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White matter neuroanatomical differences in young children who stutter

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The ability to express thoughts through fluent speech production is a most human faculty, one that is often taken for granted. Stuttering, which disrupts the smooth flow of speech, affects 5% of preschool-age children and 1% of the general population, and can lead to significant communication difficulties and negative psychosocial consequences throughout one's lifetime. Despite the fact that symptom onset typically occurs during early childhood, few studies have yet examined the possible neural bases of developmental stuttering during childhood. Here we present a diffusion tensor imaging study that examined white matter measures reflecting neuroanatomical connectivity (fractional anisotropy) in 77 children [40 controls (20 females), 37 who stutter (16 females)] between 3 and 10 years of age. We asked whether previously reported anomalous white matter measures in adults and older children who stutter that were found primarily in major left hemisphere tracts (e.g. superior longitudinal fasciculus) are also present in younger children who stutter. All children exhibited normal speech, language, and cognitive development as assessed through a battery of assessments. The two groups were matched in chronological age and socioeconomic status. Voxel-wise whole brain comparisons using tract-based spatial statistics and region of interest analyses of fractional anisotropy were conducted to examine white matter changes associated with stuttering status, age, sex, and stuttering severity. Children who stutter exhibited significantly reduced fractional anisotropy relative to controls in white matter tracts that interconnect auditory and motor structures, corpus callosum, and in tracts interconnecting cortical and subcortical areas. In contrast to control subjects, fractional anisotropy changes with age were either stagnant or showed dissociated development among major perisylvian brain areas in children who stutter. These results provide first glimpses into the neuroanatomical bases of early childhood stuttering, and possible white matter developmental changes that may lead to recovery versus persistent stuttering. The white matter changes point to possible structural connectivity deficits in children who stutter, in interrelated neural circuits that enable skilled movement control through efficient sensorimotor integration and timing of movements.

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Abbreviations: DTI = diffusion tensor imaging; IFG = inferior frontal gyrus; MTG = middle temporal gyrus; PMC = premotor cortex; SLF = superior longitudinal fasciculus; SMG = supramarginal gyrus; SSI = stuttering severity instrument; TBSS = tract-based spatial statistics

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Introduction

The ability to formulate and express language through speech production is perhaps one of the defining features of being human. Although it is often taken for granted, fluent speech production is an extremely complex feat, requiring precise coordination and timing of ~100 muscles encompassing the articulatory, laryngeal, resonance and respiratory systems (Zemlin, 1998; Barlow and Stumm, 2009). In many preschool-age children, a transient period of speech disfluency that includes speech sound repetitions, prolongations, blocks and hesitations is commonly observed. Such disfluencies can persist for >6 months in ~5% of preschool-age children (Andrews and Harris, 1964; Yairi and Ambrose, 2013), at which point most clinicians diagnose it as developmental stuttering. Among these children, ~80% would recover naturally (Yairi and Ambrose, 1999; Månsson, 2000; Dworzynski *et al.*, 2007), but the remaining children could exhibit persistent stuttering for the rest of their lives. Stuttering can severely disrupt the fluent flow of speech production, resulting in communication difficulties as well as negative psychosocial consequences.

Although the aetiology of stuttering is still unclear, there is increasing evidence of subtle functional neuroanatomical differences in both adults and children who stutter, pointing to a probable neurological basis for the onset and persistence of stuttering. The brain areas found to differentiate speakers who stutter from non-stuttering speakers have encompassed both cortical and subcortical areas, primarily those supporting sensory and motor functions relevant to motor control and timing of speech movements. Despite the different methodologies and participant demographics, some of the convergent findings reported across different labs include evidence for: (i) functional neuroanatomical anomalies in the left perisylvian regions and their connectivity. Atypical brain activity patterns in the left inferior frontal gyrus (IFG), premotor cortex (PMC), supramarginal and posterior auditory cortices (Fox *et al.*, 1996; Braun *et al.*, 1997; Chang *et al.*, 2009), and decreased functional connectivity among these aforementioned areas have been reported (Lu *et al.*, 2009; Chang *et al.*, 2011). In terms of structural connectivity, measures of white matter integrity derived from diffusion tensor imaging (DTI) have supported decreased connectivity in white matter tracts that interconnect these perisylvian regions (Sommer *et al.*, 2002; Chang *et al.*, 2008; Watkins *et al.*, 2008; Cykowski *et al.*, 2010); (ii) right-sided increases in brain activity and brain structure, in particular motor related cortical areas, including the right inferior frontal gyrus and the right motor cortex (Brown *et al.*, 2005). So far, these results have garnered two main interpretations. The first is that these differences may represent compensatory processes that have developed in response to left-sided deficits as mentioned above, and the second is that right-sided differences may also represent aetiological changes associated with stuttering (Connally *et al.*, 2013); and (iii) subcortical (basal ganglia) and cerebellar function and anatomical differences in stuttering. Several studies have shown that basal ganglia activity differs in people who stutter relative to controls during rest (Ingham *et al.*, 2012; Xuan *et al.*, 2012) and speaking conditions (Wu *et al.*, 1997; Lu *et al.*, 2010). Lesions affecting the basal ganglia thalamocortical pathway have been observed to result in symptoms similar to developmental stuttering (Ludlow *et al.*, 1987; Ciabarra *et al.*, 2000; Theys *et al.*, 2013), and dopaminergic drugs have been shown to affect stuttering symptoms (Maguire *et al.*, 2004). One study reported significant correlations between activity in the basal ganglia and stuttering severity before therapy but interestingly, not after therapy (Giraud *et al.*, 2008). Further, effective connectivity among basal ganglia, presupplementary motor area and temporal regions was found to differ significantly between controls and people who stutter (Lu *et al.*, 2010), suggesting that anomalous interaction between these structures may be present in people who stutter, and may disrupt efficient sequencing and timing of speech movements. With the exception of Chang *et al.* (2008), where children aged 8–12 years were examined, and Watkins *et al.* (2008), where adolescents were examined, most of the studies reviewed above were based on adults who stutter.

Recent studies based on children who stutter have supported anatomical differences in the putamen (11 children who stutter and 11 controls, all boys 6–12 years of age) (Beal *et al.*, 2013) and caudate (14 children who stutter and 13 controls, all boys 8–13 years of age) (Foundas *et al.*, 2013), and attenuated functional connectivity between the putamen and supplementary motor area (27 children who stutter, 29 controls, mixed gender, 3–9 years of age) (Chang and Zhu, 2013). Given increasing evidence of anomalous basal ganglia structure and function, and its connectivity with cortical motor and auditory regions, some authors have proposed that internal timing deficits might be at the core of stuttering, and posit that people who stutter compensate for this deficit with an external timing mechanism that is supported by the cerebellum and PMC (Alm, 2004; Chang and Zhu, 2013; Etchell *et al.*, 2014). Hyperactivity in the cerebellum has been commonly reported in speakers who stutter (De Nil *et al.*, 2003; Brown *et al.*, 2005), and has been interpreted as being compensatory to deficiencies in acquiring skilled movements, which, once acquired, are often automatic and internally timed.

Though neuroimaging studies have contributed to our understanding of potential neurophysiological underpinnings of stuttering, there have been important caveats in almost all of the studies conducted to date, and thus many gaps remain in our knowledge of the basis for stuttering. For instance, most studies have only examined adults who stutter, who have been stuttering for decades since onset in early childhood. At this time, there have been no neuroanatomical studies in children who stutter that have included preschool-age children close to stuttering

onset. Unfortunately, this limits interpretation of the data that clarify the basis of stuttering, versus reactions and compensations to stuttering. Two other issues include the low numbers of participants, and lack of detail in describing how stuttering severity was measured, as well as other behavioural measures relevant to speech and language function. Some of the inconsistent results that have been reported across studies could likely be attributed to insufficient power. In terms of behavioural measures, insufficient detail, for example in describing how stuttering severity, and relevant speech and language measures were assessed, is a concern as it is very likely that subtypes of stuttering exist, and thus, details and descriptions of these behavioural measures could provide an important layer of information that can elucidate brain imaging data and their relationship to stuttering behaviour.

In this study, we examined whole brain-based white matter neuroanatomical differences in children who stutter, relative to age-matched controls. These data were collected as part of an ongoing large-scale longitudinal study of childhood stuttering; initial data reporting functional connectivity and white matter tractography results focused on regions of interest in a smaller subset of participants were previously reported (Chang and Zhu, 2013). The current study focused on examining subtle differences in white matter development across the whole brain, in a larger group of children between the ages of 3 and 10. We used the well-established tract-based spatial statistics (TBSS) to examine fractional anisotropy, a measure derived from DTI.

