Neuroimaging biomarkers define neurophysiological subtypes with distinct trajectories in schizophrenia

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Abstract

Technical developments and improved access to neuroimaging techniques has brought us closer to understanding the neuropathological origins of schizophrenia. Using data-driven disease progression modelling on cross-sectional MRIs from 1124 schizophrenia patients, we characterize two distinct but stable 'trajectories' of brain atrophy, separately beginning in the Broca's area (subtype1) and the hippocampus (subtype2). The two 'trajectories' are replicated in cross-validation samples. Individuals within each subtype are further classified into two stages ('pre-atrophy' and 'post-atrophy'). These subtypes show different atrophy patterns and symptom profiles. Longitudinal data from 523 schizophrenia patients treated by antipsychotics only (APM) or adjunct transcranial magnetic stimulation (TMS) reveal that APM effects relate to phenotypic subtype (more effective in the subtype1) while TMS effects relate to the stage (superior outcomes in the 'pre-atrophy' stage). These findings suggest distinct pathophysiological processes underlying schizophrenia that potentially yield to stratification and prognostication – a key requirement for personalizing treatments in enduring illnesses.

This study was registered in the Chinese Clinical Trials Registry (number: ChiCTR2000041106).

1. Introduction

Schizophrenia is a highly disabling psychiatric disorder with a life-time prevalence of 1%, affecting about 26 million people worldwide[1]. The pathophysiological basis of schizophrenia is unclear, but more than one mechanism is suspected to play a role, given the substantial heterogeneity in clinical course[2], treatment efficacy[3], and the levels of putative biological markers[4,5]. Given this unsolved heterogeneity, currently available treatments cannot be matched to pathophysiological pathways, leading to limited long-term benefits. For more than a century, attempts have been made for clinical subtyping based on signs and symptoms[6], but this has been either unreliable or of limited therapeutic utility[7]. Biological stratification that maps on prognostic trajectories is urgently needed to promote individualized treatment decisions in schizophrenia.

At a group level, individuals with schizophrenia display compromised brain structure characterised by ventricular enlargement, cortical thinning and reduced subcortical volumes in thalamus, hippocampus and amygdala[8,9], with notable worsening of these structural aberrations being reported in the early stages[10]. However, substantial inter-individual differences exist among individuals with schizophrenia, with no consistent abnormalities at an individual level evident in radiological examinations[5,11]. These inter-individual differences in brain structure results from two distinct sources of variation: first, mechanistic differences that result in subtly different clinical features (i.e., mechanistic heterogeneity) and second, relative differences between subjects in the stage of dynamic progression (i.e., temporal heterogeneity). For example, progressive reductions in gray matter volume (GMV) are associated with longer disease duration in schizophrenia[12]. Degree of cortical thinning is linked with different illness stages[13]. First-episode patients with schizophrenia showed subtle cortical thinning mainly in frontotemporal lobes[14], whereas chronic patients showed pronounced reductions spread across the parietal and occipital cortices[13]. Furthermore, brain atrophy associated with a range of clinical syndromes in schizophrenia have also been postulated to uncover underlying distinct pathophysiological processes[15,16]. Altogether, this evidence suggests that the complex pathological progress of schizophrenia may not be explained to a single unifying pathophysiological process, but a multitude of partially independent pathophysiological profiles. Hence, a systematic characterization of brain atrophy progression which accounts for variability on an individual level is an urgent need.

Machine learning approaches are increasingly used to parse the heterogeneous features of mental disorders[17-20]. Of these, unsupervised clustering techniques and semi-supervised methods, such as HYDRA[21], provide powerful tools for disease subtyping[17-19,22]. In schizophrenia, previous subtyping studies have exclusively focused on either phenotypic heterogeneity[23,24] (i.e., individuals are clustered into distinct subgroups without considering disease stage) or temporal heterogeneity[13,25] (i.e., individuals are in different stages of disease progression without subtype differences), but not both. A novel data-driven disease progression model named Subtype and Stage Inference (SuStaIn), that requires only cross-sectional data, was proposed to identify subtypes with common patterns of disease progression and achieve individualized inference[26]. Using SuStaIn, a

recent neuroimaging study successfully detected four distinct 'trajectories' of tau deposition in Alzheimer's disease[27].

This work investigated a systematic characterization of heterogeneity in brain atrophy patterning using structural magnetic resonance imaging (MRI) from 1124 schizophrenia patients (**Supplementary Table 1**). The aims (**Extended Data Fig.1**) were (1) to identify distinct 'trajectories' of brain atrophy in schizophrenia using SuStaIn and assign individuals to biological subtypes based on their atrophy patterning; (2) to examine the associations of specific subtypes with clinical symptoms; and (3) to examine treatment response to antipsychotic medication (APM) and transcranial magnetic stimulation (TMS) in subtypes. **Supplementary Fig.2** provides a flow of statistical analyses. Such brain subtyping may provide meaningful insights into the putative pathophysiological mechanisms in subsets of patients with schizophrenia. Ultimately, accurate stratification of this enduring illness requires addressing both temporal and phenotypic heterogeneity; if successful, this approach may inform designing clinical trials differently in the future.

2. Results

2.1 Two distinct pathophysiological pathways of brain atrophy

Using the 2-folds cross-validation, the optimal clusters were determined at k=2 (**Supplementary Fig.3-5**), indicating two distinct 'trajectories' of pathophysiological progression in schizophrenia (**Fig.1b**). Regional volume loss at each stage for each subtype is visualized in **Fig.1c**, which shows a progressive pattern of spatial extension that is distinct for each 'trajectory'. Briefly, 'trajectory' 1 exhibited a cortical-predominant phenotype (i.e., cortical primacy) where atrophy began in the Broca's area while 'trajectory' 2 exhibited a subcortical-predominant phenotype (i.e., subcortical primacy) where atrophy began in the hippocampus (**Fig.1b and Supplementary Table 5-6**). **Supplementary Fig.7** also displays the 'trajectories' of cortex and subcortex across SuStaIn stages. The differences of 'trajectories' of atrophy in specific brain regions highlights potential phenotypic heterogeneity, suggesting there may be two different neuropathological pathways with distinct sites of origin in schizophrenia.

2.2 Stability of SuStaIn subtypes

Cross-validation shows that the pathophysiological progression of GMV changes in the two subtypes are reproducible, revealing a high consistency of the observed 'trajectory' (**Supplementary Fig.4**) and SuStaIn stability for the individual subtyping using different features (**Supplementary Method S6**).

2.3 Subtype-specific atrophy patterns

By disentangling both temporal heterogeneity and phenotypic heterogeneity, we further defined four subtypes (**Fig.2a**). A total of 631 (56.1%) patients with schizophrenia were assigned to 'trajectory' 1 and further classified into two phases of 'pre-atrophy' ($S1_{pre}$, n=259) or 'post-atrophy' ($S1_{post}$, n=372). The remaining 593 patients (43.9%) were assigned to 'trajectory' 2 with 'pre-atrophy' ($S2_{pre}$, n=212) and 'post-atrophy' ($S2_{post}$, n=281). The z-scores of GMV images were mapped to a glass brain template for visualization of atrophy patterns in each subtype (**Fig.2a**). Comparisons of ROI-wise z-scores between $S1_{post}$ and $S2_{post}$ showed significantly higher z-scores of cortical regions and significantly lower z-scores of subcortical regions in $S1_{post}$ compared to $S2_{post}$ ($P<0.001$, Bonferroni correction) (**Fig.2b**). In addition, comparisons between subtypes ($S1_{pre}$ vs. $S2_{pre}$, S1 vs. S2) are shown in **Supplementary Fig.8**. These results indicated more cortical reductions in subtype1 and more subcortical reductions in subtype2.

