

Dorsal language stream anomalies in an inherited speech disorder

Short title: Dorsal language stream and speech disorder

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Abstract

Speech articulation disorders are highly prevalent in the preschool years, but frequently resolve. The neurobiological basis of the most persistent and severe form, apraxia of speech, remains elusive. Current neuroanatomical models of speech processing in adults propose two parallel streams. The dorsal stream is involved in sound to motor speech transformations, while the ventral stream supports sound/letter to meaning. Data-driven theories on the role of these streams during atypical speech and language development are lacking. Here we provide comprehensive behavioural and neuroimaging data on a large novel family where one parent and eleven children presented with features of childhood apraxia of speech (the same speech disorder associated with *FOXP2* variants). The genetic cause of the disorder in this family remains to be identified. Importantly, in this family the speech disorder is not systematically associated with language or literacy impairment. Brain MRI scanning in seven children revealed large grey matter reductions over the left temporoparietal region, but not in the basal ganglia, relative to typically developing matched peers. In addition, we detected white matter reductions in the arcuate fasciculus (dorsal language stream) bilaterally, but not in the inferior fronto-occipital fasciculus (ventral language stream) nor in primary motor pathways. Our findings identify disruption of the dorsal language stream as a novel neural phenotype of developmental speech disorders, distinct from that reported in speech disorders associated with *FOXP2* variants. Overall, our data confirm the early role of this stream in auditory-to-articulation transformations.

Key words: speech; speech disorder; MRI; inherited

Abbreviations: ANCOVA, analysis of covariance; CAS, childhood apraxia of speech; DWI, diffusion-weighted imaging; EPI, echo planner imaging; FA, fractional anisotropy; fMRI, functional MRI; FWHM, full-width half maximum; IFOF, inferior fronto-occipital fasciculus; MNI, Montreal Neurological Institute

Introduction

Approximately 5% of school-aged children have a communication impairment that affects speech, language, or both (Law *et al.*, 2000; Eadie *et al.*, 2015). Family aggregation of these disorders is well known (Viding *et al.*, 2004; Newbury and Monaco, 2010). There are many subtypes of speech sound disorders (Dodd and Morgan, 2017) but the most severe is childhood apraxia of speech (CAS) that impacts sequencing of speech movements. In persistent cases, speech cannot easily be understood throughout life (Morgan *et al.*, 2017). Although CAS is rare, unravelling its neurobiological causes is likely to identify brain networks crucial to more common and less severe forms of speech disorders. Unlike adults with acquired apraxia of speech (e.g. post stroke, Trupe *et al.*, 2013), individuals with CAS have radiologically normal brain MRIs. Consequently, CAS must be caused by alterations in brain development that cannot be detected via routine radiological examination. The neurobiological pathways and networks that underlie CAS are yet to be fully characterized (Liegeois and Morgan, 2012).

Several genes have been implicated in CAS (Eising *et al.*, 2018) including *FOXP2* (Lai *et al.*, 2001; Graham and Fisher, 2015) however, for most individuals, the aetiology of their disorder remains unknown. Studies of individuals with inherited CAS provide an opportunity to gain insights into the biology and structural correlates underpinning a shared aetiology. This has been exemplified by studies of a large British family with severe, persistent CAS associated with a *FOXP2* mutation. In affected individuals, quantitative MRI techniques have revealed both structural (Watkins *et al.*, 2002b; Belton *et al.*, 2003) and functional (Liegeois *et al.*, 2003; Liegeois *et al.*, 2011) brain anomalies impacting cortical-subcortical networks (Vargha-Khadem *et al.*, 2005) that mainly alter the striatum (large volume reductions in the caudate nucleus), cerebellum, motor cortex, and inferior frontal gyrus. A recent report of an unrelated boy with *FOXP2* intragenic deletion (Liegeois *et al.*, 2016) confirmed reductions of the caudate nucleus bilaterally, as well as of the globus pallidus and hippocampus. Altogether, these findings have pointed to a crucial role of subcortical structures in *FOXP2*-related impairments. Of note, these individuals present with CAS alongside language and literacy impairments. The few available MRI studies on sporadic cases with CAS have revealed an inconsistent pattern of brain anomalies in the cortex (e.g. increased cortical thickness in the supramarginal gyrus in Kadis *et al.*, 2014) and basal ganglia (e.g. putamen and caudate nucleus reductions, see review in Liegeois *et al.*, 2014).

Models of acquisition of speech production (Terband *et al.*, 2009) suggest that somatosensory and auditory feedback contribute to the refinement of auditory and somatosensory targets

necessary for motor planning. Similarly, hierarchical models of speech production in adults (Hickok, 2012) suggest that feedforward and feedback loops, in both auditory and somatosensory modalities, provide input to the planning regions. Altogether, auditory-to-motor and somatosensory-to-motor circuits involving posterior temporal, temporo-parietal and inferior frontal regions, as well as corresponding white matter connections (superior longitudinal and arcuate fasciculi) form a dorsal stream crucial to speech production. In the acute phase of stroke, apraxia of speech in adults is associated with anatomical changes in this circuit (Hickok *et al.*, 2014). With the exception of one study (Kadis *et al.*, 2014), there has however been little evidence that changes in this dorsal route could be one of the neural phenotypes of CAS.

Here, we aimed to identify the structural and functional MRI correlates of CAS in a new large, two generation family comprising 13 affected individuals. The speech, oral motor, language and cognitive phenotype of 13 family members (two parents and 11 children) is described. Detailed MRI analysis was performed on seven affected siblings, aged 6 to 17 years. In the proband we screened three known CAS genes for mutations. We describe the neural phenotype of this large family to provide novel insights into the neurobiology of CAS.

