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FOXP2 expression during brain development coincides with adult sites of pathology in a severe speech and language disorder

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Summary

Disruption of *FOXP2*, a gene encoding a forkhead-domain transcription factor, causes a severe developmental disorder of verbal communication, involving profound articulation deficits, accompanied by linguistic and grammatical impairments. Investigation of the neural basis of this disorder has been limited previously to neuroimaging of affected children and adults. The discovery of the gene responsible, *FOXP2*, offers a unique opportunity to explore the relevant neural mechanisms from a molecular perspective. In the present study, we have determined the detailed spatial and temporal expression pattern of *FOXP2* mRNA in the developing brain of mouse and human. We find expression in several structures including the cortical plate, basal ganglia, thalamus, inferior olives and cerebellum. These data support a role for *FOXP2* in the development of corticostriatal and olivocerebellar circuits involved in motor control. We find intriguing concordance between regions of early expression and later sites of pathology suggested by neuroimaging. Moreover, the homologous pattern of *FOXP2*/*Foxp2* expression in human and mouse argues for a role for this gene in development of motor-related circuits throughout mammalian species. Overall, this study provides support for the hypothesis that impairments in sequencing of movement and procedural learning might be central to the *FOXP2*-related speech and language disorder.

Keywords: *FOXP2*; speech and language disorder; RNA expression; motor system; brain development

Abbreviations: CS = Carnegie stage; FS = fetal stage

Introduction

Despite significant advances in understanding of human brain function during the past few decades, it has not yet been possible to elucidate the precise neural mechanisms that have gone awry in developmental learning disorders such as speech and language impairment, dyslexia and autism. Research into the neural bases of such disorders initially was limited to neuroanatomical studies of post-mortem material (e.g. Bauman and Kemper, 1985; Galaburda *et al*., 1985; Cohen *et al*., 1989). More recently, the development of less invasive techniques has allowed extensive *in vivo* structural and functional neuroimaging of affected children and adults (e.g. Bailey *et al*., 1998; Clark *et al*., 1998; Shaywitz *et al*., 1998; Eckert *et al*., 2003). However, it has not been feasible previously to investigate the neural mechanisms underlying language-related disorders using the powerful tools of molecular biology. The discovery of *FOXP2*, the first case of a gene that is implicated in a developmental disorder of speech and language (Lai *et al*., 2001), now provides a unique opportunity to explore relevant neural pathways from a molecular perspective.

The link between *FOXP2* and speech and language deficits was uncovered by molecular studies of an unusual three-generation family, referred to as KE (Fisher *et al*., 1998; Lai *et al*., 2000, 2001). Half of the members of this family (15 individuals) suffer from a severe disorder, involving profound deficits in the control of complex coordinated face and mouth movements, resulting in disrupted speech (Hurst *et al*., 1990; Vargha-Khadem *et al*., 1998). This persistent orofacial

dyspraxia has its onset early in childhood and is later accompanied by impairments in the development of a wide range of linguistic and grammatical skills (Watkins *et al*., 2002*a*). Such impairments are evident in both expressive and receptive domains, whether assessed by oral or written means (Watkins *et al*., 2002*a*). Although the mean non-verbal intelligence of affected family members is lower than that of unaffected members (Vargha-Khadem *et al*., 1995), the disorder does not simply represent a general intellectual delay (Watkins *et al*., 2002*a*). A detailed review of the relationship between the various aspects of the KE phenotype is given by Fisher *et al*. (2003).

Unlike the vast majority of families affected with developmental speech and language disorders, the inheritance pattern in the KE family is compatible with mutation of a single autosomal dominant locus. Using linkage analysis, the mutated gene was mapped to chromosome 7q31 (Fisher *et al*., 1998). Further studies revealed the presence of a point mutation in *FOXP2* in all affected KE family members, but in no unaffected members (Lai *et al*., 2001). This same gene was directly disrupted by a chromosomal rearrangement in an unrelated individual (C.S.) who had a similar phenotype to that found in affected KE subjects.

