FOXP2 AND THE NEUROANATOMY OF SPEECH AND LANGUAGE

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Abstract | That speech and language are innate capacities of the human brain has long been widely accepted, but only recently has an entry point into the genetic basis of these remarkable faculties been found. The discovery of a mutation in *FOXP2* in a family with a speech and language disorder has enabled neuroscientists to trace the neural expression of this gene during embryological development, track the effects of this gene mutation on brain structure and function, and so begin to decipher that part of our neural inheritance that culminates in articulate speech.

VERBAL DYSPRAXIA
An impaired ability to perform the coordinated movements that are required for speech.

DYSPHASIA
Impairment of speech and verbal comprehension.

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Speech and language disorders have long been known to run in families, and the mutation of one or more genes has therefore been thought to be a likely cause in some of these cases. Only recently, however, was such a causative mutation identified. This discovery has opened a fascinating new chapter in the neurogenetics of our uniquely human form of communication. The new chapter began with an investigation of three generations of the KE family, half of whose members have a VERBAL DYSPRAXIA that is inherited in a pattern consistent with an autosomal dominant mutation. This verbal dyspraxia was later shown — on the basis of behavioural analysis — to be rooted in an orofacial movement disorder that is manifested most strikingly during speech. This quantitatively based description of the phenotype enabled a genetic linkage analysis, which led later to the identification of the mutated gene, *FOXP2* (REF. 1). In parallel, the behavioural phenotype helped to uncover important regions of neuropathology that are caused by the mutation. Although there is still much to be learned about this neuropathology and its functional consequences, a good start has been made in understanding how a single gene contributes to proficient human oral communication. Here, we review the behavioural phenotype in the affected KE family members, the correlated structural and functional neuropathology, and the expression of the *FOXP2* gene in normal brain tissue. These findings allow us to propose a tentative model of the portion of the neural circuitry for speech and language that is partly, but critically, dependent on *FOXP2*.

Behavioural phenotype

The KE family first came to the attention of the scientific community in 1990 with the publication of a report that characterized the affected members' speech and language disorder as a developmental verbal dyspraxia[2]. The disorder was described as one that affected the expression and articulation of language more than its comprehension, and problems were noted with organizing and coordinating the high-speed movements that are necessary for the production of intelligible speech. There were no hearing problems or neurological deficits that affected limb movements, and there was no evidence of difficulty with feeding or swallowing during infancy[2]. Later that year, several investigators published three further reports. One of these viewed the disorder as a DYSPHASIA that resulted from inflectional 'feature blindness' — an inability to use the rules of English grammar to denote tense, number, gender and so on[3]; the second suggested that it originated in the phonological and language-production systems rather than in grammar[4]; and the third classified it as a severe speech disorder that interfered non-selectively with all aspects of language, including phonology and grammar[5]. The question raised by these reports — that of the disorder's core deficits — remains unresolved.

One of the main aims behind identifying the core deficits was to obtain a reliable, quantitative index of affected status that transcended the family members' variability in speech and language performance. This search was carried out not only to advance the analysis of the behavioural phenotype but also to aid genetic

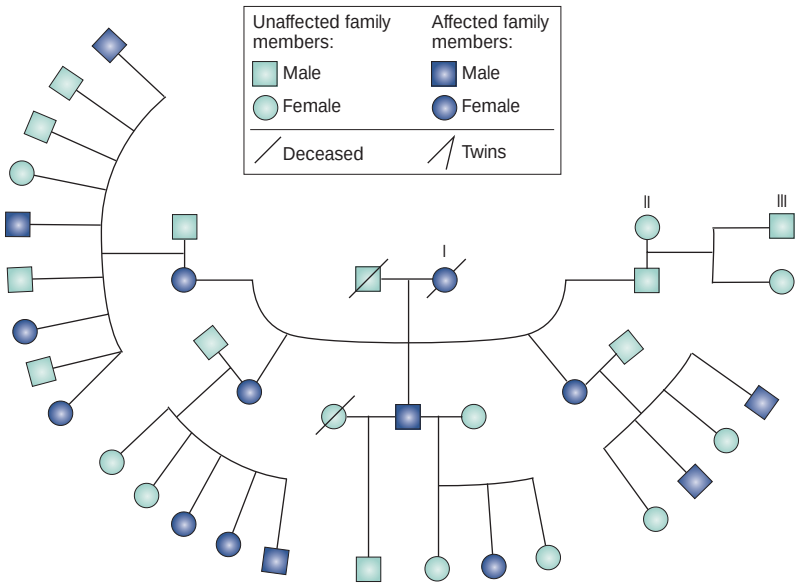

Figure 1 | **Pedigree of the KE family.** I, II and III represent the generations. Modified, with permission, from REF. 14 © (2002) Oxford University Press.

