Data supplement for Cropley et al., Accelerated Gray and White Matter Deterioration With Age in Schizophrenia. Am J Psychiatry (doi: 10.1176/appi.ajp.2016.16050610)

SUPPLEMENTARY MATERIAL

Accelerated gray and white matter aging in schizophrenia

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Supplementary Methods

Participants

Participant data was obtained from the Australian Schizophrenia Research Bank (ASRB). Exclusion criteria included any organic brain disorder, history of brain trauma followed by a long period of amnesia (> 24h), mental retardation (full-scale IQ < 70), movement disorders, current drug or alcohol dependence, as well as electroconvulsive therapy in the past 6 months. Healthy controls with a personal or family history of psychosis or bipolar I disorder were also excluded. Patients had a confirmed diagnosis of schizophrenia or schizoaffective disorder according to DSM-IV or ICD-10 diagnostic criteria.

Clinical assessment

Clinical status was assessed with the Diagnostic Interview for Psychosis (DIP) and the Scale for Assessment of Negative Symptoms (SANS). Following the method of Green et al. (1) the severity of positive symptoms was estimated by summing lifetime data for 11 DIP items assessing hallucinations and delusions. Current IQ was assessed with the Wechsler Abbreviated Scale of Intelligence (WASI).

MRI acquisition

Structural (T1-weighted) magnetic resonance imaging scans (sMRI) and diffusion-weighted imaging (DWI) scans were acquired in each participant with a Siemens Avanto 1.5-Tesla system (Siemens, Erlangen, Germany) across five different sites in Australia. Exactly the same acquisition sequence for T1-weighted and DWI scans was used across all sites. For T1-weighted images, an optimized magnetization-prepared rapid acquisition gradient echo (MP-RAGE) sequence was acquired with the following parameters: 176 sagittal slices of 1mm thickness without gap; field of view = 250 x 250 mm^2; repetition time/echo time = 1980/4.3 ms; data matrix size = 256 x 256; voxel dimensions = 0.98 x 0.98 x 1.0 mm^3. For DWI, 64 gradient-weighted volumes were acquired using a spin-echo EPI sequence with the following parameters: b-value, 1000 s/mm^2; 65 consecutive axial slices of thickness 2.4 mm; 104×104 image matrix with an in-plane voxel resolution of 2.4×2.4 mm; field of view, 25×25 cm; repetition time, 8.4 s; echo time, 88 ms; flip angle, 90 degrees. No scanner upgrades were performed during the study lifetime. Calibration scans were conducted at each site using an identical Siemens phantom and collection of MRI scans from the same individual travelling to each site using the same acquisition protocol. These procedures did not detect any site variation.

Image processing

Measurement of gray matter volume

Gray matter volume (GMV) maps were calculated for each participant by using optimized voxel-based morphometry (VBM8), as implemented in statistical parametric mapping (SPM8) software (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) running in Matlab R2014b http://www.mathworks.com.au/products/matlab/). T1 images were segmented into gray matter, white matter and cerebrospinal fluid. Gray matter segments were then spatially aligned to a high-dimensional Diffeomorphic Anatomical Registration Through Exponentiated Lie Algebra (DARTEL) template (1.5x1.5x1.5mm isotropic voxels) in the MNI space that is provided with the VBM8 toolbox. Gray matter voxels were multiplied by the non-linear components of this deformation (modulated normalized – non-linear only) to provide a measure of the absolute amount of GM tissue corrected for individual brain sizes. All images passed the VBM8 quality check based on the 'display one slice' and 'check sample homogeneity of covariance' modules. Images were smoothed with an 8mm full-width-half-maximum Gaussian kernel. Whole-brain averaged GMV and voxel-based analyses were performed on the smoothed images. For voxel-based analyses, an absolute GM mask using a threshold of 0.1 was applied.