FOXP2 encodes a novel forkhead transcription factor. These proteins are defined by the presence of a forkhead-box DNA-binding motif, and they regulate expression levels of target genes during signal transduction, cellular differentiation and pattern formation (Carlsson and Mahlapuu, 2002). Many have key functions in tissue patterning during embryogenesis, and mutations of different forkhead genes are known to cause a variety of developmental disorders, including glaucoma (Nishimura *et al*., 1998), immune deficiency (Wildin *et al*., 2001) and ovarian failure (Crisponi *et al*., 2001). The mutation in affected KE individuals alters an amino acid in a critical position of the forkhead domain of FOXP2, which is likely to have adverse consequences for protein function.

Current understanding of the neurological aspects of the disorder associated with *FOXP2* disruption is based largely on neuroimaging studies (Vargha-Khadem *et al*., 1998; Watkins *et al*., 2002*b*; Belton *et al*., 2003). MRI and PET have revealed several abnormal brain structures in affected KE family members, compared with unaffected controls. These include the caudate nucleus of the basal ganglia, which is bilaterally abnormal both structurally and functionally. Given that insufficient functional FOXP2 during embryogenesis is a likely cause of the problems of family KE and case C.S., we may gain new aetiological insights by studying the *FOXP2* expression pattern in the developing brain.

Previously, limited data on *Foxp2* expression in mouse embryos have been reported. Shu *et al*. (2001) showed that *Foxp2* is expressed in areas of the developing lungs, the intestinal system and the cardiovascular system. They also reported that *Foxp2* mRNA can be detected in the interneurons of the spinal cord at gestational day 12.5 (E12.5), and in the inner intermediate zone of neopallial cortex at E16.5. In humans, *FOXP2* mRNA has been detected in brain tissue from adults and fetuses using northern blot analysis (Lai *et al*. 2001), and in the caudate nucleus of adults using reverse transcriptase–polymerase chain reaction (RT–PCR) (Bruce and Margolis, 2002). Nevertheless, these previous studies did not acquire detailed knowledge on the temporal and spatial expression of *FOXP2* during early brain development in either mouse or human. Therefore, in the present study, we have determined the pattern of *Foxp2/FOXP2* mRNA distribution during embryonic and fetal development of the CNS in both species. We suggest that integration of these data with results from neuroimaging studies brings us closer towards a coherent explanation of the pathogenesis of this speech and language disorder.

Material and methods

Embryonic/fetal material

Foxp2 expression was characterized in random-bred CD1 mouse brain at E11.5, E13.5, E16.5 and in newborns. The distribution of *FOXP2* mRNA was studied in the human brain at Carnegie stage (CS) 17, 18, 19, 21 and 23, and fetal stage (FS) 1 (O'Rahilly and Müller, 1987). Human embryonic/fetal material was obtained from the MRC/Wellcome Trust Human Developmental Biology Resource with full ethical approval.

Production of plasmids with Foxp2/FOXP2 inserts

Two regions of *Foxp2/FOXP2* were amplified by PCR using multiple-tissue cDNA panels (Clontech). These regions were chosen for their low homology to *Foxp1/FOXP1*, a closely related forkhead gene (Shu *et al*., 2001; Banham *et al*., 2001).

The primers used were: *Foxp2* (mouse) middle probe (5′–3′) AATGGATCCCTGCTCAGCCTTCAGC and (3′–5′) AATGGATCCAGACGTTCGCGTTCC, generating a 509 bp product spanning exons 6–10; *Foxp2* 3′ probe (5′–3′) AATGGATCCAGTTTGGGCTATGGAGC and (3′–5′) AATGGATCCGCCTGTTGGTTCTGAATC, generating a 521 bp product spanning exons 15–17; *FOXP2* (human) middle probe (5′–3′) AATGGATCCCATCTGCTCAGCCTTC and (3′–5′) AATGGATCCGAAGACGTTCGCGTTC, generating a 514 bp product spanning exons 6–10; and *FOXP2* 3′ probe (5′–3′) AATGGATCCTGGCTCTTAAGGGTTC and (3′–5′) AATGGATCCAGCTCTTGGCCATGG, generating a 551 bp product containing untranslated sequence from exon 17.

PCR products were cloned into pBlueScript-KS (Stratagene), using *Bam*HI restriction sites incorporated into the primers. Insert sequences and orientations were confirmed by automated sequencing (Applied Biosystems).

Generation of Foxp2/FOXP2 riboprobes

Antisense and sense probes were generated by *in vitro* transcription using T7 and Sp6 RNA polymerases under standard procedures. Digoxigenin-dUTP was incorporated into riboprobes during *in vitro* transcription by using the DIG RNA labelling mix (Roche) according to the manufacturer's instructions.