Material and methods

Participants

The family was initially referred to our study of the inherited bases of child speech disorders as one child (II-6) was diagnosed with CAS and had four of six siblings who required speech pathology intervention. Both parents had therapy for speech disorders as children, and there is an extended family history of speech and language disorder on the maternal side. The four youngest children were born subsequently and later presented with delayed or disordered speech. Thirteen family members (two adults, 11 children) underwent detailed clinical phenotyping. Seven children (3 female) aged 6;4 to 15;7 years (mean age = 135 months, SD 45) took part in the neuroimaging study (Fig 1). All had average non-verbal cognitive skills and attended mainstream school. Two children were left-handed (1 male).

Control Group for the neuroimaging study

As there are no unaffected (i.e. without speech disorder) family members, the control group was unrelated. For each of the seven family members, two age- and gender-matched typically

developing children were recruited in order to maximise the chances of obtaining a representative distribution of typical values. These participants had taken part in other past (Liegeois *et al.*, 2013) or ongoing studies. The control participants (7 female) were aged 7;5 to 17;9 years (mean age = 145 months, SD = 39). All attended mainstream schools and had average cognitive skills, apart from one female with borderline intellect (Full Scale IQ 72). Four children were left handed (3 male). None had a history of speech problems, neurological disorders, mental disorders, epilepsy or seizures. Control group sizes varied slightly between analyses as some datasets had to be excluded due to excessive motion artefact (see statistical analyses sections).

The study was approved by the Royal Children's Hospital Human Research Ethics Committee (HREC 27053) in accordance with guidelines set by the National Health and Medical Research Council. All individuals, or their parents in the case of minors, gave written informed consent

Behavioural phenotype

Family members underwent detailed phenotyping using standardised age-appropriate assessments of speech, oral motor skills, language, phonological skills, literacy and cognition (intellectual functioning, memory and learning, see Supplementary Table 1). Audiovisual recordings of assessments were made using a Marantz PMD671 digital recorder, Countryman Isomax headset microphone, and a Sony DCR-SR85 digital camera. Standard scores were computed using normative data for each test, and speech tasks were analysed for articulation and phonological speech error patterns, and features of CAS. Speech and oral motor tasks were independently rated by two speech pathologists (SJT, ATM). Information regarding development and early speech and language skills were obtained, as well as medical records and speech pathology reports.

MRI data acquisition

MRI data were acquired on a 3T Siemens Trio Tim scanner at the Brain Research Institute in Melbourne (Australia). One hundred and sixty T1 weighted MPRAGE images were acquired approximately aligned in the AC-PC plane at a 0.9mm isotropic resolution (TR= 1900ms, TE=2.6ms, flip angle: 9⁰, matrix size: 256x256). Five of the control participants had been recruited for parallel studies and their T1-weighted scans had 1mm resolution (TR=1900ms, TE=2.55ms, flip angle: 9⁰, matrix size 256x256).

One hundred and twenty functional images were acquired using a continuous echo planner imaging (EPI) sequence with whole brain coverage (TR= 3000ms, TE= 30ms, flip angle: 85°, 44 slices, matrix size: 72x72, 3mm isotropic voxels).

Diffusion-weighted imaging (DWI) data were obtained using gradients at 64 evenly spaced directions at a b-value of 3000 s/mm² (TE/TR = 110/8300 ms, FOV = 240 × 240 mm, matrix size = 96 × 96, slice thickness = 2.5 mm (isotropic voxel size = 2.5 × 2.5 × 2.5 mm), 60 contiguous axial slices).

Functional MRI (fMRI) task

The fMRI task, where participants heard monosyllabic words and instruction cues via noise-reducing headphones, was identical to that used in a previous study (Morgan *et al.*, 2013). During the Speak condition, words were presented after the auditory cue “speak now” and participants were instructed to repeat them. During the Baseline condition, words were presented after the instruction “listen now”. A total of 30 words were presented in five Speak/Baseline cycles, with 6 stimuli per cycle. Inter-stimulus interval was 12 seconds, with 2.5s of word presentation, two seconds for the response period, followed by a 7.5s acquisition period.

fMRI preprocessing

Analysis was conducted with SPM8 software and associated toolboxes (<http://www.fil.ion.ucl.ac.uk/spm/software/spm8>). Data were initially corrected for large deviations in movement with a high pass filter and despiking technique, and corrected for atypical slices with ArtRepair toolbox (v.4). Fewer than 5% slices were corrected for each individual. fMRI data were then spatially realigned, corrected for differences in slice acquisition timing, and coregistered to structural data. Finally, fMRI data were normalised to the Montreal Neurological Institute (MNI) template and smoothed using an 8mm full-width half maximum (FWHM) Gaussian smoothing kernel. Atypical volumes were corrected with ArtRepair Volume correction function with individual participant 6 DOF motion parameters.

fMRI Statistical analysis

Speech related activation was examined using the Speak > Listen contrast. Group differences were examined using a fixed effects model, with an inclusive mask of the control group speak

> listen contrast. Age and six movement parameters were entered as variables of no interest. Voxels that survived corrected p values of .05 (Family wise error correction) were considered significant. We applied small volume correction in the putamen using a 5mm sphere centred on peak co-ordinates (-18, -6, 15 and 15, -3, 15) obtained from a previous study (Liegeois *et al.*, 2011)), as dysfunction in that region was *a-priori* hypothesized.

To better interpret fMRI group differences, we extracted mean parameter estimates during the Speak > Listen contrast in 3-mm spheres centred on peaks of underactivation, namely in the bilateral precentral gyrus and posterior superior temporal gyrus (see Supplementary Table 5 for coordinates). We also examined individual activity in these three regions in all family members using small volume correction (5mm spheres), for the Speak > Listen as well as for the Listen > Speak contrast.

Global volumes

Total brain grey and white matter volumes were extracted using tissue volume function within SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>).