Fractional anisotropy measures directionality of water diffusivity, and is an index of white matter organization in the brain (Basser and Pierpaoli, 1996), with '1' representing perfectly anisotropic diffusion and '0' representing perfectly isotropic diffusion. Fractional anisotropy can be affected by several different cellular mechanisms, such as the amount of myelin, integrity of axonal cell membrane, and coherent organization (higher fractional anisotropy) versus crossing (lower fractional anisotropy) of axon bundles. Fractional anisotropy values typically increase across the brain during development, while focal decreases in fractional anisotropy appear in demyelinating diseases such as multiple sclerosis. Region-specific fractional anisotropy increases have also been reported in relation to development during typical childhood and adolescence (Barnea-Goraly *et al.*, 2005; Giorgio *et al.*, 2008), increased skill acquisition and training (Bengtsson *et al.*, 2005; Scholz *et al.*, 2009); while fractional anisotropy decreases have been observed in neurodevelopmental (Odegard *et al.*, 2009) and psychiatric conditions (Lochner *et al.*, 2012; Wang *et al.*, 2012).

The goals of the current study were 3-fold: (i) examine whether previously reported white matter anatomical differences reported in older children and adults who stutter are present in younger children who stutter close to symptom onset; (ii) examine whether age-dependent changes in white matter development differs between children who do and do not stutter; and (iii) examine region-specific white matter changes that correlate with stuttering severity and duration. Given that we had a relatively large group of both boys and girls in both groups (stutter versus controls), we also conducted preliminary sex comparisons on the fractional anisotropy values as well as any interaction with age and other behavioural measures.

Taking into account results from previous investigations of fractional anisotropy in stuttering (Sommer *et al.*, 2002; Watkins *et al.*, 2008; Cykowski *et al.*, 2010; Connally *et al.*, 2013), including an earlier smaller-scale DTI study that involved children who stutter between the ages of 9 and 12 where focal decreases in fractional anisotropy were found in regions along the left superior longitudinal fasciculus (Chang *et al.*, 2008), in this larger study involving a wider age range (3–10 years) we expected to find reduced fractional anisotropy in children who stutter compared to age-matched controls along the left superior longitudinal fasciculus, which interconnects many of the perisylvian speech areas. Specific regions of interest included the IFG/ventral premotor region, PMC [Brodmann area (BA) 6], motor cortex (4p), supramarginal gyrus (SMG), and the posterior superior temporal gyrus. We also sought to answer whether any fractional anisotropy increases in homologous areas in the right hemisphere might be found in young children who stutter, and related increases in the corpus callosum areas, which may underlie increased interhemispheric interaction, as has been the case for adults who stutter (Choo *et al.*, 2011). In addition, we expected decreased fractional anisotropy in children who stutter relative to controls in the corticobulbar tracts that interconnect the motor cortex to subcortical, and to the brainstem areas where synapses occur with cranial nerves that support orofacial movements for speech. In all of these regions, we asked whether any group differences exacerbated or reduced with age, and whether sex and stuttering severity modulated any of these potential white matter neuroanatomical differences.

Materials and methods

Participants

A total of 89 children [47 stuttering (28 males), 42 controls (22 males)] between 3 and 10 years of age participated; all were monolingual native North American English speakers, and without concomitant developmental disorders such as dyslexia, attention deficit hyperactivity disorder, learning delay, or other confirmed developmental or psychiatric conditions. All children underwent careful screening to ensure normal speech and language developmental history except for the presence of stuttering in the experimental group. The children who stutter and controls were matched in chronological age, and did not differ in socioeconomic status (Hollingshead, 1975). Most participants were strongly right-handed on the Edinburgh handedness inventory (Oldfield, 1971); however, a total of nine children who were left-handed (four stuttering, three control) or ambidextrous (two stuttering) were included. All

participants were tested on a battery of standardized speech, language, and cognitive tests, received audiometric hearing screening, oral-motor screening, and cognitive evaluations. The tests included the Peabody Picture Vocabulary Test (PPVT-4; Dunn and Dunn, 2007), Expressive Vocabulary Test (EVT-2; Williams, 2007), Goldman-Fristoe Test of Articulation (Goldman, 2000), Fluharty Preschool Speech and Language Screening Test (Fluharty, 2000), Test of Language Development TOLD-P:3 (Newcomer and Hammill, 1997a), TOLD-I:4 (Newcomer and Hammill, 1997b), Wechsler Preschool and Primary Scale of Intelligence (WPPSI-III; for children 2:6-7:3; Wechsler, 2002), Wechsler Abbreviated Scale of Intelligence (WASI; for children aged 7 and up; Weschler, 1999), and the Purdue Pegboard Test (Tiffin, 1968). Children were excluded if scores fell below two standard deviations (SD) of the mean on any of the standardized assessments.

Stuttering severity was assessed by collecting samples of spontaneous speech, elicited through storytelling and conversational tasks with a parent and a certified speech-language pathologist. These samples were video-recorded for further off-line analyses. We calculated per cent stuttered utterances per number of syllables based on narrative samples that contained a conversation with the clinician, and a monologue elicited with storytelling with a pictures-only book ['Frog, where are you?' (Mayer, 1969)]. In addition, the Stuttering Severity Instrument (SSI-4; Riley, 2009) was used to examine the frequency and duration of disfluencies occurring in the speech sample, as well as any physical concomitants associated with moments of stuttering; all of these measures were incorporated into a composite stuttering severity rating. To determine measurement reliability of the Stuttering Severity Instrument score ratings, an intraclass correlation coefficient (ICC) was calculated based on two independent judges' ratings of SSI from a random subset (~44%) of the childrens' speech samples.

In addition to the speech-language and cognitive tests, all children were trained during a separate visit with a mock MRI scanner to familiarize them with the MRI environment and procedures, and to practice keeping still while lying down inside the bore for stretches of time. Recordings of MRI scanning noises were played during this session, so that children were aware that they would be hearing loud MRI sounds during scanning. This session was repeated in some children, as needed.

All procedures used in this study were approved by the Michigan State University Institutional Review Board. All children were paid a nominal remuneration, and were given small prizes (e.g. stickers) for their participation.

MRI acquisition

All MRI scans were acquired on a GE 3T Signa® HDx MR scanner (GE Healthcare) with an 8-channel head coil. During each session, 180 T_1-weighted 1-mm^3 isotropic volumetric inversion recovery fast spoiled gradient-recalled images (3D IRFSPGR) (10 min scan time), with CSF suppressed, were obtained to cover the whole brain with the following parameters: time of echo = 3.8 ms, time of repetition of acquisition = 8.6 ms, time of inversion = 831 ms, repetition time of inversion = 2332 ms, flip angle = 8°, field of view = 25.6 cm × 25.6 cm, matrix size = 256 × 256, slice thickness = 1 mm, and receiver bandwidth = ±20.8 kHz.

After the T_1 data acquisition, first and higher-order shimming procedures were carried out to improve magnetic field homogeneity. Then DTI data were acquired with a dual spin-echo echo-planar imaging sequence for 12 min and 6 s with the following parameters: 48 contiguous 2.4-mm axial slices in an interleaved order, field of view = 22 cm × 22 cm, matrix size = 128 × 128, number of excitations (NEX) = 2, echo time = 77.5 ms, repetition time = 13.7 s, 25 diffusion-weighted volumes (one per gradient direction) with b = 1000 s/mm^2, one volume with b = 0 and parallel imaging acceleration factor = 2.

One staff member sat inside the scanner room next to the child at all times to monitor the child's comfort and to ensure cooperation during scanning. During acquisition of volumetric T_1-weighted scans and DTI scans, the children viewed a movie to help them stay still.

DTI data analyses

Each subject's raw image data were examined before proceeding on to further analyses to detect any outliers in the data, including signal drop-outs, poor signal-to-noise ratio, and image artefacts such as banding. Any subject whose raw data contained 10 or more diffusion volumes with significant image quality issues was removed from further analyses. As a result, 12 subjects' fractional anisotropy data sets were removed from subsequent analysis. The remaining 77 high-quality DTI data sets [37 stuttering (21 males), 40 control (20 males)] were processed using FMRIB's diffusion toolbox (Smith *et al.*, 2004). Specifically, after eddy-current distortion and motion correction, diffusion metrics, including fractional anisotropy were calculated with the 'DTIFIT' software within this tool box to locally fit the diffusion tensors at each voxel.

Next, the TBSS procedure in FMRIB's Diffusion Toolbox (FDT) of FMRIB's Software Library (FSL) was applied to fractional anisotropy to assess local white matter diffusion characteristics. This method addresses alignment issues of fractional anisotropy data by using a non-linear registration of all subjects' fractional anisotropy data onto a common registration target (standardized into MNI152 space), and creating a 'skeletonized' mean fractional anisotropy image of tracts common to all subjects. TBSS projects all subjects' fractional anisotropy data onto this fractional anisotropy skeleton before applying voxel-wise across-subject statistics, to produce a more Gaussian distribution with less across-subject fractional anisotropy variability, resulting in a more robust and sensitive analysis of multiple subject diffusion imaging data (Smith *et al.*, 2006). We carried out TBSS analyses with the –n flag in the 'tbss_2_reg' script provided by FSL. This script enabled determination of the most 'typical' fractional anisotropy brain within our paediatric sample of brains, which was used as the target image to transform each subject's original fractional anisotropy image to standard space via non-linear transformations. Specifically, the script does this by taking each individual fractional anisotropy image and registering it to every other image, determining which image is the most representative of the whole data set. Once such a target image is determined, all other images are aligned to that target image. Namely, this procedure enables estimation of the average amount of warping that is necessary to align a given image to all other images to it; it then finds the one that has the smallest amount of average warping when used as a target. The script then takes this target and affine-aligns it into

1 × 1 × 1 mm MNI152 space for later skeletonization, display and coordinate reporting. Once this is done, each subject's fractional anisotropy image is warped to the target via non-linear transformation and then to MNI152 space via affine transformation, resulting in a transformation of the original fractional anisotropy image into MNI152 space. The fractional anisotropy values were thresholded at 0.2 to exclude grey matter voxels.

