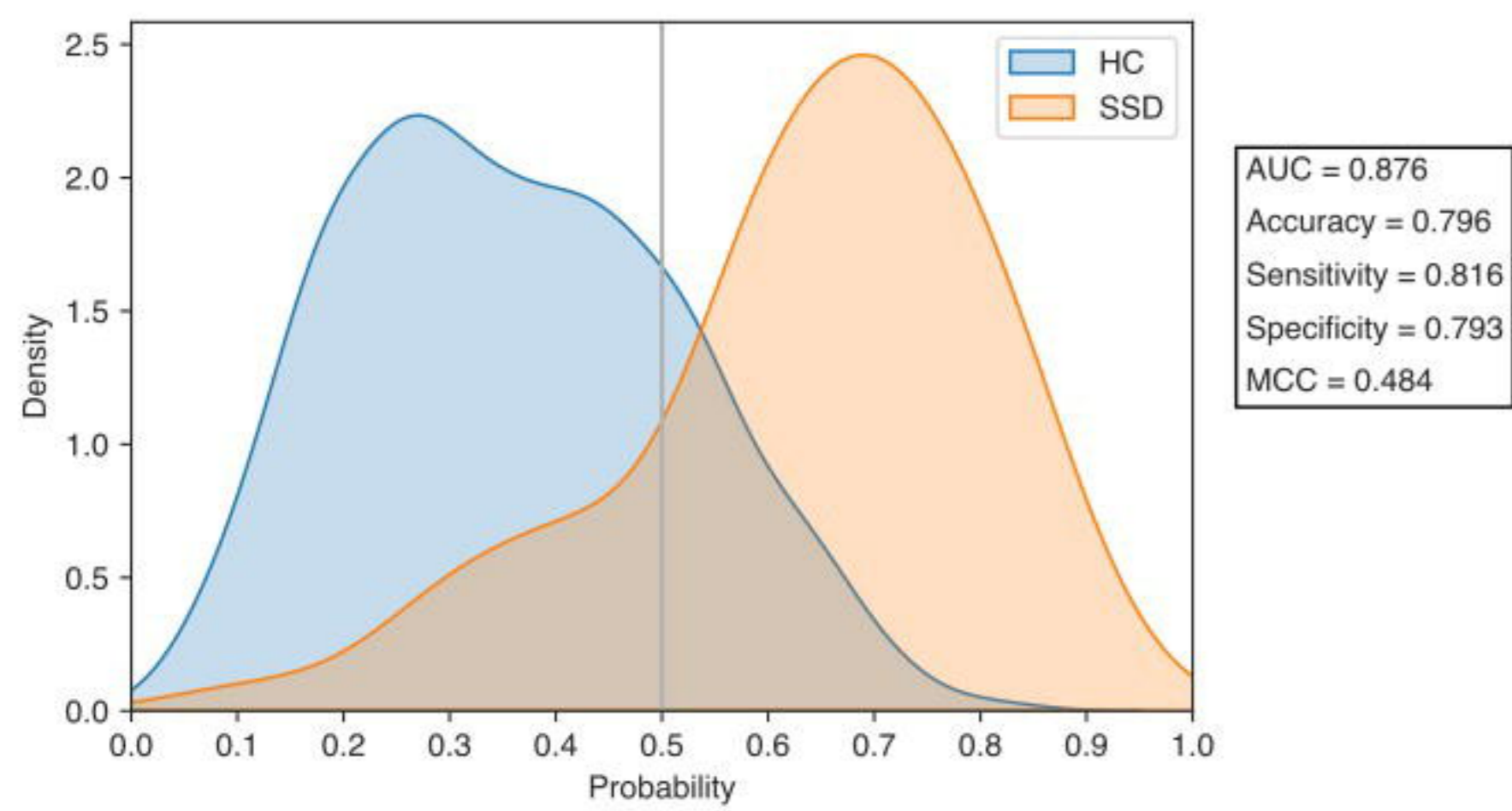


Results for LASSO Classifiers

