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Schizophrenia delays and alters maturation of the brain in adolescence

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Early-onset schizophrenia appears to be clinically more severe than the adult-onset form of the disease. In a previous study, we showed that anatomically related grey and white matter abnormalities found in adolescents patients were larger and more widespread than what had been reported in the literature on adult schizophrenia. Particularly, we found novel structural abnormalities in the primary sensorimotor and premotor systems. Here, we tested alternative hypotheses: either this striking sensorimotor-related pattern is an artefact due to a better sensitivity of the methods, or apparent greater structural abnormalities in the early-onset population are *specifically* associated with earlier disease onset. Then, if we were to find such characteristic structural pattern, we would test whether these anatomical abnormalities would remain static or, conversely, show dynamic changes in the still developing brain. To address these questions, we combined a cross-sectional study of brain structure for adolescent-onset patients ($n = 25$) and adult-onset patients ($n = 35$) and respective matched healthy subjects with a longitudinal study of adolescent-onset patients ($n = 12$, representative subset of the cross-sectional group) and matched healthy controls for >2 years. Looking at differences between adolescent and adult patients' grey matter volume and white matter microstructure abnormalities, we first confirmed the specificity (especially in motor-related areas) and the greater severity of structural abnormalities in the adolescent patients. Closer examination revealed, however, that such greater anomalies seemed to arise because adolescent patients fail to follow the same developmental time course as the healthy control group. Longitudinal analysis of a representative subset of the adolescent patient and matched healthy populations corroborated the delayed and altered maturation in both grey and white matters. Structural abnormalities specific to adolescent-onset schizophrenia in the sensori-motor cortices and corticospinal tract were less marked or even disappeared within the longitudinal period of observation, grey matter abnormalities in adolescent patients evolving towards the adult-onset pattern as defined by recent meta-analyses of adult schizophrenia. Combining cross-sectional adolescent and adult datasets with longitudinal adolescent dataset allowed us to find a unique, abnormal trajectory of grey matter maturation regardless of the age at onset of symptoms

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and of disease duration, with a lower and later peak than for healthy subjects. Taken together, these results suggest common aetiological mechanisms for adolescent- and adult-onset schizophrenia with an altered neurodevelopmental time course in the schizophrenic patients that is particularly salient in adolescence.

Keywords: early-onset schizophrenia; development; longitudinal; VBM; TBSS

Introduction

Onset of symptoms of schizophrenia is rare before puberty, but the incidence rises steeply to a plateau in the third decade (Crow, 1993). This characteristic distribution of age of onset is consistent with a neurodevelopmental aetiology (Crow *et al*., 1995). Most current evidence suggests that individuals with early- , adult- and late-onset schizophrenia manifest similar clinical deficits and neurobiological correlates (Sachdev *et al*., 1999; Kumra and Charles Schulz, 2007; Kyriakopoulos and Frangou, 2007), with early-onset schizophrenia possibly representing a more severe form of the disease (Kumra and Charles Schulz, 2007; Kyriakopoulos and Frangou, 2007).

In a previous cross-sectional study of grey matter (GM) density and white matter (WM) microstructure in adolescent patients (Douaud *et al*., 2007), we found a greater extent of changes relative to healthy controls than had previously been reported in adult-onset literature, especially in sensorimotor-related areas. Direct comparison of adolescent- and adult-onset schizophrenia datasets using the same methodology is needed to characterize whether these structural brain differences, found mainly in sensorimotor regions, are *specific* to early-onset schizophrenia and not a mere artefact due to a better sensitivity of the methods employed.

If brain abnormalities characteristic to adolescent-onset schizophrenia are indeed found, it should be determined whether these abnormalities would be stable over time or instead show a dynamic evolution reflecting an altered neurodevelopmental time course for the young patients in adolescence. Although looking at trajectories of anatomic brain development in schizophrenia, as opposed to static measures, are argued to be more informative (Arango *et al*., 2008; Giedd *et al*., 2008; van Haren *et al*., 2008), there is limited and somewhat conflicting longitudinal structural imaging data for early-onset schizophrenia. A potential concern with the three grey matter studies in adolescent-onset patients already reported (James *et al*., 2002, 2004; Reig *et al*., 2008) is that they are based on measurements from pre-specified, large anatomical regions of interest (ROI), in which different local changes over time may be averaged out (Snook *et al*., 2007). Moreover, there is to date no study addressing the longitudinal evolution of white matter microstructure in early-onset schizophrenia.

Here we wish to test three alternative hypotheses to account for apparent differences in structural neuropathology between adolescent and adult-onset schizophrenic patients (Douaud *et al*., 2007): a better sensitivity of the methods employed, a genuine greater extent of structural abnormalities with earlier onset of symptoms that remain constant over time or a reflection of an altered trajectory for brain development particularly salient during adolescence. In contrasting abnormalities in grey matter and white matter found in adolescent- and adult-onset schizophrenic patients when compared to age-matched healthy subjects, we hypothesize that apparent differences in these abnormalities are characteristic of adolescent-onset schizophrenia and not simple artefacts of methodology. Then, by investigating longitudinal grey matter and white matter changes in adolescent-onset schizophrenic patients, we predict that these specific structural differences, especially in the sensorimotor cortex and the pyramidal tract, will be related to an abnormal interaction between maturation in grey matter and white matter with the disease. We thus anticipate demonstrating a dynamic evolution over the period of observation with a fade-out of the characteristic pattern of cerebral anomalies of the adolescent patients.

Methods

The study was undertaken in accordance with the guidance of the Oxford and Berkshire Psychiatric Research Ethics Committees and written consent was obtained from all participants (and their parents if under the age of 16 years).

Subjects

Adult cohort

Thirty-five adult-onset schizophrenic patients were included in this study (age at onset of symptoms >18, 70% men of mean age 36, 30% women of mean age 32.5; see Supplementary Table S1). They were recruited by collaborating psychiatrists from Oxfordshire and Berkshire Mental Healthcare Trusts, who identified the potential participants who had primary diagnoses of schizophrenia (APA, 1994) and were confirmed for their diagnosis using the Structured Clinical Interview for DSM-IV Disorders (SCID; First *et al*., 1998). Patients additionally completed the Positive and Negative Syndrome Scale (PANSS) (Kay *et al*., 1989). All patients were taking atypical neuroleptics, except three patients who were on typical one. Thirty-five healthy control subjects, matching for age, sex and age by sex the patient group were recruited by advertisement from the general population and were screened for psychiatric disorder using the SCID. Exclusion criteria for all subjects included history of head injury or any neurological condition, substance abuse and English not being their first language. Controls were excluded if they had any history of psychiatric disorder. We will refer to these 70 subjects as the 'adult cohort' in the following.