linkage analysis. On almost every test of speech and language that was administered[6,7], the group of family members that was presumed to be affected was, on average, significantly impaired relative to the group that was presumed to be unaffected. These tests included assessments of pronunciation, grammar, semantics, verbal IQ and even non-verbal IQ, with deficits tending to be greater for measures of language production than for measures of comprehension[8]. Nonetheless, there was considerable overlap between the two groups on most of the tests. Only on two of them — word and non-word repetition[9] and OROFACIAL PRAXIS[10] — did the two groups show no overlap[7]. Performance on these two tests served as an unambiguous index of the behavioural phenotype and was used to assign affected versus unaffected status (FIG. 1). The validity of the classification was confirmed when it led to localization of the *SPCH1* gene to the long arm of chromosome 7 (REF. 11), a linkage that led, in turn, to the identification of *FOXP2* (BOX 1).

On tasks that involved repeating words and, in particular, non-words, the affected members showed a greater relative deficit when they tried to reproduce multisyllabic as compared with monosyllabic consonant–vowel combinations[12]. Tasks that involved the imitation of non-speech movements yielded the same gradient of impairment, with parallel and sequential movements showing a greater relative impairment than single orofacial actions[10]. These findings indicate that one core deficit in affected family members is a higher order orofacial motor impairment or dyspraxia that is best exemplified in speech, because speech requires the precise selection, coordination and timing of sequences of rapid orofacial movements. Affected family members showed no deficits in manual praxis[13], although this might be because only highly skilled movement sequences of the fingers and hands (such as those required for expert typing or playing musical instruments) tax motor coarticulation capabilities to the same extent as does speech.

The verbal and orofacial dyspraxia in the affected family members bears a striking resemblance to that seen in adult-onset BROCA'S APHASIA. When compared directly[12], aphasic individuals and affected KE family members are equally impaired relative to unaffected family members on tests of grammatical competence, such as those that require the production of regular and irregular past tenses, and tests of DERIVATIONAL AND INFLECTIONAL MORPHOLOGY, whether for words or non-words. The comparison indicates that the two groups show equal deficits in manipulation of morphological markers, and, therefore, that the deficit is independent of age at onset of pathology, and independent of word meaning. However, there are also important differences between the two groups. The effect of 'meaning' became apparent when word and non-word repetition were compared; as previously noted, the affected family members are severely impaired on both types of reproduction, whereas aphasic individuals are severely impaired only on repetition of non-words, presumably because they learned the articulation patterns for words before the onset of their aphasia. The difference was reversed on tests of semantic, phonemic and written fluency, on which the affected family members were significantly less impaired than the aphasic individuals, suggesting that early plasticity might have led to partial but significant compensation for word retrieval difficulties imposed by their developmental disorder.

The extensive behavioural data on the KE family, combined with the success of linkage analysis, support the proposal that there is at least one core deficit — orofacial dyspraxia — underlying the speech and language disorder of the affected members. However, it is unclear whether their associated grammatical, semantic and other cognitive impairments are all secondary consequences of this fundamental deficit, or whether they point instead to the existence of additional core deficits.

Neural phenotype

The neural basis of the behavioural abnormalities shown by affected family members has been evaluated using a combination of structural MRI and functional MRI (fMRI) techniques. Together, these approaches can provide information about both the neuropathological basis of impaired function and the neural sites that underlie preserved or reorganized function.

In line with findings from many neurodevelopmental disorders, the affected members of the KE family have no obvious focal abnormalities on conventional neuroradiological assessment of their MRI scans; any structural abnormalities are evidently too subtle to be detected using this method. For this reason, three-dimensional T1-WEIGHTED MRI datasets were acquired and analysed using voxel-based morphometry (VBM), a computational technique that was developed to identify subtle regional differences in grey or white matter

OROFACIAL PRAXIS OR ORAL PRAXIS
Oral praxis is the volitional control of skilled non-speech movements.

BROCA'S APHASIA
Severe impairment of verbal expression by speech or writing due to pathology of the left inferior frontal convolution, named after the French surgeon who discovered the relationship.

DERIVATIONAL AND INFLECTIONAL MORPHOLOGY
The part of grammar that deals with the formation of one word from another by the addition of a prefix or suffix, often to change the case, gender, number or tense.

T1-WEIGHTED MRI
MRI scans can be acquired with various types of contrast. T1-weighted images are weighted according to the so-called spin-lattice relaxation time (T1) of the protons that give rise to the MRI signals; such images provide good contrast between grey and white matter.

Box 1 | Molecular genetics of *FOXP2*

The gene that is responsible for the speech and language disorder in the KE family was originally localized to the long arm of chromosome 7 (7q31)[11]. Even at this early stage, it seemed certain that the causative gene (then named *SPCH1*) had been located, because the linkage was strong, with a LOD SCORE of 6.6 (more than 3 is statistically significant). Further analysis narrowed the region genetically, and identified an unrelated individual, C.S., with a similar disorder of speech and language and a chromosome translocation involving the *SPCH1* gene region[40]. Ultimately, it was analysis of the chromosomes of C.S. that led to identification of the *FOXP2* gene as the cause of the speech and language disorder[1].

