

Chapter 2

Genetic Pathways Implicated in Speech and Language

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Abstract Disorders of speech and language are highly heritable, providing strong support for a genetic basis. However, the underlying genetic architecture is complex, involving multiple risk factors. This chapter begins by discussing genetic loci associated with common multifactorial language-related impairments and goes on to detail the only gene (known as *FOXP2*) to be directly implicated in a rare monogenic speech and language disorder. Although *FOXP2* was initially uncovered in humans, model systems have been invaluable in progressing our understanding of the function of this gene and its associated pathways in language-related areas of the brain. Research in species from mouse to songbird has revealed effects of this gene on relevant behaviours including acquisition of motor skills and learned vocalisations and demonstrated a role for Foxp2 in neuronal connectivity and signalling, particularly in the striatum. Animal models have also facilitated the identification of wider neurogenetic networks thought to be involved in language development and disorder and allowed the investigation of new candidate genes for disorders involving language, such as *CNTNAP2* and *FOXP1*. Ongoing work in animal models promises to yield new insights into the genetic and neural mechanisms underlying human speech and language.

Keywords FOXP2 • Language genetics • Development • Speech and language • Specific language impairment • Transcription factor • CNTNAP2 • FOXP1

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Heritability of Language and Language Disorders

There is considerable evidence to suggest that genes are important for directing developmental processes necessary for the normal use of speech and language. Furthermore, disorders that disrupt speech and language development have been shown to be highly heritable, providing strong support for a genetic basis for language impairments.

A number of well-studied neurodevelopmental disorders involve speech and/or language deficits as one part of a broader profile of symptoms. Examples include autism spectrum disorders (ASD, OMIM: 209850), fragile X syndrome (FXMR, OMIM: 300624) and Angelman syndrome (AS, OMIM: 105830). However, there are developmental disorders where the central or primary deficit is in the comprehension, processing and/or use (vocal or nonvocal) of language. These disorders, such as specific language impairment (SLI, OMIM: 606711), developmental dyslexia (DD, OMIM: 127700) and developmental verbal dyspraxia or childhood apraxia of speech (DVD or CAS, OMIM: 602081) can shed light not only on the genetic and developmental underpinnings of impairments but also on pathways involved in normal language development.

The first clues to the heritability of developmental disorders of speech and language came from observations of familial clustering. Children with language disorders are much more likely to have family members displaying speech, reading or language impairments than typically developing children [1–4]. The importance of genetic influences on language impairments was further illustrated by a study showing that children who had an affected parent but that had been adopted into a language rich environment were significantly more likely to suffer from language disorders than adopted children without a family history [5].

The magnitude of the genetic contribution to a disorder or trait can be investigated using heritability estimates. These can be calculated by comparing the rate of coinheritance of the disorder in monozygotic twins (considered to be genetically near identical) to that of dizygotic twins (who, like siblings, are ~50 % genetically similar). Assuming that mono- and dizygotic twins are subject to similar levels of shared environment during development and childhood, a genetically influenced disorder should co-occur more frequently in monozygotic twins than it does in dizygotic twins. Indeed, monozygotic twins show a higher concordance of language disorders, as well as more closely matched phenotypes within these disorders, as compared to their dizygotic counterparts [6]. Concordance has been reported to be near 100 % for monozygotic twins and between ~50 and 70 % for dizygotic twins, arguing strongly for a genetic component to language disorder [7, 8].

More recently, studies have focused on longitudinal measures in the normal range of abilities. These investigations found that while early language development (~2–5 years old) could largely be accounted for by environmental factors, linguistic skills showed higher heritability (54–60 %) between the ages of 7 and 12 years and very high heritability scores (~85 %) for long-term linguistic ability (up to age 18) [9–11].

Genetic Risk Factors for Complex Language Disorders

Evidence thus far suggests that the majority of language impairments are not caused by just a single gene acting in a Mendelian manner or by only a single region of the genome [7, 12]. Rather it appears likely that, in most cases, many different risk alleles spread around the genome make small contributions to the observed language phenotypes. A number of genetic mapping studies have attempted to define relevant regions of the genome and to pinpoint the key genes, but the complex multifactorial nature of the traits and the small effect sizes involved makes identification of the genetic risk factors challenging [13].

SLI is the most common form of language disorder, with approximately 7 % of school age children reported to meet diagnostic criteria [14, 15]. SLI is classified as the failure to develop normal speech and language skills in the absence of any environmental, medical or genetic impairments (e.g. hearing loss, mental retardation or other overt neurological disorders) [15]. In the first molecular investigations of common forms of SLI, researchers used DNA from multiple families to search through the genome for genetic markers whose inheritance may be linked to the trait (referred to as 'linkage analysis'). A genome-wide analysis for linkage to quantitative measures of language in 98 UK families identified two candidate regions: SLI1 (located at chr 16q, OMIM: 606711) and SLI2 (located at chr 19q, OMIM: 606712) [16]. These findings were replicated in a follow-up study of a further 86 UK families; in particular, the SLI1 region demonstrated highly significant linkage to deficits in non-word repetition (NWR), the ability to correctly repeat nonsense words, which has been proposed as a core feature of SLI [17]. High-density screening of the SLI1 region in an expanded set of the UK families and an independent population cohort identified association to two candidate genes, one encoding a calcium-transporting ATPase, ATP2C2, the other encoding c-maf-inducing protein, CMIP [18]. A third candidate region, SLI3 (located at chr 13q21, OMIM: 607134), was identified in an independent study of language impairment in 5 Canadian families [14], and linkage of this region to reading impairment was demonstrated in a follow-up study of 22 families from the USA [19].

With rapid advances in molecular technologies, more fine-grained and wide-ranging analysis of the genome has become possible, which will likely lead to the identification of further genomic regions and candidate genes contributing to language or language-related disorders. In order to make sense of these findings, it will be necessary to understand more about the phenotypes of the different language impairments and how they relate to each other. For example, it is likely that rather than being distinct syndromes, the spectrum of disorders that involve language impairments represent overlapping groups of syndromes that share endophenotypes (measurable components on the path between global phenotype and distal genotype), each of which might present along a distribution of severity. For example, a gene that is a risk factor for SLI may also be found to be a risk factor in some but not all individuals that meet the criteria for autism (a developmental disorder primarily affecting social interaction, verbal and non-verbal social communication and

repetitive, stereotyped behaviours) or dyslexia (an impairment of reading and spelling). How disorders are classified and how subjects are chosen for inclusion in studies will greatly influence our ability to detect shared or independent genetic factors underlying language and language impairment.