Adolescent cohort

Additionally, 25 adolescent-onset schizophrenic participants (age at onset of symptoms <18, 70% men of mean age 16.5, 30% women of mean age 16) were recruited from the Oxford regional adolescent

unit and surrounding units. All were diagnosed as having DSM-IV (APA, 1994) schizophrenia, using the Kiddie Schedule for Affective Disorders and Schizophrenia (Kaufman *et al*., 1997). In addition, the participants were administered the PANSS (Kay *et al*., 1989). Age at onset of symptoms ranged from 11 to 17 years (Supplementary Table S1). All schizophrenic patients were receiving atypical antipsychotics. Twenty-five healthy control participants, matched for age, sex and age by sex to the adolescent-onset patient group, were included in this study. These adolescent control participants were recruited from the community through their general practitioners and were screened for any history of emotional, behavioural or medical problems. All participants attended normal schools. Exclusion criteria included moderate mental impairment (IQ <60), a history of substance abuse or pervasive developmental disorder, significant head injury, neurological disorder or major medical disorder (Supplementary Table S1). These 50 subjects will be referred hereafter as the 'adolescent cohort' and were the same subjects included in our previous cross-sectional study of adolescent-onset schizophrenia (Douaud *et al*., 2007).

Finally, amongst these 50 subjects of the adolescent cohort, 12 patients and 12 controls of mean age 16, matched for age, sex and age by sex, came back 2.5 (0.5) years later for a follow-up scan (65% men, 35% women) (Supplementary Table S1).

Image acquisition

The 120 participants underwent similar imaging protocol with a whole-brain T_1-weighted and diffusion-weighted scanning using the same 1.5 T Sonata MR imager (Siemens, Erlangen, Germany) with a standard quadrature head coil and maximum 40 mT m^{-1} gradient capability.

All subjects were scanned with a 3D T_1-weighted FLASH sequence using the following parameters: coronal orientation, matrix 256×256, 208 slices, $1 \times 1\,mm^2$ in-plane resolution, slice thickness 1 mm, TE/TR = 5.6/12 ms, flip angle $\alpha = 19^\circ$.

Diffusion-weighted images were obtained on the adolescent cohort (first and second time points) using echo-planar imaging (SE-EPI, TE/TR = 89/8500 ms, 60 axial slices, bandwidth = 1860 Hz/vx, voxel size $2.5 \times 2.5 \times 2.5\,mm^3$) with 60 isotropically distributed orientations for the diffusion-sensitizing gradients at a b-value of 1000 s mm^{-2} and 5 $b = 0$ images. To increase signal-to-noise ratio, scanning was repeated three times and all scans were corrected for head motion and eddy currents using affine registration before being averaged.

Fifty out of the 70 subjects of the adult cohort (25 patients and 25 controls matched for age, sex and age by sex, see Supplementary Table S1) were scanned using a diffusion acquisition sequence with the notable difference of using TE/TR = 76/5400 ms, 45 axial slices, bandwidth = 2056 Hz/vx, with 12 isotropically distributed orientations for the diffusion-sensitizing gradients at a b-value of 1000 s mm^{-2} and 1 $b = 0$ image. Scanning was repeated 12 times and averaged before being corrected for eddy currents.

Image analysis

Cross-sectional analysis contrasting adolescent- and adult-onset schizophrenic patients

The main methodological problem with directly comparing early and adult-onset patients is the different ages of the samples, as underlined by the neuropsychological findings of White and colleagues (2006). They showed that, when accounting for developmental differences in their healthy control groups, only motor performance remained poorer in adolescent patients compared with the adult patients. In the following, we therefore contrasted each adolescent and adult patient group with their respective age-matched healthy control group.

In the grey matter

We carried out an optimized VBM protocol using FSL tools (Smith *et al*., 2004, www.fmrib.ox.ac.uk/fsl) to assess where the significant differences were in the distribution of grey matter between adolescent- and adult-onset schizophrenic patients compared to their respective control group. In other words, we were interested in identifying what the grey matter abnormalities were, *characteristic* to adolescent- versus adult-onset schizophrenia.

To do so, a left–right symmetric study-specific grey matter template was built from the 50 adolescent cohort and 50 (a random subset of 25 patients and 25 controls) adult cohort grey matter-segmented native images. These 100 grey matter images were all non-linearly registered to the ICBM-152 grey matter template using FNIRT (http://www.fmrib.ox.ac.uk/fsl/fnirt), flipped along the x-axis and averaged. Then, the full set of all 120 grey matter images were non-linearly normalized onto this study-specific template. The optimized protocol also introduces a compensation (or 'modulation') for the contraction/enlargement due to the non-linear component of the transformation: each voxel of each registered grey matter image was divided by the Jacobian of the warp field. Finally, all 120 modulated registered grey matter volume images were smoothed with an isotropic Gaussian kernel with a sigma of 3 mm (~7 mm FWHM).

In the white matter

FA maps were generated using DTIFit within the FMRIB diffusion toolbox (part of FSL; Smith *et al*., 2004). Voxel-wise differences in DTI indices were assessed using Tract-Based Spatial Statistics (TBSS, also part of FSL), a recent approach which increases the sensitivity and the interpretability of the results compared with voxel-based approaches that are based purely on non-linear registration (Smith *et al*., 2006). FA native images were non-linearly registered using FNIRT onto the FMRIB58 FA template (http://www.fmrib.ox.ac.uk/fsl/data/FMRIB58_FA.html). Then, the nearest maximum FA values of each registered FA image were projected onto a white matter skeleton derived from the FMRIB58 template and thresholded at FA > 0.25. This projection step aims to remove possible residual effect of cross-subject spatial variability after the non-linear registration. No smoothing is required in this protocol.

Statistical analyses

Finally, to achieve accurate inference including full correction for multiple comparisons over space, we used permutation-based non-parametric inference within the framework of the general linear model (5000 permutations) (Nichols and Holmes, 2002). We included the four groups (ADO_CON, ADO_PAT, ADU_CON, ADU_PAT) in our statistical design and looked for where the abnormalities found between the adolescent-onset schizophrenic patients and the healthy adolescents were significantly different from the abnormalities found between the adult-onset patients and the healthy adults, i.e. (ADO_CON – ADO_PAT) – (ADU_CON – ADU_PAT).

Results in grey matter were considered significant for $P < 0.05$ (corrected) after initial cluster-forming thresholding at P-uncorrected = 0.05. Results in FA were considered significant for $P < 0.05$, corrected for multiple comparisons using threshold-free cluster enhancement (TFCE), which avoids using an arbitrary threshold for the initial cluster-formation (Smith *et al*., 2008). For both analyses, statistical testing was restricted to voxels where adolescent-onset

patients have lower grey matter density or FA values than their respective control group at *P*-uncorrected = 0.05.