Discovery dataset

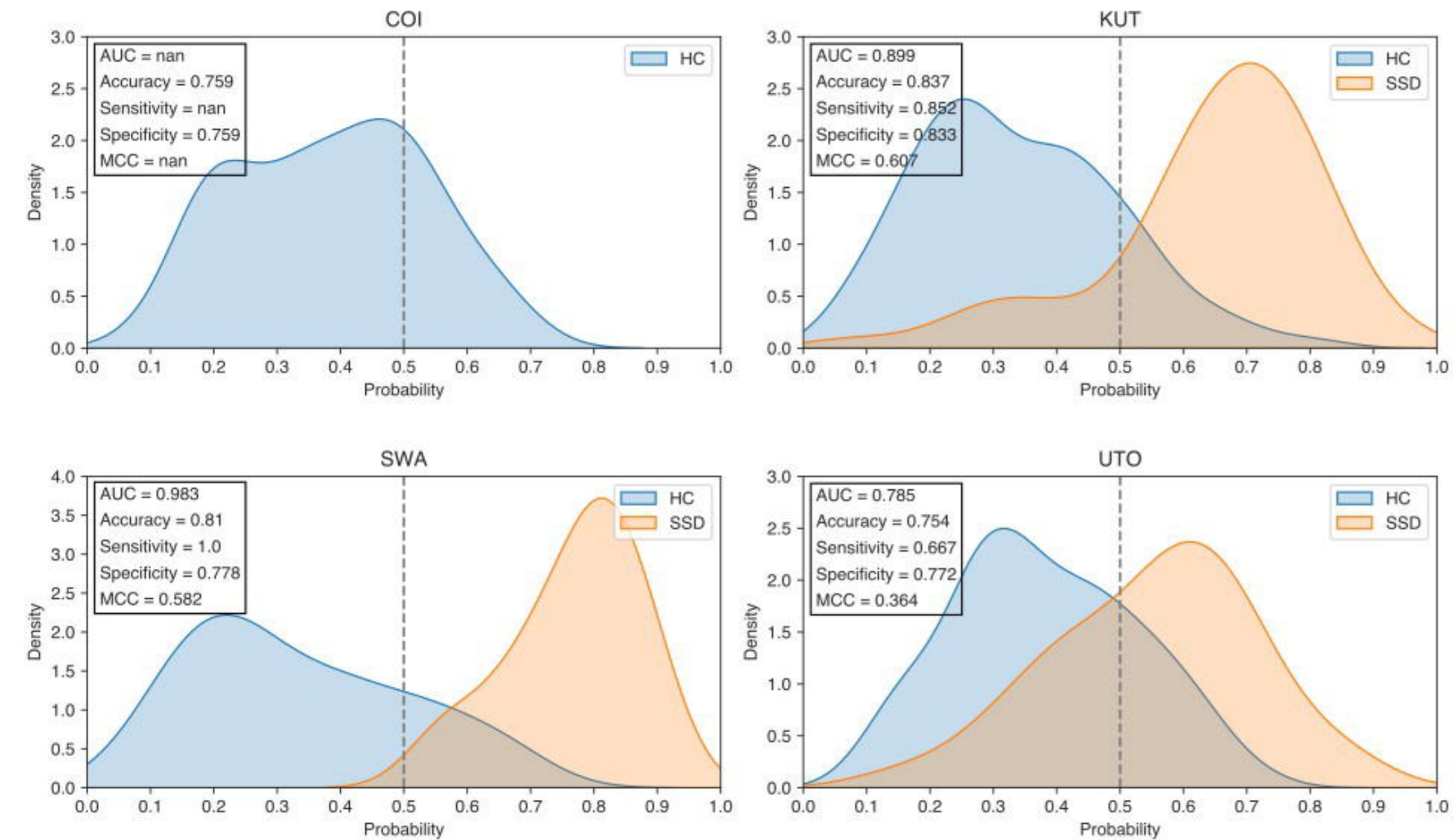
a