Studies investigating the underlying genetic factors contributing to dyslexia and autism are outside the scope of the current chapter, but there are a number of articles that comprehensively review this topic [20–24].

A Monogenic Speech and Language Disorder

As noted above, the vast majority of cases of language impairment are likely to have a complex genetic basis. However, in the late 1980s clinical geneticists came across an unusual large family showing an apparently simple inheritance pattern for their speech and language problems [25]. In this pedigree, known as the ‘KE family’, approximately half of the 30 family members, spread over three generations, suffered from a severe form of speech and language disorder [25]. The pattern of transmission observed in this family was consistent with simple autosomal dominant inheritance—highly suggestive that the disorder was monogenic that is due to disruption of just a single gene being passed from one generation to the next [25].

When researchers performed gene-mapping studies, they were able to formally demonstrate the monogenic nature of the disorder and pinpointed a small region of chromosome 7 (designated the SPCH1 locus) that was very likely to contain the causative gene [26]. The identification of an unrelated patient who had a highly similar speech and language disorder phenotype was key to determining which gene in the SPCH1 region was responsible [27]. This child (known as CS) carried a *de novo* translocation, involving a breakpoint in the SPCH1 region of chromosome 7. The investigators discovered that this breakpoint directly interrupted a previously unidentified gene known as *FOXP2*, and they hypothesised that disruption of this gene was responsible for the phenotype seen in CS. They went on to sequence the same gene in the KE family and found that all affected members carried a point mutation affecting a single nucleotide in the coding region of *FOXP2*, known as the R553H mutation (explained further below) [27]. This mutation was never found in unaffected individuals in the family or in the general population, and it was predicted to disturb the function of the gene [27]. Thus, both the CS case and the KE family carried disruptions to the *FOXP2* gene, which were potentially causative of their speech and language problems.

The KE Family

The phenotype of the KE family has been studied in detail both at the behavioural/cognitive and the neuroanatomical levels in order to dissect out the core features of

this complex disorder. Early reports posited conflicting hypotheses that the impairment seen in the KE family was largely one of articulation or conversely a grammar-specific disorder [28, 29]. However, the reality is likely to lie somewhere between the two models.

The phenotype observed in the KE family involves severe developmental verbal dyspraxia (known as DVD or childhood apraxia of speech, CAS). DVD is characterised by problems coordinating sequences of mouth/face movements when speaking, such that speech is unintelligible to the naive listener [28]. However, in the KE family, additional severe impairments are also observed in multiple areas of expressive and receptive language, affecting both spoken and written modalities. In studies that assessed a range of abilities in the KE family, tests of nonsense-word repetition (NWR) provided the most reliable metric for distinguishing between affected and unaffected family members [30]. Receptive vocabulary, lexical decision making and verbal fluency, tense production, receptive syntax at word-order level and inflectional and derivational morphology were all found to be significantly impaired in the affected members of the KE family [30, 31]. Furthermore, the orofacial dyspraxia of affected members is not entirely specific to speech. Reduced performance has also been observed in complex and sequential non-verbal oral movements, although single simple movements were unaffected [32]. Rhythm was also affected in tests of both vocal and manual timing, similar to effects reported in some other language disorders [33, 34].

Neuroimaging studies of the KE family have uncovered functional and anatomical correlates of the disorder. Magnetic resonance imaging (MRI) in ten affected and seven unaffected family members observed no overt anatomical differences differentiating the two groups [31]. However, statistical analyses using voxel-based morphometry identified subtle bilateral changes in grey matter density in affected individuals for a number of brain regions implicated in speech and language processing. Significantly reduced grey matter was observed in Broca's area, the supplementary motor area, caudate nucleus of the striatum and the ventral cerebellum, while regions of significantly increased grey matter could be seen in the thalamus, angular gyrus and parts of the cortex, including the sensorimotor and temporal cortex [31, 35]. For most individuals, language is localised to the left hemisphere of the brain. In some cases, when the left hemisphere is damaged (e.g. due to a stroke), if the right hemisphere is unaffected, it can adapt to performing language-related tasks. This process, known as relocation, is not thought to be able to occur in the KE family due to the bilateral neuroanatomical changes in grey matter density, which may help to explain why the disorder is so severe and persistent [35].

Functional neuroimaging studies have demonstrated differences in brain activation patterns in affected versus unaffected family members that are suggestive of linguistic processing defects. Aberrant bilateral activation in affected family members during semantic retrieval and articulatory planning was observed by functional magnetic resonance imaging (fMRI) [36]. In one part of this study, imaging was performed during covert (unspoken) verb generation tasks so that any signal arising from articulatory defects could be excluded. Covert verb generation tasks in an unaffected individual (unaffected KE family member or unrelated normal

individual) typically result in activation of the inferior frontal gyrus (Broca's area) in the left hemisphere and (subcortically) the putamen in the striatum [36]. Despite all participants being able to successfully perform the task overtly outside the scanner, the affected KE family members demonstrated strikingly different patterns of activation during the covert task. Significant under-activation of Broca's area and the putamen was observed in affected individuals, accompanied by significant over-activation of diffuse regions of both hemispheres, including Wernicke's area and the precentral gyrus [36].

Thus, in the KE family, a complex speech and language disorder involving receptive and expressive language impairment and associated with anatomical and functional changes in the brain was directly related to a single mutation disturbing the *FOXP2* gene.

The FOXP2 Gene

The *FOXP2* gene is located on human chromosome 7q31, made up of 25 exons (i.e. the expressed parts of the gene) that span a locus of ~600,000 nucleotides of DNA (~600 kb) (Fig. 2.1a). This gene codes for a protein (called the FOXP2 protein) that is able to act as a transcription factor, meaning that it regulates the expression (switching on and/or off) of other genes. The main version of the FOXP2 protein is 715 amino acids long, but, as with most genes and proteins, differential processing (alternative splicing) can sometimes generate alternative versions that are longer or shorter than this.