Measurement of fractional anisotropy
Skeletonized fractional anisotropy (FA) maps were calculated for each participant using tract-based spatial statistics (TBSS) (2) and the FMRIB diffusion toolkit (FDT) (3). Briefly, DWI images were corrected for linear distortions and head movement using affine registration. Gradient directions were rotated accordingly. Diffusion tensors were independently fitted to each voxel using least squares estimation to generate FA images. A white matter "skeleton" was then mapped for each participant based on voxels with locally maximal FA. The white matter skeleton delineated the centers of all major axonal pathways and was nonlinearly normalized to MNI space for each participant. Any residual misalignment remaining from the normalization process was minimized with a skeleton projection algorithm. FA images and corresponding skeletons were manually inspected for artifacts.

Supplementary Results

Medication and site effects

Additional statistical analyses were performed to examine the influence of medication and site on the age-related trajectories. Three medication categories were determined based on self-reported medication use at the time of assessment (see Table 1): i) atypical antipsychotics (patients taking atypical antipsychotics and possibly other medications); ii) typical antipsychotics (patients taking typical antipsychotics and possibly other medications); and, iii) antidepressants (patients taking antidepressants and possibly other medications). Each medication category was modelled with a separate binary covariate indicating which patients were medicated with that category. Three independent t-tests were used to assess the significance of each medication category as a predictor of GMV or FA, using Bonferroni correction. The effect of anticonvulsants, anticholinergics or anxiolytics was not assessed due to the low number of patients taking these medications. For GMV, medication class (atypicals, typicals and antidepressants) did not have a significant impact on GMV, although atypical (t=-1.9, p=.06) and typical (t=-1.88, p=.06) antipsychotics showed a trend for lower GMV (see FigureS4*a*). For FA, there was no significant effect of atypical (t=-0.81, p=.42) and typical (t=-0.81, p=.42) antipsychotics but there was for antidepressants (t=2.32, p=.02) which showed significantly increased FA compared to patients not medicated with antidepressants (see FigureS4*b*). The interaction between medication category and age was not significant. To examine the effect of site, the regression model was refit with both site and the interaction between site and diagnosis included as explanatory variables. A significant effect of site on FA (F=5.05, p=.0005) but not GMV (F=0.81, p=.52) was evident. Site showed no interaction with diagnosis for both GMV (F=0.99, p=.41) and FA (F=0.77, p=.54). Site-stratified trajectories of brain aging are shown in Figure S5. Site and the interaction between site and diagnosis were not significant predictors of age. The mean (p=.37) and variance (p=.71) in age between sites did not significantly differ.

Supplementary Tables

Table S1. Medication Breakdown of Sample

Current Medication	**Schizophrenia Patients (n=326)**		**Healthy Controls (n=197)**	
	N	%	N	%
Antipsychotics	259	86	0	0
Atypical antipsychotics	246	81.7	0	0
Typical antipsychotics	31	10.3	0	0
Antidepressants	89	29.6	8	4.2
No antipsychotics / antidepressant medication	38	12.6		
Anticonvulsant/mood stablizers	44	14.6	0	0
Anticholinergics	16	5.3	0	0
Anxiolytic/sedatives	32	10.6	1	0.5
Lithium	11	3.7	0	0
Exclusive atypical antipsychotics	108	35.9		
Typical but no atypical antipsychotics	13	4.3		
Antidepressants but no antipsychotic medication	4	1.3		
Anticonvulsant/mood stablizers but no antipsychotic medication	2	0.66		
Anticholinergic but no antipsychotic medication	1	0.33		
Anxiolytic but no antipsychotic medication	1	0.33		
No medication	10	3.3	168	91.3

