

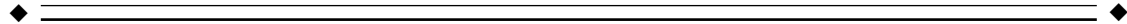
Bilateral Brain Abnormalities Associated With Dominantly Inherited Verbal and Orofacial Dyspraxia

Emma Belton,^{1*} Claire H. Salmond,¹ Kate E. Watkins,²
Faraneh Vargha-Khadem,¹ and David G. Gadian³

¹Developmental Cognitive Neuroscience Unit, Institute of Child Health, University College London, Great Ormond Street Hospital for Children, NHS Trust, London, United Kingdom

²Cognitive Neuroscience Unit, Montreal Neurological Institute, Montreal, Canada

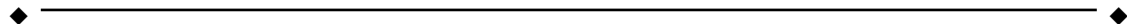
³Radiology and Physics Unit, Institute of Child Health, University College London, Great Ormond Street Hospital for Children, NHS Trust, London, United Kingdom



Abstract: The KE family is a large three-generational pedigree in which half of the members suffer from a verbal and orofacial dyspraxia in association with a point mutation in the FOXP2 gene. This report extends previous voxel-based morphometric analyses of magnetic resonance imaging (MRI) scans (Watkins et al. [2002] *Brain* 125:465–478) using a bilateral conjunction analysis. This searches specifically for areas of grey matter density that differ bilaterally in the affected members compared with both matched controls and the unaffected family members. 3-D T1-weighted MRI datasets of 17 family members (10 affected, 7 unaffected) and matched controls were compared. The most significant findings were reduced grey matter density bilaterally in the caudate nucleus, the cerebellum, and the left and right inferior frontal gyrus in the affected members. In addition, increased grey matter density was found bilaterally in the planum temporale. These results confirm that a point mutation in FOXP2 is associated with several bilateral grey matter abnormalities in both motor and language related regions. The results also demonstrate the advantages of using a conjunction analysis when bilateral abnormalities are suspected. *Hum. Brain Mapping* 18:194–200, 2003.

© 2003 Wiley-Liss, Inc.

Key words: voxel-based morphometry; bilateral abnormalities; FOXP2; caudate nucleus



INTRODUCTION

We report on voxel-based brain morphometry (VBM) in the KE family in which half of the members present with a pronounced speech and language disorder characterized as a verbal and orofacial dyspraxia [Vargha-Khadem et al., 1995; Watkins et al., 2002b]. Candidate gene analysis has indicated that a point mutation in the FOXP2 gene is responsible for this disorder [Fisher et al., 1998; Lai et al., 2001].

Previous work using VBM analysis of magnetic resonance images has shown that there are subtle brain abnormalities in the affected family members

Contract grant sponsor: Medical Research Council; Contract grant sponsor: Wellcome Trust.

*Correspondence to: Emma Belton, Developmental Cognitive Neuroscience Unit, Institute of Child Health, The Wolfson Centre, Mecklenburgh Square, London WC1N 2AP, United Kingdom.
E-mail: E.Belton@ich.ucl.ac.uk

Received 31 July 2002; Accepted 12 October 2002

DOI 10.1002/hbm.10093

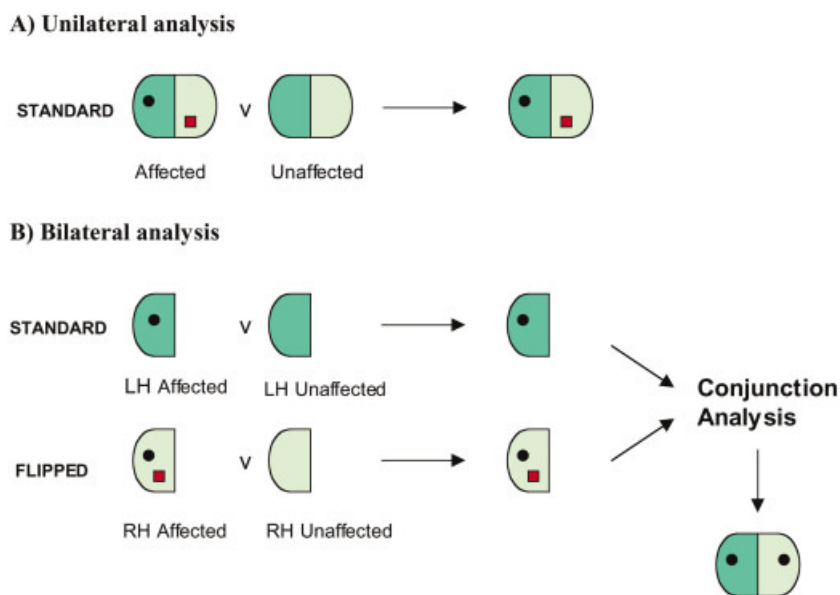


Figure 1.