The FOXP2 protein contains a number of functionally important regions, or 'domains' (Fig. 2.1b). Moving along the protein from one end (the N-terminus) to the other (the C-terminus), the following domains can be identified: a region containing a large number of glutamine (Q) residues (Q-rich), a zinc-finger/leucine zipper region (ZnF/LeuZ), a DNA-binding domain (FOX) and an acidic tail region (Acidic). The FOXP2 protein regulates gene expression by binding to regulatory regions of the genome, usually located close to the start site for the coding regions of genes, and thereby affecting the levels of transcription for these so-called 'target' genes (i.e. altering the amount of gene product that is made). FOXP2 can directly bind to these regulatory regions of DNA via the specialised section of the protein known as the forkhead-box DNA-binding domain (or FOX domain, for short) [37]. The FOX domain is a stretch of ~90 amino acids that folds into a three-dimensional structure which wraps itself around DNA [38]. The FOX domain does not wrap around just any section of DNA, but has a preference for specific sequences of nucleotide letters; thus, it binds only to particular regions of the genome, located within its target genes [39–41]. Following DNA binding, FOXP2 is able to activate (turn on) or repress (turn off) the expression of these target genes [42].

The point mutation identified in the KE family introduced an amino acid change at position 553 of the protein sequence, swapping the arginine (R) that is normally found at this position to a histidine (H) residue; thus, the mutation is known as

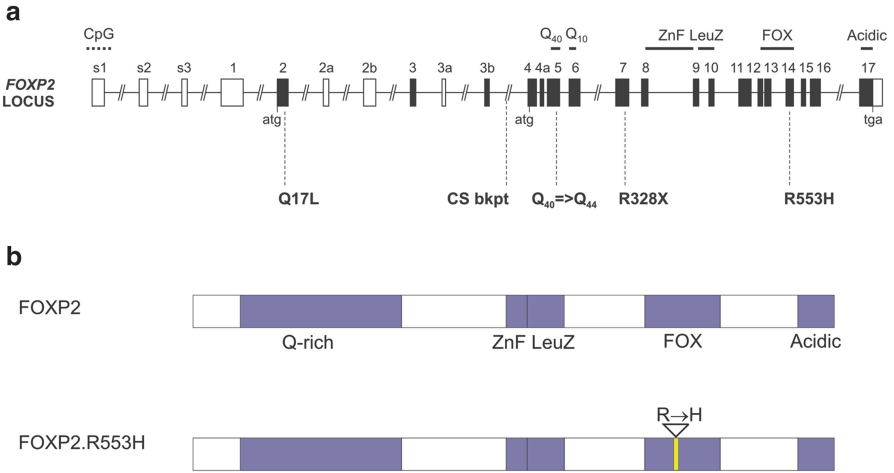


Fig. 2.1 (a) Schematic of the human FOXP2 locus, spanning >600 kb. *Black shading* indicates exons that are translated into protein; ‘atg’ and ‘tga’ denote start and end codons. Exon s1 overlaps with a type of regulatory region known as a CpG island. Additional information on features of this locus can be found in Fisher et al. [13]. Sites of coding variants reported in children with severe speech and language impairment are indicated below the locus schematic, including the R553H mutation initially identified in the KE family [27], and the three additional changes uncovered in a subsequent screening study of 49 other probands [61]. The figure also shows the site of the translocation breakpoint found in case CS, mapping between exons 3b and 4 [27]. Multiple additional translocation cases involving FOXP2 disruption have since been reported. *Adapted with permission from MacDermot et al. (2005)* [61]. (b) Schematic of the major form of the FOXP2 protein (encoded by exons 2–17) contains 715 amino acids, with polyglutamine tracts of 40 (Q40) and 10 residues (Q10) collectively known as the Q-rich region, a zinc-finger motif (ZnF), a leucine zipper (LeuZ), a forkhead domain (FOX) and an acidic C-terminus (Acidic). The location of the KE family mutation (R553H) in the protein is also shown. *Adapted from Vernes et al. (2006)*

R553H [27]. Crucially, position 553 is located in a key part of the FOXP2 DNA-binding domain (Fig. 2.1b) and an arginine is found at this location in every type of normal FOX protein that has been discovered so far (see next section), suggesting it must be important for protein function [27]. Indeed, in laboratory-based tests, introducing the R553H change into an isolated FOX domain, or into the full length FOXP2 protein, in each case abolished DNA binding to a known target sequence and affected the ability of the protein to regulate gene expression in cellular model systems [43]. These assays also suggested that the R553H form of the protein was able to interfere with the activity of normal FOXP2 protein present in the cells [43]. In addition, there was evidence of mis-localisation of the mutant protein within the cell. Normally, the FOXP2 protein is found in the nucleus of cells where it can access DNA in order to regulate gene expression [43]. However, the mutant version of the FOXP2 protein, carrying the R553H change, sometimes showed both nuclear and cytoplasmic localisation [43]. Thus, substitution of this single amino acid had severe and wide-ranging effects on the ability of the protein to function normally.

FOX Transcription Factors

FOXP2 is just one of a large group of transcription factors (known as FOX proteins), all of which carry a highly conserved version of the characteristic FOX DNA-binding domain. This group of proteins is classified into subgroups, based on comparing the sequences of the DNA-binding domain. To date 17 FOX subgroups have been identified, designated FOXA to FOXQ, in order of their discovery [44]. Accepted nomenclature for this gene family uses upper case for human genes (*FOXP*), lower case for mouse genes (*Foxp*) and upper and lower case for all other species (*FoxP*). Proteins are denoted by roman type (FOXP) [45].

FOXP2 falls into the 'P' subgroup of FOX proteins, which also includes FOXP1, FOXP3 and FOXP4 [46, 47]. The three most closely related members, FOXP1/FOXP2/FOXP4, show ~92 % similarity of amino acid sequence in their FOX domain [47], suggesting closely related function. These proteins have also been shown to interact with each other via their ZnF/LeuZ regions. Indeed, homodimerisation (interaction by two of the same FOXP molecules) and heterodimerisation (interaction between two different FOXP family members) appear to be required for efficient binding to target DNA [48]. It is also thought that the glutamine-rich regions of these transcription factors mediate interaction with other proteins to facilitate the assembly of protein complexes around gene regulatory regions. For the mouse homolog of FOXP1, the presence of the glutamine-rich region was found to be capable of fine-tuning the strength of regulation mediated by the protein [42].