All sites



b

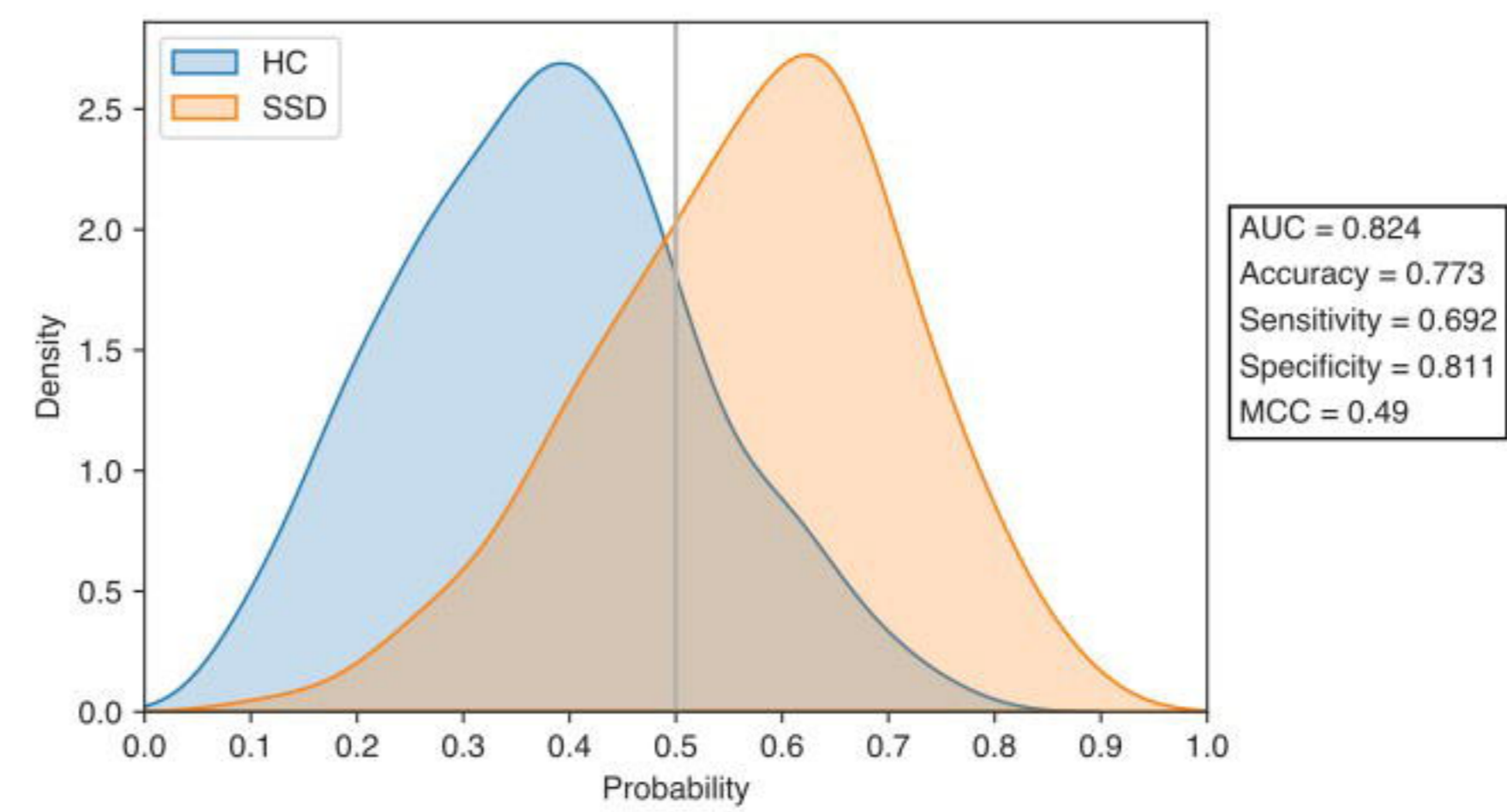
Individual sites