Voxel based morphometry

Preprocessing involved segmentation of T1-weighted images into grey matter, white matter and cerebro-spinal fluid, across all participants with the Segmentation option in the SPM12 toolbox (<http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>) using age appropriate tissue probability maps generated using the TOM8 toolbox (Wilke *et al.*, 2008; <http://www.neuro.uni-jena.de/software/tom/>). This method involves simultaneously aligning grey and white matter among the images, and generating a customized template from all participants. Images were modulated in order to preserve initial volumes, normalized to MNI space using the DARTEL template, and smoothed using 10 mm FWHM Gaussian kernel. Grey matter maps from family members and control participants were compared using analysis of covariance (ANCOVA) with age, gender, and total grey matter volume as covariates.

The resulting statistical maps therefore represent regions of focal increased or reduced grey matter. Seven family members (Mean age=135 months, SD=45, range=76-211) were compared to 14 control participants (Mean age = 145 months, SD=39; range 89-213 months). Small volume correction (5 mm radius sphere centred on peak coordinates from another study, Liegeois *et al.*, 2016) was applied to the caudate nucleus (+/-14, 3, 18) and putamen (+/-27, 3, -2). The whole superior temporal gyrus, extracted from the Neuromorphometrics atlas (<http://www.neuromorphometrics.com>) was also used for small volume correction. These three

regions were *a-priori* hypothesized to be structurally atypical based on KE family studies (Belton *et al.*, 2003).

Results were examined at a corrected threshold ($p=0.05$ family-wise error correction) with an extent threshold of 10 voxels, and also at a lower threshold ($p=0.0001$ uncorrected) in regions where we had an *a-priori* hypothesis (left superior temporal and supramarginal gyrus, putamen, and caudate).

DWI tractography of speech and language related tracts

We performed tractography in native space using MRtrix version 0.2 (Tournier *et al.*, 2012), which identifies the fiber orientation distribution at each voxel using constrained spherical deconvolution, a method proven to be advantageous in voxels where fibers cross (Tournier *et al.*, 2007). We used a probabilistic fiber tracking algorithm (Tournier *et al.*, 2004). The arcuate fasciculus and speech related motor tracts (corticobulbar tract) were tracked using seed regions in two locations of the precentral white matter, as in our previous paediatric studies (Liegeois *et al.*, 2013). For the inferior fronto-occipital fasciculus (IFOF), we used a method similar to that reported in Vandermosten *et al.*, 2012, where we delineated a seed region in the occipital lobe (coronal cross section) and two inclusion regions (one in the extreme capsule in the horizontal plane, one in the frontal lobe in the coronal plane). To ensure that metrics were calculated from the core of the arcuate and IFOF rather than from spurious streamlines, only voxels containing at least 40 streamlines were included in a binary mask. No streamline threshold was applied for the motor tracts. To reduce the number of multiple comparisons, we tracked the dorsal corticobulbar tract only, as microstructural anomalies of this tract were found to be associated with dysarthria after traumatic brain injury (Liegeois 2013) and with developmental speech disorder (Morgan *et al.*, 2018), indicating it is a major speech motor pathway. Mean fractional anisotropy (FA) was calculated by averaging values across all voxels contained within each binary mask. Total number of voxels contained within the mask was used as a measure of tract volume.

Statistical analyses for MRI tractography derived values

For each tract, we compared metrics from six family members (mean age = 130, SD=39, range 76-211, after exclusion of one dataset with excessive movement) with those of the 14 control participants using mixed model analyses of variance (group x hemisphere), controlling for age (ANCOVA). For post hoc comparisons, non-parametric Mann-Whitney tests were used. Two-tailed p values are reported as we had no *a-priori* hypothesis regarding the direction of anomaly

(increase or decrease) in the family members relative to the control group. IBM SPSS Statistics 23 software was used for analyses. To test both the alternative and null hypotheses when examining group differences, we also ran Bayesian Mann-Whitney tests and extracted Bayes factors using JASP software (<https://jasp-stats.org/>) using the default Cauchy prior width.

Regional volumes

Regional volumes were extracted from the caudate nucleus, putamen and globus pallidus using FSL (<https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) and automated segmentation method (with manual correction of segmentation errors as in a previous study (Liegeois *et al.*, 2016)). Given the wide age range in our sample, extracted volumes were expressed as percentage of total grey matter.

Direct sequencing of CAS candidate genes

Candidate genes were amplified using 20g of genomic DNA from the proband (II-6) and gene-specific primers (oligonucleotides for other genes available on request) designed to reference human gene transcripts (*FOXP2*: NM_014491). Amplification reactions were cycled using a standard protocol on a Veriti Thermal Cycler (Applied Biosystems, Carlsbad, CA). Bidirectional Sanger sequencing of all exons and flanking regions was completed with a BigDye™ v3.1 Terminator Cycle Sequencing Kit (Applied Biosystems) according to the manufacturer's instructions. Sequencing products were resolved using a 3730xl DNA Analyzer (Applied Biosystems). All sequencing chromatograms were compared to published cDNA sequence (ensembl genome browser; www.ensembl.org); nucleotide changes were detected using Codon Code Aligner (CodonCode Corporation, Dedham, MA). Coding variants were checked against genomAD (Cambridge, MA), Exome Variant Server (University of Washington, Seattle) and 1000 Genomes (Wellcome Trust, UK).

Chromosomal Microarrays

Genomic DNA (200ng at 50ng/ul) from all 13 family members was hybridized to Infinium HumanOmniExpress-12 v1.0 DNA Analysis BeadChips (Illumina, San Diego, CA) containing 733,302 non-polymorphic markers according to the manufacturer's instructions. Data analysis was performed using Illumina's GenomeStudio v2011.1 with Genotyping module 1.9.4 software.

Data availability

Please contact the corresponding author for data.