Voxel-wise statistical analyses of the whole brain fractional anisotropy maps were conducted with a permutation-based non-parametric inference *t*-test using the general linear model (10 000 permutations) with FSL's randomise program. Group and age were entered into the model to test for differences between the groups (controlling for age and handedness), main effects of age (controlling for group), and group differences in age effects (Group × Age). In addition, a separate whole-brain analysis based on only stuttering participants was conducted to examine the effect of stuttering status (stuttering severity, stuttering frequency) and sex on the fractional anisotropy measures, while controlling for age and handedness. Statistical parametric maps were thresholded at a voxel-wise $P = 0.001$ (uncorrected) with a cluster extent threshold of 10 contiguous voxels (10 mm^3). The cluster extent threshold was lowered to 5 mm^3 for regions that were determined *a priori* regions of interest (e.g. perisylvian regions along the superior longitudinal fasciculus). Although conservative corrections for multiple comparisons were not applied, this threshold is comparable to statistical thresholds used in other TBSS studies on stuttering (Connally *et al.*, 2013; Cai *et al.*, 2014b). Similar to Connally *et al.* (2013), we considered right hemisphere homologue regions along the right superior longitudinal fasciculus as significant, if the voxel-wise height threshold exceeded $P = 0.001$, and if their left hemisphere counterparts were significant.

Region of interest analyses of fractional anisotropy

In addition to whole brain-based TBSS processing, we conducted exploratory region of interest analyses to further examine the effects of sex and age in white matter voxels that were found to significantly differ between the stuttering and control groups. These regions included a number of *a priori* regions of interest along the left and right superior longitudinal fasciculus (including the IFG, premotor, motor, temporal and SMG regions) reported to differentiate between stuttering and control groups in previous investigations. We also examined other regions that were not *a priori* determined regions of interest, along white matter tracts such as the cingulum, external capsule, and cerebellum, which differed between the two groups of children based on the whole brain TBSS analysis. In addition to the stuttering-control group comparisons, we examined the effects of stuttering severity and stuttering frequency on the fractional anisotropy measures, within the stuttering group. To create the regions of interest, we first generated a spherical binary mask with a 6 mm radius, centred on the peak skeleton voxel derived from the statistical comparisons between the groups. This sphere was multiplied to the white matter skeleton mask, creating a region of interest comprising white matter tract voxels restricted within the 6 mm radius sphere, with the centre on the peak statistical voxel based on the group contrasts of fractional anisotropy. Thus the regions of interest were kept approximately equal in size across the different regions. Fractional anisotropy values were extracted from the mean value averaged across voxels in each region of interest from each subject in MNI152 space. These regions of interest included the white matter regions underlying the left IFG (BA44), left PMC (BA6), left motor cortex (M1; 4p), left posterior superior temporal gyrus, left supramarginal gyrus (SMG), left and right middle temporal gyrus (MTG), left and right external capsules, left cingulate and bilateral cerebellum. We also examined voxels where the stuttering group exhibited greater fractional anisotropy compared to the control group, such as the right IFG (BA45), left insula/MTG, and right cerebellum. We then used these regions of interest to extract fractional anisotropy values from each individual subjects' normalized skeleton fractional anisotropy data. The averaged fractional anisotropy measures from the regions of interest from each child's fractional anisotropy data were entered into a multivariate analysis of variance to examine the effects of sex (male, female), and age on fractional anisotropy values in the regions of interest, with covariates of no interest entered into the model (e.g. behavioural measures). For the region of interest analyses within the stuttering group, we created regions of interest based on clusters exceeding statistical thresholds for those regions that showed a significant negative effect of stuttering severity instrument on fractional anisotropy measures. We conducted a separate MANCOVA with sex entered as an independent factor and the total score derived from the stuttering severity instrument (SSI-4), and age, stuttering duration entered as covariates in the statistical model.

Results

Behavioural assessments

The stuttering and control groups' demographic data and results of standardized testing are shown in Table 1. As shown here, the children from the two groups did not differ in age, socio-economic status, or handedness; however they did differ on some of the standardized speech-language measures such as the Expressive Vocabulary Test, Peabody Picture Vocabulary Test (receptive vocabulary), receptive language, and verbal/full IQ. These findings are in line with other studies that involved large cohorts of children who stutter and age-matched control groups: children who stutter have been reported to exhibit speech language ability within the normal range, but with consistently decreased scores relative to age-matched controls (Ratner and Silverman, 2000; Anderson *et al.*, 2005; Coulter *et al.*, 2009). Note that our inclusion criteria required that all children score within normal range for their age. Apart from stuttering, none of the children exhibited speech/language performance considered to be delayed or atypical in development, with the exception of one child in the stuttering group and two children in the control group who scored below norm (−1 SD) on the Goldman-Fristoe Test of Articulation; and two other children who stutter and one other control child who scored below norm (−1 SD) in the receptive language test. None of the children scored below −2 SD of the norm on any

Table 1 Subject demographic information

Measure	Control ($n = 40$, 20 females)	Stuttering ($n = 37$, 16 females)	t	df	P (two-tail)
Age (years)	6.34 (2.07)	6.35 (2.07)	−0.030	75	0.976
Edinburgh handedness quotient	55.18 (59.22)	60.22 (55.37)	−0.385	75	0.701
Mother's education	6.39 (0.64)	6.28 (0.78)	0.708	72	0.481
Weschler Abbreviated Scale of IQ (WASI) Full IQ	114.69 (0.96)	103.86 (22.23)	2.509	74	0.014*
WASI Performance IQ score	111.38 (16.50)	106.92 (13.46)	1.289	74	0.201
WASI Verbal IQ score	116.97 (15.32)	105.54 (15.01)	3.284	74	0.002**
Peabody Picture Vocabulary Test (PPVT-4)	117.03 (13.06)	109.76 (13.38)	2.411	75	0.018*
Expressive Vocabulary Test (EVT-2)	115.64 (14.24)	106.62 (12.51)	2.928	74	0.005**
Goldman-Fristoe Test of Articulation (GFTA-2)	104.70 (9.20)	104.49 (8.65)	0.105	75	0.917
Receptive Language Quotient (based on TOLD or TACL)	104.50 (12.06)	97.71 (10.79)	1.825	37	0.076
Per cent stuttering-like disfluencies (SLD)	1.12 (0.89)	5.95 (5.61)	−5.312	74	<0.00001**
Per cent other disfluencies (OD)	4.83 (2.41)	5.59 (2.67)	−1.303	74	0.1966
Stuttering Severity Instrument (SSI)	N/A	21.05 (8.02)	N/A	N/A	N/A

Age, socioeconomic status, standardized speech/language, cognitive assessment scores, and fluency assessment data. TACL = Test for Auditory Comprehension of Language; TOLD = Test of Language Development. *$P < 0.05$ **$P < 0.01$.

standardized test administered. Although the present findings of decreased performance on speech/language measures in children who stutter parallel previous reports, we thought it important to control for these measures when examining group differences in the brain data. Hence, we examined the relationship between each of these speech/language dimensions and the fractional anisotropy measurements. Additionally, behavioural measures exhibiting significant correlation(s) with the dependent variables were entered as covariates of no interest into any subsequent statistical comparisons of fractional anisotropy between the groups.

For children who stutter, average time since stuttering onset (duration of stuttering) was 39.3 months (SD =24.4; range 6–90 months) and stuttering onset age according to parent report was at an average of 38.7 months (SD = 15.1; range 18–77 months), which is consistent with previously reported typical onset age for stuttering (Yairi and Ambrose, 1992). Children who stutter exhibited average per cent stuttering per syllables at 5.08 (SD = 4.65, range 1–25.7). Children who stutter had an average SSI score of 21.1 (SD = 8.02; range 10–48) corresponding to a 'moderate' stuttering severity. The stuttering severity among the participants ranged from very mild to very severe. The intra-class correlation coefficient for the overall SSI measurement between two independent judges was 0.98. Per cent stuttered disfluencies (e.g. sound-syllable repetitions, monosyllabic word repetitions, audible and inaudible sound prolongations), and normal disfluencies (e.g. interjections, phrase repetitions, revisions, etc.) were also examined in both groups.

Decreased fractional anisotropy in children who stutter compared to age-matched controls

Relative to controls, children who stutter exhibited decreased fractional anisotropy values along the left superior longitudinal fasciculus, including the IFG (BA 44), premotor (BA6), motor (4p), STG/MTG, and inferior parietal areas (BA39/40). Right-sided decreases were also noted, although smaller in extent than group differences found in the left hemisphere homologues, in the right IFG (BA44), MTG/STG, and SMG. There was also decreased fractional anisotropy noted in the cerebellum, brainstem and the corpus callosum (genu, body and splenium) for stuttering compared to the control group (Table 2 and Figs 1 and 2).