Illustration of unilateral (**A**) and bilateral (**B**) methods of VBM analysis. LH, left hemisphere; RH, right hemisphere.

[Vargha-Khadem et al., 1998, Watkins et al., 1999, 2002a]. VBM is an automated procedure that compares grey matter density (i.e., proportion of tissue volume ascribed to grey matter) on a voxel-by-voxel basis between two groups using statistical parametric mapping (SPM) techniques [Ashburner and Friston, 2000]. The abnormalities found in the affected members were identified using a VBM contrast that detects regions of difference in grey matter density in each hemisphere separately [Ashburner and Friston, 2000] (see Fig. 1A). Results of this unilateral analysis revealed that there were regions of abnormally reduced or increased grey matter density in speech- and motor-related areas [Watkins et al., 2002a]. Some of these regions, in particular the caudate nucleus, were abnormal in both hemispheres, whereas others, such as the inferior frontal gyrus and supplementary motor area, were abnormal only on the left. On the basis of these findings, it was concluded that the brain abnormality underlying the behavioural phenotype was in the frontostriatal system, parts of which were affected bilaterally.

Unilateral brain abnormalities of developmental origin, affecting the neural substrates of speech and language, rarely result in persistent and chronic dysphasia, and are likely to result in a much milder syndrome than if the same region is abnormal bilaterally [Landau and Kleffner 1957; Vargha-Khadem et al., 1985]. This is presumably because of the capacity to reorganize function after early unilateral damage, provided a viable neural substrate is available intra- or inter-hemispherically [Booth, 1999; Isaacs et al., 1996; Vicari et al., 2000]. When selective and chronic deficits

are encountered, bilateral pathology of the systems subserving speech and language are then suspected [Vargha-Khadem et al., 1985]. Therefore, in the case of the KE family, the severe and persistent phenotype of verbal and orofacial dyspraxia leads us to suspect bilateral pathology of these cortical and subcortical systems.

A recent development in the use of VBM allows the selective investigation of symmetrical bilateral abnormalities (see Fig. 1B). This is achieved by producing two statistical parametric maps (SPMs), one comparing two groups of standard images, and the other comparing the same two groups but with the images flipped across the midline from left to right. A conjunction analysis is then used to find regions of significant difference that are common to both SPMs [Salmond et al., 2000].

Here we report on the use of this bilateral method of analysis to confirm previous findings, and to determine whether the left lateralized abnormalities that had been identified using the unilateral method were in fact bilateral. Moreover, a second conjunction analysis allowed us to differentiate between abnormalities common to the entire family vs. those specific to members with the FOXP2 mutation.

SUBJECTS AND METHODS

Subjects

This study involved the 17 family members and controls previously reported by Watkins et al. [2002a]. Ten affected family members were included (5 men, 5

women, mean age 28.1 years). Four were from the first and second generations, the remaining six from the third generation aged between 9 and 21 years. Seven unaffected members were also scanned (3 males, 4 females, age range 9–27 years; mean age 17.1 years). Seventeen normal controls were selected to match the family members on age and sex.

Data acquisition

All participants were scanned using a 1.5 T Siemens SP system with a T1-weighted 3D MPRAGE sequence (sequence TR = 10 msec, TE = 4 msec, TI = 200 msec, flip angle = 12 degrees, matrix size = 256×256 , field of view = 250 mm, partition thickness = 1.25 mm, 128 sagittal partitions, voxel size = $0.98 \times 0.98 \times 1.25$ mm, acquisition time = 8.3 min).

Image processing

The 3D data sets were processed and analysed using SPM99 software (Wellcome Department of Imaging Neuroscience, UK). First, the images were spatially normalized to a T_1 template (MNI). For the bilateral analysis, a symmetrical template was calculated from this T_1 template, thus reducing asymmetries. The second stage segmented the images into grey matter, white matter, and cerebrospinal fluid compartments (again using a symmetric probabilistic template for the bilateral analysis). The grey matter partitions were then selected and duplicated for each data set, and then flipped in the transverse plane along the anterior–posterior axis [Salmond et al., 2000]. All images were smoothed using a 12-mm FWHM isotropic Gaussian kernel. The voxel values thus provide an index of the amount of grey matter per unit volume under the smoothing kernel [Ashburner and Friston, 2000].