Results

Family Pedigree

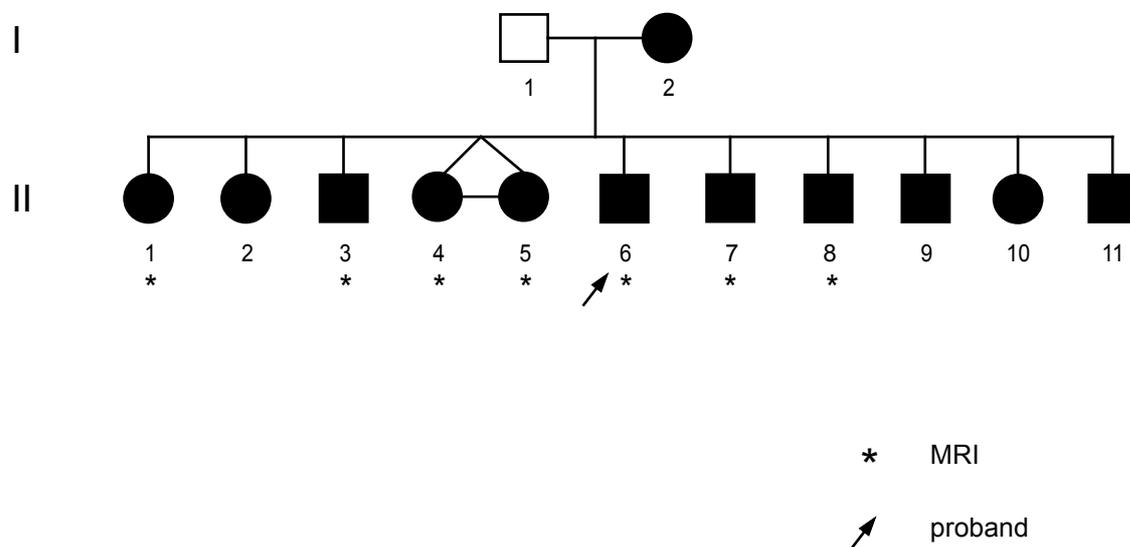


Figure 1. Family pedigree

Clinical phenotype

The dominant profile in this family was speech disorder. Six children had CAS characterised by speech sequencing difficulties, error inconsistency, and prosodic impairment (ASHA 2007; see Supplementary Table 2). All family members had difficulty repeating multisyllabic real words and nonsense words. Speech impairment was more severe in the younger siblings (II-8, II-9, II-10, II-11) who had unintelligible speech. The five older children and mother had articulation and phonological errors in speech, and features of CAS. Reduced, imprecise or asymmetrical lip, tongue and jaw movement was observed in some individuals. Eight family members had oral structural anomalies including retrognathia: II-4, II-5, II-6, II-10; bifid uvula: II-4, II-5, II-11; and high arched palate: I-2, II-1, II-2, II-10. The father (I-1) had a distinct speech phenotype, including reduced and imprecise tongue movement and phonological speech errors, but not CAS.

All 10 individuals tested had non-verbal intelligence within the normal range (normative mean: 100 ± 15 ; family range: 84 to 116), three had verbal working memory impairment (II-1, II-3, II-6) and four had language impairment (receptive: II-2, II-8, II-11, expressive: II-6, II-8; see Supplementary Table 3). Seven individuals had early reading and spelling difficulties that

required classroom support (I-1, II-1, II-2, II-3, II-6, II-8, II-9), but literacy scores were within the normal range at the time of assessment (see Supplementary Table 3).

All seven siblings who underwent MRI scanning had (i) CAS errors, (ii) oral motor impairment, (iii) phonological speech errors, and (iv) impairments repeating nonsense words and multisyllabic words. Two also had language impairment (expressive-receptive for II-8, expressive for II-6), and none had spelling/reading impairments at the time of testing (see Supplementary Table 3). These co-occurring features are often present in individuals with CAS (Morgan *et al.*, 2017).

Table 1. Key diagnostic features in 13 family members.

Individual	I-1	I-2	II-1	II-2	II-3	II-4	II-5	II-6	II-7	II-8	II-9	II-10	II-11
Sex	M	F	F	F	M	F	F	M	M	M	M	F	M
Oral motor impairment	Y	-	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
CAS errors	-	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Articulation errors	-	Y	Y	Y	-	Y	Y	Y	Y	-	Y	Y	-
Phonological speech errors	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Repetition of nonsense words/ multisyllabic real words impaired	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Language impairment	-	-	-	Y	-	-	-	Y	-	Y	-	-	Y
Early literacy difficulties	Y	-	Y	Y	Y	-	-	Y	-	Y	Y	-	-

Y, feature present; M, male; F, female; CAS, childhood apraxia of speech. Individuals highlighted in grey underwent MRI scanning

Genetic analysis to interrogate known genes

FOXP2 was sequenced in the proband (II-6), and no pathogenic variant was identified. Chromosomal microarrays on all 13 family members excluded a copy number variant genome-wide, including at the loci of *FOXP2*.

MRI group differences

Global brain volumes are intact

There were no group differences in either grey matter (family median = 213880, interquartile range or IQR= 13732; control median = 214220, IQR=16664; Mann-Whitney's U=48.0, p=0.97) or white matter (family median = 143182, IQR= 12954; control median = 145340, IQR= 10459; U=34.0, p=0.29) volumes.

Grey matter differences

No region of reduced grey matter was detected in the family relative to controls, using a threshold of p=0.05 with family-wise error correction. Using a less stringent threshold (p=0.0001 uncorrected) however, the largest reduction (in voxels) was located in the superior temporal gyrus/planum temporale extending into the supramarginal gyrus (cluster size=114 voxels, T=5.96, peak coordinates -60, -33, 18), which survived small volume correction (T=5.0, cluster size =74 voxels, p=0.009; Fig. 2a).