Validation dataset

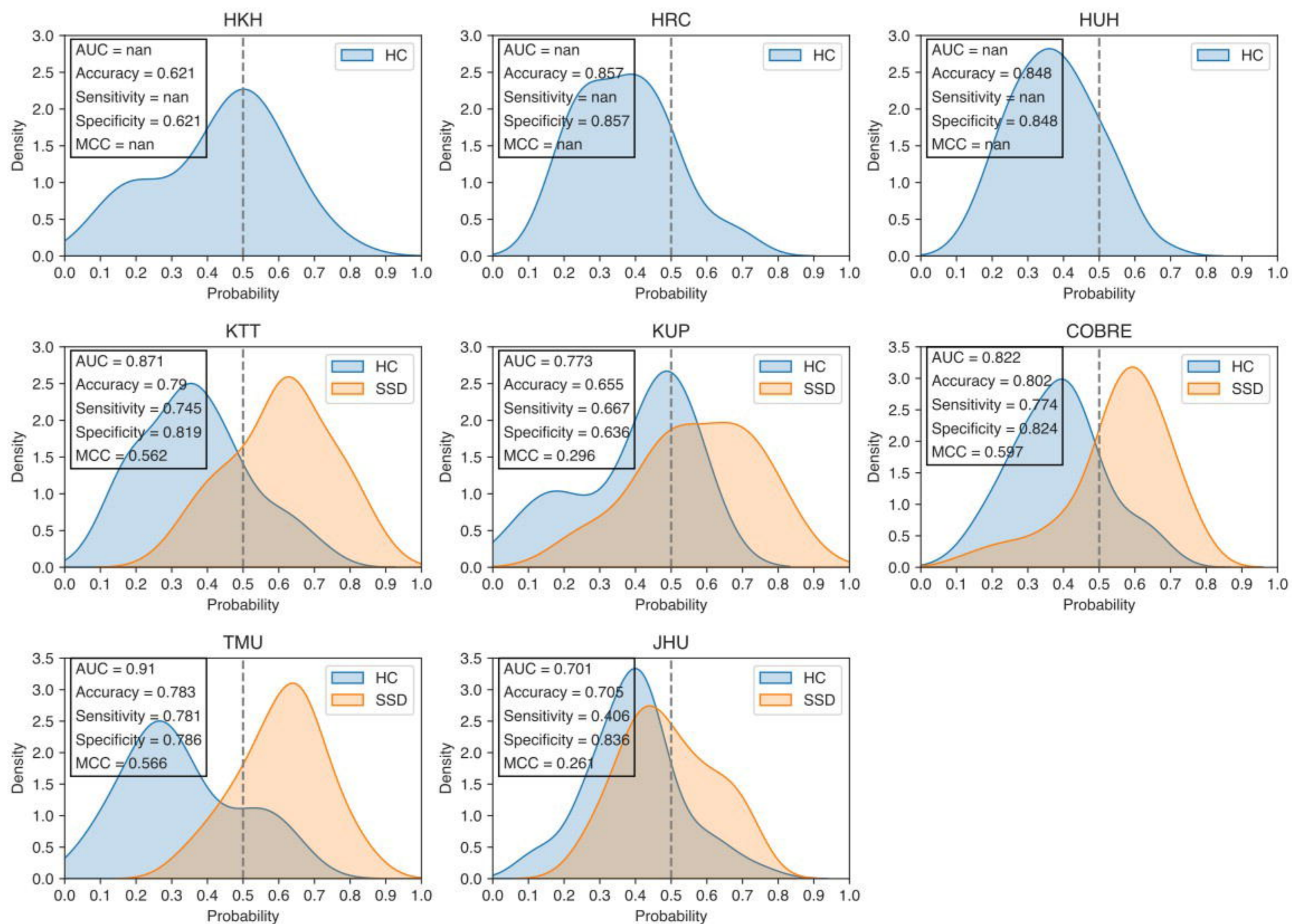