The translocation breakpoint in C.S. mapped onto a single BACTERIAL ARTIFICIAL CHROMOSOME (BAC) clone and, in this BAC, investigators found part of a new gene, *FOXP2*, with homology to other FORKHEAD GENES. The translocation breakpoint in C.S. disrupted the genetic structure of *FOXP2*. Moreover, a mutation elsewhere in the *FOXP2* sequence was found in members of the KE family. This mutation occurred in every affected family member, but not in unaffected members, nor in 364 chromosomes from unrelated control subjects, showing that the mutation was not simply a polymorphic variant. Importantly, the mutation substituted a histidine for an arginine at site 553 in the FOXP2 sequence. This arginine is invariant among *FOX* genes, suggesting that it has a crucial functional role, and lies adjacent to a histidine in the third helix of the forkhead domain, where the protein contacts the DNA during transcriptional control. A mutation at the corresponding residue, R127H, in FOXC1 has severe consequences for protein function *in vitro*[41].

So, it seems certain that the amino acid substitution in the KE family leads to a loss of function of one copy of the *FOXP2* gene, and that the one copy that remains is insufficient for normal brain development (haploinsufficiency), leading to the speech and language disorder.

between groups of scans. The images are compared on a voxel-by-voxel basis, with the final outcome displayed as statistical parametric maps that show regions where the local amounts of grey or white matter differ between groups. This method can also be used to correlate behavioural or other variables with regional grey or white matter density. An important feature of VBM is that the analysis covers the whole brain; specific regions of interest identified with VBM can then be subjected to further (for example, volumetric) analysis.

Given the large numbers of multiple statistical comparisons that are associated with VBM analyses, it is important to embark on these analyses with prior hypotheses. On the basis of the behavioural phenotype of affected family members, it was proposed that their underlying neuropathology would involve one or more components of the motor system. It was also proposed that the pathology would be bilateral — were it unilateral the expectation, for a neurodevelopmental disorder such as this, would be that reorganization to homologous regions of the contralateral hemisphere would have occurred, resulting in the preservation of basic speech functions.

The findings supported these hypotheses. The initial VBM analyses[7,14] showed bilateral abnormalities in several motor-related regions, including the caudate nucleus, which was of particular interest because this structure also showed functional abnormalities in a related positron emission tomography (PET) study[7]. A more detailed volumetric analysis confirmed that both caudate nuclei were reduced in volume (by about 25%) in the affected family members compared with the unaffected members and age-matched controls. Moreover, the volume of the caudate nuclei correlated significantly with the performance of affected family members on a test of ORAL PRAXIS, a test of non-word repetition and the coding subtest of the Wechsler Intelligence Scale[14]. The correlations on the first two tests suggest a relationship between the abnormal development of this nucleus and the impairments in oromotor control and articulation seen in the KE family.

Further analyses[15] used a modification of the VBM method that searches explicitly, and with increased sensitivity, for bilateral brain abnormalities[16]. They found abnormally low levels of grey matter in the inferior frontal gyrus (Broca's area), the precentral gyrus, the temporal pole, the head of the caudate nucleus and the ventral cerebellum (lobules VIIB and VIIIB) (FIG. 2). By contrast, there were abnormally high levels of grey matter in the posterior portion of the superior temporal gyrus (Wernicke's area), the angular gyrus and the putamen.

Functional neuroimaging studies have also been carried out using two fMRI language protocols, one involving covert (silent) verb generation and the other overt (spoken) verb generation and word repetition[17]. The unaffected family members showed a typical left-dominant pattern of activation involving Broca's area in the verb generation tasks and a more bilateral distribution in the repetition task, whereas the affected members showed a more posterior and more extensively bilateral pattern of activation in all tasks. Consistent with the morphological findings, the functional findings during both the covert and overt tasks indicated that, compared with the unaffected family members, the affected members had significantly less activation in Broca's area and its right-hemisphere homologue, as well as in the putamen. Abnormally low activation was seen in other speech-related cortical regions, but no abnormal functioning of the head of the caudate nucleus was detected, perhaps because the tasks used did not reliably activate this region in either the control or the unaffected family groups. By contrast, affected individuals showed overactivation in regions that are not usually involved in language, including the postcentral, posterior parietal and occipital regions. This overactivation might reflect recruitment of compensatory circuits, use of an alternative strategy or simply extra cognitive effort or attention that the affected family members required to perform the tasks.

These structural and functional imaging studies provide a crucial link in our understanding of the chain of events through which a point mutation in the *FOXP2* gene results in the speech and language disorder shown by the KE family. They indicate that *FOXP2* might be important for the development of putative frontostriatal and frontocerebellar networks that are involved in the learning, planning and execution of orofacial and, particularly, speech motor sequences, similar to the networks involved in learning and performing manual and other motor sequences.