Note. Medication data was unavailable for 25 cases and 13 controls

Supplementary Figures

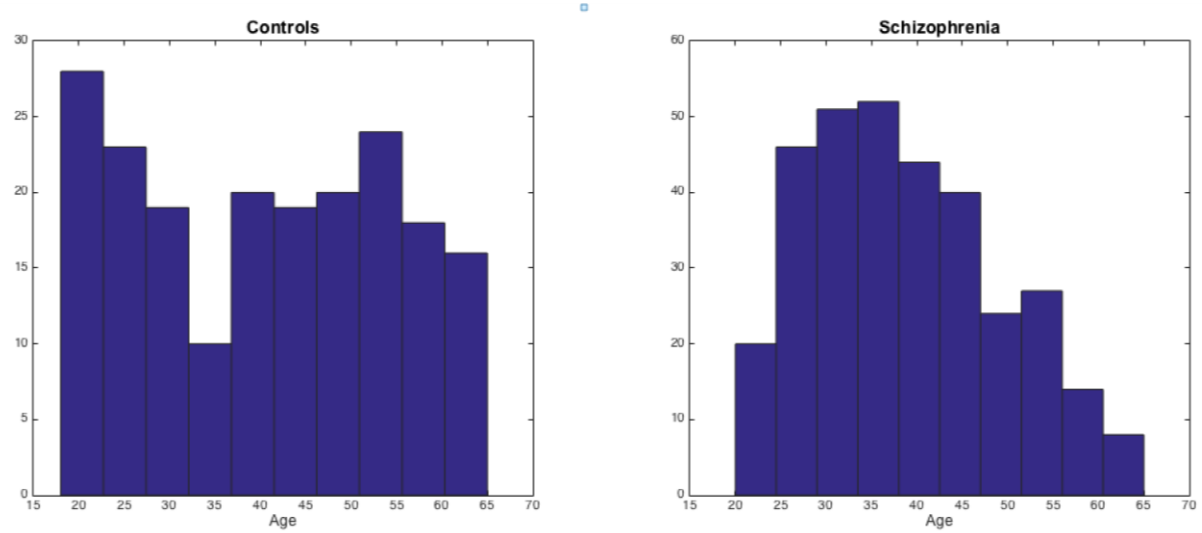

Figure S1. Distribution of age in controls and schizophrenia patients. Age did not differ in distribution between patients and controls in the range 25 to 50 years (Kolmogorov-Smirnov test, $p = 0.12$.