Data analysis

Statistical analysis was carried out in SPM99. The hypothesis for this analysis was that the affected members would have grey matter density abnormalities in regions involved in the speech–motor network.

In order to identify areas of significant abnormality specific to the affected members of the KE family and to identify areas of abnormality common to all the members of the KE family, two basic contrasts were used in interaction and conjunction analyses. These contrasts were: 1) Affected members vs. their controls; and 2) unaffected members vs. their controls.

Areas of abnormality specific to the affected members of the family

Unilateral analysis

An interaction analysis was carried out with the four groups (affected members, controls for the affected members, unaffected members, controls for the unaffected members) with the contrasts entered as $-1\ 1\ -1\ 1$ (increased grey matter in the affected members) and $-1\ 1\ 1\ -1$ (decreased grey matter in the affected members) in SPM99.

Bilateral analysis

A conjunction analysis was carried out with the interaction contrasts for the flipped and unflipped data as described in Salmond et al. [2000].

Areas of abnormality common to both affected and unaffected members of the KE family

Unilateral analysis

A conjunction analysis was carried out with the conjoined contrasts as $(1\ -1\ 0\ 0; 0\ 0\ 1\ -1)$ to look at increased grey matter density in both the affected and unaffected family members, and $(-1\ 1\ 0\ 0; 0\ 0\ -1\ 1)$ to look at decreased grey matter density in these family members.

Bilateral analysis

A two-level conjunction was carried out with one level constraining the search to bilateral abnormalities [See Salmond et al., 2000] and the second level constraining the search to common family abnormalities.

Statistics

Findings of abnormalities in the affected family members, with respect to both unaffected members and controls, in the bilateral conjunction and the unilateral contrasts are reported at a significance level of $P < 0.05$ after correction for multiple comparisons, or at an uncorrected level of $P < 0.001$ if the region fell within the a priori hypothesis.

Regions of abnormality in grey matter density that were found to be common to both affected and unaffected members in comparison with controls are reported if they reached a corrected level of $P < 0.05$. As there was no a priori hypothesis, regions reaching an uncorrected level of significance only are not reported.

TABLE I. Bilateral and unilateral conjunction analyses

Region	Coordinates (mm)			Corrected <i>P</i>	Uncorrected <i>P</i>
	<i>x</i>	<i>y</i>	<i>z</i>		
Unilateral conjunction analysis					
Affected members: less grey matter					
L head of caudate nucleus	-14	-16	9		<0.001
R head of caudate nucleus	16	18	9		0.003
L dorsal inferior frontal gyrus	-42	38	14		0.001
L precentral gyrus	-52	-2	26		0.001
R temporal pole	22	10	-36		<0.001
Affected members: more grey matter					
L superior temporal gyrus	-44	-36	12		0.004
R superior temporal gyrus	58	-32	9		0.001
L angular gyrus	-51	-64	24	<0.001	
L cerebellum lobule VIIIB	-42	-51	-45		<0.001
Affected and unaffected: less grey matter					
L anterior cerebellum	-30	-44	-50	0.023	
Affected and unaffected: more grey matter					
L inferior temporal lobe	-36	-2	-32	0.025	
R inferior frontal sulcus	52	21	34	0.025	
Bilateral conjunction analysis					
Affected members: less grey matter					
Head of caudate nucleus	15	16	10		<0.00001
Dorsal inferior frontal gyrus	45	36	16		<0.0001
Precentral gyrus	56	-4	26		0.001
Ventral cerebellum lobule VIIIB	21	-86	-51		0.001
	34	-78	-57		0.001
Ventral cerebellum lobule VIIIB	21	-50	-64		0.001
	30	-45	-58		0.001
	18	-52	-66		0.001
	24	-46	-62		0.001
Temporal pole	26	15	-40		0.001
Affected members: more grey matter					
Posterior superior temporal gyrus	56	-30	8		<0.001
Angular gyrus	48	-62	27		<0.001
Affected and unaffected: less grey matter					
Anterior calcarine sulcus	21	-56	8	0.012	
Dorsal thalamus	15	-27	14	0.028	
Affected and unaffected: more grey matter					
Inferior temporal lobe	34	-3	-33	0.001	
Frontal pole	16	63	-2	0.009	

Corrected *P* values included when they reach significance ($P < 0.05$).