FOXP1/2/4 display distinct but overlapping expression patterns in the brain. FOXP2 follows a highly restricted pattern of expression in a range of structures of the brain during development. During foetal development in humans, FOXP2 was shown to be expressed (at around 9–14 weeks gestation) in the thalamus; hypothalamus; developing striatum (caudate-putamen); areas of the cortex including the perisylvian regions and frontal, parietal and occipital cortices; the medulla; and the cerebellum [49, 50]. As development progresses, FOXP2 expression becomes restricted to specific subpopulations of neurons in these regions, for example, to the deep layers of the cortex (layers V/VI), the inferior olivary complex of the medulla and Purkinje cells of the cerebellum [49, 50].

Studies in rodents, which show the same expression patterns as seen in the human tissue tested, have illustrated the combinatorial expression of the *Foxp* subfamily in the brain. *Foxp1* expression overlaps with *Foxp2* in a number of regions as both genes are expressed in the developing cortical plate, striatum, thalamus and inferior olives of the medulla [49]. However, while expression overlaps in the developing cortex, in the mature (six-layer) cortex, *Foxp2* is restricted to the deepest layers (layer V/VI), while *Foxp1* is found throughout layers III–V [49]. By comparison, *Foxp4* expression is spread throughout layers II–VI [51].

Unlike *Foxp2*, *Foxp1* and *Foxp4* can be found in the developing and adult hippocampus, and while *Foxp2* is strongly expressed in the cerebellum, amygdala and olfactory bulb, *Foxp1* is completely absent from these regions [49]. *Foxp4* expression overlaps with *Foxp2* in the developing striatum, olfactory bulb and Purkinje cells of the cerebellum [51, 52]. *Foxp2* and *Foxp4* are both expressed in the

amygdala but are largely found in different neuronal subtypes. Expression of *Foxp2* and *Foxp4* switches on earlier in development than *Foxp1* and postnatally *Foxp4* expression is severely downregulated in the forebrain, while *Foxp1* and *Foxp2* expression persists [51]. *Foxp3* is not expressed in the brain. It appears that in the normal brain, a precisely controlled and coordinated pattern of expression is orchestrated, and the requirement of these closely related family members in different regions may give clues to the different functions they perform during brain development.

In addition to the contributions of *FOXP2* to human language development, *FOXP* proteins have been shown to play functional roles in diverse processes ranging from organ development to tumorigenesis [44]. Both *FOXP1* and *FOXP2* have been implicated in cancer progression [53, 54]. Furthermore, studies of mouse models in which these genes are ‘knocked out’ have implicated *Foxp1/2* in lung development and *Foxp1/4* in heart development. *Foxp1* has shown to be crucial for determining motor neuron identity in the spinal cord [55, 56]. *Foxp4* is a key regulator of foregut development [57–59] and also appears to be important for Purkinje cell arborisation and connectivity [52]. *FOXP3* is the most divergent member of the family in terms of sequence and function and has been shown to be important for immune system development [60].

FOXP2 Mutations in Other Cases of Language Disorder

Since the original identification of the point mutation in the KE family, further evidence has come forward, supporting the role of *FOXP2* in language disorders. A number of inherited and de novo mutations have been identified that disrupt the *FOXP2* locus in various ways. In some cases these mutations yield a speech and language disorder that matches very closely with the phenotype observed in the KE family. However, other cases display mixed phenotypes that incorporate elements of DVD, ASD, intellectual disability (ID) and other neurodevelopmental disorders. This heterogeneity can usually be attributed to the size of the mutation, as larger disruptions can often disturb multiple neighbouring genes on chromosome 7, in addition to their effect on *FOXP2*.

Point Mutations of *FOXP2*

The first report to identify independent mutations of *FOXP2* focused on a panel of 49 cases of DVD, chosen for their phenotypic similarity to the disorder observed in the KE family [61]. The study screened the entire coding region of *FOXP2* (Fig. 2.1a) and identified three novel heterozygous variants, in different cases of DVD. Two of these changes (Q17L and Q₄₀→₄₄) were each found in an affected proband but not an affected sibling. As such, although these changes were not found when screening large numbers of control chromosomes, it was unclear if they represented functional mutations or merely rare coding variants [61]. The third variant

was a heterozygous C to T transition in exon 7 of *FOXP2*. This change was predicted to introduce an early stop codon into the *FOXP2* protein sequence (R328X), severely truncating the protein such that most of the functional domains including the leucine zipper/zinc finger and DNA-binding domains were predicted to be lost. This variant was not observed in any of 252 control chromosomes tested, but was present in the other affected members of the proband's family. Specifically, the proband's sister had a similar diagnosis of DVD, while his mother suffered from expressive/receptive language difficulties and had shown speech delay in childhood; each carried one copy of the R328X mutation, whereas the phenotypically normal father did not [61]. Functional studies demonstrated that truncation of the encoded *FOXP2* protein via introduction of the R328X mutation resulted in severe mis-localisation of the protein to the cytoplasm [43]. This early stop codon also appeared to result in nonsense-mediated decay and/or an unstable protein product, such that little or no protein could be detected [43]. Thus, the mutation found in this pedigree appears to be effectively a 'null' mutation, completely knocking out one copy of *FOXP2*.

Chromosomal Alterations Affecting the *FOXP2* Locus: Translocations

Many probands have also been identified that carry chromosomal rearrangements such as translocations or deletions involving the *FOXP2* locus. As described above, pivotal to the original identification of *FOXP2* was case CS who carried a balanced translocation of chromosome 7 that interrupted the coding region of the gene between exons 3b and 4 [27]. This proband displayed a phenotype that was highly similar to that observed in the KE family including severe DVD and substantial expressive and receptive language impairment [27].

A balanced translocation was also found in a mother and daughter with a mixed speech and language disorder with features of spastic dysarthria and DVD [62, 63]. The breakpoints of this translocation were located within the *FOXP2* gene on chromosome 7 and the *RFC3* gene on chr13 [63]. For both these genes, the translocation was predicted to introduce frameshift mutations resulting in early stop codons that would truncate the protein products. In fact the resulting *FOXP2* fusion protein was predicted to be very similar to that produced by the R328X mutation and was thus suggested to be non-functional [43, 61, 63]. This study performed a detailed phenotypic comparison with the KE family and observed a similar impairment of speech, consistent with apraxia of speech (CAS/DVD) but also similar expressive and receptive language deficits—particularly in grammar. This provides supporting evidence for the effects of *FOXP2* disruption on the normal development of language, in addition to motor impairment related to vocalisation [63].