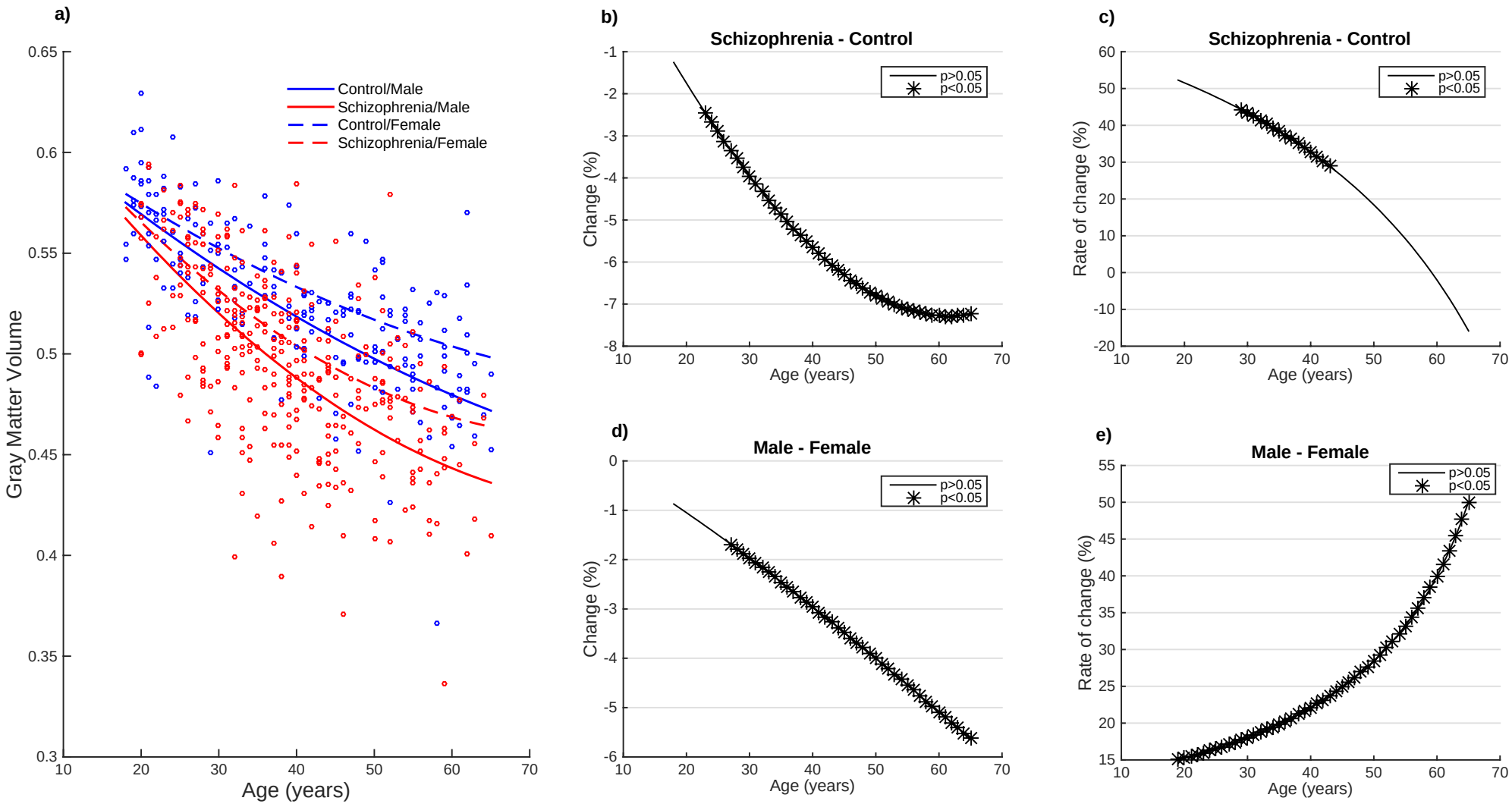

Figure S2. Sex-stratified quadratic model of whole-brain averaged GMV changes with age in schizophrenia and healthy controls.
a) Fitted curves modeling age-related loss of GMV in controls (blue curve) and schizophrenia (red curve) stratified by sex (males solid curve; females dashed curve). Two additional explanatory variables were added to the regression model: the interaction between age and sex and the three-way interaction between age, sex and diagnostic status. *b)* and *c)* shows the between-group difference in the amount (*b*) and rate (*c*) of GMV loss in patients as a function of age, as described in the original regression model. *d*) Difference in the amount of GMV loss as a function of age between males and

females (irrespective of diagnostic status). Negative percentages indicate a greater GMV loss in males. e) Difference in the rate of GMV loss as a function of age between males and females, with positive percentages indicating a faster rate of loss in males compared to females. Age epochs at which the amount (*b* and *d*) and rate (*c* and *e*) of GMV loss significantly differed between groups (patients versus controls or males versus females) are denoted with an asterisk ($p<0.05$). The interaction between diagnostic status and sex, and the three-way interaction between age, sex and diagnostic status were not significant predictors of GMV.