One region of significant grey matter increase was detected in the left cingulate cortex (cluster size=129 voxels, T=7.17, peak coordinate -8, 12, 29, p=0.037 with FWE correction; Fig. 2b). Increases in the right putamen (cluster size= 140 voxels, T=6.74, peak coordinates 30, -12, 8, FWE corrected p=0.066) and left putamen (cluster size= 45 voxels, T=5.54, peak coordinates -27, -9, 6, corrected p=0.32) were detected at the lower threshold (p<0.0001 uncorrected) but were *a-priori* hypothesized (Fig. 2c). When using small volume correction, an increase in the right putamen (30, -6, 2; T=5.09, p=0.004, cluster size 7 voxels) was also detected. No group differences were detected in the caudate nucleus. Of note, we ruled out systematic registration errors in the family by inspecting subtraction maps (created in FSL <https://fsl.fmrib.ox.ac.uk/fsl/fslwiki>) between individual grey matter images and the custom template.

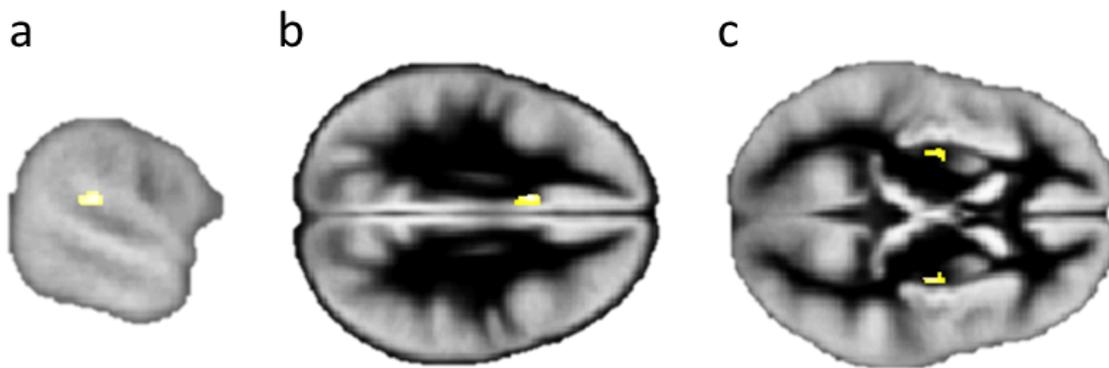


Figure 2. Grey matter differences between family members and control participants.

Basal ganglia volumes are intact

There were no significant group differences in volumes for the caudate (left, $U=25$, $p=0.079$; right, $U=34$, $p=0.29$), putamen (left, $U=44$, $p=0.74$; right, $U=41$, $p=0.59$) or globus pallidus (left, $U=27$, $p=0.11$; right, $U=31$, $p=0.20$; Supplementary Fig. 1). No differences were detected either when comparing scans of identical resolution (seven family members vs. nine controls).

Differences in the arcuate fasciculus, but not in the IFOF or motor tracts (Fig. 3)

FA was reduced in both the direct and anterior segments of the left arcuate fasciculus in the family relative to the control group (Fig. 3d and 3e; see Supplementary Table 4 for ANCOVA results with post hoc non-parametric group comparisons). In contrast, there were no group differences in FA between the family members and the control group in the motor tracts (Fig. 3a and 3b) or in the IFOF (Fig. 3c).

No tract volumetric differences were significant ($p>.29$ in all cases) except for the IFOF, where family members showed larger volumes than the control group ($F=5.11$, $p=0.037$). No hemispheric differences or group by hemisphere interaction effects were significant for any tract volumes.

An ANCOVA examining group differences in FA for the dorsal (arcuate) and ventral (IFOF) language tracts within the same model confirmed a tract by group interaction for the anterior segment ($F=6.54$, $p=0.020$) but not for the direct segment ($F=3.51$, $p=0.079$). Bayes factors

(see Supplementary Table 7) indicated that, where we reported no group differences in diffusion metrics, the data were either more likely under the null hypothesis or provided insufficient evidence. When we reported significant differences, the data were more likely under the alternative hypothesis (moderate to strong evidence).