RESULTS

Unilateral method

Using a unilateral conjunction it was found that the affected family members have reduced grey matter density in several areas relative to unaffected members and controls, the most significant being the left caudate nucleus (see Table I). Less grey matter was

also seen at closely homologous coordinates in the right caudate nucleus at a lower threshold. In addition, significant unilateral abnormalities were found in the left dorsal inferior frontal gyrus, the left precentral gyrus, and the right temporal pole (see Table I).

There were also regions of increased grey matter density in the affected members, with indications of a bilateral abnormality in one area, the posterior superior temporal gyrus, whereas additional abnormalities

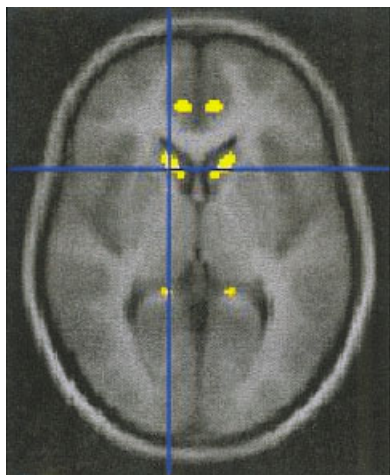


Figure 2.

Result of the bilateral conjunction analysis. Less grey matter density in the caudate nuclei in the affected members. Left and right caudate nuclei, uncorrected $P < 0.001$ at coordinates $\pm 13\ 20\ 3$.

in the angular gyrus and cerebellar lobule VIIB [Schmahmann et al., 1999] were found only in the left hemisphere (see Table I).

Bilateral model

The most significant region of reduced grey matter density bilaterally in the affected members was in the caudate nucleus (see Fig. 2), confirming the results of the unilateral conjunction. The bilateral conjunction analysis also showed that both the dorsal inferior frontal gyrus (corresponding to BA 45) [Tomaiuolo et al., 1999] and the precentral gyrus (lower primary motor cortex) were significantly abnormal in both hemispheres. These regions were only significant in the left hemisphere in the unilateral analysis (see Table I). In addition, a decrease in grey matter density was seen in the temporal pole region in the bilateral analysis, whereas with the unilateral model this area only reached significance in the right hemisphere.

A bilateral decrease in grey matter density was also seen in several regions of the ventral cerebellum (lobules VIIB and VIIIB) [Schmahmann et al., 1999], which were not seen in the unilateral conjunction analysis (see Table I).

An increase in grey matter density was found bilaterally in the posterior region of the superior temporal gyrus, which confirms that those regions of abnormality found with the unilateral model are symmetrical. The angular gyrus was also found to be abnormal bilaterally, whereas the unilateral conjunction sug-

gested this was lateralized to the left hemisphere (see Table I).

Familial abnormalities in grey matter density

A conjunction between comparisons of affected members and matched controls and unaffected members and matched controls revealed areas of abnormality that are common to both family groups. Only those surviving correction for multiple comparisons are reported. Reductions in grey matter density were found in several posterior regions, none of which were present in both the unilateral and bilateral models (see Table I). In terms of increased grey matter density, a region common to both affected and unaffected members was found in a region of the temporal lobe, adjacent to the temporal horn, in both the bilateral and unilateral models. Additional regions were found in the right prefrontal cortex (see Table I). None of these regions were in language-related areas.

DISCUSSION

This study has confirmed that the most consistent morphometric finding in the affected KE family members is a bilateral reduction in grey matter density in the caudate nucleus. This is common to both unilateral and bilateral conjunction analyses, and confirms previous findings reported by Watkins et al. [2002a] using both VBM and volumetric measurements. The association of the caudate nucleus with motor planning and sequencing, and with cognitive function [Abdullaev et al., 1998; Jueptner et al., 1997] is suggestive of the role that this structural abnormality may play in the phenotype of the affected members [Watkins et al., 2002b].

The bilateral conjunction analysis also confirms that the regions of cortical abnormality that appeared to be bilateral using the standard unilateral model are symmetrically abnormal, for example, the posterior superior temporal gyri. This region has been implicated in speech perception and developmental language disorders. Scott et al. [2000] showed that the left superior temporal sulcus was specifically activated with the presentation of intelligible speech. In addition, studies have suggested that individuals with specific language impairment and developmental dyslexia have an abnormal asymmetry of the planum temporale, also in this area [Foster et al., 2002; Galaburda et al., 1985; Leonard et al., 2001]. Bilateral abnormalities were also confirmed in the cerebellum, a structure that has many connections to the prefrontal cortex, and has been highly implicated in motor and cognitive func-

tions [Diamond, 2000; Jueptner and Weiller, 1998; Leiner et al., 1993; Rao et al., 1997].