LOD SCORE
A mathematical function that provides a measure of the strength of linkage between genetic loci in a breeding study. A lod score of 3 or more is considered to provide initial evidence that linkage exists.

BACTERIAL ARTIFICIAL CHROMOSOME
(BAC). A vector containing an origin of replication that enables genomic or other DNA fragments, inserted into the vector, to be grown in bacteria.

FORKHEAD GENES
A family of evolutionarily related genes, the FOX genes. FOX proteins regulate the transcription of target genes by binding their regulatory DNA sequences. This binding is performed by a special protein structure, the winged helix, which is encoded by the forkhead DNA sequence in the FOX gene.

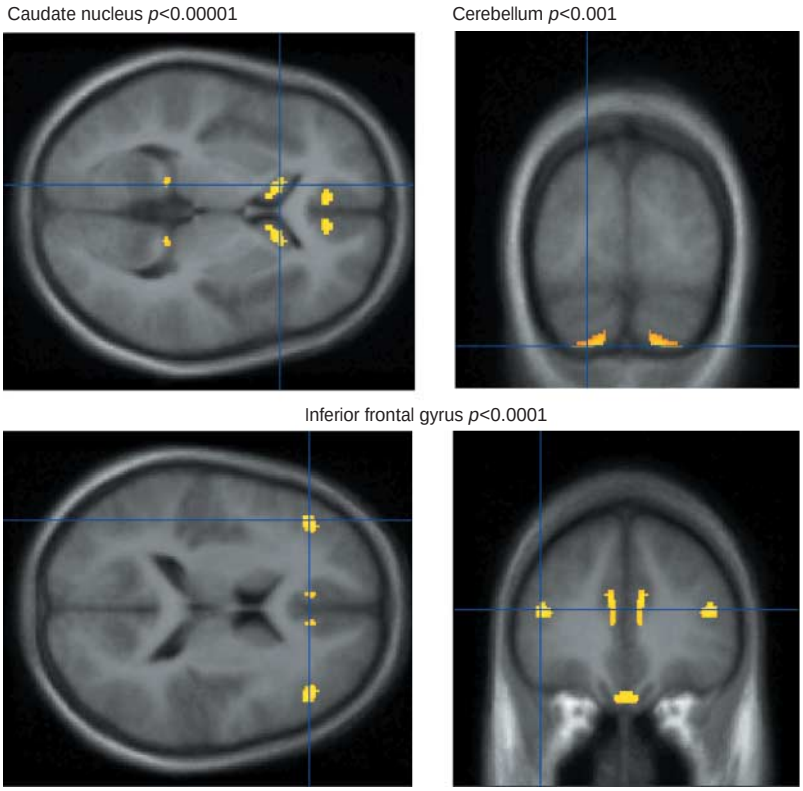

Figure 2 | **Bilateral voxel-based morphometry (VBM) analyses showing (in colour) some of the regions in which affected KE family members have significantly reduced grey matter.** Results were reported[15] at a significance level of $p<0.05$ after correction for multiple comparisons, or at an uncorrected level of $p<0.001$ if the regions fell within the *a priori* hypothesis. Significance levels (uncorrected) for the regions shown here were $p<0.00001$ for the caudate nucleus, $p<0.0001$ for the inferior frontal gyrus and $p<0.001$ for the cerebellum. The display threshold in the figure is $p<0.001$ for the images showing the caudate nucleus and inferior frontal gyrus, and $p<0.005$ for the image showing the cerebellum.

LEUCINE ZIPPER
A structural feature of some proteins in which two alpha helical regions, one from each protein monomer, are held together by hydrophobic interactions between leucine residues. The leucine zipper allows protein dimerization (pairing), which is necessary for the DNA-binding activity of some transcription factors.

PATCH COMPARTMENTS
These are distinguishable histochemically from the surrounding 'matrix' compartments of the neostriatum, the patches being rich in opiate receptors and substance P, and the matrix containing cholinergic neurons and a rich plexus of somatostatin-immunoreactive fibres.

Neural expression of *FOXP2*

The *FOXP2/Foxp2* gene (in humans and other mammals, respectively) encodes a multi-domain transcription factor that belongs to a large class of DNA-binding proteins known as winged-helix or forkhead proteins[18]. The FOXP2 protein interacts with the regulatory regions of downstream target genes and controls their expression by repressing the level and/or rate of transcription[19,20]. *Foxp2* is expressed not only in the brain, but also in other organs, including the lungs, heart and gut[1,19]. It functions in the lungs during embryonic development to inhibit the expression of genes associated with the differentiation of pulmonary epithelial cells. Hence, *Foxp2* might have an important role in the specification and differentiation of lung epithelial tissue[19].