In situ hybridization

In situ hybridization was carried out as described by Wilkinson (1992). Briefly, embryos/fetuses at selected stages were dissected and fixed in 4% paraformaldehyde (PFA) in phosphate-buffered saline (PBS) overnight at 4°C. Following fixation, tissues were dehydrated and embedded in paraffin wax. Sections of 8–10 μm were cut using a standard microtome and attached to slides coated with 3-aminopropyl-triethoxysilane or Superfrost Plus microscopic slides (BDH).

Before hybridization, tissue sections were de-waxed, hydrated, fixed in 4% PFA/PBS and rinsed twice with PBS. Proteins were removed by incubation with proteinase K (20 μg/ml) in PBS. After washing with PBS, the sections were re-fixed in the same PFA solution, and treated with 0.1 M triethanolamine containing 0.25% acetic anhydride. Slides were dehydrated through an alcohol series and air-dried.

Hybridization solution contained riboprobe (1/100 dilution), RNAguard (1 μl/ml) and tRNA (0.5 mg/ml) in hybridization buffer (50% formamide, 0.3 M NaCl, 20 mM Tris–HCl pH 7.5, 5 mM EDTA pH 8.0, 10% dextran sulfate and 1× Denhardt's solution). A 100 μl aliquot of hybridization probe was added to each slide, which was incubated in a sealed chamber moistened with 50% formamide/1 × standard saline citrate (SSC) overnight at 65°C.

Stringency washes were performed in the following order: 2× SSC (twice at 65°C); 50% formamide/2× SSC (twice at 65°C); 2× SSC (twice at 65°C); 0.2× SSC (65°C) and 0.2× SSC (65°C cooled to room temperature). Slides were then incubated for 1 h in 150 mM NaCl and 100 mM Tris–HCl pH 7.5 containing 10% fetal calf serum (FCS). For antibody detection, slides were incubated in anti-digoxigenin antibody conjugated with alkaline phosphatase (anti-Dig antibody diluted 1 : 1000, containing 2% FCS) overnight at 4°C. Expression patterns were visualized using the NBT/BCIP system (Roche). Sections were mounted in VectaMount (Vector Labs) and analysed using the Axioplan 2 imaging system (Zeiss). Hybridization experiments were repeated in at least three mouse embryos and one human embryo at each developmental stage.

Results

We characterized *Foxp2*/*FOXP2* expression in mouse brain at E11.5–E16.5 and newborn, and in human brain at CS17–23 and FS1, as described in Material and methods. Two probes from different areas of *Foxp2/FOXP2* were used, with identical results in each case.

We first observed *Foxp2* expression in the developing mouse brain at E11.5 in the myelencephalic part of the rhombencephalon, an area destined to form the future medulla oblongata (data not shown). While no signal was detected in human brain at CS17 (~41 days gestation), *FOXP2* mRNA was identified at the midline of the hindbrain by CS18 (~45 days gestation) (data not shown), with an expression domain similar to that in mouse. This indicated high conservation of timing and tissue distribution at onset of *Foxp2/FOXP2* transcription in the CNS of the two species. As development progressed to the mid–late embryonic period, *Foxp2/FOXP2* expression became more complex, with signals detected in several brain regions (Fig. 1). At E13.5/CS23, expression was detected at the medullary raphe and in confined areas of the medulla oblongata (Fig. 1H and I). Strong signals were also localized to the alar plate of the cerebellar primordium (Fig. 1A and B). In the diencephalon, *in situ* hybridization labelled the medial region of the hypothalamus and the thalamus, in both mouse and human (Fig. 1C–E). A diffuse signal was found in the caudate nucleus, adjacent to the internal capsule (Fig. 1F and G). The expression pattern in human continued to strongly resemble that in mouse.

Despite an increase in relative strength of hybridization later in development, at E16.5 in mouse (Fig. 2) and FS1 in human (Fig. 3), the basic neural structures positive for *Foxp2/FOXP2* expression remained unchanged. In mouse at E16.5, signals became stronger in the hypothalamus and thalamus (Fig. 2E, F, I and J). *Foxp2* expression in the caudate–putamen was more intense compared with that observed at E13.5 (Fig. 2I and J). In the hindbrain, hybridization signal was observed in the developing cerebellum, including the deep nuclei (Fig. 2A and B). While the signal in the medulla remained strong (Fig. 2M and N), *Foxp2* transcripts were also found at E16.5 in the substantia nigra (Fig. 2F), the inferior colliculus, the habenular nucleus, the lateral lemniscus nucleus and the zona incenta (data not shown).