Surprisingly, a balanced translocation of 7q31 and 10p14 that did not disrupt the *FOXP2* coding region was also found in a patient displaying severe speech impairment and moderate mental retardation [64]. The authors suggest that although the coding region of *FOXP2* is intact (*FOXP2* is located ~500 kb from the breakpoint), the translocation may produce a position effect that changes the expression of *FOXP2*, but this hypothesis has not been functionally tested [64].

Chromosomal Alterations Affecting the *FOXP2* Locus: Deletions

A range of deletions spanning 7q31 of various sizes and associated phenotypes have been reported. Five patients with hemizygous deletions spanning the *FOXP2* locus (sized from 11 to 15 Mb) and displaying a phenotype that included (but was not limited to) DVD were reported by Feuk and colleagues [65]. In addition to DVD, four of these patients also displayed symptoms of ASD or developmental delays. The additional phenotypic features observed in these patients are likely to be related to the large deletions observed in which multiple genes in addition to *FOXP2* were also lost.

All five of these individuals carried deletions affecting the paternal copy of 7q31 (i.e. on the chromosome inherited from the father of the proband). In addition, this study reported a further seven probands that inherited two copies of chromosome 7 from their mother, instead of a copy from each parent (a phenomenon known as maternal uni-parental disomy of chromosome 7 or matUPD7) and who presented with features of DVD and Silver-Russell Syndrome (SRS). SRS is a developmental disorder characterised by intrauterine and postnatal growth retardation, craniofacial dysmorphism and musculoskeletal abnormalities. It was observed that *FOXP2* expression levels were significantly lower in lymphoblast cells from patients with 7q31 deletions or with matUPD7, as compared to cells from unaffected controls. The researchers proposed that the reduced expression of the *FOXP2* gene in individuals with matUPD7 may be due to a 'parent-of-origin effect' [65]. This kind of effect has been observed for other genetic loci where only one copy of the gene is normally expressed (in this case hypothesised to be the paternally derived copy of the gene), and the other copy is normally 'imprinted' or silenced (here hypothesised to be the maternally derived allele). Under this hypothesis, loss of the maternally derived copy should not severely affect gene expression or phenotype, but loss of only the paternally derived version would be expected to produce a severe effect, similar to that observed when one or both copies of the gene are lost.

In keeping with this parent-of-origin theory, a paternally inherited 16 Mb deletion of 7q31 spanning the *FOXP2* locus was later identified in a proband with a severe expressive and receptive communication disorder including DVD, dysmorphic features and mild developmental delay [66]. In addition, this proband carried a separate inversion of 7q11, although this inversion did not interrupt the coding region of any genes.

However, subsequent reports have identified that deletions of maternal *FOXP2* also cause speech and language problems and thus call into question the parent-of-origin hypothesis. One proband was reported with DVD, expressive/receptive language disorder, language delay, dysmorphic features and moderate mental retardation, due to a maternally inherited 9.1 Mb deletion spanning 7q31.1–7q31.31 [67]. Moreover, members of two additional pedigrees were reported to carry 8.3 and 6.5 Mb deletions of 7q31 [68]. Family members carrying the deletion demonstrated speech problems in addition to a range of other defects, including developmental delay, some autistic features and dysmorphic features. Importantly, one of these families included independent cases of maternal and paternal transmission within the same pedigree, with no discernable difference in the severity of phenotype,

suggesting no parent-of-origin effect for the deletion, in contrast to the findings of Feuk et al. [65].

The smallest *FOXP2*-spanning deletion identified thus far was found in a pedigree in which a 1.57 Mb deletion was transmitted from mother to son, both of whom were affected with DVD [69]. This submicroscopic deletion encompassed only three genes: *FOXP2*, *MDFIC* and *PPPIR3A*. Dysmorphic features were not observed in the family, and they did not meet the criteria for ASD, although the mother and son were classified with pervasive developmental disorder-not otherwise specified (PDD-NOS). The proband displayed severe DVD, expressive language impairment and motor planning defects, while his mother presented with a more moderate phenotype. Thus, in this pedigree, the maternally inherited deletion (in the proband) produced a very severe phenotype of DVD, in contrast to the suggestion of parent-of-origin effects [65].

Finally, a proband was described showing the first example of mosaicism for a *FOXP2* deletion [70]. This was a large de novo deletion of 14.8 Mb, spanning multiple genes, which was only observed in ~50 % of (blood) cells. Despite this, a strong phenotype was observed, including severe DVD, mild mental retardation and language disorder. Thus, a 7q31 deletion in only ~50 % of cells appears sufficient to produce the severe phenotype usually associated with complete loss of one copy of *FOXP2* in all cells. It is worth noting that this deletion involves a number of additional genes that may be affecting the phenotype and that the level of mosaicism in the brain may not reflect the pattern observed in blood cells [70]. At present, little is known regarding patterns of mosaicism in different regions of the brain and how accurately this is represented by the mosaicism observed in blood cells.

In sum, the large number of unrelated individuals in which *FOXP2* disruptions are associated with a language-related phenotype provides strong support for the involvement of *FOXP2* in language disorder. Although only accounting for a small percentage of cases worldwide, it is likely that as DNA technologies advance, smaller (submicroscopic) deletions, copy number variants (CNVs) and further mutations affecting *FOXP2* expression and/or function will be identified. In addition, more precise phenotype definitions and standardised testing will be valuable in unravelling the different genetic causes of speech and language disorders.

Evolution of FOXP2

Although it is clearly involved in speech and language, a human-specific trait, the *FOXP2* gene, is not exclusive to humans. The gene is found in many vertebrate species throughout the animal kingdom, and ancestral forms of *FOXP2* have also been identified in the genomes of invertebrates. Furthermore, orthologues of the *FOXP2* protein found in species such as chimpanzee, mouse or songbird are remarkably similar to the protein produced in humans.