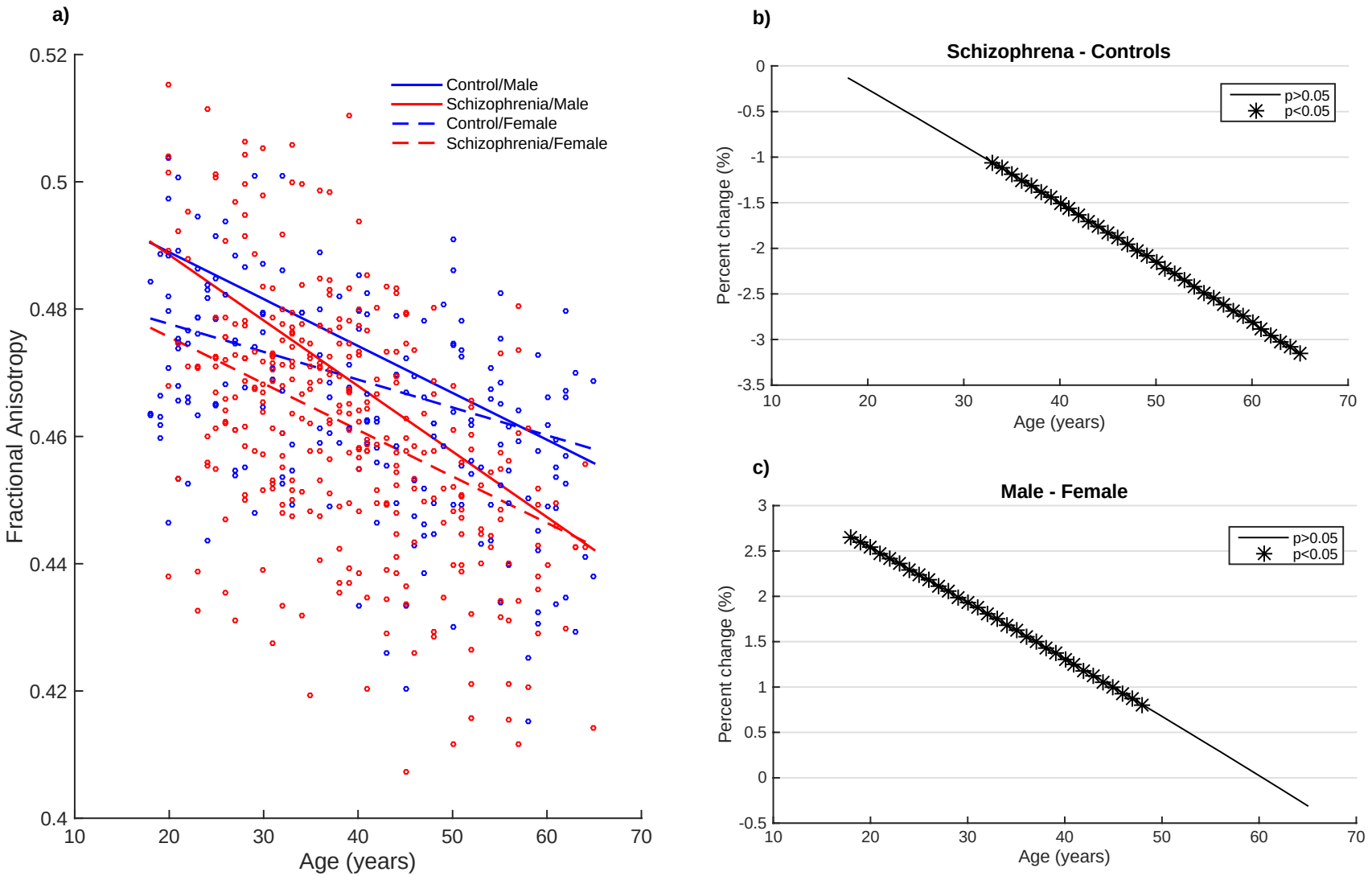

Figure S3. Sex-stratified linear model of whole-brain averaged FA changes with age in schizophrenia and healthy controls.
a) Fitted linear curves modeling age-related loss of FA in controls (blue curve) and schizophrenia (red curve) stratified by sex (males solid curve; females dashed curve). Two additional explanatory variables were added to the regression model: the interaction between age and sex and the three-

way interaction between age, sex and diagnostic status. *b)* Between-group difference in the amount of FA deterioration in patients as a function of age, as described in the original regression model. Negative percentages indicate lower FA in patients. *c*) Difference in the amount of FA loss as a function of age between males and females (irrespective of diagnostic status). Positive percentages indicate higher FA in males in comparison to females. Age epochs at which the amount of FA deterioration significantly differed between schizophrenia and controls (*b*) and males and females (*c*) are denoted with an asterisk ($p<0.05$). The interaction between diagnostic status and sex, and the three-way interaction between age, sex and diagnostic status were not significant predictors of FA.