c

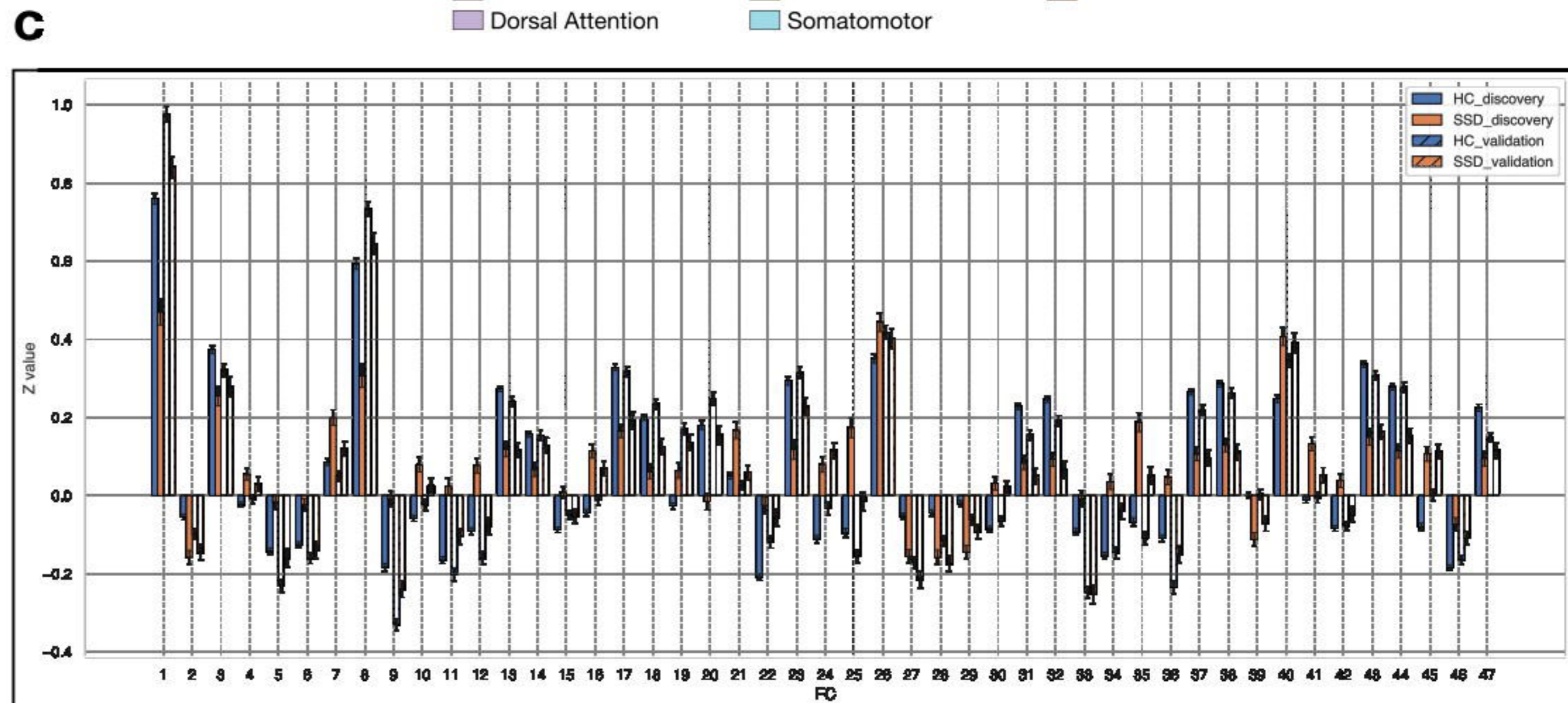
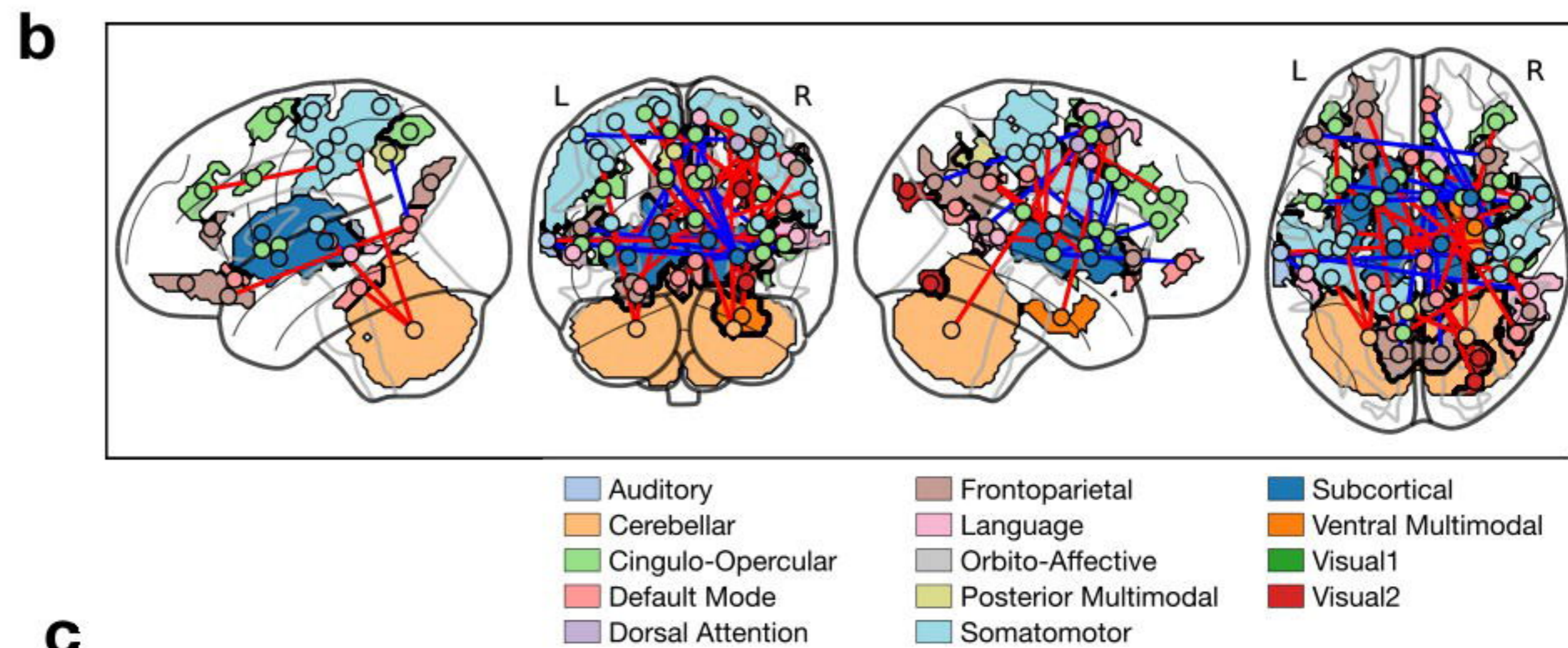