FOXP2 transcription was found correspondingly in the cerebellum, thalamus, caudate nucleus and medulla (Fig. 3) in transverse sections through a human brain at FS1. Compared with the pattern at CS23, an increase in both the intensity and the extent of the *FOXP2* expression domain in the thalamus was observed. Hybridization in the caudate nucleus was weak but detectable (Fig. 3D). Intense staining was still seen in the cerebellum (Fig. 3E and F) at FS1, while an equally prominent signal was located in the developing inferior olivary nuclei of the medulla (Fig. 3G and H).

Due to ethical limitations and restricted availability of material, studies of human embryonic expression were confined to early gestation; data from later gestation were obtained only for mouse. In newborn mice, the majority of neural structures are in their mature form and readily identifiable. Strong *Foxp2* signal was seen in the piriform layer of the cerebellum (Fig. 2C and D), which consists of large Purkinje cells. Weaker staining was found in the caudate nucleus (Fig. 2K and L), the cortical plate (Fig. 2K and L), the

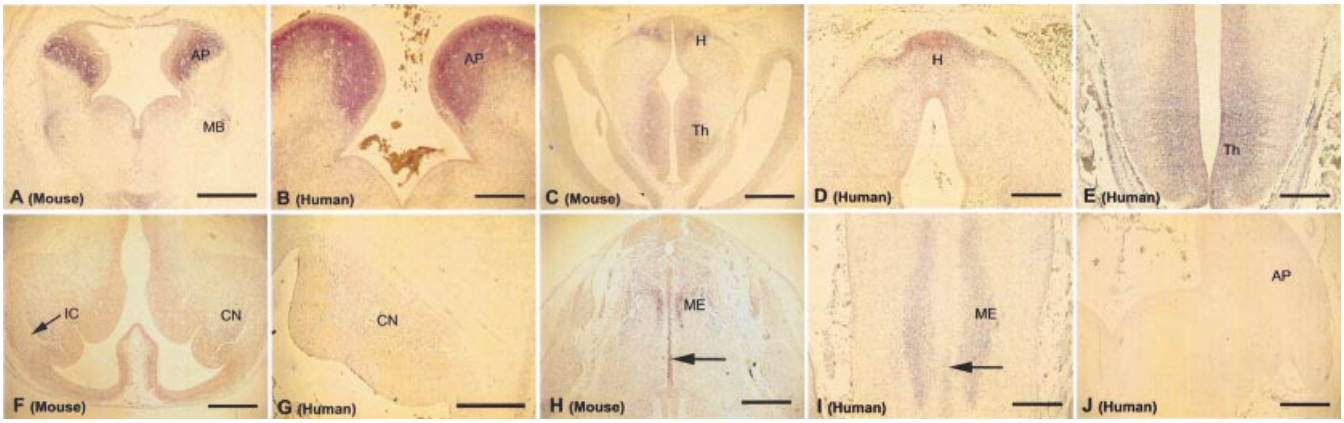

Fig. 1 Localization of *Foxp2/FOXP2* by *in situ* hybridization in the developing mouse brain at E13.5 and in a human brain at a comparable embryonic stage (CS23). (**A–I**) Transverse sections hybridized with an antisense probe generated from the 3′-untranslated regions of *Foxp2/FOXP2*. (**A**) In mouse, an intense hybridization signal is detected in the alar plate of the developing cerebellum. A weaker signal is also observed in the mantle layer of the midbrain. (**B**) A very similar expression pattern in the early cerebellum is seen in a corresponding section from a human brain at CS23. (**C–E**) In the mouse (**C**) and the human (**D** and **E**) diencephalon, the mamillary area of the hypothalamus and the dorsal thalamus are labelled by the *in situ* hybridization technique. (**F** and **G**) Diffuse signal is found in the caudate nucleus, adjacent to the internal capsule in both species. (**H** and **I**) In the mouse and human hindbrain, *Foxp2/FOXP2* transcripts are detected in the medullary raphe (arrows) and the medulla oblongata. (**J**) No signal is seen in the hybridizations performed with a 3′ sense control to human. A similar result was obtained with mouse sense controls (data not shown). AP = alar plate; MB = midbrain; H = hypothalamus; Th = thalamus; CN = caudate nucleus; IC = internal capsule; ME =medulla oblongata. Scale bars: (**A**, **C**, **F** and **H**) 1 mm, (**B**, **D**, **E**, **G**, **I** and **J**) 0.5 mm.