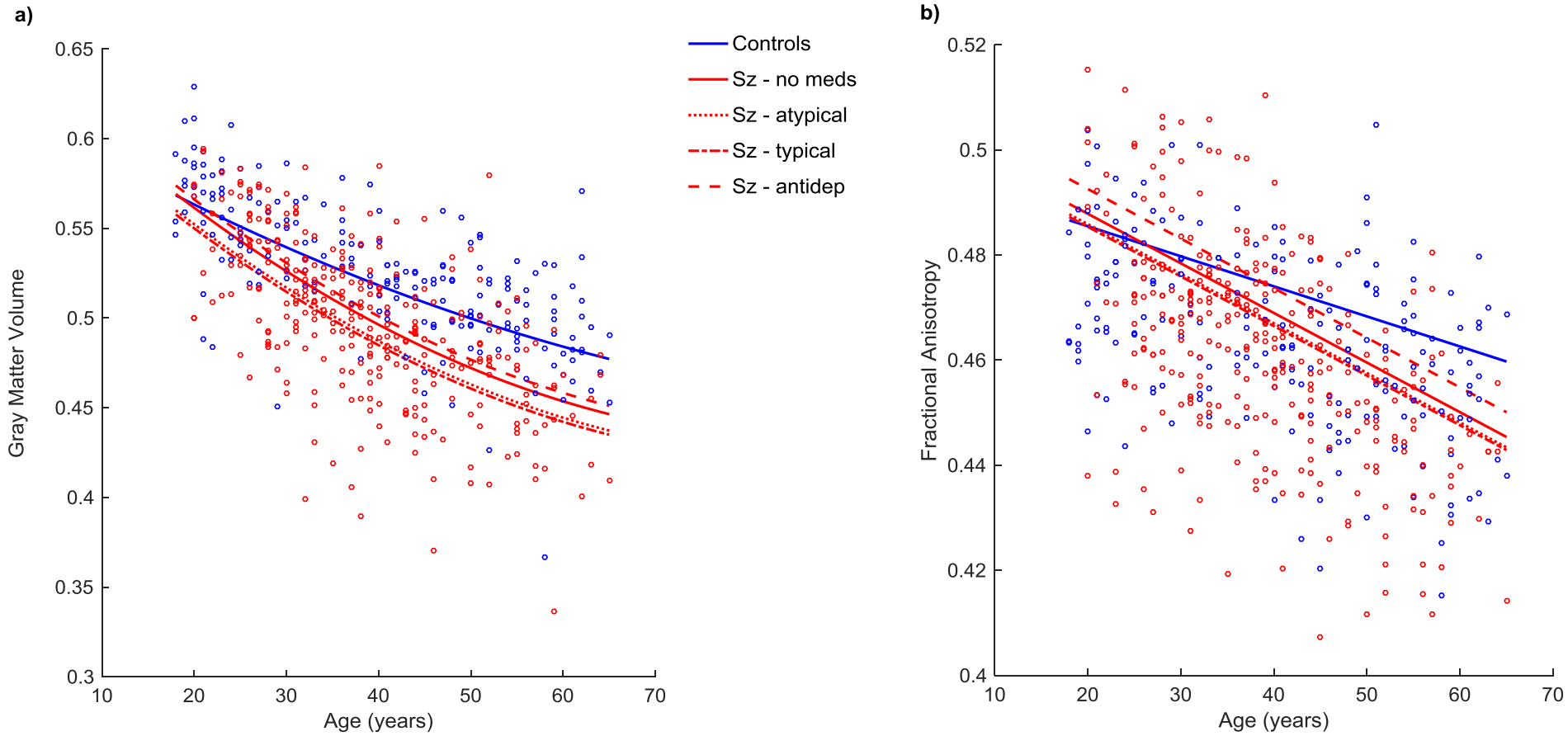

Figure S4. Medication-stratified models of whole-brain averaged GMV and FA changes with age in schizophrenia and healthy controls. Fitted models showing age-related loss of GMV (*a*) and FA (*b*) in controls (blue curves) and schizophrenia (red curves) stratified by medication groups.