2.4 Longitudinal examination of SuStaIn trajectories

In the preceding analyses, we used SuStaIn on cross-sectional individual MRI data to make pseudo-longitudinal inferences about the pathophysiological 'trajectories' of brain atrophy. To verify the truth, we collected longitudinal samples from our previous study[14] that included a total of 127 individuals who were drug-naive FES and scanned MRI at both baseline and 12-weeks follow-up (**Supplementary Methods S7**). Longitudinal data show that the $S1_{pre}$ (i.e., schizophrenia patients whose baseline GMV belong to the earliest stage of 'trajectory' 1 [before stage I]) had the fastest GMV reduction in the Broca's area and

insula (**Supplementary Fig.9**). In the $S2_{pre}$ individuals (i.e., their baseline GMV belong to the earliest stage of 'trajectory' 2), the fastest GMV reduction occurred in the hippocampus (**Supplementary Fig.9**). These findings are consistent with the expectation that these $S1_{pre}$/$S2_{pre}$ individuals were going from before stage I to stage I. Remarkably, mirroring the cross-sectional findings, the longitudinal observations support the ground of SuStaIn 'trajectories' (mainly the early part of the 'trajectory') of brain atrophy in schizophrenia.

2.5 Relationships between regional atrophy and clinical symptoms

We examined the relationships between regional atrophy and clinical symptoms for each subtype. As expected, higher z-score of GMV (that is, more reduction of GMV) was positively associated with increasing PANSS negative subscale score (that is, worse negative symptoms) in many cortical, subcortical and cerebellar regions (**Extended Data Fig.2, Supplementary Table 7-8**). Negative relationships between GMV z-scores, positive symptoms and general psychopathology were observed in subtype 1, indicating a lower burden of non-negative symptoms in individuals with more atrophy. Interestingly, this pattern of relationship with non-negative symptoms was reversed in individuals within subtype2 (**Extended Data Fig.2, Supplementary Table 7-8**), suggesting that associations between brain atrophy and symptoms are subtype-specific in schizophrenia.

2.6 Different clinical profiles among subtypes

We compared demographic, clinical and brain variables (**Table 1**) across the two stages and the two subtypes. The pre-atrophic subjects with subcortical-primacy subtype ($S2_{pre}$) had shorter illness duration compared to all other individuals including the $S1_{post}$ and $S2_{post}$ (**Fig.3a**). The pre-atrophic subjects with cortical-primacy subtype ($S1_{pre}$) had worse positive symptoms compared to all other individuals including the $S1_{post}$ and $S2_{post}$ (**Fig.3b**). Further, as expected, the $S1_{post}$ and $S2_{post}$ showed smaller total GM volume and larger total CSF volume compared to $S1_{pre}$ and $S2_{pre}$ (**Table 1**). We also compared the differences between the subtype1 ($S1_{pre}$ and $S1_{post}$) and subtype2 ($S2_{pre}$ and $S2_{post}$) and found worse positive symptom in subtype1 compared with subtype2 (**Supplementary Table 9**).

We also found a relationship between SuStaIn stage scores and illness duration, symptoms, GM and CSF volume. As expected, increasing SuStaIn stage scores was positively associated with longer illness duration (r=0.208, P<0.001, **Fig.3c**), higher burden of negative symptoms (r=0.127, P=0.008, **Fig.3d**), larger CSF volume (r=0.353, P<0.001, **Fig.3e**) and smaller GM volume (r=-0.250, P<0.001, **Fig.3f**) were observed.

In addition, we compared the differences of positive and negative symptoms among individuals belonging to different SuStaIn stages using the ANOVA and post-hoc tests. In the cortical-primacy subtype, individuals belonging to the later stage VI (higher atrophy) showed a higher score of negative symptoms compared to individuals in stages I, II, III and pre-stage I (i.e., individuals without atrophy in any regions) (corrected P<0.05) (**Fig.3g**). In subcortical-primacy subtype, individuals belonging to pre-stage I (no atrophy) showed a higher score of positive symptoms compared to stage I individuals (corrected P<0.05) (**Fig.3h**).

2.7 Treatment outcomes and subtypes

We examined whether subtype classification will relate to differential treatment response to APM and TMS using a longitudinal independent cohort. As for APM sample, we found a significant positive correlation between the probability belong to subtype1 and PANSS positive score reduction ratio (r=0.127, P=0.014, **Fig.4a**), indicating that individuals who have a higher probability assigned to subtype1 showed better treatment outcomes. This significant correlation remained consistent even controlling the factors including baseline PANSS scores, sites, education, sex, age, illness stage and CPZ (**Supplementary Table 11**). As for TMS follow up sample, we observed a significant negative correlation between the SuStaIn stages and PANSS reduction ratio in terms of positive score (r=-0.370, P<0.00001, **Fig.4b**), general score (r=-0.237, P=0.003) and total score (r=-0.279, P<0.001), indicating that individuals who have an earlier SuStaIn stage showed better treatment outcomes. The significant correlation between SuStaIn stage and symptom remission remained consistent even controlling the factors including baseline PANSS scores, sites, education, sex, age and illness stage (**Supplementary Table 12**), and was observed in both subtype1 (r=-0.351, P=0.001) and subtyp2 (r=-0.395, P<0.001).

We also compared the differences of follow up PANSS between the subtype1 and subtype2. For individuals who were treated with APM, we found that compared with the subtype2, the subtype1 showed significant better positive symptoms remission after controlling the baseline PANSS (**Fig.4c, Supplementary Table 10**). This difference remained consistent even after controlling the effects of CPZ and illness stage. However, as for the individuals who were treated with TMS, we did not observe a significant difference in symptom reduction between two subtypes.

In addition to the two phenotypic subtypes, we compared the differences of follow up PANSS among the four subgroups ($S1_{pre}$, $S1_{post}$, $S2_{pre}$ and $S2_{post}$). As for APM, the $S1_{pre}$ exhibited better treatment outcomes, especially to the positive and general symptoms (**Table 2, Fig.4d**), compared with the other subgroups after controlling the baseline PANSS, CPZ and illness stage. As for TMS, the $S1_{pre}$ and $S2_{pre}$ showed more PANSS positive score reduction compared with the $S1_{post}$ and $S2_{post}$ (**Supplementary Table 13, Fig.4e**), indicating better TMS outcome to treating positive symptoms for these individuals with less brain atrophy. These findings remained consistent even controlling the factors of baseline PANSS, illness stages and TMS targets. We observed that in the $S2_{pre}$, TMS exhibited better improvement to treat negative symptoms (**Supplementary Table 13, Fig.4e**).

Altogether, antipsychotics are more effective in the cortical-primacy type (subtype1) while superior outcomes with TMS is seen in pre-atrophic stage (for both $S1_{pre}$ and $S2_{pre}$). One specific case where TMS may benefit is the treatment of negative symptoms in subcortical-primacy type before atrophy sets in ($S2_{pre}$). Once ‘atrophy’ sets in, antipsychotics work better for reducing positive symptoms.