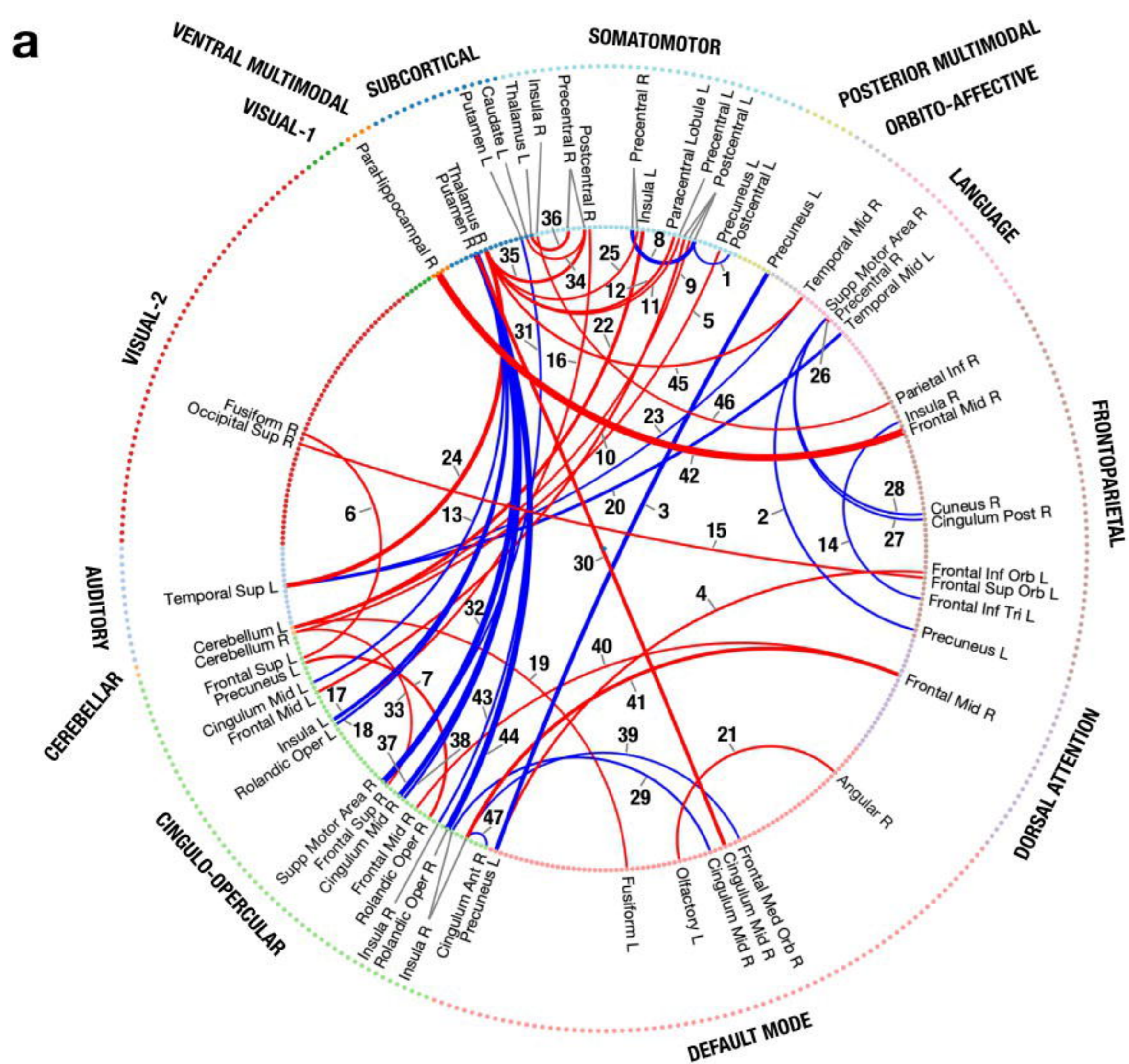
All sites