In addition to confirming previous results, the bilateral conjunction also provided some new findings. In many of the regions where left lateralized abnormalities in cortical areas had been found using standard VBM contrasts, a symmetrical abnormality in both hemispheres was found using the bilateral model. These additional findings can be attributed to the greater specificity and selectivity of the bilateral model [Salmond et al., 2000]. This was the case for the inferior frontal gyrus region, the precentral gyrus, and the angular gyrus. These regions are also known to be highly involved in language, with BA 44/45 and the primary motor cortex relating particularly to speech production, paralleling the core features of impaired articulatory and orofacial sequencing in this phenotype [Lotze et al., 2000; Price, 2000; Watkins et al., 2002b]. The angular gyrus is also thought to be involved in language, specifically in semantic processing [Hart and Gordon, 1990; Price, 2000].

The bilateral nature of these cortical and subcortical abnormalities may explain the severe phenotype in the affected members of the KE family. Children with early focal brain lesions of the left hemisphere show a remarkable level of language proficiency with only subtle deficits, suggesting that speech and other language functions have reorganized to other brain regions [Isaacs et al., 1996; MacWinney et al., 2000; Vargha-Khadem et al., 1985; Vicari et al., 2000]. In the case of bilateral abnormalities in these regions, reorganization would be compromised. The two reported cases with developmental bilateral perisylvian pathology support this conclusion, showing a failure to develop intelligible speech [Landau and Kleffner, 1957; Vargha-Khadem et al., 1985].

In addition to selectively searching for bilateral abnormalities, the use of a conjunction analysis in this study has one further application. It allows regions of grey matter density that are common to both affected and unaffected family members, and different from controls, to be detected. In the case of a standard contrast between the affected group and normal controls, such abnormalities may be erroneously interpreted as being related to the specific genetic mutation in the affected family members. None of the regions found to be abnormal in both the affected and unaffected members were in language-related areas.

In conclusion, this study highlights the striking nature of the bilateral abnormalities, particularly the reduction in grey matter density in the caudate nuclei that have been found in this autosomal dominant speech and language disorder.

ACKNOWLEDGMENTS

We thank the members of the KE family and control subjects for their continued cooperation with our research programme. We also thank Frederique Liegeois for assistance with acquiring the data and, along with Torsten Baldeweg, for valuable input into this manuscript. Research at the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust benefits from the Research and Development funding from the NHS Executive.

REFERENCES

- Abdullaev YG, Bechtereva NP, Melnichuk KV (1998): Neuronal activity of human caudate nucleus and prefrontal cortex in cognitive tasks. *Behav Brain Res* 97:159–177.
- Ashburner J, Friston KJ (2000): Voxel-based morphometry: the methods. *NeuroImage* 11:805–821.
- Booth JR (1999): Functional organization of activation patterns in children: whole brain fMRI imaging during three different cognitive tasks. *Prog. Neuro-Psychopharmacol Biol Psychiat* 23: 669–682.
- Diamond A (2000): Close interrelation of motor development and cognitive development and of the cerebellum and prefrontal cortex. *Child Dev* 71:44–56.
- Fisher SE, Vargha-Khadem F, Watkins KE, Monaco AP, Pembrey ME (1998): Localisation of a gene implicated in a severe speech and language disorder. *Nat Genet* 18:168–170.
- Foster LM, Hynd GW, Morgan AE, Hugdahl K (2002): Planum temporale asymmetry and ear advantage in dichotic listening in developmental dyslexia and attention-deficit/hyperactivity disorder (ADHD). *J Int Neuropsychol Soc* 8:22–36.
- Galaburda AM, Sherman GF, Rosen GD, Aboitiz F, Geschwind N (1985): Developmental dyslexia: four consecutive patients with cortical anomalies. *Ann Neurol* 18:222–233.
- Hart-Jr, Gordon B (1990): Delineation of single-word semantic comprehension deficits in aphasia, with anatomical correlation. *Ann Neurol* 27:226–231.
- Isaacs E, Christie D, Vargha-Khadem F, Mishkin M (1996): Effects of hemispheric side of injury, age at injury, and presence of seizure disorder on functional ear and hand asymmetries in hemiplegic children. *Neuropsychologia* 34:127–137.
- Jueptner M, Frith CD, Brooks DJ, Frackowiak RS, Passingham RE (1997): Anatomy of motor learning. II. Subcortical structures and learning by trial and error. *J Neurophysiol* 77:1325–1337.
- Jueptner M, Weiller C (1998): A review of differences between basal ganglia and cerebellar control of movements as revealed by functional imaging studies. *Brain* 121:1437–1449.
- Lai CS, Fisher SE, Hurst JA, Vargha-Khadem F, Monaco AP (2001): A forkhead-domain gene is mutated in a severe speech and language disorder. *Nature* 413:519–523.
- Landau WM, Kleffner FR (1957): Syndrome of acquired aphasia with convulsive disorder in children. *Neurology* 10:915–921.
- Lotze M, Seggewies G, Erb M, Grodd W, Birbaumer N (2000): The representation of articulation in the primary sensorimotor cortex. *Neuroreport* 11:2985–2989.
- Leiner HC, Leiner AL, Dow RS (1993): Cognitive and language functions of the human cerebellum. *Trends Neurosci* 16:444–447