Longitudinal analyses of adolescent-onset schizophrenic patients

We wanted to determine if the adolescent-onset schizophrenic patients would show a structural development different from the healthy adolescents', having in mind that the characteristic abnormalities in these adolescent patients could possibly evolve towards the adult-onset schizophrenia pattern of cerebral changes. This could only be verified with a longitudinal study.

Preprocessing

We therefore performed an optimized 'FSL-VBM' analysis of the grey matter of 12 adolescent-onset patients (out of the 25 of the adolescent cohort) and 12 matched controls (out of the 25 of the adolescent cohort) that were scanned at two time points t_0 and t_1 ($t_0 + 2.5$ years). To take advantage of the pairing of the subjects, we developed a specific protocol for the preprocessing of the grey matter-segmented images: for each subject, we calculated the two 'halfway' affine matrices (seven DOF) between the t_0 and the t_1 brain-extracted scans. We then segmented both t_0 and t_1 scans into grey matter, applied the transformation matrices to the respective grey matter images and averaged them. These 24 averaged grey matter images were then non-linearly registered using FNIRT onto a study-specific template built from the 48 grey matter-segmented native images. The resulting non-linear transformations were applied to the 'halfway' t_0 and t_1 grey matter images, which were then modulated accordingly and smoothed with an isotropic Gaussian kernel with a sigma of 3 mm.

Additionally, a TBSS analysis was performed on the 48 FA images which were all non-linearly registered onto the FMRIB58 template. Then, the nearest maximum FA values of each registered FA image were projected onto the FMRIB58 skeleton.

Statistical analyses

Similarly to the modelling described above, we included the four groups (CON_t_0, PAT_t_0, CON_t_1, PAT_t_1) in our statistical design and looked for where the abnormalities found between the adolescent-onset schizophrenic patients and the healthy adolescents were differently evolving over the 2.5 years separating the two scanning time points: (CON_t_0 − PAT_t_0) − (CON_t_1 − PAT_t_1) = (CON_t_0 − CON_t_1) − (PAT_t_0 − PAT_t_1). We used permutation-based non-parametric inference. Results in grey matter were considered significant for $P < 0.05$, after correction for multiple comparisons using an initial cluster-threshold at $P = 0.001$ uncorrected. Results in FA were considered significant for $P < 0.05$, after correction for multiple comparisons using the TFCE approach (Smith *et al*., 2008).

Adolescent- and adult-onset schizophrenia: A continuous trajectory of grey matter maturation, different from that for normal development and ageing?

Using the same preprocessing employed in the cross-sectional study described above, we analysed the grey matter density of all 60 schizophrenic patients from both adolescent- and adult-onset groups pooled together and compared them directly with all 60 matched healthy subjects with permutation-based non-parametric inference. Results were considered significant for $P < 0.05$, after correction for multiple comparisons using the TFCE approach (Smith *et al*., 2008). This led to the creation of a ROI representing where the schizophrenic group globally differed from the healthy group. We then extracted the ROI-averaged grey matter density for both groups depending on the age of the subjects and tested various models (linear, quadratic, logarithmic, exponential, bi-exponential and other polynomial models in MATLAB 7.3) for qualitatively well describing the data and to define a common trajectory that would unify adolescent and adult healthy participants on the one hand, adolescent and adult schizophrenic patients on the other hand.

Moreover, among the subjects of the adolescent follow-up cohort, seven patients and seven healthy controls were at an age >18 years (controls: 18.2–22.1; patients: 18.2–21.9), allowing us to obtain a continuous sample of grey matter density across all ages. We were therefore able to verify whether these 14 subjects would follow the respective curves describing the volume of grey matter depending on the age of the subjects. To do so, we spatially normalized these 14 subject's grey matter-segmented images onto the study-specific template created in the first, cross-sectional study. We then used the ROI determined by the significant results of the FSL-VBM analysis of combined adolescent and adult cohorts to get the corresponding grey matter density values of the 'new' 14 longitudinal subjects.

Finally, we processed as described above the grey matter images corresponding to 11 adult patients who had an onset of symptoms between 16 and 18 years and whose disease duration ranged from 1 to 29 years (age at scanning: 18–45). These 11 additional patients were recruited and scanned following the 'adult cohort' protocol (comprising then 35 adult subjects with adult-onset of symptoms and 11 adult subjects with adolescent-onset of symptoms) but were only taken into account at this stage of the study.

All results were identified using a combination of three complementary atlases: the Harvard-Oxford structural cortical probability maps based on MRI T_1-weighted images, Jülich cytoarchitectonic probabilistic maps based on post-mortem brains and Talairach Daemon labels corresponding approximately to Brodmann areas (Eickhoff *et al*., 2005; Lancaster *et al*., 2007).

Results

Cross-sectional analysis contrasting adolescent- and adult-onset schizophrenic patients

We performed an FSL-VBM analysis of adolescent- and adult-onset schizophrenic patients and age-matched healthy subjects. Separated cross-sectional results of abnormalities in the adolescent cohort and in the adult cohort can be found in Supplementary Fig. S1.

More interestingly, we looked for where the abnormalities found between the adolescent patients and healthy adolescents would be significantly different from the abnormalities found between adult patients and healthy adults. Significantly lower grey matter density in adolescent-onset patients relative to adolescent controls than between adult-onset patients and their respective control group was found bilaterally in regions including the pre- and post-central gyri (only the right side survived after correction for multiple comparisons), as well as medially in the SMA and pre-SMA. Significant grey matter reductions specific to

adolescent-onset schizophrenic patients were also observed in regions encompassing the pars opercularis (BA44) in the left hemisphere and bilaterally in the parietal operculum and in Heschl's gyrus (Fig. 1; Supplementary Table S1).

We then used the significant, corrected results of this FSL-VBM analysis to define a large ROI from which we extracted ROI-averaged grey matter volume over all 120 participants. Remarkably, plotting these values against age revealed that the differences in grey matter abnormalities found in the two contrasts of patients with age-matched healthy controls were not explained by lower grey matter density in the adolescent-onset patients (mean density ± SD: 0.62 ± 0.03) than in adult-onset patients (0.58 ± 0.05), but by reduced grey matter density in healthy adult controls (0.58 ± 0.04) relative to the healthy adolescents in this ROI (0.71 ± 0.04) (Fig. 1).