How *Foxp2* acts as a gene regulator in the brain is still unknown, but where it acts has been described in both the mouse[19,21,22] and rat[23] at several stages of development from embryo to adult, and in the human embryo/fetus[21,22,24] between the ages of 6 and 22 weeks. These studies were based on the identification of *Foxp2/FOXP2* mRNA by *in situ* hybridization[19,21,22,24] and detection of the FOXP2 protein by immunohistochemistry[22]. For descriptions of where the gene acts in the brains of songbirds[24,25], see BOX 2. The expression patterns of *Foxp2/FOXP2* in the fetal mouse and human brains show striking similarities at comparable stages (FIG. 3). The *FOXP2/Foxp2* gene is widely expressed in the brain[19,21–24], and is present in sensory nuclei, limbic nuclei, the cerebral cortex and several motor structures.

The neural expression patterns of *Foxp2*'s closest relatives, *Foxp1* (REFS 19,22,23,24) and *Foxp4* (REF. 26), overlap partly with that of *Foxp2*, an important finding in view of the demonstration that all three proteins can heterodimerize through their LEUCINE ZIPPER motifs[20,27]. Hence, the transcriptional repressor functions of FOXP1, 2 and 4 might depend on synergistic molecular functions. Perhaps the relative balance of the different *FOXP* genes is crucial to ensure normal brain expression with respect to the development of speech and language capability. Reduced FOXP2 function might compromise the formation of sufficient heterodimers of a type required to activate downstream patterns of development. Nevertheless, the striking speech and language phenotype seen in humans with *FOXP2* mutations provides evidence against significant redundancy of function, at least in this aspect of neural activity.

Sensory nuclei. The *Foxp2/FOXP2* gene is expressed in the olfactory bulb of the adult mouse[22], the superior and inferior colliculi, and the lateral and medial geniculate bodies (midbrain and thalamic visual and auditory structures) of the adult mouse and human fetus[21,22]. It is also expressed in the ventral posterior lateral and ventral posterior medial nuclei (thalamic somatosensory relays) of mouse and human fetuses[19,23].

Limbic nuclei. In limbic nuclei, the gene is expressed in the amygdala, septal areas and paraventricular nuclei of both the hypothalamus and thalamus of fetal and adult rodents[21–23], and in the anterior and medial dorsal thalamic nuclei of the human fetus[24].

Cerebral cortex. Interestingly, gene expression in the cortex is limited to tissue below the granule cells of cortical layer IV; that is, to the infragranular layers VI and, to a lesser extent, V. The *Foxp2* mRNA signal appears embryologically in the inner layer of the cortical plate of all fetal mammals that have been investigated[19,21–24], with a trend towards greater expression in lateral than in medial aspects of the plate[22]. Subsequently, in newborn and mature rodents, *Foxp2* is expressed in a sub-population of neurons that is located mainly in layer VI (REFS 22,23).

Motor structures. In the motor system, *Foxp2* is expressed at many levels of the neuraxis. In the forebrain, the gene is expressed in the caudate nucleus and putamen of all mammals and at all ages that have been investigated[19,21–24]. Other basal ganglia structures that express *Foxp2* are the nucleus accumbens in the fetal and adult rat[23], the globus pallidus and subthalamic nucleus in the human fetus[24], and the substantia nigra in the mouse at all ages[21,22]. The gene is also expressed in basal ganglia-related subdivisions of the thalamus, including