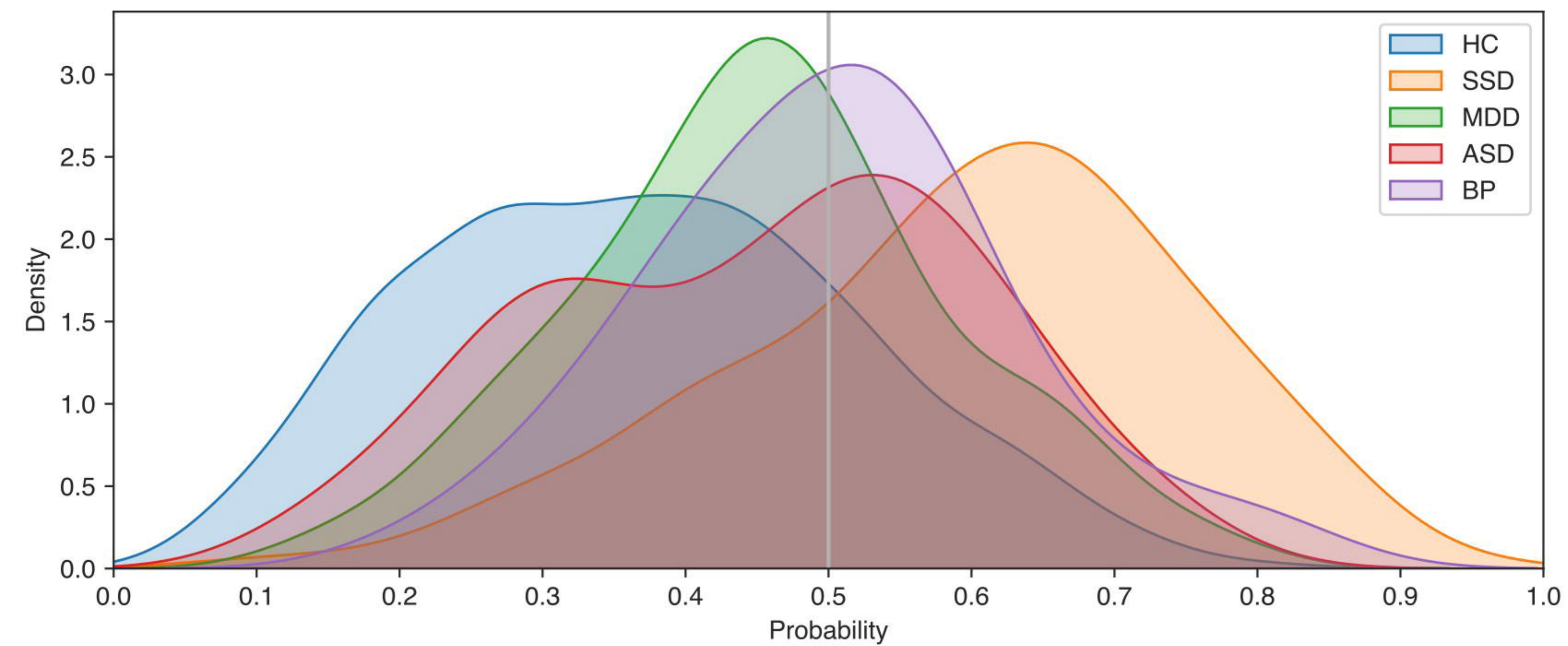
d

Individual sites

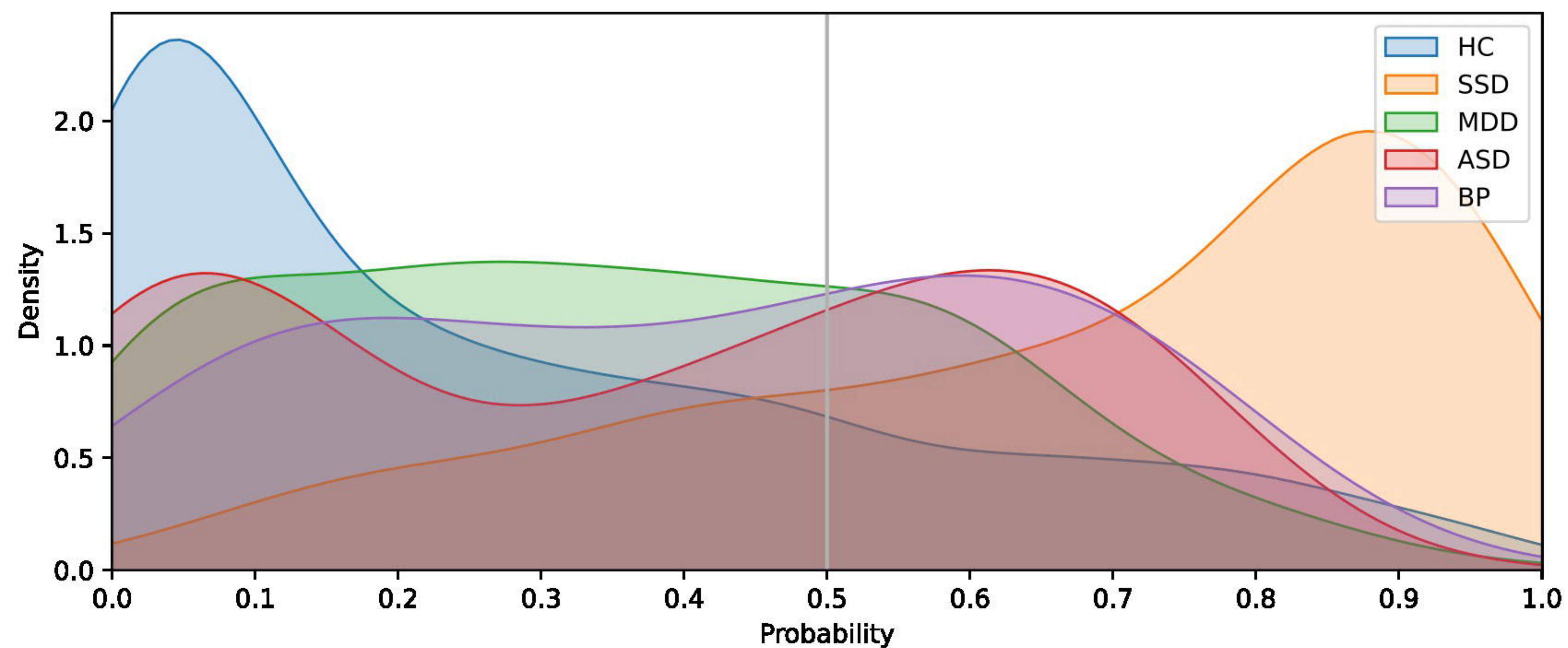