Age and sex effects

The whole brain TBSS analyses examining group differences in fractional anisotropy with age (i.e. Group × Age interaction) revealed that controls consistently exhibited greater fractional anisotropy increases with age compared to children who stutter in most areas of the brain, including the left superior longitudinal fasciculus areas (Table 3). There were no regions where children who stutter exhibited greater fractional anisotropy increases with age compared to controls.

Fractional anisotropy values were extracted from each subject's fractional anisotropy skeleton maps, from regions of interest defined on the white matter voxels centred on the peak statistical voxel that showed significant group differences (control versus stuttering) noted above. Multivariate analysis of variance (MANCOVA), with the covariates of no interest, Peabody Picture Vocabulary Test, Expressive Vocabulary Test, full-scale IQ, Receptive Language Score, and handedness were performed to confirm the effects of age and sex on fractional anisotropy values from each region of interest, shown in Fig. 2. (Full IQ was entered as a covariate instead of the verbal IQ, as both scores are highly correlated. Full IQ was favoured over verbal IQ, to reduce possible effects of multicollinearity in the model as verbal IQ is also correlated with other speech-language measures).

Table 2 Regions showing significant group differences in fractional anisotropy based on the whole brain (TBSS) analyses

Regions (approximate Brodmann areas) (left/right)	Cluster size (mm^3)	max t	x	y	z
Controls > Stuttering					
Precentral gyrus (4a/6) (L)	36	4.76	−42	−7	46
CC—body/cingulate gyrus (24) (L)	33	4.55	−15	−9	36
MTG/STG/SLF (41/22) (L)	32	4.48	−44	−42	7
CC—body/cingulate gyrus (24/32/6) (L)	29	4.05	−16	2	40
Precentral gyrus/postcentral gyrus/ SLF (4p) (L)	19	4.60	−36	−12	38
Cerebellum (tonsil) (L)	19	4.14	−24	−57	−35
Precentral gyrus/paracentral lobule (4a/6) (L)	19	3.76	−13	−21	63
MTG/SLF (R)	15	3.81	45	−43	2
MTG/STG (19/22) (R)	15	3.62	34	−57	18
Anterior cingulate/CC—genu (24) (L)	10	4.01	−9	27	10
Cerebellum (tonsil) (R)	10	3.58	19	−34	−30
MTG/AG/STG/SMG/SLF (39) (L)	9	3.40	−34	−58	29
IFG—p.o./SLF (44) (L)	8	4.32	−48	8	17
STG/MTG/AG (22/39) (L)	8	3.57	−34	−52	21
Posterior cingulate/CC—splenium (23) (L)	7	3.74	−11	−33	23
IFG—p.o./SLF/insula (44) (R)	5	3.76	46	8	18
IPL/SMG/SLF (40) (R)	5	3.34	38	−34	32
IFG—p.o./MFG (44/9) (R)	3	3.64	46	12	29
SMA/SFG (6) (L)	3	3.48	−10	0	67
Postcentral gyrus/precentral gyrus/SLF (3/4) (L)	3	3.42	−37	−19	35
IFG—p.t.(45) (L)	3	3.22	−38	36	6
Stuttering > Controls					
Insula/STG (13/41) (L)	14	3.54	−31	−37	18
IFG—p.t.(45) (R)	14	3.52	43	24	16
Cerebellum (culmen)/substantia nigra (L)	8	4.03	−8	−27	−14
Cerebellum (uvula) (R)	8	3.46	2	−65	−30

AG = angular gyrus; CC=corpus callosum; IPL = inferior parietal lobule; MFG = middle frontal gyrus; p.o.= pars opercularis; p.t.= pars triangularis; SFG = superior frontal gyrus; SMA = supplementary motor area; L = left; R = right.

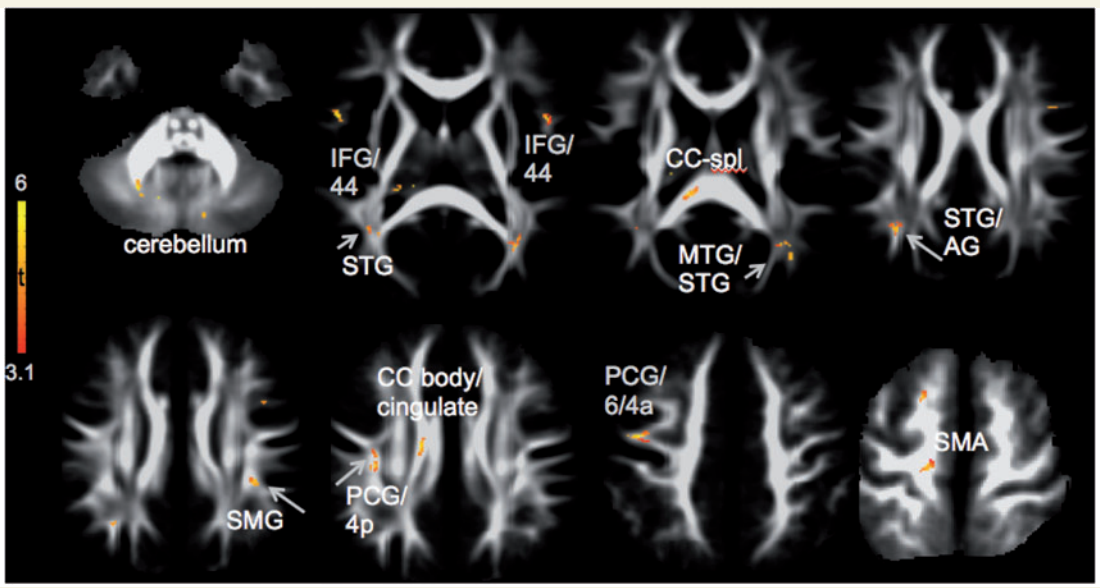

Figure 1 White matter regions showing significant group differences in fractional anisotropy based on whole-brain analysis (TBSS). Coloured highlights show areas with significantly decreased fractional anisotropy in children who stutter compared to controls. AG = angular gyrus; CC-spl = corpus callosum (splenium); PCG = precentral gyrus; SMA = supplementary motor area.