substantia nigra (data not shown) and a number of thalamic nuclei (Fig. 2G and H). Transcription of *Foxp2* was also detected in the inferior colliculus and the lemniscus nuclei (data not shown). At this stage, the distinctive expression seen in the medulla oblongata at E13.5/E16.5 could be clearly identified as the developing inferior olives (Fig. 2O and P), homologous to the expression pattern observed in human.

Discussion

Our detailed study of *Foxp2/FOXP2* mRNA distribution during development of the mammalian CNS indicates that the gene is not uniformly or diffusely expressed, but neither is its expression limited to just one brain area. Instead, we find that it shows restricted expression in a number of related brain structures. It is of note that there are many brain regions in which we did not detect *FOXP2* expression, including the developing and mature hippocampus. Moreover, as the brain matures, *FOXP2* expression is refined to specific substructures within positive regions. For example, early diffuse expression in the medulla becomes confined to the inferior olives, while cerebellar expression is restricted to the piriform layer by the time of birth. Thus, *FOXP2* transcription appears to be tightly regulated both spatially and temporally during CNS development.

FOXP2 is expressed in motor-related circuits during brain development

In addition to expression in the developing cortical plate, *FOXP2* transcription during CNS development is found predominantly in a series of neural circuits that have been implicated in motor control, including the basal ganglia, the thalamus, the inferior olives and the cerebellum. These structures are intricately interconnected to subserve motor-related functions; the basal ganglia modulate activity of premotor and prefrontal cortical areas via complex connections projecting through the globus pallidus, substantia nigra and thalamus, while the cerebellum plays an important role in regulating motor coordination, receiving input from the inferior olives. Our data implicating *FOXP2* in the development of corticostriatal and olivocerebellar motor-related circuits during embryogenesis may account for the persistent oromotor problems of humans with *FOXP2* mutation (Vargha-Khadem *et al*., 1998).

It is possible that the accompanying linguistic and grammatical impairments observed in the KE family are secondary consequences of basic deficits in motor planning and sequencing. However, it is equally plausible that the motor and cognitive problems arise simultaneously. There is growing appreciation that areas traditionally considered to be purely motor related also contribute to cognitive and complex behaviour (Middleton and Strick, 2000). The exclusively motoric nature of the caudate nucleus is challenged by data supporting roles in procedural learning and memory (Packard and Knowlton, 2002). Similarly, it is now recognized that the cerebellum and prefrontal cortex form neural circuits with both motor and cognitive capabilities (Diamond, 2000). Thus, our data are consistent with the emerging view that subcortical structures play a significant role in linguistic functioning.