3. Discussion

Using a data-driven modelling technique, we show that pathological atrophy of schizophrenia is better characterized by two distinct pathophysiological 'trajectories': a cortical-predominant phenotype that begins in the Broca's area/frontoinsular cortex and a subcortical-predominant phenotype that begins in the hippocampus. These subtypes showed different illness duration, symptom profiles and treatment outcomes. These findings raise critical research questions for stratified clinical trials in schizophrenia and indicate biological plausibility and therapeutic relevance of the identified subtypes.

Two distinct pathophysiological 'trajectories' of brain atrophy were identified as cortical-predominant phenotype and subcortical-predominant phenotype, indicating two possible sites of pathophysiological origin: cortical atrophy begins in the Broca's area and the frontoinsular cortex while subcortical atrophy begins at the hippocampus. Broca's area abnormalities have been widely found in schizophrenia[28]. Further, our previous studies found that individuals at high risk of psychosis exhibited functional connectivity changes primarily in the Broca's area[29], suggesting that dysfunction of Broca's area, possibly influenced by distinct genetic pathways[30], has emerged even before the first psychotic episode. Current results also provide direct structural imaging evidence that the Broca's area and adjacent frontoinsular cortex may be one of the 'site of origins' of brain abnormalities in schizophrenia. These findings contribute to a key neuropathological role of Broca's area, in line with Crow's linguistic primacy hypothesis[31] and the frontoinsular-cingulate cortex, in line with the salience network model[32].

In subtype2, subcortical atrophy began at the hippocampus, another possible 'site of origin' identified here. Some studies have highlighted hippocampal atrophy as one of the first regions to show volumetric loss in schizophrenia[8,33]. Recently, a longitudinal clinical high-risk psychosis study reported that for individuals who experience a prodromal stage to syndromal psychosis, hippocampal pathology (such as glutamate excess and hypermetabolism) leads to volume loss and expands to other regions of the hippocampal circuit and other connected areas along with illness progress[34]. Together, these findings challenge the notion that there is a single unifying pathophysiological process in schizophrenia, although this will require validation.

We note that as the degree of atrophy progresses with longer illness, individuals exhibited worse negative symptoms irrespective of their subtypes. Interestingly, both the cortical- and subcortical-primacy subtypes showed worse positive symptoms when atrophy was limited (positive symptom correlation in subtype1 [**Extended Data Fig.2**]; pre-stage I vs stage I comparison for subtype2, [**Fig.3h**]). While this may appear counterintuitive at the outset, this lends support to the emerging notion that the progressive grey matter changes in schizophrenia may indeed be a feature of cortico-subcortical reorganization in response to positive symptoms[10,14]. Thus, unlike a degenerative process in which tissue reduction will predict worse clinical symptoms, in schizophrenia such reduction may alleviate the positive symptoms, at the cost of worsening negative symptoms[35]. This notion may also contribute to understanding why, in general, antipsychotic exposure hastens brain tissue

loss in schizophrenia[14,36], although it is still a complex and fiercely debated topic. We also found that patients with longer illness duration had lower positive subscale and higher negative subscale. It was consistent with a previous longitudinal work[37], revealing that the positive symptoms exhibited a general pattern of improvement while negative symptoms showed less reduction over time. We also noticed the inconsistency of inter-subtype symptom difference between the cross-sectional and longitudinal samples, which may be due to the heterogeneity of psychotic symptoms and stages of illness[38,39].

The combined subtyping and staging approach employed here also highlights the prognostic potential of MRI. By MRI subtyping, we found that 'what kind of brain' and 'at which stage' is more likely to benefit from specific treatments, providing preliminary support for the prognostic potential of schizophrenia biotypes. Our data revealed that antipsychotics outcome is related to phenotypic subtype while TMS is associated with stage subtype. These results are consistent with studies reporting that schizophrenia patients with specific brain features may benefit from specific interventions. For example, volume increase in the hippocampus predicted negative symptom improvement for TMS[40]. Our study observed that patients who better respond to APM had stronger cortico-cortical connectivity compared to non-responders[14]. But it should be noted that medication use of the current sample is highly heterogeneous, involving monotherapy and combined therapy, and including up to ten antipsychotic drugs. Although identifying potential mechanisms is still challenging, the subtypes we report parse the heterogeneity of the brain features, and map them to specific treatments. These results suggest that prediction of treatment outcome may benefit from stratification based on biological subtypes of schizophrenia.

This study has several limitations. First, the SuStaIn create pseudo-longitudinal sequences using cross-sectional data. The fitted pathophysiological 'trajectories' do not directly reflect the real illness progression. Although longitudinal data support the truth of SuStaIn 'trajectories' (mainly the early stage of the 'trajectory'), future work needs to verify the pathophysiological 'trajectories'. Not all individuals with schizophrenia had quantitative medication information, which limited to eliminate the medication impact. Second, it is unclear if MRI-based estimates reflect true tissue atrophy. In addition to GMV, cortical thickness or gyrification could also be considered. The current mixed sample had confounding factors from different cohorts, scanners and sites. Harmonization methods[41] should be used to alleviate differences across MRI acquisition protocols. Patients undergoing TMS also received APM. Ideally, randomizing individuals with comparable illness duration to APM or TMS, and reducing variations in medication choice and the site of TMS stimulation would have improved the out-of-sample generalizability of our findings. Robust demonstration of clinical utility of the subtypes requires prospective trials in the future. Finally, while clustering/subgrouping may aid in stratified interventions, considerable variability may still exist among individuals within a cluster; dimensional approaches to personalisation may be more appropriate to address this issue[42]. We have not tested the extent to which the subtypes identified here could account for the heterogeneity; a continuous representation of neurobiological changes may be superior in this regard, but this needs to be tested.

In conclusion, we describes two distinct but stable pathophysiological ‘trajectories’ of brain atrophy of schizophrenia, separately beginning in Broca’s area and the hippocampus. These subtypes exhibit different atrophy patterns, clinical symptom profiles and treatment outcomes. Antipsychotics are more effective in the cortical-primacy type while superior outcomes with TMS are seen in the pre-atrophic stage of illness irrespective of the phenotypic subtypes. These findings suggest distinct pathophysiological processes underlie schizophrenia and they potentially yield to stratification and prognostication which are key requirements for personalizing treatments in enduring illnesses.

4. Methods

4.1 Sample characteristics.

Cross-sectional sample. The primary sample consisted of cross-sectional T1-weighted magnetic resonance imaging (MRI) scans from 2239 individuals (1168 patients with schizophrenia) from 4 hospitals including Shanghai Mental Health Centre (dataset #1), First Affiliated Hospital of Zhengzhou University (dataset #2), Taipei Veteran General Hospital (dataset #3) and Clinical Hospital of Chengdu Brain Science Institute in Chengdu (dataset #4) and from another 5 publicly available datasets, i.e., COBRE (dataset #5), NMorphCH (dataset #6), FBIRN (dataset #7), NUSDAST (dataset #8) and DS000115 (dataset #9). All individuals with schizophrenia were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). Individuals were excluded from the study if they were (1) diagnosed with schizoaffective disorder, mood disorders, or other major medical or neurologic disorders; (2) alcohol/drug dependence; (3) had a history of electroconvulsive therapy within six months; (4) other contraindications to MRI scanning. Individuals with illness duration less than 2 years were defined as first-episode schizophrenia (FES). The data quality control steps are described in the **Supplementary Method S4**. After data quality control, 2170 individuals were included, of which 1124 were schizophrenia patients (479 females, age=31.1±12.8 years) and 1046 healthy subjects (498 females, age=32.6±12.4 years). Symptom severity was assessed with the Positive and Negative Syndrome Scale (PANSS) for individuals from datasets #1, #2, #3, #4 and #5, with the Brief Psychiatric Rating Scale (BPRS) for individuals from dataset #8, or with the Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS) for individuals from datasets #6, #8 and #9. Detailed information of each cohort is provided in the **Supplementary Method S1**. A summary of demographics of subjects are shown in **Supplementary Table 1**.