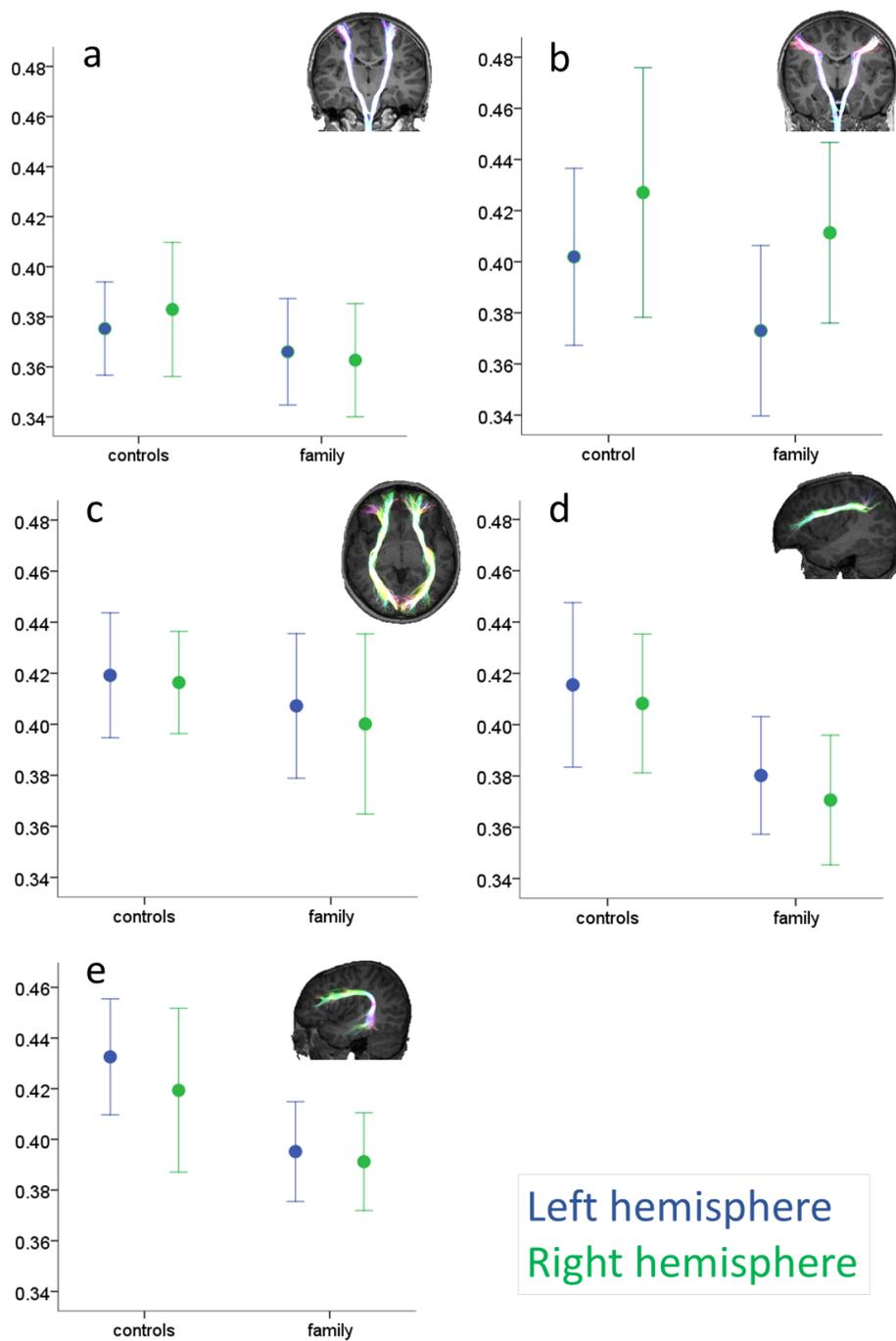


Figure 3. White matter metrics in each group.

fMRI reduced activation during speech in the family relative to a matched control group

Recording of verbal responses confirmed that all participants performed the task in the scanner, including the family members. When repeating words, the family showed reduced activation relative to the control group bilaterally in the pre- and postcentral gyrus, superior temporal gyrus extending into the supramarginal gyrus, cuneus, cerebellum, thalamus, and globus pallidus (Supplementary Table 5 and Fig. 4). The bilateral precentral and left posterior temporal regions will be referred to as the “three underactive regions of interest”.

The control group showed bilateral activation in the precentral gyrus extending into the insula, superior temporal gyri, supplementary motor area, and bilateral cerebellum (Fig. 4a, Supplementary Table 6). In contrast, there were no supra-threshold voxels for the family at this threshold. Activation in the precentral gyrus bilaterally was detected at a lower threshold ($p=0.001$ uncorrected; Fig. 4b).

Small volume correction ($p=0.05$ at peak level) revealed no detectable activation in any family member in the three underactive regions of interest. Left precentral and right precentral activation was however detected at the group level (left: peak at -44, -16, 42, $T=2.34$; right: peak at 52, -6, 36, $T=2.16$).

Parameter estimates from these three regions revealed individual variability (Supplementary Fig. 4c) and weaker activation than in the control group (Supplementary Fig. 4a). There was no detectable difference in these regions for the Listen>Speak contrast in the family members, as a group or individually. Overall, individual and group data for the family members indicate weak activation (see Supplementary Fig. 4a and 4c) in motor and left posterior temporal regions. Group map for seven control participants, in contrast, shows detectable activation ($p=0.05$, corrected, see Supplementary Fig. 4b).

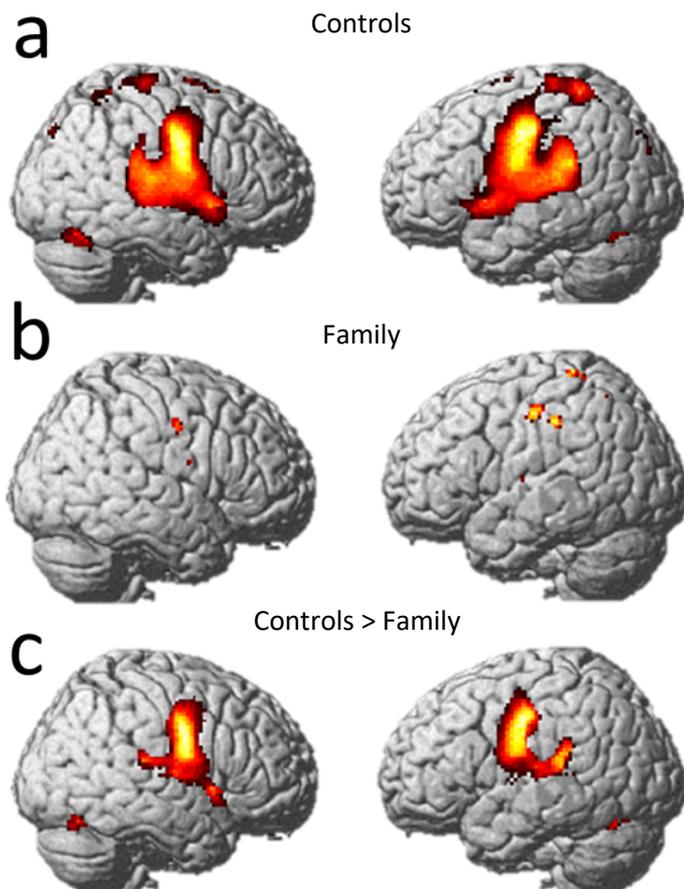


Figure 4. fMRI activation during speech

Discussion

The core speech phenotype in this family was CAS. In twelve family members, speech sequencing impairments were evident on tasks designed to stress the phonological system, namely the repetition of pseudowords and multisyllabic words. Encoding phonological representations into motor plans is therefore a persistent core deficit in this family. Repetition deficits that increase with word length and difficulty are a typical feature of both familial (Watkins *et al.*, 2002a; Lewis *et al.*, 2004; Peter *et al.*, 2013) and non-familial (Turner *et al.*, 2013; Nijland *et al.*, 2015) CAS.