Analysis of white matter FA in the same four groups using TBSS revealed a greater decrease of FA in the adolescent patients than in adult patients relative to their corresponding control groups bilaterally in the pyramidal tract (Fig. 2). Slight differences in image acquisition between the adolescent and the adult cohort precluded direct comparison of absolute FA values as presented in Fig. 1 for the grey matter, but did not impact on our results (see the statistical model used in our TBSS analysis, contrasting two by two comparable datasets).

Longitudinal analyses of adolescent-onset schizophrenic patients

With a VBM longitudinal study of a representative subgroup of the adolescent-onset patients and healthy subjects (Supplementary Fig. S2), we confirmed that there were widespread, highly significant regions in which adolescent patients showed differences in longitudinal changes (over the mean 2.5 year observation period) relative to the healthy adolescent controls (Fig. 3). Adolescent patients and healthy controls showed significantly different trajectories of grey matter development bilaterally in many regions identified by the above cross-sectional analysis, including the pre- and post-central gyri, SMA/pre-SMA, parietal operculum and Heschl's gyrus (Supplementary Table S2).

Plotting grey matter density against age in the ROI defined by significantly different longitudinal change between patients and

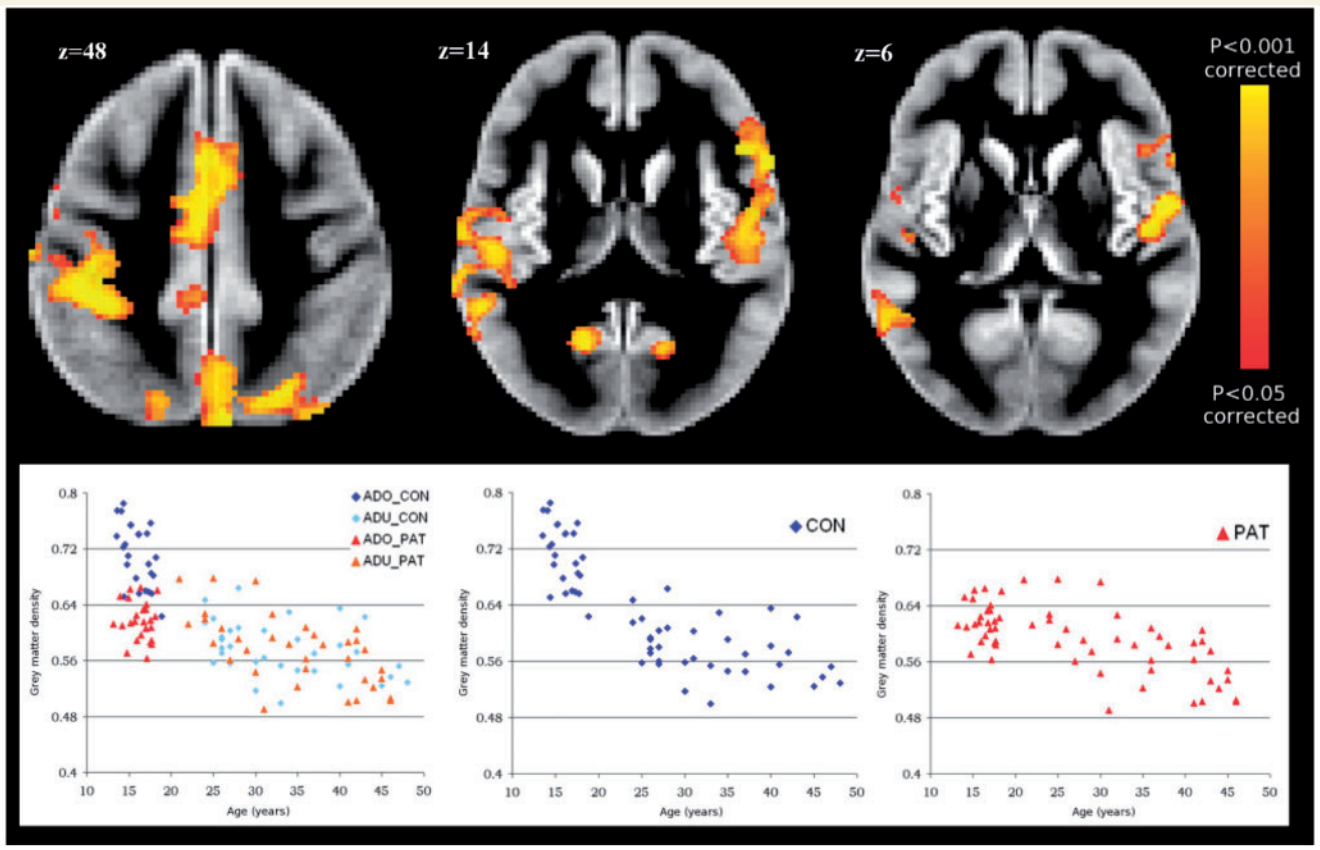

Figure 1 FSL-VBM results of contrasted adolescent and adult cohorts. Top panel: contrast showing where grey matter abnormalities are different between adolescent- and adult-onset patients ($P < 0.05$ corrected for multiple comparisons). Radiological convention (L is R). Bottom panel: plots representing grey matter density extracted in the ROI determined by the results of the whole-brain FSL-VBM analysis combining adolescent and adult cohorts (*y*-axis) depending on the age of the subjects (*x*-axis). This clearly shows that the voxel-wise differences between the adolescent and the adult cohorts are explained by substantial decrease of grey matter density in the healthy subjects, not by a more severe pattern of abnormalities in adolescent-onset patients compared with adult-onset patients.

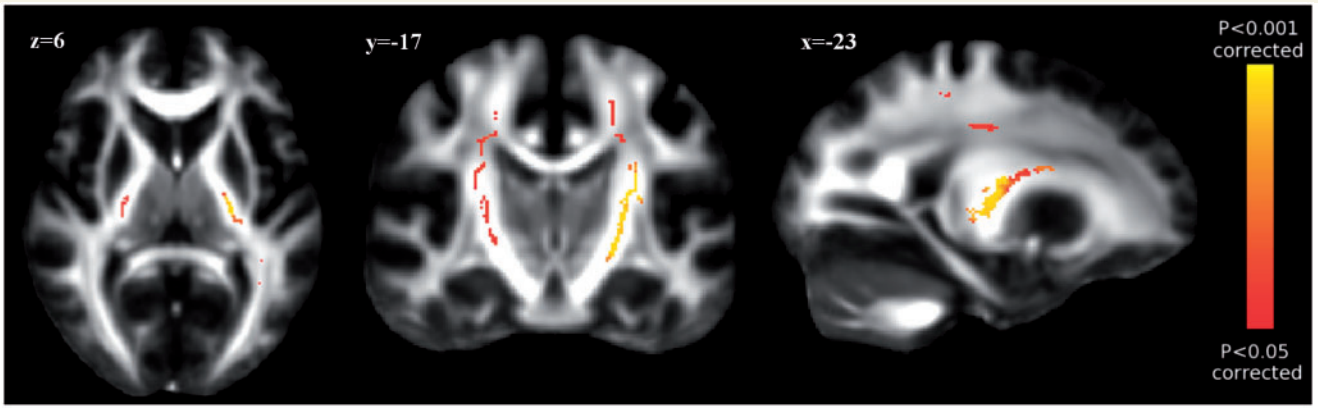

Figure 2 TBSS results of contrasted adolescent and adult cohorts. Contrast showing where FA abnormalities are significantly different between adolescent- and adult-onset patients ($P<0.05$ corrected for multiple comparisons). Radiological convention (L is R).