Longitudinal sample. A total of 373 patients with schizophrenia (190 females, age=26.4 ±9.0 years), from four hospitals (Shanghai Mental Health Center [Shanghai], N=180; Peking University People's Hospital [Beijing1], N=102; Peking University Sixth Hospital [Beijing2], N=65; Clinical Hospital of Chengdu Brain Science Institute [Chengdu], N=26), were treated with antipsychotic medication (APM) and included in the longitudinal analyses (**Supplementary Table 2**). All individuals met DSM-IV diagnostic criteria for schizophrenia, and no other comorbid Axis I disorders. Inclusion and exclusion criteria of subjects are provided in our previous study[14]. At baseline, 294 of them were treatment-naïve FES. Following baseline MRI, schizophrenia patients received antipsychotic medications. 300 of 373 received monotherapy: amisulpride (n=26), aripiprazole (n=58), blonanserin (n=3), clozapine (n=7), olanzapine (n=85), paliperidone (n=15), paliperidone palmitate injection (n=4), quetiapine (n=8), risperidone (n=87), ziprasidone (n=3), unknown (n=4). 73 received combined therapy (⩾two antipsychotic drugs). The daily dosage of drugs was converted to chlorpromazine equivalents (CPZ). The mean CPZ during medication was 378.9±210.0 mg/day. The severity of symptoms was evaluated based on PANSS administered by the same psychiatrist. Symptom relief indicated in PANSS total and subscale scores (reduction ratio = (baseline-follow up)/baseline x 100%) were used to measure treatment response.

The average duration of PANSS follow up was 9.6 weeks. At follow up, 267 of 373 (71.6%) were considered as APM responders whose symptoms relief (percentage of reduction ratio in PANSS total score) >25%. Information on antipsychotic medication usage is provided in **Supplementary Table 3**.

A total of 150 patients with schizophrenia (66 females, age=30.1±12.3 years), from four hospitals (First Affiliated Hospital of Anhui Medical University [Anhui], N=38; Fourth Military Medical University [Xi'an], N=36; Clinical Hospital of Chengdu Brain Science Institute [Chengdu], N=27; Harbin First Specialized Hospital [Harbin], N=49), were treated with TMS under stable dosage of antipsychotics and included in the longitudinal analyses. At baseline, 100 of them were treatment-naïve FES. The inclusion criteria and TMS parameters are detailed in the **Supplementary Method S3**. In brief, stimulation target was set at the left temporoparietal junction (TPJ) for 74 individuals and the left dorsolateral prefrontal cortex (DLPFC) for 27 individuals and the right orbitofrontal cortex (OFC) for 49 individuals. PANSS assessments were performed at baseline and at follow-up by the same psychiatrist. The average duration of PANSS follow up was 4.0 weeks. At follow up, 82 of 150 (54.7%) were considered as TMS responders whose percentage of reduction ratio in PANSS total score >25%. A summary of demographics of subject who treated by antipsychotic medication or TMS are shown in **Supplementary Table 2.** We use the naturalistic data from APM and TMS samples collected during routine clinical care; this is not a report of a randomized trial. The TMS study was registered in the Chinese Clinical Trials Registry (number: ChiCTR2000041106) and the TMS protocol was available (http://www.chictr.org.cn/showproj.aspx?proj=65566). Written informed consent was obtained from all participants and/or their legal guardians. Participants received travel compensation and remuneration up to 300 Chinese Yuan based on the study they participated in.

Ethics and Inclusion statement.

The study included local researchers throughout the research process - study design, study implementation, data ownership, intellectual property and authorship of publications. The relevant roles and responsibilities were agreed amongst collaborators ahead of the research. The study has been approved by the Medical Research Ethics Committees of the local hospitals (ethics number: 2017-36R[dataset#1], 2018-KY-88[dataset#2], YM105091F[dataset#3], CDFH2014030501[dataset#4], 2017-36R[Shanghai], 2008-2[Beijing1], 2017-16[Beijing2], CDFH2014030501[Chengdu], 2016003[Anhui], XJYYLL-2015047[Xi'an] and IRB2019-004[Harbin]).

4.2 Image acquisition and processing.

T1-weighted MRI acquisition procedures (including the longitudinal sample) for each cohort have been described previously[12,14,43,44]. T1-weighted images were processed using the Computational Anatomy Toolbox (http://www.neuro.uni-jena.de/cat/) within SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Briefly, a fully automated procedure for standard voxel-based morphometry (VBM) (including spatial registration, tissue segmentation and bias correction of intensity non-uniformities) was conducted, resulting in GMV images. The GMV images were parcellated based on the automated

anatomical (AAL) atlas. These parcellations were used to extract mean GMV values within different regions of interest (ROIs) for each subject.

4.3 SuStaIn.

Traditional data-driven subtyping of brain imaging in schizophrenia has low replicability due to the confounding effect of illness stage. Most clustering methods classify individuals based on their symptoms, cognitive scores or structural or functional neuroimaging features. All of these features change with disease progression; thus, the assumption that all individuals are at the same stage of illness when measurements are obtained is fallacious. Here, we model disease progression in schizophrenia as a linear deviation from normality of brain structure (see **Supplementary Method S5** for further discussion on this assumption). A novel approach–SuStaIn generates clustering solutions across subjects while accounting for disease progression[26] (**Fig.1a**). SuStaIn has demonstrated ability to identify diverse but distinct progression patterns using cross-sectional neuroimaging data for brain disorders[26,27].

The SuStaIn approach has been presented in detail in a previous publication[26]; we briefly describe the major features here. The z-score model underlying SuStaIn is a development of the original event-based model[45]. Event-based model regards disease progression as a series of events, where each event corresponds to a switch from a normal to an abnormal level for a biomarker/feature[45]. The linear z-score SuStaIn model reformulates the events that represent the continuous linear accumulation (more biologically plausible) of a biomarker/feature from one z-score to another, rather than a discrete event-related transition towards an abnormal state[26]. Please see **Supplementary Method S5** for a discussion on the validity of this assumption in schizophrenia.

4.3.1 z-scores

The data for SuStaIn needs to be z-scored relative to a control population. The z-scores represent the severity of an abnormality for a specific feature/biomarker of interest, in this case MRI-derived grey matter volume (GMV). Higher z-scores represent larger deviations from the normal (i.e., more severe atrophy in this case). In this study, the ROI-wise GMV values were first adjusted by regressing out the effects of sex, age, the square of age, total intracranial volume (TIV) and sites as dummy covariates using a regression model. We did not include ethnicity into the regression model due to similar covarying tendencies of ethnicity and site (dataset#1-4 for Han Chinese; dataset #5-9 for not Han Chinese). Subsequently, the adjusted GMV values were normalized relative to control population using z-scores. Finally, these z-scores representing normative deviations were multiplied by -1 so as the regional brain volumes decrease in patients with schizophrenia, the z-scores increase.