Interestingly, most family members scored in the appropriate range on language, nonverbal intelligence, phonological awareness, and verbal memory tests. Similarly, literacy difficulties were not detectable in adolescence or adulthood, despite being reported in the early school

years for some. An inherited phenotype of CAS sparing language and literacy in the long term appears rare, as most reported individuals have some degree of persistent language difficulties affecting receptive grammar, vocabulary and sentence production, whether in the setting of a *FOXP2* mutation (Morgan *et al.*, 2017) or not (Lewis *et al.*, 2011; Carrigg *et al.*, 2016; Fedorenko *et al.*, 2016; Morgan *et al.*, 2018). The neural phenotype identified here cannot therefore be explained by co-occurring language or literacy impairments.

Neural phenotype: A dorsal route impairment

Reduced FA in the arcuate fasciculus bilaterally, combined with structural and functional reductions in the left temporoparietal cortex (superior temporal/planum temporale/supramarginal gyrus) are strong indicators of a developmental disorder affecting the dorsal language route (Fig 5). This route links auditory input/representation to articulatory systems (Hickok, 2012; Fridriksson *et al.*, 2016) and transforms phonological representations into motor programs (Kummerer *et al.*, 2013; Cogan *et al.*, 2014). This dorsal route is therefore also crucial to phonological memory (Buchsbaum and D'Esposito, 2008; Papagno *et al.*, 2017). In contrast, the speech *execution* white matter pathway (corticobulbar) and the ventral language route (IFOF) were not altered in this family, showing anatomical specificity of our findings.

Our hypothesis of a dorsal route impairment is consistent with findings from adult studies showing that direct stimulation of the arcuate fasciculus induces phonemic paraphasias, while stimulation of the fronto-parietal segment induces apraxia of speech (Duffau, 2008). Indeed, both sequencing and phonemic errors were observed in family members. Our findings are also consistent with lesion studies showing the importance of the temporo-parietal region for both word and nonword repetition (Rogalsky *et al.*, 2015), and of the supramarginal gyrus for speech praxis in adults with acute stroke (Hickok *et al.*, 2014) and children with idiopathic CAS (Kadis *et al.*, 2014). Intact phonological representations in this family suggest that only *transformation* of these representations into motor programs is affected. Without longitudinal data in this family, we cannot establish whether white matter alterations precede or follow grey matter dysfunction within the dorsal route during development. Their development could be jointly altered.

In the hierarchical model of speech production proposed by Hickok (2012), there are two feedback loops, one somatosensory and one auditory. Although at weak levels, sensori-motor activation was detected during speech in the family at the group level, and we had no evidence of de-activation in motor regions. Finally, we had no evidence of morphological alteration in the somatosensory (S1) or motor (M1) cortices, and no evidence of altered primary motor

pathways. We cannot rule out that early in speech development, alterations in somatosensory feedback in this family were the cause of poorly specified somatosensory targets. Without MRI evidence to support a somatosensory or motor deficit however, we suggest that the primary deficit in this family is in auditory to motor transformations.

Comparison with FOXP2-related neural phenotypes

Despite phenotypic differences, there were some commonalities between the neural phenotype of our family and that reported in individuals with *FOXP2* anomalies. Increased grey matter in the putamen bilaterally has been reported in the KE family (Watkins *et al.*, 2003), but not in case A-II (Liegeois *et al.*, 2016). Decreased activation in the wide speech network during speech tasks, including the globus pallidus, is another commonality with *FOXP2*-related CAS (Liegeois *et al.*, 2003; 2011; 2016). This overlap can be explained by the role of the putamen and globus pallidus in the late phases of sensorimotor learning (Lohse *et al.*, 2014; Hardwick *et al.*, 2013) and initiation/execution of motor plans (Price, 2010), especially in the selection of motor programs (Gil Robles *et al.*, 2005; Argyropoulos *et al.*, 2013). One major difference was that volumetric reductions in the caudate nucleus bilaterally, a major phenotypic marker of *FOXP2* disruption, were not observed in the new family. If we consider the caudate nucleus as embedded in language selection (rather than speech selection, Argyropoulos *et al.*, 2013 or cognitive control, Gil Robles *et al.*, 2005) networks, this difference could be due to the much less severe language impairment in the family reported here. Finally, the grey matter reduction in the superior temporal gyrus observed here was not seen in the KE family, who instead showed increases bilaterally in that region (Watkins *et al.*, 2002a; Belton *et al.*, 2003). Both increases and decreases could be associated with atypical development of the speech processing network.

Possible compensation mechanisms

We suggest that impairment of the dorsal language system can be compensated for via the ventral route for language as well as literacy. Specifically, as lexical knowledge increases, direct mapping between semantic concepts and speech sound maps can develop. For speech sound production, reliance on this ventral route is however suboptimal. It results in errors when repeating nonsense and complex words because the high sequential load cannot be coded without using sublexical (i.e., phoneme level) programming. We speculate that disruption of the arcuate fasciculus in our family members has delayed literacy acquisition via the dorsal phonological route (grapheme-phoneme decoding), but has been compensated for by the ventral orthographic route (Vandermosten *et al.*, 2012) via a larger IFOF.

It is noteworthy that our suggestion of the ventral or direct route as a compensatory mechanism is consistent with the hierarchical model of speech production proposed by Hickok (2012). In this model, there is a direct route from semantic/conceptual levels, to word level, to motor programs (PM/IFG in Fig. 5). In a developmental condition such as CAS, this route may need to over-develop to compensate for a dorsal impairment - hence larger IFOF volumes in the family.

A developmental neuroanatomical model of CAS

During the babbling phase (delayed in children with CAS), projections from auditory and sensorimotor maps to feedback control maps are believed to undergo progressive tuning (Guenther, 2006; Terband *et al.*, 2009). At birth, this dorsal route is immature: only short connections between auditory and premotor cortices are present (Skeide and Friederici, 2016), and they support speech repetition (Saur *et al.*, 2008). We propose that if these mappings do not develop appropriately, feedback and feedforward control maps cannot form during the imitation phase, resulting in CAS as in the family reported here. This scenario fits with current computational models of CAS (Terband *et al.*, 2009). Here we provide the first direct neuroimaging evidence to show the importance of the dorsal section of this model in speech disorders.