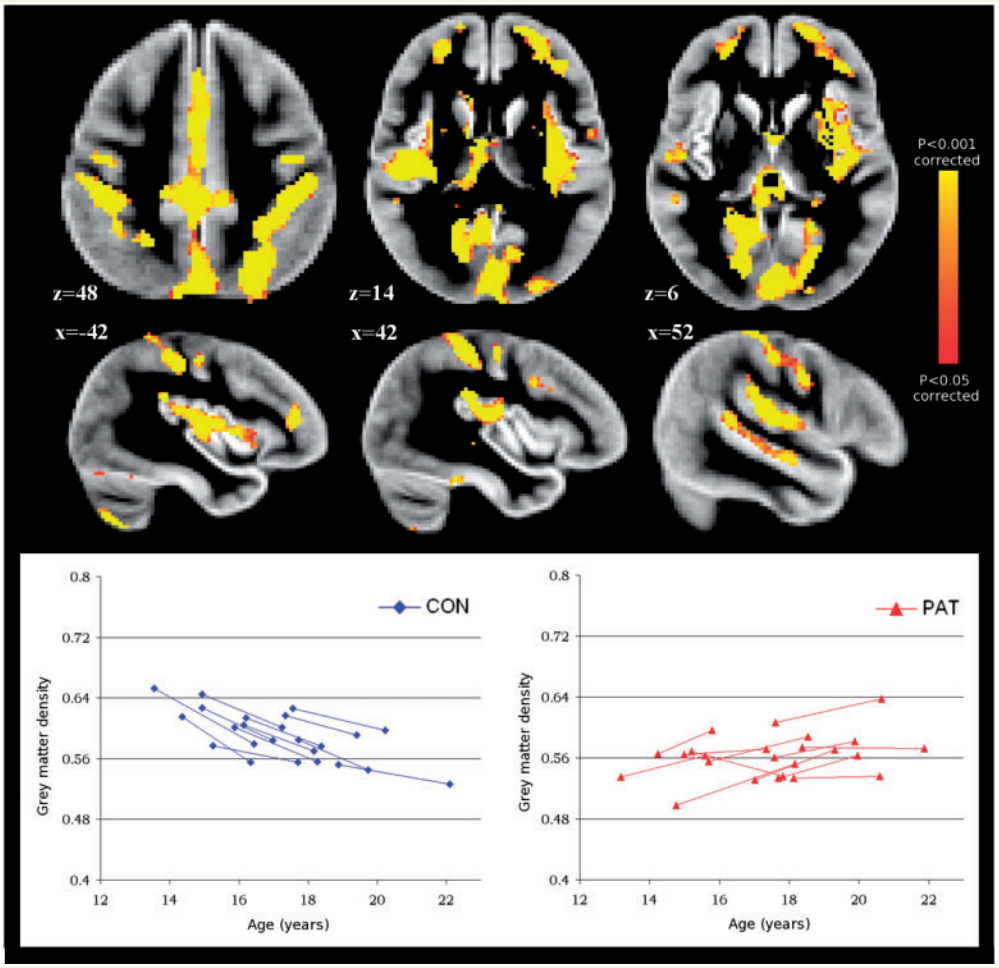

Figure 3 Longitudinal FSL-VBM results of the adolescent cohort. Top panel: significant clusters ($P<0.05$ corrected) showed widespread differences in the grey matter evolution between the patient and the control groups. The pattern was very similar to the one found in the cross-sectional FSL-VBM analysis of combined adolescent and adult cohorts including: pre- and post-central gyrus, SMA/pre-SMA, parietal operculum, Heschl's gyrus, precuneus, calcarine fissure and left lateral occipital cortex. Radiological convention (L is R). Bottom panel: plots representing grey matter evolution in the ROI determined with the FSL-VBM analysis (*y*-axis) depending on the age of subjects (*x*-axis). Differences in grey matter development are related to substantial reduction of grey matter density in the healthy adolescents while the patient's grey matter density has increased.

controls revealed that these differences were essentially explained by a reduction of grey matter density in the healthy adolescents, as grey matter density in patients was increasing over the same period of time (Fig. 3).

In contrasting the t-maps for the evolution of grey matter density of each group, we mapped the regions of the brain according to where patient and control groups had reducing or increasing grey matter density over the 2.5 year period. As opposed to results shown in Fig. 3, this was carried out in all grey matter voxels regardless of significance, allowing us to compare developmental trajectories between the two groups directly across the whole brain (Fig. 4). The most widespread pattern was where controls had reducing grey matter while patients had increasing grey matter ($n = 75\ 642$ voxels, in light blue), and the second most widespread pattern of longitudinal change was where controls had greater reduction of grey matter than patients ($n = 47\ 430$ voxels, in green). The region defining where patients had greater reduction of grey matter than the controls was restricted to $n = 11\ 940$ voxels (in red).

Notably, all regions with significantly different trajectories (as presented in Fig. 3) were confined to the light blue region with the only exception being part of the cuneus, which also extended into the green region.

TBSS analysis of longitudinal white matter changes revealed that divergence in FA trajectory between adolescent healthy subjects and patients was also widespread, with the highest significance bilaterally located in the pyramidal tract and the medial lemniscus (Fig. 5).

Post hoc analyses of those voxels which had significantly different trajectories showed that healthy subject's FA was roughly constant over the period of observation, while FA was increasing in patients (Fig. 5).