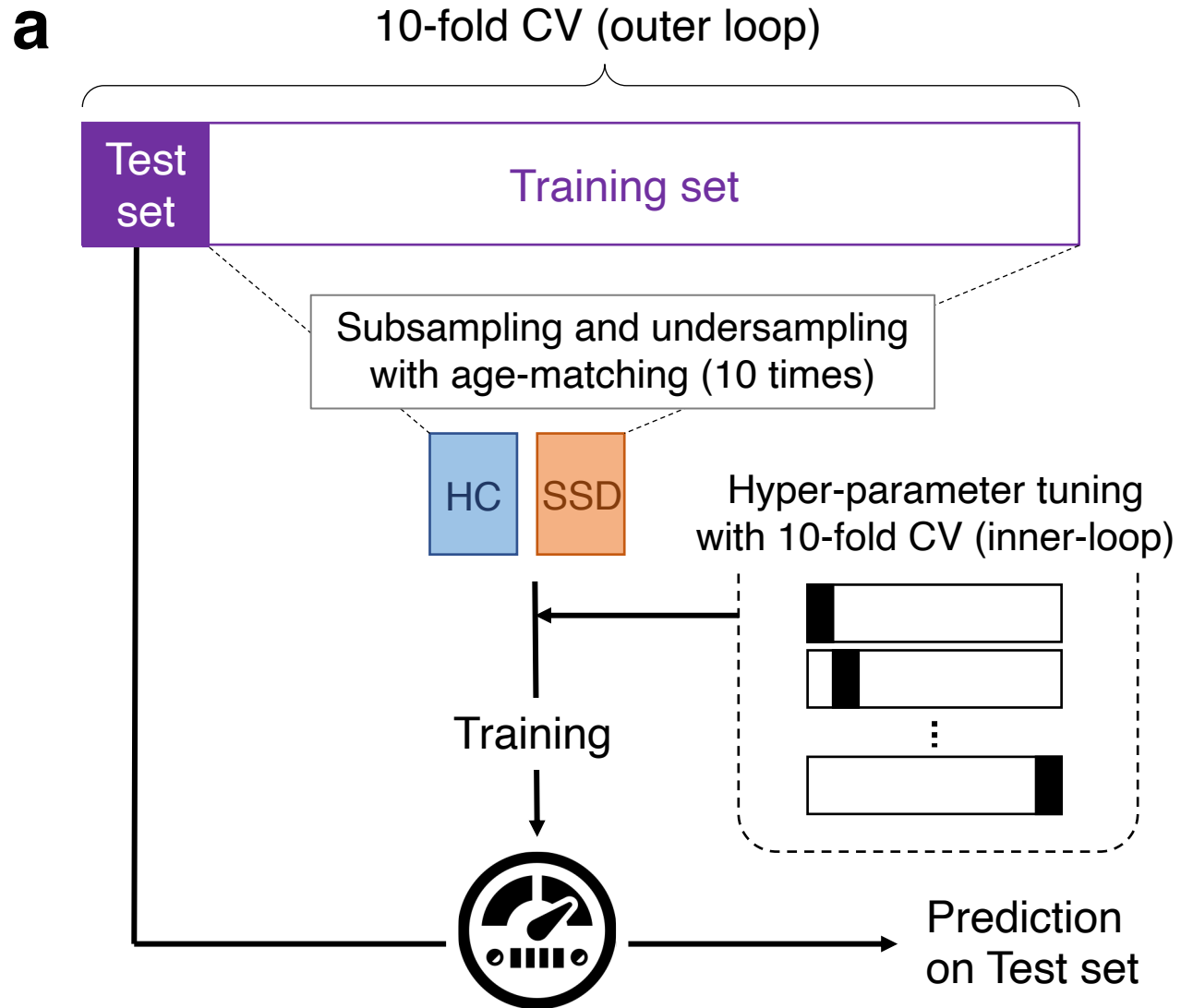


a LASSO Classifiers



b Voting Classifiers





10-fold CV × 10 subsampling = 100 classifiers

b