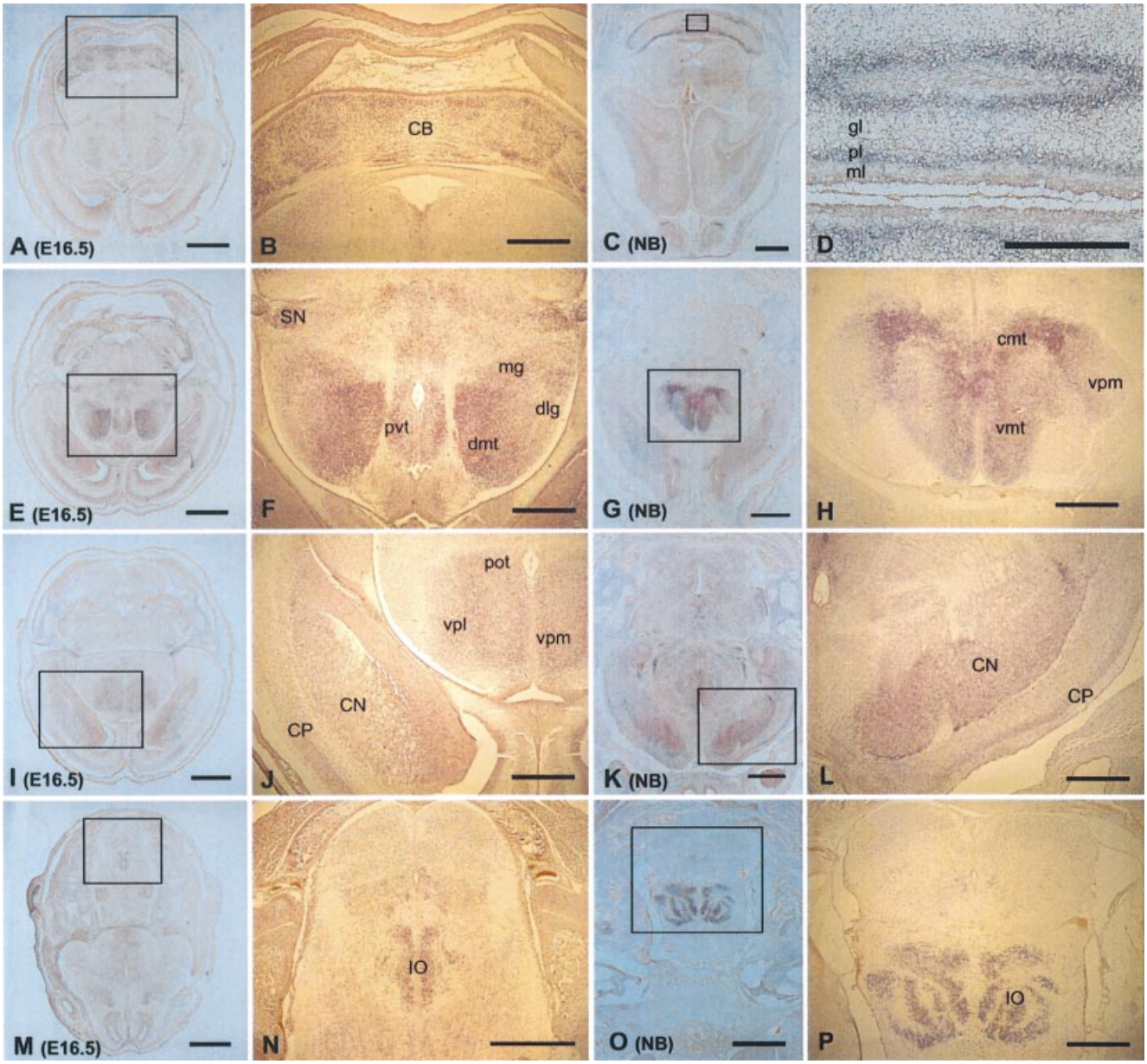

Fig. 2 *Foxp2* mRNA expression in the embryonic mouse brain at E16.5 and in the newborn. Sequential transverse sections, from anterior to posterior, hybridized with antisense *Foxp2* 3′ probe, are shown in the first and the third columns. Boxed areas are shown magnified in adjacent panels. (**A**–**D**) Strong *Foxp2* signal is observed in the cerebellum at E16.5 (**A** and **B**) with restriction in the newborn to the piriform layer, but not the molecular layer or the granular layer (**C** and **D**). (**E**–**L**) *Foxp2* is strongly expressed in the thalamus at E16.5 and in the newborn. Various thalamic nuclei express *Foxp2* (**E**, **F**, **I** and **J**), including the periventricular thalamic nuclei, ventral posterior thalamic nucleus, posterior thalamic nucleus and the lateral dorsal thalamic nucleus (data not shown). Additionally, the medial geniculate body and the dorsal lateral geniculate body show conspicuous *Foxp2* staining. *Foxp2* mRNA is also detected at the dorsalmedial hypothalamic nucleus, the substantia nigra and the lateral lemniscus (data not shown). (**G** and **H**) Intense *Foxp2* signal is seen in the thalamus of the newborn. High intensity *Foxp2* mRNA can be detected in the centromedial thalamic nucleus, the ventral posterior thalamic nucleus and the ventromedial thalamic nucleus. (**I**–**L**) Besides the thalamus, *Foxp2* mRNA is detected at both E16.5 and in the newborn in the caudate nucleus, and expression is present in the developing cortical plate. In posterior brain sections of the newborn, *Foxp2* expression in the substantia nigra and lateral lemniscus can be identified (data not shown). (**M** and **N**) *Foxp2* expression in the medulla oblongata is intense at E16.5. (**O** and **P**) In the newborn, this domain of *Foxp2* expression is localized to the inferior olivary complex (IO). CB = cerebellum; pl = piriform layer; ml = molecular layer; gl = granular layer; pvt = periventricular thalamic nucleus; vpl = ventral posterior thalamic nucleus (lateral); pot = posterior thalamic nucleus; mg = medial geniculate body; dlg = dorsal lateral geniculate body; dmt = dorsalmedial hypothalamic nucleus; SN = substantia nigra; cmt = centromedial thalamic nucleus; vpm = ventral posterior thalamic nucleus (medial); vmt = ventromedial thalamic nucleus; CN = caudate nucleus; CP = cortical plate; IO = inferior olivary complex. Scale bars: (**A**, **C**, **E**, **G**, **I**, **K**, **M** and **O**) 1 mm, (**B**, **D**, **F**, **H**, **J**, **L**, **N** and **P**) 0.5 mm.