4.3.2 Input Features

Input for SuStaIn requires an M x N z-score matrix. M represents the number of patients with schizophrenia (M=1124 in this study). N represents the number of SuStaIn features/ROIs (N=17 here). In this case, SuStaIn features represent the mean z-scores of GMV within different ROIs. Due to computational complexity (**Supplementary Method S6**)

and sufficient power of sample size, SuStaIn models typically used approximately 15 ROIs in previous studies[26,27]. Here, all of the AAL ROIs of whole brain were separated into 17 features (frontal lobe, temporal lobe, parietal lobe, occipital lobe, insula, cingulate, sensorimotor, Broca's area, cerebellum, hippocampus, parahippocampus, amygdala, caudate, putamen, pallidum, nucleus accumbens and thalamus) (**Supplementary Fig.1a**). See **Supplementary Table 4** for a summary of the features used in the SuStaIn modelling. On the basis of previous literature[26], we used z-scores = 1 (that is 1 s.d. from normal), 2 and 3 as severity cutoffs indicating waypoints of disease progression for the included features.

4.3.3 Sequence estimation (i.e., the trajectories of pathophysiological progression)

We imported 17 ROIs, each ROI having 3 severity cutoffs (z=1, 2, 3), to the model of SuStaIn, yielding a total of 51 events to be sequenced. The most probable sequence ($\boldsymbol{S_k}$=[e_1, e_2, … , e_{51}]) of spatial progression (i.e., 'trajectory') for each subtype was then evaluated using SuStaIn (see details in[26]). SuStaIn assumes a uniform prior that all combinations of subtype and stage equally likely. The model is initialized with an expectation–maximization algorithm and repeated for 25 different random start points to find the maximum likelihood solution. The number of all possible sequences is too large so we evaluated the relative probability (uncertainty) of all possible sequences for each subtype using a 10,000 Markov Chain Monte Carlo (MCMC) sampling (see details in[26]). The cumulative probability for each feature/ROI to reach a particular z-score over time is presented in **Fig.1b**.

4.3.4 Number of subtypes

To establish the clustering tendency within the data, we employed Hopkins statistics[46] which provided a robust support for the existence of clusters (H=0.8026, indicating a high clustering tendency at the 90% confidence level). SuStaIn could identify the potential distinct 'trajectories' of pathophysiological progression with a given subtype number K. Prior clustering studies without progression-based modelling have reported 2 to 6 morphological subtypes of schizophrenia[19,47,48]. We used this range to estimate SuStaIn models separately. To determine the optimal number of subtypes with distinct trajectories, we measured the reproducibility of SuStaIn subtype by a 2-fold cross-validation method. Specifically, the cohort was randomly split into two non-overlapping subfolds (50% of the patients as one subfold and left 50% as the other subfold). Above procedure was repeated ten times to avoid the occasionality of one split. For each non-overlapping subfold, the SuStaIn model was trained on one of non-overlapping folds, separately for each k=1-6 subtypes, and further tested using the other non-overlapping subfold. The optimal subtype number was determined using three metrics: (1) Consistency of individual subtype assignment (**Supplementary Fig.3**). For each individual, the subtype label was estimated separately in two non-overlapping subfolds. As the classification label may change for independent SuStaIn modelling (For example, Label 1 of train set may correspond to Label 6 of test set), the subtype label vector was transformed to an adjacent matrix. Dice coefficient was used to measure the consistency of the adjacent matrix between two non-

overlapping subfolds. (2) Consistency of the SuStaIn 'trajectory' (**Supplementary Fig.4**). In each non-overlapping subfold, SuStaIn estimated the 'trajectory' (i.e., the most probable sequence (S_k) of regions) for each subtype. The mean Kendall's tau coefficient between the S_k from paired subfolds was used to quantify the consistency in the SuStaIn 'trajectory'. (3) Silhouette clustering evaluation criterion (**Supplementary Fig.5**). In each non-overlapping subfold, Silhouette value was used as another evaluation indicator for subtype number range from 2 to 6. **Supplementary Fig.3-5** show that the optimal subtype number K=2 is consistency by cross-validation, indicating the best fit to the data included two subtypes with two distinct pathophysiological progressions of GMV changes in schizophrenia. The two-cluster model of SuStaIn was fitted to the whole sample.

4.3.5 Visualization of distinct trajectories of gray matter atrophy

To visualize the pathophysiological progression of gray matter atrophy across SuStaIn stages, we calculated the mean z-score images for individuals belonging to the following stage bins: I(e_1, e_2); II(e_3, e_4); III(e_5, e_6); IV(e_7, e_8) for both subtypes; the last two bins were comprised of V(e_9, e_{10}) and VI(e_{11} to e_{51}) for subtype1, V(e_9 to e_{12}) and VI(e_{13} to e_{51}) for subtype2. Regions with mean z-score > 0.7 for regional volume loss are displayed. Two distinct trajectories of gray matter atrophy are displayed in **Fig.1c**.

4.3.6 Subtyping and staging at the individual level

For each individual with schizophrenia, SuStaIn calculated the likelihood of belonging to a subtype, and a stage based on the average position over the posterior distribution on the sequence via 10,000 MCMC iterations. Individuals were assigned to their maximum likelihood subtype first, and then the stage with the highest likelihood was determined. The proportion in each subtype and stage is provided as **Supplementary Fig.1b**. Note that SuStaIn assigned individuals who do not deviant GMV in any feature/ROI (here z scores of all features < 1) into 'stage 0', which was defined as a 'pre-atrophy' stage. As the SuStaIn classifies all individuals into clusters according to distinct sequences of GMV reductions in different brain regions, rather than clustering the individuals based on their current atrophy degree, SuStaIn can categorize these individuals with similar atrophy sequences, even if the brain of some of these individuals has not atrophied to a significant degree (i.e., z=1 defined in this study). **Supplementary Fig.6** provides an example showing how 'pre-atrophy' and 'post-atrophy' individuals could be classified into the same subtype.

4.4 Subtype characterization

4.4.1 Subtype-specific atrophy patterns

To visualize atrophy patterns of whole gray matter for each subtype, we calculated the mean z-score of GMV for each AAL atlas ROI. We compared the ROI-wise z-scores between subtypes using independent samples t-test (two sided). Multiple comparisons were corrected by Bonferroni correction P<0.001. The ROI-wise z-score images were further mapped to a glass brain template for visualization using BrainNetViewer (https://www.nitrc.org/projects/bnv/).

4.4.2 Association between regional atrophy and clinical symptoms

Within each subtype, we examined the relationships between regional atrophy and symptoms by deriving the Spearman coefficient between PANSS (positive, negative and general psychopathology subscales) and mean z-score of GMV for each ROI, after adjusting for sex, age, square of age, TIV and sites. To correct for multiple comparisons, a permutation-based procedure was applied to control the family wise error (FWE) rate[49].