Adolescent- and adult-onset schizophrenia show a continuous trajectory of grey matter maturation, different from that for normal development and ageing

The FSL-VBM global analysis, contrasting the entire patient group (adolescent- and adult-onset, $n = 60$) with all healthy controls

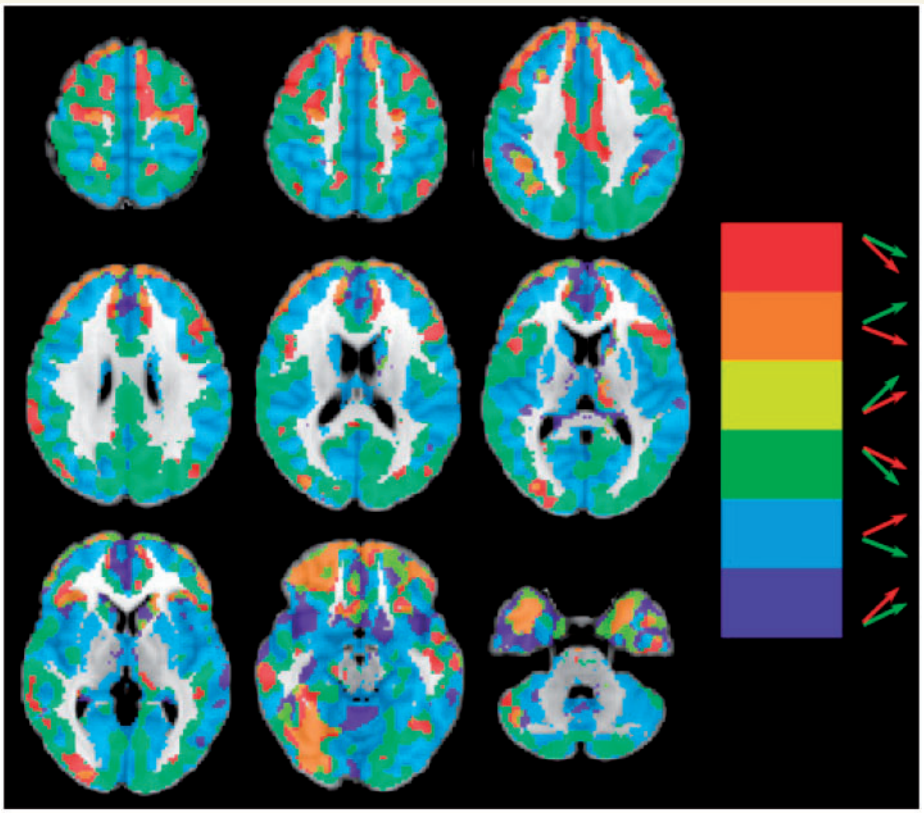

Figure 4 Binarized representation of all the combinations of grey matter development over 2.5 years between the healthy adolescents (green arrows) and the schizophrenic patients (red arrows). For *significant* differences between the trajectories of healthy and schizophrenic subjects, please refer to Fig. 3. Axial views of this representation are overlaid onto the non-linear ICBM-152 template. Radiological convention (L is R). The rainbow colour bar codes for the combinations of grey matter trajectories between controls (green arrows) and schizophrenic patients (red arrows): violet–grey matter density has increased more rapidly in patients than in controls; light blue–grey matter density has increased in patients when it has decreased in controls; green–grey matter density has decreased less rapidly in patients than in controls; yellow–grey matter density has increased at a greater rate in controls than in patients; orange–grey matter density has increased in controls when it has decreased in patients; red–grey matter density has decreased less rapidly in controls than in patients.

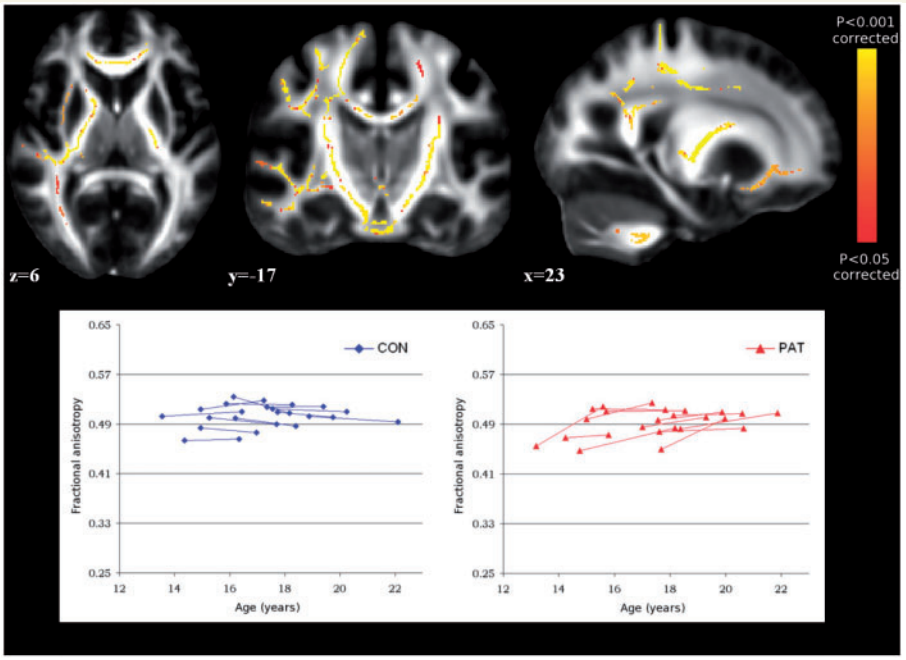

Figure 5 Longitudinal TBSS results of the adolescent cohort. Top panel: significant clusters ($P<0.05$ corrected) showed widespread differences in white matter microstructural evolution between the patient and the control groups. The strongest effect was observed in the corticospinal/cortico-pontine tract, but differences were also seen bilaterally in the medial lemniscus, the superior longitudinal fasciculus, the entire corpus callosum and the superior, middle and inferior cerebellar peduncles and in various tracts of the right hemisphere. Radiological convention (L is R). Bottom panel: plots representing FA evolution in the ROI determined with the TBSS analysis (*y*-axis) depending on the age of the subjects (*x*-axis). Differences in FA are related to substantial increase of FA in the patients while the FA of the healthy adolescents has remained more or less constant.

(adolescents and adults, $n=60$), confirmed extensive grey matter abnormalities in the brains of the patients (Supplementary Fig. S4). These significantly altered cerebral regions were used as an ROI to extract grey matter density information for each subject, which we plotted against age (Fig. 6).

Monotonically decreasing and decelerating change in grey matter density was found for healthy controls across the age-range studied. By contrast, patient's grey matter time course followed a non-monotonic curve, with an increase of grey matter density reaching an apparent maximum around 18 years and showing progressive decrease in older age that was more pronounced than for the controls. Grey matter density values of those 11 additional adult patients with adolescent onset of symptoms fit the bi-exponential model well (in green on Fig. 6). These results imply a common, abnormal trajectory of grey matter density for patients with schizophrenia.