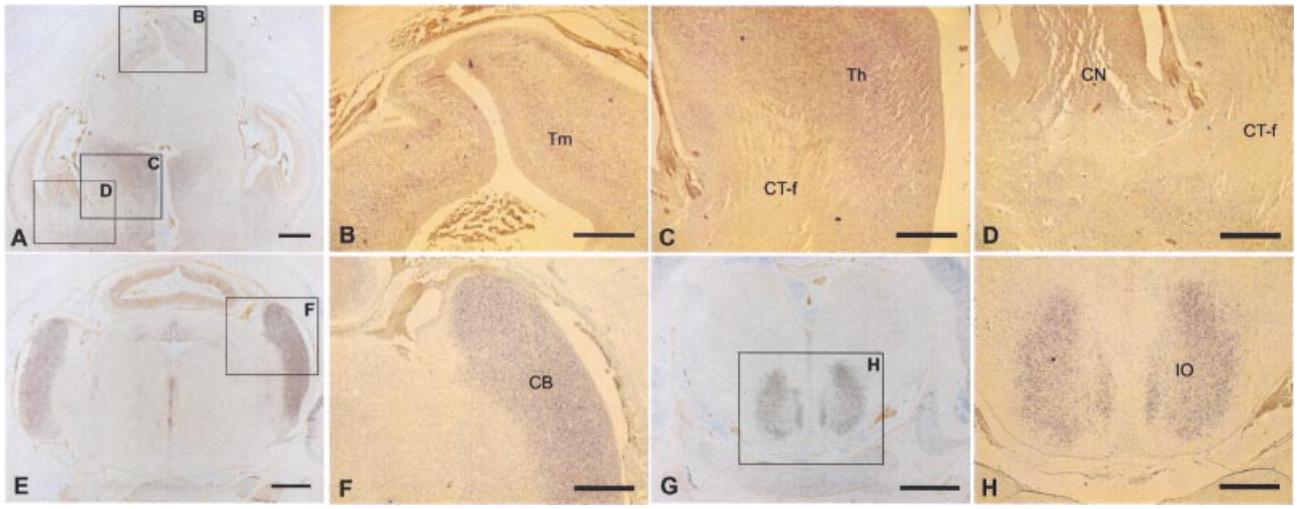

Fig. 3 *FOXP2* mRNA expression pattern in human brain at FS1 (~9 weeks post-fertilization). (**A**, **E** and **G**) Sequential transverse sections of the fetal brain. Boxed areas are shown magnified in adjacent panels (**B**, **D, F** and **H**). *FOXP2* expression is observed in the tectum (**B**) and the thalamus (**C**). (**D**) The caudate nucleus and the putamen of the basal ganglia are also positive for *FOXP2* expression. (**E** and **F**) Bilateral signal is observed in the cerebellum, in concordance with the pattern of mouse *Foxp2* expression. (**G** and **H**) Intensive staining in the medulla labels the developing inferior olivary nuclei. CN = caudate nucleus; Th = thalamus; CB = cerebellum; IO = inferior olivary complex; Tm = tectum; CT-f = cortico-thalamic tract. Scale bars: (**A**, **E** and **G**) 1 mm, (**B–D**, **F** and **H**) 0.5 mm.