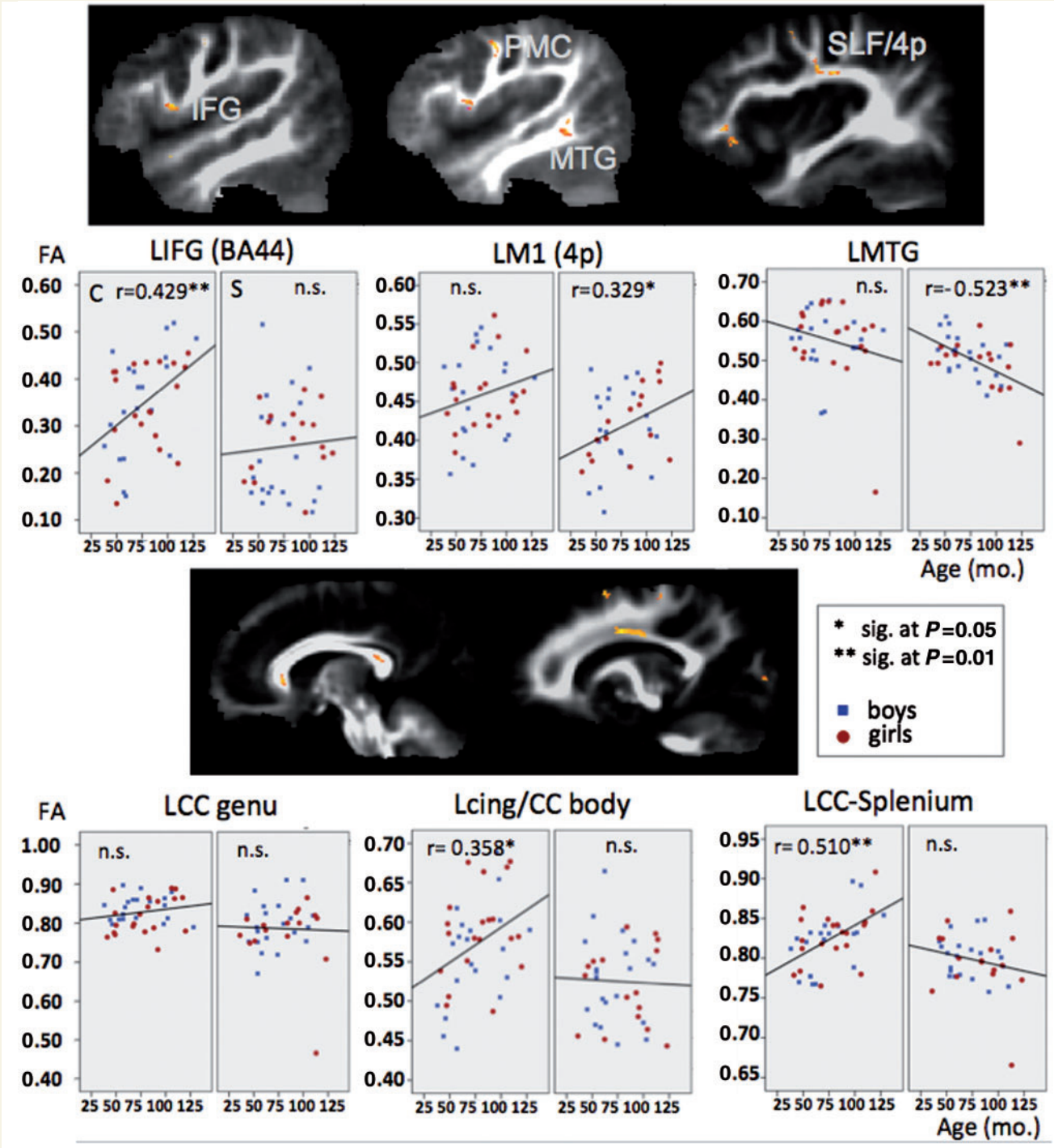

Figure 2 Scatter plots of fractional anisotropy values extracted from left hemisphere regions of interest, shown in relation to age (25–125 months). In each scatter plot, the *left* panel shows the control children's data; the *right* panel, the data for children who stutter. LCC = left corpus callosum; Lcing = left cingulate; LM1 = left primary motor cortex; L = left; mo = months; SLF = superior longitudinal fasciculus; LIFG = left IFG; FA = fractional anisotropy; n.s. = not significant.

Multivariate tests (Wilks' λ) revealed a significant effect of age ($F = 2.970$, $P = 0.003$). Sex effect was not significant ($F = 0.561$, $P = 0.863$). The Group × Sex effect was not significant ($F = 0.614$, $P = 0.820$). None of the speech measures (Peabody Picture Vocabulary Test: $F = 0.835$, $P = 0.614$; Expressive Vocabulary Test: $F = 0.773$, $P = 0.674$; receptive language: $F = 1.670$, $P = 0.103$; full IQ: $F = 1.079$, $P = 0.398$), or handedness were significant ($F = 0.324$, $P = 0.981$). Given the significant effect of age, we subsequently conducted exploratory univariate ANOVAs to

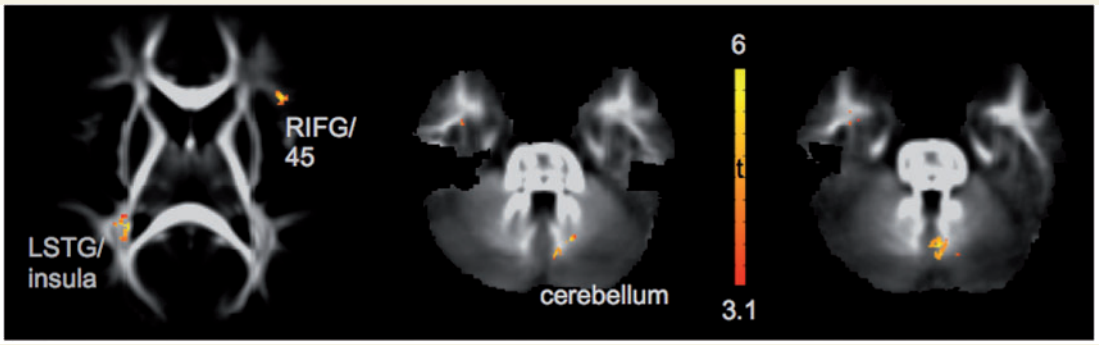

Figure 3 White matter regions showing significantly increased fractional anisotropy in children who stutter compared to controls, based on whole brain analysis (TBSS). LSTG = left superior temporal gyrus; RIFG = right IFG.

Table 3 Regions showing significant group by age interactions based on whole brain TBSS analyses

Regions (approximate Brodmann areas) (left/right)	Cluster size (mm^3)	max *t*	*x*	*y*	*z*
Controls > CWS: age					
Cingulate gyrus/CC-body (24/31) (R)	53	4.18	21	−20	38
Insula/claustrum (13) (L)	51	3.91	−26	24	6
Cingulate gyrus/CC-body (31) (R)	29	4.39	20	−23	36
Cingulate gyrus/CC-body (24) (L)	29	4.06	−9	12	28
Anterior cingulate/MFG (32/10) (L)	29	3.97	−12	47	−6
SPL/precuneus (7/5) (R)	26	5.23	20	−45	57
STG/MTG (38/21) (R)	20	4.82	40	7	−26
Thalamus/caudate (R)	20	4.34	9	−5	17
Posterior cingulate gyrus/CC-splenium (31) (L)	20	4.03	−14	−39	21
Superior occipital gyrus/cuneus (18) (L)	18	4.54	−14	−84	26
Insula/putamen/IFOF (13) (L)	18	4.29	−24	21	−2
Postcentral gyrus/precentral gyrus RO (6/4/3) (R)	16	4.26	57	−2	19
Superior medial gyrus/SFG (9) (R)	16	3.93	13	55	29
Precentral gyrus (4/6) (R)	15	4.00	16	−24	59
ITG/MTG (21/20) (R)	14	4.71	58	−24	−15
Medial FG/Forceps minor (10) (L)	14	4.23	−10	51	−7
Calcarine gyrus/cuneus (17/18) (L)	13	4.31	−17	−75	15
Fusiform gyrus/inferior occipital gyrus (R)	13	4.05	32	−76	−5
Postcentral gyrus/precentral gyrus (1/2/3/4) (L)	13	3.67	−29	−32	63
IFG-p.o./p.t. (44/45) (L)	13	3.63	−45	31	23
MTG (21/22) (L)	11	4.46	−45	−34	−1
Posterior cingulate/CC-splenium (23) (L)	11	3.96	−4	−28	24
SFG/SMA/medial FG (6/24) (R)	10	3.70	18	2	50
MTG/AG/STG (22/39/13) (R)	10	3.66	38	−48	21
Postcentral gyrus/ precentral gyrus (3/4) (R)	10	3.55	34	−21	34
Medial FG	10	3.39	28	31	15

AG = angular gyrus; CC = corpus callosum; FG = frontal gyrus; IFOF = inferior frontal occipital fasciculus; ITG = inferior temporal gyrus; MFG = middle frontal gyrus; p.o.= pars opercularis; p.t.= pars triangularis; RO = rolandic operculum; SFG = superior frontal gyrus; SMA = supplementary motor area; SPL = superior parietal lobule.

examine the effects of age on each of the regions of interest. Age effect was not significant at a Bonferroni corrected $P = 0.004$ in any of the areas examined, however the L4p ($F = 5.613$, $P = 0.021$), left IFG ($F = 4.886$, $P = 0.030$), left MTG ($F = 8.020$, $P = 0.006$), right MTG ($F = 4.814$, $P = 0.031$) reached significance at an uncorrected $P = 0.05$.

Given the significant Group × Age interaction shown in many of these same areas based on the whole brain analysis, we conducted correlation analyses to derive Pearson correlation coefficients within each group to explore fractional anisotropy changes with age. The scatter plots revealed group differences between the stuttering and

control groups with regard to fractional anisotropy changes with age (Fig. 2).

Heightened fractional anisotropy in children who stutter compared to age-matched controls

Whole brain-based analyses showed that children who stutter exhibited increased fractional anisotropy relative to controls in the right IFG (BA45), adjacent to the right IFG (BA44) region which was found to be decreased compared to controls (see above). Children who stutter also showed increased fractional anisotropy in the left superior temporal gyrus/posterior insular junction, and in the vermis and right cerebellum compared to the controls (Table 2).

The multivariate analyses of variance based on exploratory region of interest analyses to determine the effects of age and sex with behavioural covariates (see above) found that neither age ($F = 0.909$ $P = 0.442$) nor sex effects ($F = 0.416$, $P = 0.742$) were significant.

Relationship between fractional anisotropy and stuttering severity

A separate whole brain TBSS analyses was conducted within the stuttering group, to examine the effects of stuttering severity on fractional anisotropy in each subject. There was significant negative effect of SSI on fractional anisotropy values, especially along major left white matter tracts including the left IFG (BA44), left precentral gyrus/M1 (4p) and left SMG; fractional anisotropy values in the left cingulate, bilateral cerebellar tonsils, and bilateral external capsules also exhibited negative correlation with fractional anisotropy (Fig. 4 and Table 4). Similar findings were observed when stuttering frequency, rather than SSI values were entered into the general linear model as an explanatory variable for fractional anisotropy changes (Supplementary Fig. 1 and Supplementary Table 1; cluster overlap in areas showing significant effect of both stuttering frequency and severity bolded in Table 4 and Supplementary Table 1).