The common ancestors of humans and mice diverged over ~65 million years ago, but the versions of FOXP2 protein observed in these two species only differ by three amino acids (equating to ~99.5 % similarity) [71]. This makes FOXP2 one of the most highly conserved proteins shared by these two species [71, 72]. Interestingly, these three amino acid changes are found in exon 3 (E80D) and exon 7 (T303N and N325S) and thus outside the known functional domains of the protein [71]. The fact that the DNA-binding domain is identical in the different species suggests that mouse and human FOXP2 protein are capable of binding to the same target DNA sequences. Moreover, consistent patterns of expression observed in the mouse and human brain suggest similar functions during brain development in both species [49, 50]. Sequence conservation remains high when making comparisons with more distant species; only 8 amino acid changes are found between the human and zebra finch proteins (99 % similarity) and again none of these changes are located in the DNA-binding domain. It is not until one compares human FOXP2 protein to the corresponding fish orthologue that amino acid conservation drops to ~75 % similarity [73]. Even some invertebrates, such as the fruit fly (*D. melanogaster*), worm (*C. elegans*) or sponge (*A. queenslandica*), have an orthologous ancestral protein. However, unlike vertebrates, where FOXP2 is a member of a subgroup of 4 proteins (FOXP1–4), invertebrates have so far only been found to carry a single FoxP molecule that displays ~62–67 % amino acid similarity with the human FOXP family [74]. Given the high degree of conservation of the FOXP2 protein, it follows that model organisms will be highly beneficial in helping us understand how this gene contributes to neural development and function, particularly at a molecular level.

Remarkably, against this background of little change in the protein over millions of years of evolution, two amino acid substitutions in FOXP2 occurred on the human lineage, after splitting from the chimpanzee lineage, at some point within the last 6 million years. The evolutionary time separating humans from chimpanzees is less than a tenth of that separating human and mouse. Yet, in this short period, two of the three amino acid changes that distinguish the human and mouse orthologues arose in the human FOXP2 protein sequence [71]. This rapid fixation of amino acid substitutions on the human lineage is thought to be due to positive selection and may point to altered functions for FOXP2 in the human brain that are subtly different from that in other closely related species [71]. As noted above, the strict constraints on FOXP2 protein sequence over long periods of evolution argue for important role(s) in brain development across a wide range of species. How can we reconcile this observation with its demonstrated impact on complex spoken language, a human-specific phenotype? Human communication involves coordination of a range of sensorimotor, auditory and cognitive components and it is likely that the capacity for language evolved from existing systems in the brain, rather than as a completely novel system [75]. Thus, FOXP2 may have been involved in directing the development of aspects of the ancestral brain that have been later co-opted to subserve language processing during human evolution. If this is true, we can learn a great deal about the neurological basis of language by studying such systems in animal models.

Mouse Models of FOXP2 Mutations

A number of different mouse models have been generated to investigate functions of FOXP2. These are providing complementary insights into neural mechanisms that are normally mediated by FOXP2 as well as the effects of aetiological mutations that cause human disorder (reviewed by [76]). Current mouse models include animals (a) with a complete loss of the protein, (b) carrying changes that mimic the aetiological mutations implicated in speech/language disorder and (c) engineered with evolutionary substitutions that are specific to the human protein (i.e. a mouse that is partially ‘humanised’ at this locus).

Groszer and colleagues generated two mouse lines (*Foxp2-S321X* and *Foxp2-R552H*) that carried distinct point mutations in *Foxp2* akin to those found in humans with FOXP2-related speech and language disorder [77]. The *Foxp2-S321X* allele introduced an early stop codon that results in a truncated protein product highly similar to that observed for a small pedigree segregating verbal dyspraxia (FOXP2-R328X) [61]. *Foxp2-R552H* mimics the aetiological missense mutation originally found in the KE family (FOXP2-R553H) [27]. Note that although the amino acid numbering system of the human and mouse proteins is slightly different, the *Foxp2-R552H* change in mouse matches exactly the FOXP2-R553H change in humans, yielding an arginine-to-histidine substitution at the same position in the DNA-binding domain. As such, mice carrying the S321X or R552H mutations were assessed for phenotypic abnormalities that might shed light on pathways that go awry in the human disorder. Homozygous mutant mice (carrying two mutant copies) were smaller, showed abnormal motor function (e.g. in tests of ‘righting reflex’—the ability of a mouse to regain its footing when laid on its back) and survived ~3–4 weeks postnatally, before dying for unknown reasons [77]. The only gross brain abnormality that could be observed was a disproportionately small cerebellum with reduced foliation, indicative of a delayed maturation of this structure [77].

The heterozygous mice (one mutant copy, one normal copy—as in humans with FOXP2-related speech disorders) appeared to be overtly normal, showing none of the developmental delays or reduced viability observed in the homozygotes [77]. These mice did however display subtle phenotypes that point to abnormalities in *Foxp2*-related areas of the brain. Despite normal baseline motor abilities, the heterozygous S321X and R552H mice demonstrated significantly impaired motor-skill learning on voluntary running wheel and accelerating rotarod tasks. Furthermore, altered synaptic plasticity was observed in two key areas of *Foxp2* expression that are already established to be important for motor-skill learning, the striatum and the cerebellum; in particular, there was a dramatic reduction of long-term depression (LTD) in corticostriatal circuits [77]. More recently, *in vivo* electrophysiological recordings in awake behaving mice have shown *Foxp2*-mediated effects on striatal plasticity while these mice are actively acquiring a motor skill [78]. In heterozygous R552H mice, the normally low resting firing rate of medium spiny neurons (MSNs)—thought to be important for normal action selection and movement—was elevated. During learning trials on an accelerated rotarod, MSN firing rate typically

increases in wild-type mice, but by contrast showed negative modulation in *Foxp2* heterozygous mutants, in a manner that could not be explained by the increased resting rate. There was also a clear reduction in plasticity of MSN firing during training sessions. Finally, the temporal coordination of striatal input was observed to be different between the wild-type and heterozygous mutant mice [78].

Taken together, these results highlight the importance of *Foxp2* for the activity and function of the neural circuits in which it is expressed, particularly those involving neuronal subpopulations of the striatum and cerebellum. Interestingly, Groszer et al. [77] and French et al. [78] observed that heterozygous R552H mice showed greater disruptions of motor learning than their heterozygous S321X counterparts. This is consistent with data from *in vitro* human studies that observed a potential dominant negative effect for the FOXP2-R553H protein in functional cell-based assays, beyond a simple loss of function [43]. Other studies have uncovered additional subtle differences between R552H and S321X heterozygous mice. Although gross hearing appears normal in both mouse models, sound-evoked auditory brainstem responses in S321X heterozygotes did not differ from wild-type littermates, whereas those from R552H mice showed some small but systematic alterations, suggesting potential roles for *Foxp2* in auditory processing and auditory system development [79]. To our knowledge, detailed audiometry has not been described for the KE family, so it is not known if there are subtle alterations in the auditory system of the affected individuals and whether this contributes to the severity of their speech and language disorder.