- Leonard CM, Eckert MA, Lombardino LJ, Oakland T, Kranzler J, Mohr CM, King WM, Freeman A (2001): Anatomical risk factors for phonological dyslexia. *Cerebral Cortex* 11:148–157.
- MacWhinney B, Feldman H, Sacco K, Valdés-Pérez R (2000): Online measures of basic language skills in children with early focal brain lesions. *Brain Lang* 71:400–431.
- Nakahara H, Doya K, Hikosaka O (2001): Parallel cortico-basal ganglia mechanisms for acquisition and execution of visuomotor sequences: a computational approach. *J Cogn Neurosci* 13:626–647.
- Price C (2000): The anatomy of language: contributions from functional neuroimaging. *J Anat* 197:335–359.
- Rao SM, Harrington DL, Haaland KY, Bobholz JA, Cox RW, Binder JR (1997): Distributed neural systems underlying the timing of movements. *J Neurosci* 17:5528–5535.
- Salmond CH, Ashburner J, Vargha-Khadem F, Gadian DG, Friston KJ (2000): Detecting bilateral abnormalities with voxel based morphometry. *Hum Brain Mapp* 11:223–232.
- Schmahmann JD, Doyon J, McDonald D, Holmes C, Lavoie K, Hurwitz AS, Kabani N, Toga A, Evans A, Petrides M (1999): Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. *Neuroimage* 10:233–260.
- Scott SK, Blank CC, Rosen S, Wise RJS (2000): Identification of a pathway for intelligible speech in the left temporal lobe. *Brain* 123:2400–2406.
- Tomaiuolo F, MacDonald JD, Caramanos Z, Posner G, Chiavaras M, Evans AC, Petrides M (1999): Morphology morphometry and probability mapping of the pars opercularis of the inferior frontal gyrus: an in vivo MRI analysis. *Eur J Neurosci* 11:3033–3046.
- Vargha-Khadem F, Watters GV, O’Gorman AM (1985): Development of speech and language following bilateral frontal lesions. *Brain Lang* 25:167–183.
- Vargha-Khadem F, Watkins K, Alcock K, Fletcher P, Passingham R (1995): Praxic and nonverbal cognitive deficits in a large family with a genetically transmitted speech and language disorder. *Proc Natl Acad Sci USA* 92:930–933.
- Vargha-Khadem F, Watkins KE, Price CJ, Ashburner J, Alcock KJ, Connelly A, Frackowiak RSJ, Friston KJ, Pembrey ME, Mishkin M, Gadian DG, Passingham RE (1998): Neural basis of an inherited speech and language disorder. *Proc Natl Acad Sci USA* 95:12695–12700.
- Vicari S, Albertoni A, Chilosi AM, Cipriani P, Cioni G, Bates E (2000): Plasticity and reorganization during language development in children with early brain injury. *Cortex* 36:31–46.
- Watkins KE, Gadian DG, Vargha-Khadem F (1999): Functional and structural brain abnormalities associated with a genetic disorder of speech and language. *Am J Hum Genet* 65:1215–1221.
- Watkins KE, Vargha-Khadem F, Ashburner J, Passingham RE, Connelly A, Friston KJ, Frackowiak RSJ, Mishkin M, Gadian DG (2002a): MRI analysis of an inherited speech and language disorder: structural brain abnormalities. *Brain* 125:465–478.
- Watkins KE, Dronkers NF, Vargha-Khadem F (2002b): Behavioural analysis of an inherited speech and language disorder: comparison with acquired aphasia. *Brain* 125:452–464.