4.4.3 Distinct clinical profiles between subtypes

Demographic, clinical and global brain variables available for our discovery cohort included age (n=1124), sex (n=1124), illness duration (n=388), PANSS (n=750), TIV (n=1124), total GM volume (n=1124), total white matter (WM) volume (n=1124) and total cerebrospinal fluid (CSF) volume (n=1124). To determine subtype-specific characteristics, these variables were statistically compared between subtype1 and subtype2 by using a regress model with sex, age, age^2, site and SuStaIn stage as covariates. Furthermore, individuals within each subtype were further divided into two subgroups ('pre-atrophy' and 'post-atrophy') based on the degree of atrophy. Thus, the statistical comparison among the four subgroups ($S1_{pre}$, $S1_{post}$, $S2_{pre}$ and $S2_{post}$) involved two steps: (1) comparison to all other subgroups (one-versus-all comparison). A one-versus-all approach was used to compare each subgroup to all individuals of other three subgroups to determine the subgroup-specific characteristics, and (2) each subgroup was compared directly to each other subgroup (one-versus-one comparison) to assess the differences between subgroups. After testing for assumptions of normality and equal variances, the statistical comparisons were conducted using ANOVA with appropriate post-hoc tests (two sided), with FDR correction for the number of variables assessed.

We also investigated the relationship between the staging scores from SuStaIn and age, illness duration, symptoms, total GM volume and total CSF volume using Spearman's correlation across the whole sample and stratified by subtype. Two sided p values were FDR-corrected for the number of variables assessed.

4.5 Treatment outcomes across subtypes

In this exploratory analysis, we examined whether subtype classification based on baseline brain features will relate to differential treatment response to antipsychotic medications (APM) and TMS. A total of 373 patients with schizophrenia treated by APM and 150 patients with schizophrenia treated by TMS were included in the longitudinal analyses (**Supplementary Table 2**).

Based on the baseline MRI data, the SuStaIn model first assigned each individual with schizophrenia to one of two subtypes (i.e., phenotypic subtype1 or subtype2) according to the probability belong to which 'trajectory'. Then, individuals within each phenotype were further assigned to one of the stages based on the SuStaIn 'trajectory'. The SuStaIn probability score of subtype1 membership and the estimated SuStaIn stages were used as two quantitative indicators to measure their association with follow up treatment outcomes. Following baseline MRI, individuals with schizophrenia received APM or TMS (Details in the **Supplementary Method S3**). At follow up, treatment outcome was measured by the reduction ratio (reduction ratio=(baseline-follow up)/baseline x 100%) for PANSS total and subscale scores. Spearman correlation analysis between the above SuStaIn quantitative

indicators and treatment outcomes was performed after controlling the baseline PANSS. We also compared the differences of follow up treatment outcomes between the two phenotypic subtypes (i.e., subtype1 and subtype2) using the one-versus-all and one-versus-one statistical comparisons. In addition to the two phenotypic subtypes, we further classified individuals within each phenotype into two subgroups ('pre-atrophy' and 'post-atrophy'), based on the intra-phenotypic differences (i.e., temporal subtype). By disentangling both temporal heterogeneity and phenotypic heterogeneity, we further obtained four subgroups ($S1_{pre}$, $S1_{post}$, $S2_{pre}$ and $S2_{post}$). After testing for assumptions of normality and equal variances, we compared the differences of follow up treatment outcomes among the four subgroups using ANOVA with appropriate post-hoc tests (two sided). A permutation-based FWE procedure was employed for controlling for multiple comparisons[49].

4.6 Replication analysis

To establish the SuStaIn validity for an alternative atlas, another three commonly used atlases (BN246 atlas, Schaefer200 atlas, and HCPMMP360 atlas) were applied for ROI extraction and SuStaIn modelling. To evaluate the stability of SuStaIn at a relative higher spatial resolution, the 17 AAL features were expand to 22 and 27 features (AAL22 and AAL27) by a data-driven hierarchical clustering procedure. A total of five validation sets of features were generated to further verify the stability of SuStaIn (**Supplementary Method S6**). In addition, we also examined the stability of SuStaIn 'trajectories' using leave one-site out resampling (**Supplementary Fig.10**). Finally, we performed post-hoc power analyses for the primary results of this study using G*Power (https://www.psychologie.hhu.de/arbeitsgruppen/allgemeine-psychologie-und-arbeitspsychologie/gpower) (**Supplementary Method S9**).

Data availability

Data of COBRE, NMorphCH, FBIRN and NUSDAST were obtained from the SchizConnect, a publicly available website (http://www.schizconnect.org/documentation#by_project). The NMorphCH dataset and NUSDAST dataset were download through a query interface at the SchizConnect (http://www.schizconnect.org/queries/new). The COBRE dataset was download from the Center for Biomedical Research Excellence in Brain Function and Mental Illness (COBRE) (https://coins.trendscenter.org/). The FBIRN dataset was download from https://www.nitrc.org/projects/fbirn/. The DS000115 dataset was download from OpenfMRI database (https://www.openfmri.org/). Data from the other datasets (cross-sectional datasets #1, #2, #3, #4, longitudinal AMP and TMS data) are not publicly available for download, but access requests can be made to the respective study investigators: cross-sectional data (dataset #1, #2, #3, #4) -- J.Feng; APM data -- J.Wang, X.Yu, W.Yue and C.Luo; TMS data -- J.Wang, G.Ji, L.Cui and C.Luo. Requests for raw and analyzed data can be made to the corresponding author (J.Feng, jffeng@fudan.edu.cn) and will be promptly reviewed by the Fudan University Ethics Committee to verify whether the request is subject to any intellectual property or confidentiality obligations.

Code availability

Python of the SuStaIn algorithm are available on the UCL-POND GitHub (https://github.com/ucl-pond). T1-weighted images were processed using the Computational Anatomy Toolbox (http://www.neuro.uni-jena.de/cat/) within SPM12 (https://www.fil.ion.ucl.ac.uk/spm/software/spm12/). The visualization of ROI-wise z-score images was conducted using BrainNetViewer (https://www.nitrc.org/projects/bnv/). Statistical analyses, including correlation analysis, t-test, ANOVA etc., were conducted using MATLAB (version: R2018b) and SPSS Statistics (version: 26.0). Other custom codes developed in the current study are available at GitHub (https://github.com/YuchaoJiang91/Disease-Progress-Model).

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Author Contributions Statement

J.Feng led the project. Y.Jiang, W.Cheng and J.Feng were responsible for the study concept and the design of the study. J.Wang and E.Zhou provided crucial advice for the study. Y.Jiang, E.Zhou, C.Xie, W.Zhang, J.Lv, D.Chen, C.Shen, X.Wu, B.Zhang, N.Kuang, Y.J.Sun and J.Kang analyzed the data and created the figures. Y.Jiang wrote the manuscript. J.Wang, E.Zhou, L.Palaniyappan and W.Cheng made substantial contributions to the manuscript and provided critical comments. J.Wang, E.Zhou, C.Luo, G.Ji, J.Yang, Y.Wang, Y.Zhang, C.C.Huang, S.J.Tsai, X.Chang, J.Zhang, H.Huang, H.He, M.Duan, Y.Tang, T.Zhang, C.Li, X.Yu, T.Si, W.Yue, Z.Liu, L.B.Cui, K.Wang, J.Cheng, C.P.Lin and D.Yao contributed to the data acquisition.

Competing Interests Statement

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Table 1. Comparison of variables between subtypes in the cross-sectional discovery sample.