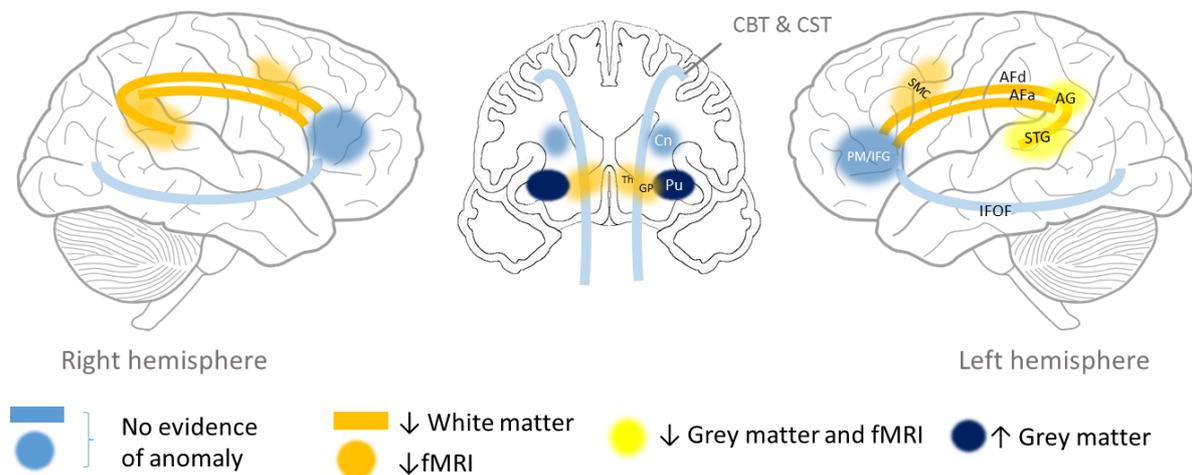


Figure 5. Dorsal and ventral routes in the family.

Prosodic impairments are one of the key features of CAS (ASHA, 2007). Vowel errors were seen across the family, whereas stress errors were mainly shown by the youngest members of the family (Supplementary Table 2). Hickok (2017) proposes that the human laryngeal control

circuit provides “prosodic frame” to the planning of speech. Dorsal stream disruption (including in temporo-parietal regions) would therefore also be consistent with prosodic impairments shown by the family.

Implications for speech and language disorders

We have demonstrated that a developmental communication disorder can be associated with cortical and white matter anomalies. This hypothesis complements the more widespread view that language disorders may be of subcortical origin (Krishnan *et al.*, 2016). We posit that disruption or delayed maturation of the dorsal language network could also explain less severe and more common forms of speech disorders and speech delay. Similarly, we suggest that integrity of the dorsal language stream should be examined in speech disorders caused by other genes, and associated with acquired neurological disorders.

In conclusion, we have identified brain anomalies associated with CAS, in a family for whom the underlying molecular basis has not yet been found. We suggest that speech and phonological memory impairment in this family arises from atypical development of the dorsal language network responsible for auditory-motor transformations. Taken together, our data support a novel neurobiological network for an inherited speech disorder.

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Scholarship 1017773), M.B. (Senior Research Fellowship 1002098 and Program Grant 1054618) and I.E.S. (Program grant 1091593 and Practitioner fellowship 1104831). F.L.'s research is supported by the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London (London, UK). S.J.T.'s research is also supported by a Speech Pathology Australia Nadia Verrall Memorial Research grant and Australian National University Gowrie Scholarship.

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Figure Legends

Fig. 1. Family pedigree. Individuals in black have CAS errors (see Supplementary Table 2 for details).

Fig. 2. Regions of decreased grey matter in the (a) left planum temporale/supramarginal gyrus, and increased grey matter in the (b) left cingulate and (c) bilateral putamen in family members relative to the control group. Images are displayed at $p=0.0001$, uncorrected for multiple comparisons and overlaid onto the group specific grey matter template. See text for peak coordinates. Left hemisphere is on the top on axial views.

Fig. 3. White matter differences between the family and control group. Group values (mean \pm 1SD) for mean Fractional Anisotropy (FA) within the motor tracts (a, hand-related corticospinal; b, speech-related corticobulbar), the ventral language-related tract (c, inferior fronto-occipital fasciculus or IFOF) and dorsal language-related tracts (d, arcuate anterior segment and e, arcuate direct segment). Inserts: examples of tractography output superimposed onto a control participant's T1-weighted dataset. Tracts are projected onto each slice to allow visualisation of full length. See **Supplementary Fig. 2** for a scatterplot of the relationship between age and FA in the IFOF and corticospinal tracts.

Fig. 4. Functional MRI during speech. Average group activation in the control group (a) and family (b), and map of activation difference (Control > family, c) for the Speak vs. Listen contrasts. Results are displayed at the FWE corrected .05 significance level (a and c), and at the uncorrected $p<.001$ significance level for the family (b) as no activation was detected with FWE correction. Results are projected onto the SPM8 rendered image.

Fig. 5 Proposed impaired bilateral dorsal network (yellow/orange) in this family, based on functional and structural imaging results. Abbreviations: CBT, corticobulbar tract; CST, corticospinal tract; IFOF, inferior fronto-occipital fasciculus; AF, arcuate fasciculus (d, direct segment; a, anterior segment); SMC, sensori-motor cortex; Pu, putamen; Cn, caudate nucleus; Th, thalamus; GP, globus pallidus; STG, superior temporal gyrus; AG, angular gyrus; PM/IFG, premotor cortex/inferior frontal gyrus. ↓, reduced; ↑, increased.