FOXP2 expression in sites of pathology identified by brain imaging

Previous studies of the neuroanatomical basis of the disorder in the KE family have been necessarily limited to brain imaging analyses (Vargha-Khadem *et al*., 1998; Watkins *et al*., 2002*b*; Belton *et al*., 2003). Such investigations have yielded insight into structural and functional neural abnormalities that ultimately result from *FOXP2* mutation, but are unable to shed light on the developmental course that has led to these abnormalities. The situation is complicated by compensatory reorganization of abnormal neural systems, and it is often difficult to determine the relationship between the size of a neural structure, variation in its activation and its overall contribution to disorder (Watkins *et al*., 2002*b*). By highlighting regions of normal *FOXP2* expression, we offer the first glimpse of how disruption of this gene might impact on development of particular brain systems in the human embryo.

There is an intriguing level of concordance between structures implicated by our study and those suggested by complementary investigations of affected individuals with *FOXP2* mutation. Most notably, our observation of *FOXP2* expression in the developing caudate nucleus of the embryo is paralleled by neuroimaging findings in the KE family. A bilateral reduction of grey matter density in the caudate nucleus has been found in affected individuals using morphometric and volumetric techniques (Vargha-Khadem *et al*., 1998; Watkins *et al*., 2002*b*; Belton *et al*., 2003). In addition, a PET study detected over-activation of the caudate nucleus in two affected individuals when performing a word repetition task (Vargha-Khadem *et al*., 1998). The convergence of molecular, structural and functional data strengthens the case that the caudate nucleus is an important site of pathology in this disorder.

The caudate nucleus is not the sole site of *FOXP2* expression, and it is also not the only region of abnormality uncovered by neuroimaging studies of the KE family. For example, *FOXP2* mutation is also associated with significant structural anomalies in the cerebellum (Vargha-Khadem *et al*., 1998; Watkins *et al*., 2002*b*; Belton *et al*., 2003), a structure in which we have found striking *FOXP2* expression during embryonic development. Studies of unrelated patients with acquired lesions have highlighted a cerebellar role in procedural learning, particularly in detection and generation of event sequences (Molinari *et al*., 1997), and in linguistic functions (Schmahmann and Sherman, 1998). Indeed, impairments in the ability to sequence movement or in procedural learning recently were proposed as potential core deficits underlying the KE phenotype (Watkins *et al*., 2002*a*).

In addition to the Purkinje cells of the cerebellum, high levels of *FOXP2* mRNA were detected in the developing inferior olives of the medulla. The climbing fibres of inferior olivary neurons provide strong synaptic excitation to the Purkinje cells, forming a system that plays an important role in coordination and timing of motor control (Welsh *et al*., 1995; Yarom and Cohen, 2002). In relation to this, it has been reported that affected KE subjects are deficient in perception and production of rhythm, both vocally and manually (Alcock *et al*., 2000). Our expression data, therefore, draw attention to a possible link between the olivocerebellar system and timing deficits in the KE family, highlighting a neural circuit that warrants further examination.

FOXP2 expression patterns are highly concordant in mouse and human brain development

A key finding of our study is the high degree of similarity between mouse and human *FOXP2* expression patterns in the developing CNS. We have not found any evidence for regions of *FOXP2* expression that are only observed in humans in early brain development. Despite the high level of *FOXP2* coding sequence conservation in mammals, evolutionary studies indicate that human-specific changes in FOXP2 protein sequence underwent positive selection recently in human history, at a time that is compatible with the emergence of spoken language (Enard *et al*., 2002). Our data suggest that *FOXP2* might be generally implicated in aspects of motor control in mammalian species, and was already playing a role in the development of motor-related brain regions in the human–mouse common ancestor. Thus, positive selection of FOXP2 protein changes in recent human history probably involved modifications to pre-existing brain systems, rather than acquisition of novel ones.

Towards an integrated explanation of speech and language disorder aetiology

In conclusion, our study demonstrates the potential of integrating molecular genetic data with those obtained from other approaches, including neuropsychological investigations and brain imaging. We provide evidence, from the perspective of developmental biology, for involvement of the caudate nucleus, thalamus, inferior olives and cerebellum in the *FOXP2*-associated speech and language disorder. Future work, including mouse models in which the *Foxp2* gene is disrupted, should provide additional insight into the role of this gene in development of specific brain regions.

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