Effects of sex and stuttering duration

We examined whether regions showing significant negative effects of SSI on fractional anisotropy based on the whole brain TBSS analyses were maintained when age and other behavioural measures were controlled for. We conducted the region of interest analyses using multivariate analyses of variance with age, Peabody Picture Vocabulary Test, Expressive Vocabulary Test, full IQ, and receptive scores as covariates of no interest. We also examined whether the effects were modulated by stuttering duration. The multivariate tests were significant for sex ($F = 3.875$, $P = 0.012$), and stuttering duration ($F = 4.604$, $P = 0.006$). None of the other behavioural measures or the age effect were significant. *Post hoc* univariate ANOVAs showed that none of the regions examined survived Bonferroni correction at $P = 0.005$ for stuttering duration. There was a significant sex effect in the left external capsule ($F = 10.515$, $P = 0.003$), with left IFG ($F = 5.566$, $P = 0.025$), left SMG ($F = 5.336$, $P = 0.028$), left cerebellum ($F = 4.301$, $P = 0.046$) and right cerebellum ($F = 6.572$, $P = 0.015$) reaching significance at an uncorrected $P = 0.05$ (Fig. 4). When scatterplots were constructed to explore the correlation (partial correlation with age partialled out) between stuttering severity and fractional anisotropy values in regions of interest within each sex, it was revealed that correlation coefficients were influenced by a large extent to the three most severe cases (all boys). When these cases were excluded from analysis, only the correlation involving the left SMG and left external capsule remained significant (Fig. 4). When stuttering frequency (per cent stuttered utterances /total syllables) was entered for correlation analysis with fractional anisotropy, with the same extreme cases excluded, the left red nucleus ($r = -0.449$, $P = 0.035$) and left cingulate ($r = -0.434$, $P = 0.041$) showed significant negative correlation between stuttering frequency and fractional anisotropy values.

Discussion

Summary of main group differences

In the largest paediatric neuroimaging study of stuttering to date, we examined differences in white matter development to ascertain possible neuroanatomical differences underlying childhood stuttering. Whole brain-based, as well as exploratory region of interest based comparisons of white matter development were conducted to investigate differences between children who do and do not stutter. The effects of age, sex, and stuttering severity on white matter measures were also examined. We used TBSS to extract fractional anisotropy, a measure that reflects white matter development. In the context of brain development and stuttering, a lower fractional anisotropy value in certain areas may reflect less robust development and connectivity among regions that form critical networks for efficient and rapid interaction that support complex skills such as speech production.

The whole brain-based comparison of groups showed significant decreases in fractional anisotropy in children who stutter compared to age-matched controls; strongest differences were found in the left hemisphere white matter regions underlying sensorimotor cortical regions such as the IFG, PMC, motor cortex (M1), middle/superior temporal gyri (MTG/STG), and inferior parietal areas. There were also subtle fractional anisotropy decreases in homologous right hemisphere regions encompassing frontal and temporoparietal areas. Additional significant fractional anisotropy decreases in children who stutter were found in the corpus callosum (genu, body and splenium), cingulum and the

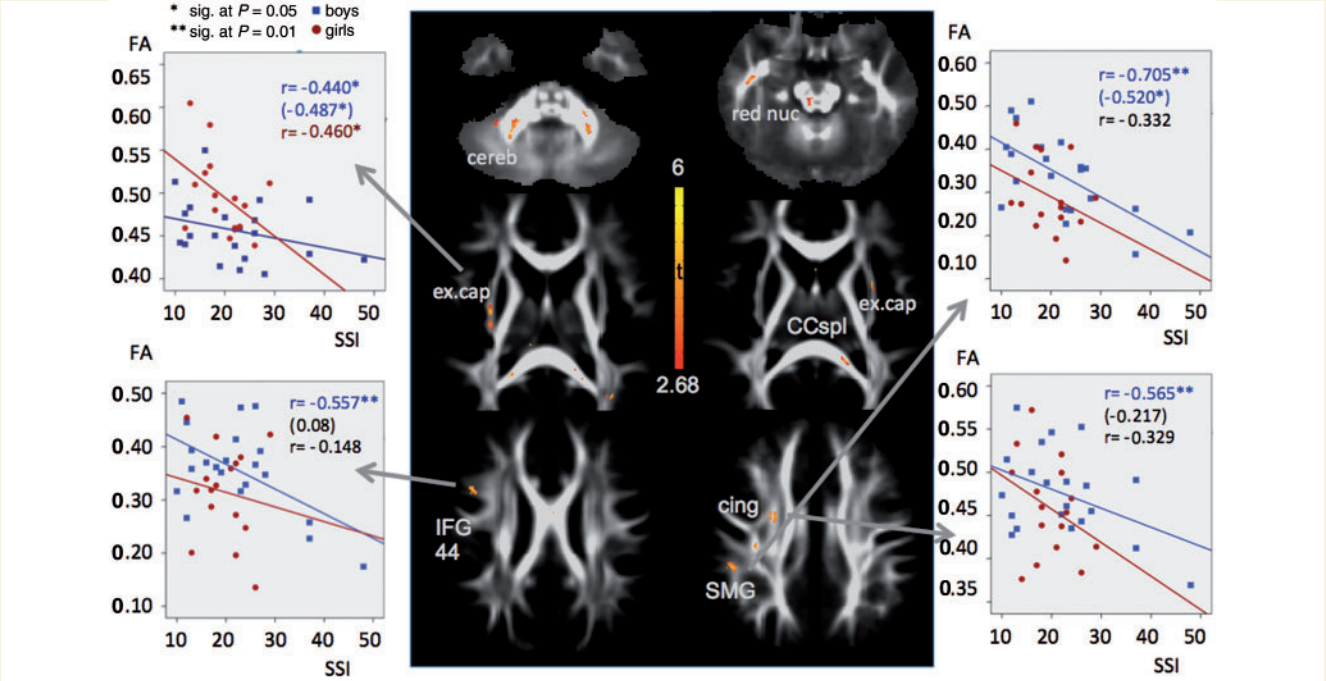

Figure 4 White matter regions showing significant negative effect of stuttering severity scores (as measured with the stuttering severity instrument) on fractional anisotropy. Scatter plots show fractional anisotropy (FA) values extracted from regions of interest based on the whole brain analyses, to explore sex differences by age (see text for more details). Boys who stutter generally drove the negative correlation between fractional anisotropy and stuttering severity. The r-values shown in upper rows in each scatterplot represent partial correlation coefficients derived from boys only, and the lower value represents those from the girls only. The r-values within parentheses show r-values based on calculations excluding the three most severe cases that occurred among boys who stutter. When these values were removed, only the left external capsule (ex. cap) and the left SMG remained significant. cereb = cerebellum; CCspl = corpus callosum (splenium); cing = cingulate.

cerebellum. Notably, given our relatively large sample size we were also able to find previously unreported fractional anisotropy increases in children who stutter compared to controls, in the right IFG (BA45), left STG/posterior insula, and in the right cerebellum.

These results show extensive white matter structural differences in children who stutter compared to age-matched controls, suggesting deficits in long-range connectivity that support efficient sensorimotor and interhemispheric integration, and cortical-subcortical interaction for skilled movement control.

White matter development differentiates children who stutter from non-stuttering peers

Many of the areas that showed significant group differences (control > stuttering), were also those areas where children who stutter exhibited altered patterns of age-related fractional anisotropy changes. In many regions (in particular the left IFG), group differences tended to become greater with age. These results suggest white matter developmental changes involving regions supporting sensory-motor and interhemispheric integration in children who stutter are affected, which may further affect brain networks that support fluent speech acquisition across development (during later school-age and beyond). The age-matched children who do not stutter seemed to show consistent fractional anisotropy increases across most areas examined, whereas children who stutter exhibited discrepancies in white matter development across these same regions, some showing accelerated fractional anisotropy increases, and in others significant decreases or no changes at all. For example, in the left IFG (BA44), controls showed linear fractional anisotropy increases with age, whereas children who stutter did not, suggesting that this white matter difference may become more prominent with persistent stuttering. Similar findings were shown in the right MTG, and the corpus callosum (body and splenium). Children who stutter exhibited generally decreased fractional anisotropy compared to children who do not stutter in the left M1 (4p) but showed accelerated fractional anisotropy increases with age compared to controls. Children who stutter also showed fractional anisotropy decreases with age in the left middle temporal gyrus. This apparent discrepancy among brain areas in the developmental trajectory of fractional anisotropy (i.e. general stagnation or even decreases in fractional anisotropy in many regions examined, but accelerated fractional anisotropy increases in certain areas

Table 4 Regions showing significant negative effects of stuttering severity (measured with SSI) on fractional anisotropy