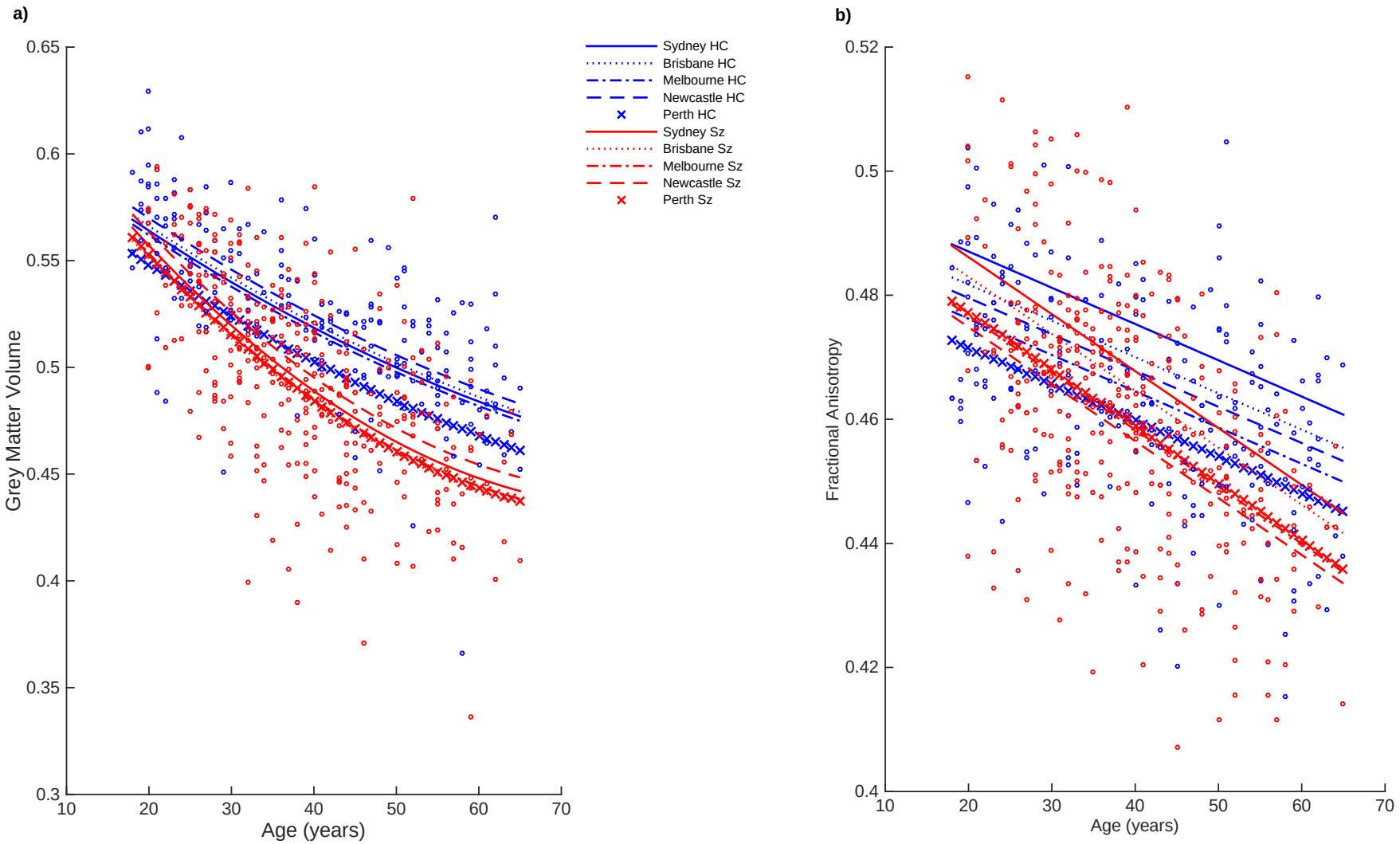

Figure S5. Site-stratified models of whole-brain averaged GMV and FA changes with age in schizophrenia and healthy controls.
Fitted models showing age-related loss of GMV (*a*) and FA (*b*) in controls (blue curves) and schizophrenia (red curves) stratified by scanning site.

References

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4. Skudlarski P, Schretlen DJ, Thaker GK, Stevens MC, Keshavan MS, Sweeney JA, et al. Diffusion tensor imaging white matter endophenotypes in patients with schizophrenia or psychotic bipolar disorder and their relatives. Am J Psychiatry. 2013;170(8):886-898.