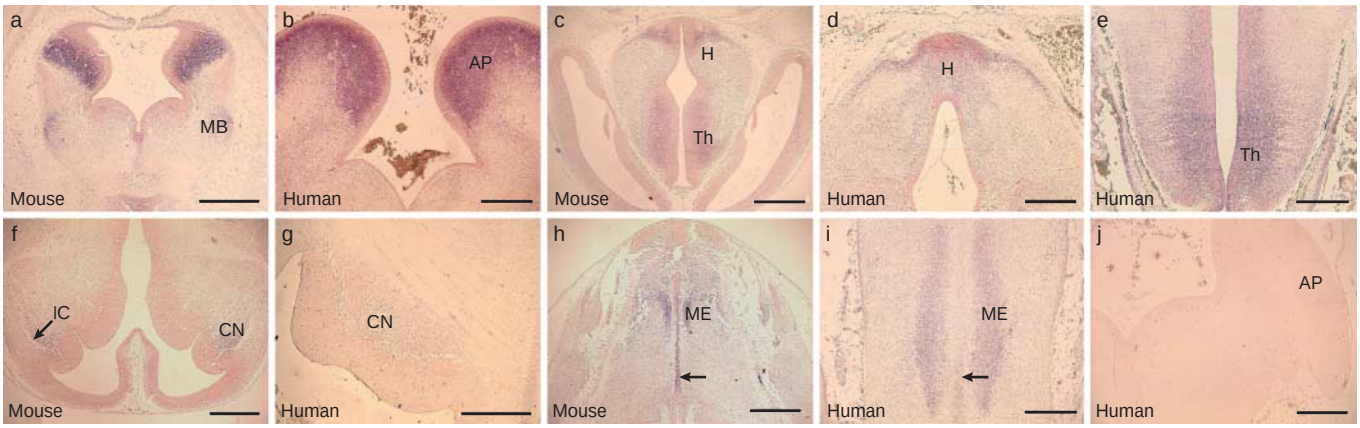

Figure 3 | **Expression of mouse *Foxp2* and human *FOXP2* are similar in the brain at embryonic day 13.5 (mouse) and Carnegie stage 23 (56 days post-fertilization in human).** Expression of *FOXP2*/*Foxp2* mRNA was detected by *in situ* hybridization on transverse histological sections, using an antisense cRNA probe generated from the 3′-untranslated regions of *FOXP2* and *Foxp2*. **a** | In the mouse, an intense hybridization signal is detected in the alar plate (AP) of the developing cerebellum. A weaker signal is also observed in the mantle layer of the midbrain (MB). **b** | A similar expression pattern is seen in the cerebellum of the early fetal human brain. **c**–**e** | In the mouse (**c**) and human (**d**,**e**) diencephalon, the mamillary area of the hypothalamus (H) and the dorsal thalamus (Th) express *Foxp2*/*FOXP2* in a similar way. **f**,**g** | A diffuse signal is found in the caudate nucleus (CN), adjacent to the internal capsule (IC) in both species. **h**,**i** | In the mouse and human hindbrain, *Foxp2*/*FOXP2* transcripts are detected in the medullary raphé (arrows) and the medulla oblongata (ME). **j** | No signal is seen in the hybridizations performed with a 3′ sense control probes. Scale bars: **a**,**c**,**f**,**h**, 1 mm; **b**,**d**,**e**,**g**,**i**,**j**, 0.5 mm. Reproduced, with permission, from REF. 21 © (2003) Oxford University Press.

the ventral medial, centromedian and parafascicular nuclei of the newborn mouse and human fetus[21,24]. In the hindbrain, it is expressed in the cerebellum and inferior olivary complex of all species studied[21–23], as well as in other cerebellum-related structures, including the red nucleus and ventral lateral nucleus of the thalamus in the human fetus[24]. *Foxp2* mRNA has also been found in the spinal cord of the embryonic mouse[19].

Box 2 | FOXP2 in songbirds

Songbirds, like humans, learn vocalizations through imitation, raising the question of whether there are any similarities between the different versions of *FOXP2* in such widely divergent species. Recent reports[24,25] indicate that there are. Although mammals and birds separated from a common line more than 300 million years ago, the FoxP2 protein in the zebra finch differs from the FOXP2 protein in mice at only five amino acid positions and from human FOXP2 at eight positions; these differences yield a figure of more than 98% identity of the protein, even between songbirds and humans[25]. Moreover, the overall pattern of *FoxP2* expression in the brain of the zebra finch[24] is remarkably similar to the pattern in mammalian brains, including the brain of the human fetus (see text).

Of particular interest is the expression of *FoxP2* in the avian song circuit. The more rostral of the two pathways that make up this circuit forms a loop homologous to the frontal–basal ganglia loop shown in FIG. 4. Specifically, neurons in a pallial or 'cortical' area (high vocal centre) send axons to a striatal/pallidal subdivision (area X, possessed only by vocal learners), which projects, in turn, to a thalamic region (medial nucleus of the dorsolateral thalamus, DLM), and from there back to the 'cortex' (lateral magnocellular nucleus of the anterior neostriatum, LMAN). LMAN then projects to the circuit's caudal pathway, which constitutes a motor path serving song production (see REF. 24 and other references therein). Not only is *FoxP2* strongly expressed in basal ganglia area X and thalamic region DLM, but in addition its expression in area X increases during the critical age (post-hatch days 35–50) at which the bird learns to imitate song[25]. Also noteworthy is the finding that the expression of *FoxP1*, unlike that of *FoxP2* (with which *FoxP1* can dimerize), is sexually dimorphic, showing enhanced expression in area X of the song-learning male but not in the comparable region of the non-song-learning female. This finding indicates that *FoxP1* could also be crucial for human speech.

Although many motor regions express *Foxp2*, this expression is often specific to certain subdivisions or types of neuron. For example, the *Foxp2* signal is restricted to the PATCH COMPARTMENTS of the neostriatum (particularly the caudate nucleus)[22], the shell region of the nucleus accumbens[23], the internal segment of the globus pallidus[24], the Purkinje cells and deep nuclei of the cerebellum[21–23], and the interneurons that are dorsal to the motor neurons of the spinal cord[19].