Regions (approximate Brodmann areas) (left/right)	Cluster size (mm^3)	max *t*	*x*	*y*	*z*
IFOF/forceps major (17) (R)	90	5.49	31	−69	5
Cerebellum (tonsil) (L)	58	4.69	−22	−49	−33
SLF/Precentral gyrus (L)	57	3.77	−26	−1	35
IFOF (R)	28	3.74	30	−26	−2
MTG/ILF/IFOF (21) (L)	20	3.42	−46	−12	−12
Cerebellum (tonsil) (R)	15	3.20	21	−45	−34
External capsule/SLF/insula (13) (R)	14	3.32	32	2	15
ILF/UF (R)	14	3.20	37	8	−31
IFOF (L)	13	3.41	−31	−23	0
IPL/SMG (40) (L)	11	4.41	−50	−32	38
Red nucleus/substantia nigra (L)	10	4.09	−5	−27	−11
Cingulate gyrus (31) (R)	9	3.93	20	−46	22
Precentral gyrus/postcentral gyrus (6/4/3) (L)	9	3.12	−46	−4	29
Cerebellar (tonsil) (L)	8	4.40	−30	−43	−36
IFG-p.t., p.o./MFG (45/44/9) (L)	8	4.18	−47	17	28
SLF/EC (L)	8	3.67	−30	−15	21
IFOF/ILF (R)	8	3.62	35	−57	21
IFOF/STG (41) (L)	8	3.40	−34	−36	13
EC/claustrum (L)	8	3.38	−28	10	22
IFOF/ILF (R)	8	3.37	29	−31	1
IFOF/ILF/Insula (13) (L)	8	3.32	−42	−13	−7
IFOF/ILF/lentiform nucleus/putamen/caudate (R)	8	3.27	33	−16	−7
IFOF/MTG (39) (R)	8	3.11	41	−50	6
SLF (L)	8	3.11	−35	−19	38

The bold regions indicate those areas that were also significant when stuttering frequency rather than SSI was used as an explanatory variable. EC = external capsule; IFOF = inferior frontal occipital fasciculus; ILF = inferior longitudinal fasciculus; IPL = inferior parietal lobule; p.o.= pars opercularis; p.t. = pars triangularis; SLF = superior longitudinal fasciculus; SMA=supplementary motor area; SMG = supramarginal gyrus; UF = uncinate fasciculus.

such as the motor cortex), points to the possibility of uneven growth in the white matter tracts critical for speech production in children who stutter. It is likely that these differences may become more exaggerated with age as stuttering persists.

These developmental differences, compounded by existing aberrant speech networks, may be exacerbated with continued stuttering and likely result in adaptive changes. Such adaptations and compensatory processes are likely to be individual-specific, and hence could lead to variable changes in white matter development with age. Some of the inconsistent results that have been reported in the past based on adults who stutter may partly be due to such individual specific compensatory processes, in addition to subject characteristics, scanning parameters, and analyses that differed across the different studies.

White matter changes in some regions may support more fluent speech in children who stutter

In addition to examining overall group and age-related differences, we also examined whether white matter development as reflected through fractional anisotropy was associated with stuttering severity. In more severe cases of stuttering, lower fractional anisotropy values were found in the left IFG, left M1, left cingulate, left inferior parietal lobule (SMG), and corpus callosum (splenium). These results plausibly suggest that 'normalized' connectivity in these areas supports more fluent speech within the stuttering group. Additional areas that did not differentiate the two groups in the main group contrast emerged as being negatively correlated with fractional anisotropy; these areas included the bilateral cerebellar tonsils, left red nucleus, and the bilateral external capsules. The most severe cases tended to show the lowest fractional anisotropy values in these regions. These results were driven to a large extent by three of the most severe cases (all boys) within our participant pool. When the three most severe cases were excluded from analysis, only the left external capsule and left SMG remained significant in their negative correlation with stuttering severity. The left external capsule contain fibres that interconnect posterior auditory regions to inferior frontal areas through a ventral pathway (Kelly *et al.*, 2010). In a previous report using probabilistic tractography, the white matter tracts in the external/extreme capsule were found to be significantly decreased in tract density in children who stutter compared to controls (Chang and Zhu, 2013). While children who stutter as a group exhibit decreased white matter coherence in this tract compared to controls, greater development here seems to

correlate with better fluency among children who stutter. With regard to SMG, Connally *et al.* (2013) reported similar findings where a neighbouring inferior parietal region, the angular gyrus, was found to be negatively correlated with stuttering severity. SMG is a somatosensory cortex that borders the inferior parietal cortex; both regions have interconnections with the laryngeal motor cortex, which is important for integration of proprioceptive and tactile feedback from the orofacial, respiratory, and laryngeal regions during voice production (Simonyan and Horwitz, 2011). Greater white matter coherence in this region, as well as in the left external capsule mentioned above, within the stuttering group may predict better fluency outcomes, and may indicate important regions that may support successful recovery. Furthermore, because the currently reported stuttering severity-related analyses are based on cross-sectional samples in this study, a future investigation involving longitudinal tracking of these same subjects is expected to clarify whether fractional anisotropy increases in some areas are specifically associated with achieving more fluent speech, or are associated with less successful adaptations to stuttering.

The negative correlations between stuttering severity and fractional anisotropy were predominantly found in boys but not in girls who stutter, with the exception of the left external capsule, where girls who stutter showed greater negative correlation with stuttering severity than in their male counterparts; and in the left SMG, where both boys and girls who stutter showed a trend toward negative correlation between the two measures. For the reason that we had fewer numbers of girls than boys who stutter, and the girls in our sample did not exhibit the full range of stuttering severity as observed in the boys who stutter, it would be of interest to confirm the current findings with a larger sample of girls who stutter who manifest severity levels comparable to their male counterparts. We also expect that more girls than boys will recover from stuttering with age (Seider *et al.*,1983; Ambrose *et al.*, 1997), and hence our planned longitudinal analyses of these same subjects is expected to elucidate sex-specific patterns of white matter development that are associated with recovery from stuttering versus persistent stuttering.

In addition to examining fractional anisotropy changes based on stuttering severity measures (SSI), we also explored fractional anisotropy changes when stuttering frequency was entered as an explanatory variable. The results were largely comparable with that found with severity measures, however with some interesting differences that may warrant further examination in future studies. Stuttering severity is based on consideration of stuttering frequency, average duration of the three longest stuttering instances, and physical concomitants. This measure likely provides a more comprehensive profile of a child's stuttering status, especially when one considers the inherent variable nature of stuttering frequencies depending on day, conversational partner, and setting. However, an argument can be made that stuttering frequency may provide a more direct measure that correlates with brain function and anatomy. Jiang *et al.* (2012) have attempted to differentiate between different stuttering symptoms (e.g. prolongations versus word repetitions) based on brain activity. Future studies that examine potential differences in brain anatomy and function within the stuttering group, based on the frequency of different types of disfluencies, may provide insights into possible subtypes of stuttering.

Relation to previous literature and insights into possible pathophysiological bases of developmental stuttering

The majority of previous investigations of brain differences in stuttering have focused on adults. Because stuttering onset typically occurs during early childhood, examining children who stutter near stuttering onset can provide a way to interpret previous reports about what may be attributed to aetiological factors of stuttering, versus compensatory, or adaptations to stuttering itself. Strikingly, many of the previously reported white matter differences found in older children and adults who stutter were confirmed in younger stuttering children for the first time in this study. The present results confirm previously reported decreases in fractional anisotropy (reflecting white matter integrity) in white matter tracts interconnecting perisylvian cortical areas that support fluent speech, such as the left IFG, PMC, M1, MTG/STG, and inferior parietal lobule (SMG and angular gyrus). While the group differences were greatest along the left hemisphere white matter tracts, we also found evidence to support subtle fractional anisotropy decreases in regions along the homologous right hemisphere tracts including the right IFG, M1, and SMG. Furthermore, fractional anisotropy was decreased compared to controls in corpus callosum, cingulum and cerebellar regions.

The white matter regions showing the most significant group differences comprised those regions established in previous studies to support auditory-motor and somatosensory-motor integration for speech motor production (left IFG, BA6, M1, STG, inferior parietal lobule). The integration between motor areas (inferior frontal, premotor and motor areas) and posterior secondary auditory areas is crucial for acquiring and maintaining fluent speech production. Raushecker and Scott (2009) proposed feed-forward and feedback loops between these regions, with the inferior parietal lobe acting as an integrating area for speech processing (Rauschecker and Scott, 2009). During the initial stages of speech acquisition, motor speech production is heavily reliant on sensory feedback, but as speech becomes an established skill and motor programs are stored with sensory expectations associated with them, the feed-forward processes become predominant, consequently obviating heavy reliance on delayed feedback signals (Guenther, 2006; Golfinopoulos *et al.*, 2009). Tight

interconnections between the auditory and premotor regions are demonstrated during sensory perturbation tasks where increased activity in both regions are associated with better online modification and control through self-monitoring of one's own vocalizations (Chang *et al.*, 2013). These studies provide an explanation as to why typically fluent individuals are able to effortlessly adjust their speech to unexpected perturbations. In such experiments involving auditory as well as somatosensory perturbations, speakers who stutter show attenuated or slower compensation responses, which may reflect less efficient interaction between the sensorimotor areas (Cai *et al.*, 2011, 2014a). It is not clear whether this might be the case in children who stutter as well, however, given the current findings of widening fractional anisotropy changes with age, these behavioural differences may also diverge more with age, showing greater differences in adults.

Attenuated connectivity among auditory-motor regions reveals only part of the picture of stuttering. It is well known that regardless of stuttering severity, most people who stutter can, from time to time, speak completely fluently, suggesting that fundamental auditory-motor integration for speech production is present and functional, albeit with low thresholds for breakdown. Hence, while strong evidence exists for attenuated interconnectivity between motor and auditory regions in the left hemisphere, these are likely influenced by connectivity with subcortical areas as well as homologous regions in the right hemisphere.