	Subtype1 (n=631)		Subtype2 (n=493)	
	Pre-atrophy ($S1_{pre}$)	**Post-atrophy ($S1_{post}$)**	**Pre-atrophy ($S2_{pre}$)**	**Post-atrophy ($S2_{post}$)**
Number	259	372	212	281
Age (year)	31.1(12.1)	31.8(13.1)	30.0(12.2)	31.1(13.3)
Sex (female/male)	115/144	155/217	94/118	115/166
Ethnicity(Han Chinese/Hispanic/Not Han Chinese & Not Hispanic/Unknown)	187/8/38/26	251/20/69/32	163/8/33/8	213/8/43/17
Race(Black or African American/Asian/White/Others/ Unknown)	14/188/38/3/16	36/252/57/3/24	13/163/28/2/6	18/215/33/2/13
Illness duration(year) (n=388)	9.1(10.2)	12.2(11.7) [c]	7.4(10.5) [*,b,d]	11.1(11.7) [c]
PANSS scores (n=750)				
Positive scale [#]	18.5(6.8) [*,b,d]	16.9(7.0) [a]	17.2(6.7)	16.1(7.0) [a]
Negative scale [#]	15.1(7.4)	16.3(7.7)	15.9(7.8)	16.0(8.1)
General Scale [#]	34.2(11.1) [*,b,d]	33.3(10.4) [a]	33.8(10.8)	32.7(11.3) [c]
Total scores [#]	67.9(22.0) [*,d]	66.5(20.80)	66.9(21.0)	64.9(22.1) [a]
Total intracranial volume (cm^3)	1500.8(163.1)	1498.6(162.6)	1500.1(168.3)	1488.4(177.2)
Total GM volume (cm^3)	674.2(72.4) [*,b,d]	635.3(72.2) [*,a,c]	687.3(82.6) [*,b,d]	643.8(74.2) [*,a,c]
Total WM volume (cm^3)	516.6(59.6) [*,d]	509.6(65.6)	512.4(59.5) [d]	500.7(64.4) [*,a,c]
Total CSF volume (cm^3)	308.0(65.7) [*,b,d]	354.2(71.8) [*,a,c]	299.3(62.8) [*,b,d]	343.6(79.9) [*,a,c]

[#] Variables statistically compared among four groups after controlling sex, age, age2, site and illness stage. * Corrected P<0.05 (versus all other subtypes); [a] Corrected P<0.05 (versus pre-atrophy of subtype1); [b] Corrected P<0.05 (versus post-atrophy of subtype1); [c] Corrected P<0.05 (versus pre-atrophy of subtype2); [d] Corrected P<0.05 (versus post-atrophy of subtype2). P values are two-sided and corrected by multiple comparisons. Standard deviations are given in parentheses where relevant. GM, gray matter; WM, white matter; CSF, cerebrospinal fluid.

Table 2. Comparisons of treatment outcomes among four SuStaIn subgroups in a longitudinal sample of 373 schizophrenia patients treated with antipsychotic medications.

	Subtype1 (n=202)		Subtype2 (n=171)	
	Pre-atrophy ($S1_{pre}$)	**Post-atrophy ($S1_{post}$)**	**Pre-atrophy ($S2_{pre}$)**	**Post-atrophy ($S2_{post}$)**
Number	96	106	70	101
Age (years)	25.0(7.5) [d]	26.4(9.0)	25.1(7.7) [d]	28.5(10.6) [a,c]
Sex (female/male)	49/47	54/52	36/34	51/50
Education (years)	12.9(2.7)	13.2(2.8)	12.6(2.7)	12.8(3.0)
Illness duration (years)	2.8(4.7) [d]	4.7(7.3)	3.3(5.1)	5.2(8.3) [a]
CPZ (mg/day)	384.8(219.3)	383.9(200.6)	368.9(229.7)	375.3(207.1)
Responders (%) [#]	79.17%	66.98%	71.43%	69.31%
Baseline PANSS				
Positive subscale	22.5(5.1)	22.4(7.0)	23.3(4.7)	22.6(6.4)
Negative subscale	17.9(6.1) [*,c,d]	19.4(7.4)	20.1(6.5) [a]	21.1(6.9) [*,a]
General subscale	38.2(6.8)	38.8(8.6)	39.9(7.2)	40.2(8.4)
Total score	78.6(12.9) [*,c,d]	80.5(18.3)	83.3(12.7) [a]	84.2(15.5) [*,a]
Follow up PANSS				
Positive subscale	11.6(4.6)	11.3(4.7) [c]	13.1(4.5) [*,b]	12.5(4.5)
Negative subscale	13.2(5.4) [*,c,d]	14.8(8.4)	15.0(5.2) [a]	16.3(6.4) [*,a]
General subscale	25.3(6.0) [*,c,d]	26.3(6.6)	27.8(6.4) [a]	27.6(5.9) [a]
Total score	50.1(13.3) [*,c,d]	52.1(14.5) [d]	56.0(13.5) [a]	56.2(13.6) [*,a,b]
PANSS Reduction Ratio (%)				
Positive subscale	46.7(20.1) [*,c,d]	45.2(23.4) [d]	42.3(20.7) [a]	41.1(22.1) [*,a,b]
Negative subscale	22.1(30.8)	15.5(46.1)	23.1(20.3)	19.9(28.5)
General subscale	32.7(15.6) [*,c,d]	29.6(20.3)	29.6(14.0) [a]	29.5(16.4) [a]
Total score	35.6(15.5) [*,c,d]	32.9(20.5)	32.4(14.2) [a]	31.9(16.5) [a]

* Corrected $P < 0.05$ (versus all other subtypes); [a] Corrected $P < 0.05$ (versus pre-atrophy of subtype1); [b] Corrected $P < 0.05$ (versus post-atrophy of subtype1); [c] Corrected $P < 0.05$ (versus pre-atrophy of subtype2); [d] Corrected $P < 0.05$ (versus post-atrophy of subtype2); P values are two-sided and corrected by multiple comparisons. Standard deviations are given in parentheses where relevant. CPZ, Chlorpromazine equivalence for antipsychotic dose; PANSS, Positive and Negative Syndrome Scale. [#] At follow up, patients whose symptom burden measured as percentage reduction ratio in PANSS total score dropped > 25% were defined as responders.

Figure Legends/Captions

Fig.1| Pathophysiological progression of brain atrophy in schizophrenia. (a) Data-driven disease progression model (i.e., SuStaIn) was used to identify population subtypes with common pattern of pathophysiological 'trajectory' by disentangling phenotypic heterogeneity and temporal heterogeneity on cross-sectional individual data. (b) Atrophy sequences of specific brain regions obtained using SuStaIn. The positional variance diagrams visualize the cumulative probability that each brain region has reached a particular z-score using different colors. The color indicates the level of severity of gray matter volume (GMV) loss: red is mildly affected (z-score=1, i.e., 1 standard deviation unit from healthy control average); magenta is moderately affected (z-score=2); and blue is severely affected (z-score=3). Colour in density represents the proportion of the posterior distribution in which events (y-axis) appear in a particular position in the sequence (x-axis). *f* is the proportion of individuals assigned to each phenotype. Arabic Numbers 1-10 marked against brain regions in the variance diagram indicate the order of the first ten events with brain region atrophy, estimated by the SuStaIn. (c) Pathophysiological 'trajectory'. The mean z-score images of GMV were derived across schizophrenia patients belonging to stage bins (I, II, III, V, IV and VI) and mapped to a glass brain template for visualization using BrainNetViewer (https://www.nitrc.org/projects/bnv/). Regional volume loss at each stage bin shows a progressive spatial expansion pattern but differs between trajectories. In 'trajectory' 1, volume loss is first observed in Broca's area and the insula (stage I), and then the anterior cingulate, prefrontal, and lateral temporal cortices (stage II), and then the orbitofrontal and sensorimotor cortices (stage III), and then the occipital, parietal and temporal cortices (stage IV and V), and finally to the cerebellum and subcortical regions (stage VI). In 'trajectory' 2, volume losses occur first to the hippocampus and amygdala (stage I) and then involve the parahippocampus, thalamus and accumbens (stage II), and then the caudate and insula (stage III), followed by the putamen, cingulate, frontal and temporal lobe (stage IV and V), and finally to the other cortical areas (stage VI).