Just as it is unclear why mutation of *FOXP2* in the KE family affects the development and maintenance of brain tissue but not, apparently, that of other tissues in which it is expressed, so is it unclear why the KE mutation seems to affect some brain regions in which *FOXP2* is expressed but not others. It is important to note that the structural and functional imaging studies might not have identified all areas with abnormalities; less stringent statistical criteria than the ones adopted in these studies might have led to the inclusion of other areas of abnormality. Nevertheless, several of the regions that strongly express the gene — notably a subset of lateral frontal and lateral temporoparietal cortical areas, and several components of the basal ganglia and cerebellum — are those that are abnormal in the affected KE family members. These concordances encourage the proposal that is advanced below.

A model of *FOXP2*-dependent circuitry

An assumption that is consistent with the neural expression pattern of *FOXP2*/*Foxp2* is that the basic neural circuitry that underlies normal speech is similar, in broad outline, to that determined for other motor functions (FIG. 4). This circuitry enables the motor cortex to be modulated and controlled by other frontal cortical areas both directly, through cortico-cortical pathways,

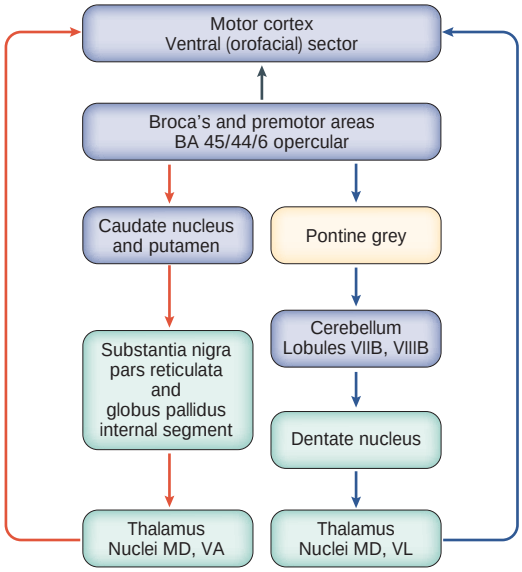

Figure 4 | **Proposed circuit for FOXP2-dependent speech and language.** Red arrows, inferior frontal–basal ganglia loop; blue arrows, inferior frontal–cerebellum loop. Blue and green boxes indicate structures that express *FOXP2*; blue boxes indicate the structures that have been found, using neuroimaging, to be abnormal either structurally, functionally, or both in affected KE family members. Besides the structures shown here, other components of the basal ganglia circuit that express *FOXP2* include the subthalamic nucleus and the ventral medial, centromedian and parafascicular nuclei of the thalamus; similarly, other cerebellum-related structures that express this gene include the inferior olivary complex and the red nucleus. BA, Brodmann areas; MD, medial dorsal thalamic nucleus; VA, ventral anterior thalamic nucleus; VL, ventral lateral thalamic nucleus.

and indirectly, through two parallel cortico-subcortical pathways — one frontostriatal and the other frontocerebellar[28–30].

Every component of the circuitry shown in FIG. 4, with the exception of the pontine grey, has been shown to express *Foxp2*. Several other structures belonging to the two parallel loops shown in the figure also express *Foxp2* (see figure legend). It is not known whether such widespread expression in motor-related structures reflects the importance of this gene in the functioning of all body musculature or only parts of it, but the KE family gene mutation clearly impairs the function of the orofacial musculature, particularly for movement sequences. Consistent with these findings, neuroimaging studies of affected family members show frontal lobe abnormalities caudally, in a ventral portion of the precentral gyrus (orofacial motor cortex), and more rostrally, in a portion of the inferior frontal gyrus (Broca's area). The abnormalities in these two areas lie on either side of, and possibly involve, the ventral premotor cortex in the region of the frontal operculum. All three areas — Broca's area, the ventral motor cortex and the opercular premotor cortex — are important for the sequencing of orofacial movements, especially speech[31–35]. By contrast, there were no apparent abnormalities in the supplementary motor area on the medial surface[36], an area that is important for the sequential programming of limb movements[37–39] (but, as noted above, the imaging studies might not have identified all areas of abnormality). The proposed model differs, in this respect, from a model for skilled sequential movements of the limbs.

Besides Broca's area and the ventral precentral cortex, which are abnormal presumably because of neuronal pathology in cortical layer VI, two other components of the frontostriatal and frontocerebellar loops from which they receive inputs also show abnormalities in the affected members of the KE family. These components include the head of the caudate nucleus, in which the abnormality is probably due to pathology in the striosomal/patch compartments, and cerebellar lobules VIIB and VIIIB, which are presumably abnormal as a result of pathology in the Purkinje cells. In addition, Broca's area receives inputs from language-related areas in the superior temporal and angular gyri, both of which are also affected by the KE mutation. Because Broca's area is likely to send projections directly or indirectly through premotor areas to the ventral (orofacial) portion of the motor cortex, it is in a position to transmit the normal or abnormal influence of all of its cortical and subcortical inputs to the orofacial musculature.