The finding of decreased fractional anisotropy in children who stutter relative to controls in the homologous right hemisphere dorsal stream regions was somewhat unexpected. Given findings of hyperactivity in the right hemisphere areas in adults who stutter, one might expect greater but not less corpus callosum and right hemisphere involvement in children who stutter. A recent study involving resting state functional MRI with children who stutter also found that functional connectivity between the IFG and posterior auditory regions was decreased bilaterally in children who stutter, particularly in boys (Chang and Zhu, 2013). Collectively, structural evidence for bilateral decreases in areas supporting sensorimotor function for speech in children who stutter, but left-sided decreases in the face of right-sided increases in adults who stutter may indicate a lack of age-appropriate increases in left-sided laterality in children who stutter. In typically developing children, it has been shown that left IFG development becomes more lateralized with age (Lu *et al.*, 2006; Szaflarski *et al.*, 2006; Holland *et al.*, 2007), possibly supporting better phonological and other speech related skills as the child develops.

The attenuated corpus callosum findings in children who stutter lends additional support for a weak left lateralization for speech and language function: in one study examining normally fluent speakers, the midsagittal area of the corpus callosum was shown to be associated with greater left lateralization for language (Josse *et al.*, 2008). Two studies examined the corpus callosum area and volume in children and adults who stutter. The first study with children between 9 and 12 years of age did not find differences in the corpus callosum area or volume among those with persistent stuttering, those who recovered from stuttering and typically developing children (Choo *et al.*, 2012). However, the overall area of the corpus callosum, and area of the rostrum and anterior midbody [regions which connect the (pre)frontal, (pre)motor and supplementary motor cortices] were larger in adults with persistent stuttering compared to controls; additionally, adults with persistent stuttering also featured an increased cluster of white matter in the rostrum (Choo *et al.*, 2011). These results pointed to region-specific differences in corpus callosum morphological measures being greater in adults who stutter than in children who stutter, suggesting that interhemispheric integration may become more aberrant with age in stuttering speakers. The present results based on DTI may not be readily comparable to the morphology studies mentioned here, which also had a tighter age range and much lower *n*. However, based on present results showing attenuated age-related increases in the left speech motor and auditory regions as well as corpus callosum segments, we posit that greater right-sided involvement, supported by greater corpus callosum area and volume in mostly genu and mid-body regions, may be required with increasing age, leading to heightened group differences as adults in the right homologue.

Aberrant white matter development in the corpus callosum may also suggest deficient interhemispheric connectivity that supports integration of articulation with suprasegmental features such as prosody and rhythm (Karniol, 1995); the latter of which is supported by right hemisphere structures (Gunji *et al.*, 2007; Lappe *et al.*, 2013). In a recent investigation we found that children who stutter performed significantly worse on a rhythm discrimination task compared to age-matched controls (Wieland *et al.*, 2014). This indicates that children who stutter may have an inherent difficulty with rhythm perception and production, which relies on internal timing. The ability to internally generate rhythmic and precisely timed movements, such as in the case of speech production, relies not only on the sensorimotor networks but also on the subcortical systems that form a functional circuit with these cortical regions. In fact, those who displayed better ability in the internal generation of rhythm showed greater activity in a network of regions that included the putamen, supplementary motor area, PMC, insula, and posterior superior temporal gyrus (Grahn and McAuley, 2009). Interestingly, it is well known that people who stutter can be completely fluent when given an external rhythm (e.g. metronome) to pace their speech; a functional MRI study reported that such fluency-inducing conditions enhanced activity in all of these network regions shown to support rhythm processing (Toyomura *et al.*, 2011). Based on functional connectivity analyses of resting state functional MRI data, we reported that children who stutter had reduced functional connectivity among the supplementary motor area, putamen,

and auditory areas compared to age-matched controls (Chang and Zhu, 2013). However, the present study was focused on white but not grey matter, and as such, we are unable to confirm fractional anisotropy differences in the putamen. We did, however, find subtle group differences in the supplementary motor area, which has known substantial connectivity with the putamen, as well as the other areas supporting rhythm processing.

There were some mixed results involving the cerebellum in the present study that warrant some discussion. There was reduced fractional anisotropy in the left cerebellum in children who stutter relative to controls but increased fractional anisotropy relative to controls in the right cerebellum. The cerebellum is engaged in online error correction mechanisms, and in optimization of acquired motor sequences (Penhune and Steele, 2012). The cerebellum also contributes to organizing sequential movements into chunks, which enable rhythmic movements that are characteristic of skillful motor actions (Sakai *et al.*, 2004). De Nil *et al.* found that people who stutter had smaller decreases in chunk timing with practice compared to controls (Smits Bandstra and De Nil, 2013), and a trend towards hyperactivity in the cerebellum, which was attenuated with therapy (De Nil *et al.*, 2003). Interestingly, De Nil also found increased activity in the right cerebellum in people who stutter compared to controls who tended to have greater activity in the left cerebellum (De Nil *et al.*, 2001). The fact that children who stutter are already displaying increased right cerebellar fractional anisotropy compared to their age-matched peers, suggests that this difference is present even during early stages of stuttering, with implications for how these aberrations may influence corticocortical and cortical-subcortical interactions for speech acquisition and adjustments to speech error during development.

In sum, the present results based on younger children who stutter support the possibility of a complex and dynamic system that is affected in multiple areas (Ludlow, 2000). The sensorimotor cortical areas relevant for speech and language remain plastic throughout childhood, and are likely affected by their connections with subcortical regions such as the basal ganglia and cerebellum. The current white matter differences found in children who stutter compared to age-matched peers seem to support structural bases of deficient connectivity among neural circuits that underlie precise timing of movements (Rao *et al.*, 1997), including bilateral cortical auditory and motor areas, as well as subcortical structures and cortical areas they connect to (such as the supplementary motor area) and sensorimotor processing areas (cerebellum, PMC) that support efficient integration of timing and rhythm for speech sequencing.

Caveats and future directions

Most of the white matter tracts of interest that are relevant to supporting speech and language contain major areas of crossing fibres that affect fractional anisotropy values. Measures such as mean diffusivity and the principal component of the diffusion vector (L1), which are commonly derived from DTI along with fractional anisotropy, are also not free of this limitation (Jbabdi *et al.*, 2010). Thus, interpretation of fractional anisotropy data can be tricky in areas with crossing fibres. Recently, the TBSS method was extended to include crossing fibre models, which takes into account partial volume fractions in the voxel-wise white matter data. This allows the modelling of two distinct orientations of fibres and ensures that these fibres are consistently assigned across subjects. TBSS can then be performed based on these two distinct fibres for each voxel. In a preliminary analysis, we used this method (TBSS-X; Jbabdi *et al.*, 2010), to show corroborating results based on the two (F1, F2) distinct fibre tracts to the fractional anisotropy results reported in this study (Chang *et al.*, unpublished data). Future studies that use diffusion spectral imaging and/or other techniques that use high angular resolution diffusion imaging (HARDI) (Tuch *et al.*, 2003; Jones *et al.*, 2013) may further elucidate these issues.

The significant group differences in speech/language and cognitive measures were initially a concern, although further analyses determined that none of these measures were correlated with fractional anisotropy across the brain. While stuttering is oftentimes considered as pertaining to a speech motor control issue and not attributed to higher order language or cognitive deficits, many studies have found that children who stutter exhibit subtle decreased performance in standardized language tests (Ratner and Silverman, 2000; Coulter *et al.*, 2009). These findings suggest the existence of subgroups within the stuttering population (Seery *et al.*, 2007), possibly suggesting that there may be a group of children who stutter with concomitant subtle language difficulties, or dissociation among language skill development. In other developmental conditions such as specific language impairment and dyslexia, decreased language scores are associated with poorer rhythm perception (Przybylski *et al.*, 2013). Additionally, music training has been reported to shape structural brain development in many of the regions found to differ between children who do and do not stutter in this study (Hyde *et al.*, 2009). Music ability has also been linked to attention (Seither-Preisler *et al.*, 2014), which may be relevant to stuttering given the high comorbidity with attention deficit hyperactivity disorder in this population (Anderson *et al.*, 2003; Blood *et al.*, 2003; Ajdacic *et al.*, 2009). These results will need to be examined in future studies to unravel the relationship between language development, rhythm processing, related basal ganglia-thamocortical circuits, and stuttering.

This study was conducted as part of a larger longitudinal study on developmental stuttering. It is likely that we will be able to further elucidate developmental brain trajectory changes associated with persistent stuttering that may differ between the sexes. Multimodal data analyses are underway, which include not only DTI data, but also other morphometric measures (grey matter volume, density, area) as well

as functional MRI data that will likely provide a more comprehensive explanation of the aetiology of developmental stuttering. Through the synthesis of various structural and functional connectivity data we expect to further examine possible deficiencies in the interrelated neural circuits that have been found in the present study to be associated with childhood stuttering, and track how they change with symptom improvement versus chronicity. These discoveries are expected to lead to better prognostic indicators, and prompt new interventions that seek to modulate aspects of brain development toward better fluency in young children who stutter.

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Supplementary material

Supplementary material is available at *Brain* online.

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