Given the importance of human FOXP2 for spoken language, a capacity that obviously involves vocal output, several studies have assessed the impact of *Foxp2* disruptions on mouse vocalisation. A complete homozygous knockout of *Foxp2* yields a lack of ultrasonic isolation calls, the innately specified cries that mouse pups make when they are separated from their mother [80, 81]. However, it has been argued that the absence of isolation calls may be a secondary consequence of the severe general developmental and motoric impairments that these homozygous mice suffer from [77, 82]. Crucially, vocalisations made by heterozygous S321X and R552H mouse pups (in the absence of general developmental delay) are produced with similar frequency to wild-type littermates and have largely normal acoustic properties [77, 82]. The use of innate pup vocalisations of mice as a proxy for human speech and language is problematic at best. Innate mouse pup calls are relatively simple and produced without any requirement for voluntary control or auditory feedback (they begin before the animal is able to hear); these vocalisations are more akin to the crying of a baby than to human speech. Furthermore there is evidence from primate studies that innate and learned vocalisations utilise different neural pathways [82, 83].

Moving beyond models of gene dysfunction, a mouse line was engineered to carry certain human-specific changes in the FOXP2 gene, to explore the functional significance of evolutionary differences between human and chimpanzee FoxP2 proteins. This mouse model (which is sometimes referred to as a 'humanised' line) carries two amino acid changes (T303N and N325S), encoded by exon 7, which distinguish the human FOXP2 protein from its chimpanzee counterpart (see also

earlier section on evolution of the gene) [84]. For clarity, we refer to the ‘humanised’ form as FoxP2 as it does not completely match the human or mouse protein. It should be noted that an N325S change also occurred independently during evolution of carnivores and thus this substitution is not unique to humans [85]. Furthermore, a third amino acid difference between human and mouse (E80D, found in exon 3) was left unchanged in this mouse model, so the encoded protein is only partially humanised, and the regulatory regions that control its expression were also unaltered. Nevertheless, intriguing phenotypic differences could be observed in this mouse model as compared to wild-type littermates. Although a large phenotypic screen observed no gross effects on FoxP2 expression, or on anatomy or physiology for any of the tissues tested, including the brain, the partially humanised mice displayed reduced exploratory behaviour and reduced dopamine levels [84]. Since FoxP2 is not expressed in dopaminergic neurons, the authors hypothesised that this transcription factor may indirectly regulate dopamine levels, possibly via its expression in striatal MSNs, which are major targets of dopaminergic neurons [84]. In these mice, the synaptic plasticity of MSNs was also found to be altered, with significantly increased LTD [84]; this contrasted with prior observations in mice carrying disruptive mutations, where LTD was significantly reduced [77]. Lastly, the human-specific amino acid changes were found to have an effect on the length of dendrites in certain FoxP2-expressing areas of the brain. In the striatum, the thalamus and bipolar cells from deep layers of the cortex, dendrites were longer in the ‘humanised’ mouse than in wild-type littermates [86, 87]. In other FoxP2-expressing areas, such as Purkinje cells of the cerebellum or pyramidal cells from deep layer cortical regions, no significant effects could be observed [87]. In combination, these data suggest that the human form of the FOXP2 protein contributes to the connectivity and function of corticostriatal circuitry, in ways that are subtly different from the murine version [87].

It is not yet clear whether the predominant mode of action for Foxp2 in the brain is developmental or if there is a continued requirement for the protein in circuits of the mature CNS. Studies of songbirds have provided evidence for the importance of FoxP2 in the postnatal brain. Haesler and colleagues selectively knocked down FoxP2 expression in a key song-related nucleus of the juvenile zebra finch brain. Remarkably, this resulted in inaccurate and incomplete imitation of tutor songs, with significantly lower accuracy per song motif, indicating a generalised lack of copying precision compared to controls [88]. The generation of a conditional knock-out mouse in which Foxp2 expression can be selectively disrupted at specific developmental time points (or in particular regions of the brain) will also allow investigation of the continued requirement for Foxp2 in the mouse brain [89].

Molecular Networks Underlying Speech and Language

Although *FOXP2* is the most well-defined and extensively studied gene contributing to human speech and language, the molecular mechanisms underlying language development in the brain are likely to involve complex interactions between large

numbers of genes, potentially acting in a range of different neural circuits, and at varying developmental time points. *FOXP2* has been proposed as a ‘molecular window’ by which we can gain a better understanding of these networks [76]. Indeed, studies exploiting the role of *FOXP2* in regulating the expression of downstream target genes have allowed identification of a large number of genes for further investigation.

FOXP2-Related Molecular Networks

The first high-throughput screens for *FOXP2* target genes searched for regions of the genome bound by this protein in human neuronal cell lines and human foetal brain tissue [90, 91]. More than 300 predicted targets of *FOXP2* were identified in each study, with highly significant overlap observed between the two reports [90]. When a subset of targets were assayed individually, the effect of *FOXP2* binding to these promoter regions could be observed. In cell-based assays, the transcription factor typically acted to reduce the expression of the majority of targets tested, although there were some genes that increased their expression in response to the presence of *FOXP2*. Thus, it appears that *FOXP2* largely acts as a repressor but in a small proportion of cases is able to activate gene expression [90, 91]. Given the large number of *FOXP2* target genes, it was possible to get an indication of the types of processes that *FOXP2* is involved in by understanding the previously identified functions (also known as ‘gene ontology’) of these target genes. By looking for functional categories that are significantly overrepresented in the target list, it was hypothesised that *FOXP2* regulates pathways including the growth and guidance of axons, signalling pathways important for brain development such as ‘Wnt/notch signalling’ as well as organ morphogenesis [90, 91].