By integrating the evidence about the neural phenotype of the KE family with the neural expression pattern of *FOXP2*, the proposed circuitry provides a tentative account not only of how the KE mutation has resulted in the affected members' orofacial and verbal dyspraxia, but also of a way in which the normal *FOXP2* gene might have contributed to the emergence of proficient spoken language (BOX 3).

Suggestions for further research

Many of the findings that we have discussed in this review lead to suggestions for further research. Perhaps the most important outstanding behavioural question is whether all the deficits seen in the affected members of the KE family arise from the single root cause of orofacial and verbal dyspraxia. The possibility that there are one or more further core deficits merits careful investigation. Given the behavioural phenotype of the KE mutation, candidates for independent core deficits include rule-based learning, lexical acquisition and retrieval, and non-verbal cognition. However, the verbal dyspraxia itself needs to be examined further using such methods as electropalatography — a system for recording tongue–palate contact during speech — to identify precisely the sources of misarticulation. It is also unclear whether the misarticulation is primary or whether the primary problem lies upstream from the motor system in defective phonological representation, or even further upstream in basic acoustic processing. Until issues such as these are investigated, we cannot be confident that current interpretations regarding the behavioural effects of the *FOXP2* mutation are correct.

The structural and functional MRI studies outlined above have made substantial contributions to our understanding of the links between the genetic abnormality and the behavioural profiles of affected KE family members. Nevertheless, much remains to be elucidated. For example, longitudinal MRI investigations conducted early in

Box 3 | Evolution of *FOXP2*

Great interest has centred on the evolution of *FOXP2*, given the abnormal speech and language development observed in members of the KE family who have a mutation in one copy of the gene. A comparison of the nucleotide and amino acid sequences of the *FOXP2* genes of humans, other primates and other placental mammals[42,43] shows that FOXP2 is among the most highly conserved 5% of proteins, indicating that it has a fundamental role in mammals. Moreover, different human populations show essentially no variations in amino acid sequence, indicating that the present FOXP2 sequence is fixed in modern humans.

Although a number of nucleotide changes have accumulated in *FOXP2* since the human and mouse lineages diverged, around 70 million years ago, only three amino acids have changed in the FOXP2 sequence. Strikingly, two of these three changes (threonine to asparagine at position 303 and asparagine to serine at position 325) are present uniquely in humans, but not in chimpanzees, gorillas or orangutans. Hence, these amino acid substitutions arose and became fixed in the *FOXP2* sequence since the human lineage diverged from the chimpanzee lineage, only 4 to 6 million years ago. This rate of amino acid change is significantly greater than that expected by chance, given this period of evolutionary time[42,43]. Moreover, the two amino acid changes in the FOXP2 sequence satisfy all the criteria for a relatively recent selective 'sweep', in which this putatively advantageous genotype spread rapidly in all human populations. Indeed, it has been estimated that the spread was completed within the past 100,000–200,000 years[42,43], close to the time that anatomically modern humans appeared.

These findings of the *FOXP2* nucleotide sequence analysis predict that the two 'human-specific' amino acid changes will prove to have a consequence for the function of FOXP2. The change at amino acid 325 creates a potential phosphorylation site, which could affect how the protein functions as a transcriptional repressor, although whether the human FOXP2 sequence is functionally related to the speech and language capability of modern humans remains to be determined.

the lives of individuals at risk of *FOXP2* mutations or deletions might be able to chart the first indications of any structural brain abnormalities in postnatal life and document possible changes in abnormal regions as a function of brain and language development. In addition, the temporal resolution provided by electrophysiological measurements obtained at different stages of postnatal development could be combined with the structural resolution of MRI to provide a more comprehensive picture of the pattern of brain abnormalities. Moreover, diffusion-based MRI tractography techniques, which continue to be developed, might provide information about abnormalities of fibre tracts and connectivity associated with the pattern of structural brain abnormality.

As for further gene expression studies, it would be valuable to determine the upstream regulatory mechanism that governs the localization of *Foxp2* expression, an issue that involves identifying the promoter elements and the proteins that bind to them. The same is true for the downstream molecular events that are regulated by *Foxp2*. The latter can only be identified once a knockout mouse is available — gene expression events in the null mouse could then be 'subtracted' from those in a wild type, showing which events depend on *Foxp2*. A further level of refinement would be to create 'conditional' null mutant mice in which *Foxp2* could be inactivated in particular brain regions. This would allow investigators to determine the role of FOXP2 in the development of neural circuits on a region-by-region basis. Of course, the potential difference in function between human and mouse *FOXP2/Foxp2* remains, and would not be revealed by the above analysis.

Many of these issues have implications for the neuroanatomy of *FOXP2*-dependent speech and language, and many such studies must, therefore, be undertaken to revise and improve the proposed model. However, the evidence obtained should repay the effort, inasmuch as *FOXP2* is likely to continue to cast new light on the still mysterious neural mechanisms of human oral communication.

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Competing interests statement
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