Fig.2| Atrophy patterns in four subtypes of schizophrenia. (a) All individuals with schizophrenia were firstly classified into two phenotypes by distinct 'trajectories' from SuStaIn, based on the inter-phenotypic differences (i.e., phenotypic heterogeneity). Individuals within each phenotype were further assigned into two subgroups according to which stage of the 'trajectory' they belong to, based on the intra-phenotypic differences (i.e., temporal heterogeneity). By disentangling temporal heterogeneity and phenotypic heterogeneity, we identified four subtypes of 'trajectory' 1 pre-atrophy ($S1_{pre}$, n=259), 'trajectory' 2 pre-atrophy ($S2_{pre}$, n=212) and 'trajectory' 2 post-atrophy ($S2_{post}$, n=281). The atrophy pattern of whole gray matter (revealed by the mean z-score of GMV) in four subgroups was mapped to a glass brain template for visualization. **(b)** Comparison of the mean z-score of GMV across all ROIs, after adjusting for sex, age, the square of age, total intracranial volume and sites. The adjusted GMV values were normalized relative to the control population to derive z-scores (i.e., a value of z=0 represents the normal level in the control population). These z-scores were multiplied by -1 so that the z-scores would

increase as the regional volumes decrease in patients with schizophrenia. Note that a higher z-score (or T-value) indicates a larger reduction of GMV; cortical GMV is extensively reduced post-atrophy in $S1_{post}$ than $S2_{post}$; but subcortical GMV reduction is more pronounced post-atrophy in S2post than S1post. Data are presented as mean values +/- SEM. * Indicates significant differences between the $S1_{post}$ (n=372) and $S2_{post}$ (n=281) using two-sample t-test (two-sided P<0.001, Bonferroni correction). Exact p values are provided in the Supplementary Table 14. CING, cingulate cortex; INS, insula; SM, sensorimotor; BA, Broca's area.

Fig.3| Subtypes characterized by clinical variables. (a) Subtype differences of disease duration among the $S1_{pre}$(n=77), $S1_{post}$(n=124), $S2_{pre}$(n=83) and $S2_{post}$(n=104). Data are presented using a box-plot (center line, median; box limits, upper and lower quartiles; whiskers, 1.5×interquartile range [IQR]; points, outliers). **(b)** Subtype differences of positive symptom burden among the $S1_{pre}$(n=167), $S1_{post}$(n=242), $S2_{pre}$(n=153) and $S2_{post}$(n=188). Data are presented as mean values +/- SD. Increasing SuStaIn stage was associated with **(c)** longer illness duration (r=0.208, $p=4.6\times10^{-4}$), **(d)** worse negative symptoms (r=0.127, p=0.008), **(e)** larger CSF volume (r=0.353, $p=1.7\times10^{-22}$) and **(f)** less GM volume (r=-0.250, $1.1\times<10^{-11}$) across all subtypes, by FDR correction. **(g)** In 'trajectory' 1 (pre-stage I, n=167; stage I, n=71, stage II, n=39, stage III, n=37, stage IV-V, n=46, stage VI, n=49), individuals belonging to stage VI showed a higher score of negative symptoms compared to individuals belonging to stage I, II, III and pre-stage I (S0, i.e., individuals without obvious atrophy in any regions) (corrected P<0.05). Data are presented as mean values +/- SEM. **(h)** In 'trajectory' 2 (pre-stage I, n=153; stage I, n=62, stage II, n=39, stage III, n=24, stage IV-V, n=35, stage VI, n=28), individuals belonging to later stages (especially stage I; corrected P<0.05) showed a lower score of positive symptoms compared to the stage before any atrophy is detectable. Data are presented as mean values +/- SEM. The asterisk (*) in figures (b, c, h and i) indicates significant differences between the two subgroups using ANOVA with post-hoc tests (two sided P<0.05, correction for multiple comparisons).

Fig.4| Treatment outcome and subtypes of schizophrenia in patients with follow-up data. (a) The SuStaIn probability of belonging to subtype 1 correlates significantly with the reduction ratio of PANSS positive symptoms scores when using antipsychotic medications (APM) by Spearman correlation test (r=0.127, p=0.014, two-sided). **(b)** The progressive SuStaIn stages relate to a significantly lower PANSS positive symptoms reduction ratio when administering TMS by Spearman correlation test (r=-0.370, $p=3.1\times<10^{-6}$, two-sided). **(c)** Differences in APM-related PANSS reduction ratio across domains (p=0.003 for PANSS positive scale; p=0.019 for PANSS total score) between the subtype1 (n=202) and subtype2 (n=171) in schizophrenia. **(d)** Differences in APM-related PANSS reduction ratio among the $S1_{pre}$ ('pre-atrophy' stage of subtype1, n=96), $S1_{post}$ ('post-atrophy' stage of subtype1, n=106), $S2_{pre}$ ('pre-atrophy' stage of subtype2, n=70) and $S2_{post}$ ('post-atrophy' stage of subtype2, n=101). From left to right, the significant differences are marked by the

asterisk (PANSS positive subscale: p=0.033 for $S1_{pre}$>$S2_{pre}$, p=0.005 for $S1_{pre}$>$S2_{post}$, p=0.037 for $S1_{post}$>$S2_{post}$; PANSS general subscale: p=0.038 for $S1_{pre}$>$S2_{pre}$, p=0.017 for $S1_{pre}$>$S2_{post}$; PANSS total score: p=0.028 for $S1_{pre}$>$S2_{pre}$, p=0.006 for $S1_{pre}$>$S2_{post}$). **(e)** Differences in TMS-related PANSS reduction ratio among the $S1_{pre}$(n=47), $S1_{post}$(n=38), $S2_{pre}$(n=29) and $S2_{post}$(n=36) for patients with schizophrenia receiving TMS. From left to right, the significant differences are marked by the asterisk (PANSS positive scale: p=0.0004 for $S1_{pre}$>$S1_{post}$, p=0.0006 for $S1_{pre}$>$S2_{post}$, p=0.004 for $S2_{pre}$>$S1_{post}$, p=0.005 for $S2_{pre}$>$S2_{post}$; PANSS negative scale: p=0.037 for $S2_{pre}$>$S1_{pre}$; p=0.003 for $S2_{pre}$>$S2_{post}$; PANSS total score: p=0.019 for $S1_{pre}$>$S2_{post}$, p=0.037 for $S2_{pre}$>$S1_{post}$, p=0.005 for $S2_{pre}$>$S2_{post}$). Data in figures (c, d and e) are presented as mean values +/- SD. The asterisk (*) in figures (c, d and e) represents significant difference between the two subgroups using ANOVA with post-hoc tests (two sided P<0.05, correction for multiple comparisons). P, PANSS positive subscale; N, PANSS negative subscale; G, PANSS general subscale; T, PANSS total score.

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