We directly tested this interpretation of the cross-sectional data with the grey matter density obtained from the longitudinal data in adolescents. Grey matter density for the adolescent patients and healthy adolescents studied longitudinally fit the shape of each respective curve well, without substantially modifying the equation of the bi-exponential models (in turquoise for controls and orange for patients on Fig. 6).

Discussion

We have previously reported widespread grey matter and white matter structural differences in adolescent-onset schizophrenic patients with age-matched healthy controls (Douaud *et al*., 2007). Here, we report a comprehensive series of analyses extending these observations. Using identical analytical methods, we have confirmed that the extent of cerebral abnormalities in adolescent-onset patients is substantially greater than in adult-onset schizophrenic patients. However, more detailed examination strikingly shows that such greater anomalies do not arise because of substantial differences between the brains of adolescent- and adult-onset patients, but rather because adolescent patients fail to follow the same developmental time course as the healthy control group. Considered cross-sectionally, grey matter density measures in the adolescent- and adult-onset patients suggest a maturational trajectory markedly different from that of the control subjects. This delayed and altered time course of grey matter maturation in schizophrenia provides evidence for developmental differences in brain structure in adolescence and a possible enhanced rate of brain atrophy in later life. Longitudinal follow-up of the adolescent-onset patients was consistent with the delayed and abnormal developmental pattern suggested from the cross-sectional data and supported the hypothesis of a maturational continuum between adolescent- and adult-onset patients. Together, these results suggest common disease mechanisms altering brain maturation in schizophrenia.

One question raised in our previous, cross-sectional, study of adolescent-onset schizophrenia (Douaud *et al*., 2007) was whether the greater extent of grey matter and white matter abnormalities found compared with the adult-onset schizophrenia literature, especially in the sensorimotor regions, was attributable

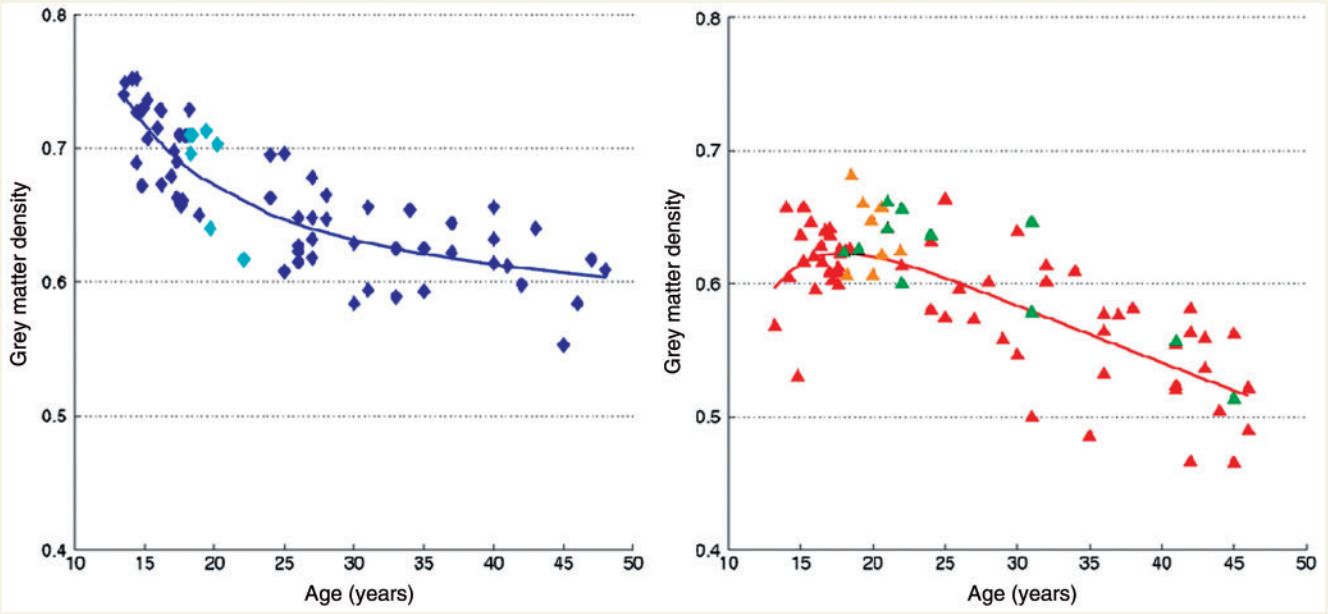

Figure 6 Plots representing grey matter density in the ROI determined with the FSL-VBM analysis combining adolescent and adult cohorts (*y*-axis) depending on the age of the subjects (*x*-axis). Longitudinal control's grey matter density is represented in light blue. Longitudinal patient's grey matter is in orange, additional schizophrenic patients with adolescent onset of symptoms in green. Qualitatively, the bi-exponential model was best describing these grey matter trajectories: we found that one unique bi-exponential curve fit well the time course of the entire healthy group ($1.0–0.1 \times e^{-x/1.0} + 1.0 \times e^{-x/8.5}$, norm of residuals: 0.30) and of the adolescent- and adult-onset patient groups taken together ($0.3–0.5 \times e^{-x/13.4} + 0.7 \times e^{-x/44.5}$, norm of residuals: 0.28).

to the high sensitivity of the novel methods used, to differences in a potentially pathologically more severe adolescent-onset form of the disorder (Kyriakopoulos and Frangou, 2007) or to an abnormal neurodevelopmental time course in schizophrenia with greater manifestation during adolescence. The new results reported here are most consistent with the last of these hypotheses. Grey matter density and white matter microstructure voxel-wise analyses of adolescent and adult cohorts confirmed the hitherto pre-supposed specificity of anatomical abnormalities with an earlier age at onset, which remarkably coincided with the pattern of sensorimotor changes found in the adolescent cross-sectional study (Douaud *et al*., 2007). However, by looking at the grey matter values as a function of age in these significant regions, the apparent characteristic differences between adolescent- and adult-onset schizophrenia appear to be related primarily to the still-developing grey matter in healthy adolescence and the normal substantial reduction of grey matter observed between adolescence and adulthood (Giedd *et al*., 1999; Gogtay *et al*., 2004*a*; Paus, 2005; Lenroot and Giedd, 2006).

Further support for altered neurodevelopment in schizophrenia was obtained by direct study of longitudinal structural changes in the adolescent patients. Highly significant regions of divergence in grey matter development between healthy and patient groups notably co-localized with the abnormalities in the adolescent cross-sectional study, especially in sensorimotor-related areas (Douaud *et al*., 2007). This co-localization demonstrates that differences between adolescent patients and healthy adolescents are primarily related to a divergence in their brain development trajectories. The pattern was highly consistent: all but one of the patients showed grey matter increases in the regions of significant divergence with the healthy controls, arguing in favour of a neurodevelopmental delay in the adolescent schizophrenic patients. As pointed out in our previous study (Douaud *et al*., 2007), alterations in the complexity of cortical folding in adolescent patients with psychosis may also underlie differences in grey matter revealed by a VBM approach (White *et al*., 2003; Harris *et al*., 2007; Voets *et al*., 2008). Our data do not support the finding of an accelerated rate of loss of grey matter in the most dorsal parts of the lateral and medial cortex in childhood-schizophrenic patients ($n = 12$) (Thompson *et al*., 2001; Vidal *et al*., 2006), but our cohort shows changes similar to those reported in the largest longitudinal study of early-onset schizophrenia to date ($n = 70$) (Greenstein *et al*., 2006) (Supplementary Fig. S5). This highlights the value of adopting a voxel-based approach to study brain development in schizophrenia: pathological changes are widespread and non-uniform so ROI-based approaches alone could lead to misinterpretations of the main effect or Type II error (James *et al*., 2002, 2004; Sporn *et al*., 2003; Gogtay *et al*., 2004*b*; Reig *et al*., 2008).

Interestingly, Fig. 4 first demonstrates that all regions showing a significant alteration and delay of maturation, including the sensorimotor cortex, fall into the light blue region which reflects an increasing grey matter in schizophrenic adolescents and decreasing grey matter in healthy adolescents (except for a part of the cuneus). In addition, Fig. 4 also shows that the maturational time course of the whole brain in the patients does not follow the same spatial pattern as healthy adolescents. Indeed, it would seem that in regions that are known to normally develop later than the sensorimotor regions (Gogtay *et al*., 2004*a*; Shaw *et al*., 2008), adolescent patients have already reached a peak and subsequently

have decreasing grey matter (regions in red, orange and green of Fig. 4 encompassing parietal and occipital cortices).

Significant differences between grey matter trajectories for healthy subjects and patients during adolescence also strongly overlapped with regions of structural abnormalities distinguishing adolescent- and adult-onset schizophrenia, suggesting a dynamic progression of brain development in adolescent patients towards the pattern of the adult-onset disease. Consistent with this, these abnormalities specific to adolescent-onset schizophrenia were less marked or even disappeared over the period of observation (Supplementary Fig. S2). In particular, there was no difference in the pre- and post-central gyri or in the SMA after 2.5 years. Regions that remained abnormal at follow-up were similar to those consistently found to be abnormal in adult-onset schizophrenia, as emphasized by recent VBM meta-analyses (Honea *et al*., 2005; Glahn *et al*., 2008).

Analogous results were found for the white matter: white matter abnormalities in the pyramidal tract specific to adolescent-onset schizophrenia almost entirely disappeared after only 2.5 years (Supplementary Fig. S3). While the FA of healthy subjects was stable in these tracts (consistent with previous observations of healthy white matter maturation) (Lebel *et al*., 2008), FA in all adolescent patients showed progressive increases towards normal values. Interestingly, therefore, the most significant differences between healthy subjects and patients over the longitudinal period of observation were detected in grey matter and white matter regions with the greatest on-going neurodevelopmental changes in healthy adolescence: the pyramidal tract in the white matter (Paus, 2005; Lebel *et al*., 2008) and the dorsal primary sensorimotor areas in grey matter (Gogtay *et al*., 2004*a*; Lenroot and Giedd, 2006).

Several hypotheses can be suggested to explain pathophysiological processes underlying these non-uniform (Fig. 4), widespread yet focal, structural abnormalities in schizophrenia. Alterations in neuroplasticity (Frost *et al*., 2004) or abnormal cortico–cortical connections—possibly mediated by a defect in synaptic pruning, synaptic plasticity or myelination (Feinberg, 1982; Randall, 1983; Innocenti *et al*., 2003; Davis *et al*., 2003; Stephan *et al*., 2006)—could both reconcile the observation of widespread structural abnormalities with the clinical specificity of the syndrome. Unfortunately, *in vivo* imaging lacks the resolution to test alternative mechanisms (e.g. dendritic remodelling, cell death, synaptic pruning or myelination) at the origin of such grey matter and white matter changes (Toga *et al*., 2006). However, it seems that our results do not support the hypothesis that delayed myelination alone explains all abnormalities observed in schizophrenia. On-going myelination in the grey matter should reduce grey matter-white matter contrast and lead to a subsequent apparent decrease of grey matter density following tissue segmentation, rather than the grey matter increase observed in the patients, for instance in the primary sensorimotor cortical regions. A selective developmental lesion of the heteromodal cortex has also been proposed as unique structural substrate for schizophrenia (Pearlson *et al*., 1996), but the regions showing altered brain development in this study prove to extend beyond heteromodal association areas (e.g. pre- and post-central gyrus, corticospinal tract and medial lemniscus). An interaction of these neurodevelopmental processes with factors such as substance abuse, stress or environmental factors including medication may also take part in the distribution of brain changes (Pantelis *et al*., 2005; van Haren *et al*., 2008).

The common abnormal trajectory of significant change in grey matter density defined by our data, regardless of the age at onset of symptoms and of disease duration (Fig. 6), suggests an increase of grey matter up to an apparent maximum that is lower and reached later than for healthy subjects (Giedd *et al*., 1999, Gogtay *et al*., 2004*a*) and accelerated disease-related later-life atrophy. One hypothesis is that factors responsible for impaired neurodevelopment may also contribute to increased susceptibility to neurodegeneration (Harrison, 1995; Lieberman, 1999; Bramon and Sham, 2001; Church *et al*., 2002, Perez-Neri *et al*., 2006). Extensive follow-up of adolescent-onset patients throughout adulthood, as well as screening of high-risk subjects during adolescence, would be needed to indisputably confirm the continuity of altered brain maturation in schizophrenia. The observation that grey matter density of patients with wide range of disease and treatment duration fit well this single curve may be more consistent with a lack of substantial effects related to medication.

To conclude, our results converge to demonstrate abnormal, delayed brain development during adolescence in the brains of schizophrenic patients. Grey matter and white matter abnormalities that are greater and more widespread in adolescent-onset schizophrenia compared with adult-onset schizophrenia are an epiphenomenon attributable to this delay, a 'snapshot' testifying of the great deviation in brain development in adolescence, when timing is critical. We have shown a continuum between adolescent- and adult-onset schizophrenia with a progressive fading of the 'specific', mainly sensorimotor-related, grey matter and white matter anomalies in adolescent patients and with a single, altered maturational trajectory for both adolescent- and adult-onset patients regardless of age at onset of symptoms and disease duration.

Supplementary material

Supplementary material is available at *Brain* online.

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