One of the identified *FOXP2* targets, the *uPAR* gene, caught the attention of researchers working on other disorders involving disrupted language [90, 92]. The *uPAR* protein (also known as *PLAUR*) forms a complex with the protein encoded by *SRPX2*, a gene mutated in epilepsy of the rolandic speech areas of the brain [92]. *SRPX2* mutations may also produce symptoms of DVD and/or perisylvian polymicrogyria—a disorder involving abnormal cortical development associated with motor control deficits, cognitive impairment and in some cases seizures and/or language disorder [92]. Given the shared endophenotypes and neurobiological features of syndromes involving *FOXP2* and *SRPX2* mutations, researchers hypothesised that the molecular pathways might be linked. Indeed, functional cell-based assays demonstrated that the *FOXP2* protein can bind to the promoter regions of both *uPAR* and *SRPX2* to downregulate their expression [92]. Interestingly, when these same assays were carried out using a mutant version of *FOXP2*, carrying the R553H substitution from the KE, there was a loss of repression for both target genes. This led Roll and colleagues to screen *FOXP2* in people with disorders of the speech cortex, similar to those caused by *SRPX2* mutations. A screen of 32 patients identified a heterozygous missense mutation of *FOXP2* (M406T) in a proband displaying focal epilepsy, polymicrogyria of the left rolandic operculum and cognitive and

speech defects [92]. Although this change was also observed in one other family member, with no neurological problems, functional assays demonstrated that the amino acid substitution affected the normal activity of the FOXP2 protein. The M406T change resulted in increased mis-localisation of FOXP2 to the cytoplasm and reduced its ability to regulate the *SRPX2* target gene, while uPAR regulation remained unaffected [92]. These data suggest that the mutation of FOXP2 in this patient contributes to the observed phenotype but that it is not directly causative. Further genomic analysis of this proband may uncover mutations in other genes that contribute to the penetrance of the disorder.

Mouse models of *Foxp2* have facilitated more in-depth investigation of the crucial molecular networks, using methods that would be difficult to apply to human cases of speech/language disorder. *Foxp2* targets in the embryonic mouse brain were inferred from experiments assessing promoter binding across the entire genome. These efforts were coupled with whole genome expression analysis in the developing striatum, a region of high *Foxp2* expression that has been implicated in speech and language-related networks in humans and which shows altered function in people with language disorders [93]. The data from this study implicated *Foxp2* in a range of developmental processes including cell migration, G-protein-coupled receptor signalling, dopamine signalling, neuron projection morphogenesis and, as before, wnt signalling and axon guidance [90, 93].

Neurite outgrowth and axon guidance are functional categories that reflect the ability of neurons to connect to each other by developing and directing the growth of cellular projections (known as axons and dendrites). These categories were consistently observed across multiple independent FOXP2 studies [84, 90, 93] prompting researchers to investigate this pathway in more detail. A number of putative targets of *Foxp2* that were known to be involved in neurite outgrowth were shown to be regulated by *Foxp2* in vivo in the developing mouse brain and/or in neuron-like cells in vitro [93]. Furthermore in cultured primary neurons taken from the developing mouse striatum, the loss of functional *Foxp2* significantly affected the growth of neurites [93]. Cells expressing normal *Foxp2* showed significantly longer neurites with more branch points than the cells expressing mutated *Foxp2*, suggesting that in the developing brain, *Foxp2* may contribute to setting up neural networks in language-related areas of the brain by affecting their connectivity [93].

Another interesting finding from this study was that *Foxp2* could regulate the expression of microRNA (miRNA) molecules [93]. miRNAs are short (~22 nt) non-coding RNA molecules that mediate post-translational regulation of gene expression [94]. Mature miRNAs recognise target mRNA molecules via complementary base pairing with a target site in the 3'-UTR of genes and this process generally results in inhibition of translation and/or degradation of mRNA [94]. MicroRNAs such as mir-137, mir-9 and mir-216 that have previously been implicated in brain development and neuronal differentiation were shown to be directly regulated in the developing mouse brain, suggesting that *Foxp2* may fine tune gene expression during brain development via the control of miRNA levels [93].

New Candidate Disease Genes: CNTNAP2

A striking proof of principle of the utility of FOXP2 as a molecular window into wider networks of genes involved in language development and disorder came with the identification of *CNTNAP2* as a directly regulated FOXP2 target [95]. In early, low-throughput studies of FOXP2 transcription factor activity, a site within the first intron of the *CNTNAP2* locus was identified as being bound by the FOXP2 protein in human neuronal models [95]. *CNTNAP2* expression was also significantly repressed by FOXP2 [95]. *CNTNAP2* is a large gene that encodes Caspr2, a member of the neurexin superfamily. This protein is localised to the axon initial segment (AIS) and juxtaparanodal regions of myelinated nerve fibres and is involved in regulating the clustering of potassium channels in this region [96, 97]. Given that *CNTNAP2* had previously been implicated in language-related disorders, such as ASD, cortical dysplasia and focal epilepsy (CDFE) with language regression and Tourette's syndrome [98–101], its function in neuronal recognition and adhesion [101, 102] and its enriched expression in language-related circuitry [103], this presented an excellent candidate gene for language development and disorder [95]. Analysis of SLI families with quantitative measures of SLI endophenotypes demonstrated significant association between 'non-word repetition' and a cluster of genetic markers (single nucleotide polymorphisms, or SNPs) towards the end of the *CNTNAP2* coding region (between exons 13–15) [95]. Some of these same SNP alleles had also previously shown association with a different language-related measure, 'age at first word', in a cohort of autistic children [100]. Since any individuals displaying features of ASD were excluded from the SLI cohort, these findings suggests that similar susceptibility factors at the *CNTNAP2* locus may influence language-related endophenotypes of these different disorders [95]. This work demonstrated that knowledge of a rare Mendelian disorder (speech/language disorder caused by high penetrance *FOXP2* mutations) could inform the genetic basis of more complex language phenotypes (such as SLI or ASD) to highlight shared neurogenetic pathways (FOXP2-CNTNAP2) between clinically distinct syndromes.

New Candidate Disease Genes: FOXP1

FOXP1 was initially considered a good candidate gene underlying language pathways given that it is the most closely related gene to *FOXP2* in the genome. The two protein products have very high amino acid similarity and show conserved and overlapping patterns of expression in regions of the brain, such as the striatum, thalamus and developing cortical plate [49]. Furthermore, the FOXP1 and FOXP2 proteins have been shown to interact to form heterodimers and cooperatively regulate target gene expression [48, 57]. Studies in songbird models have also pointed to a functional role for FOXP1 in the brain as the songbird orthologue of human FOXP1

(FoxP1) shows sexually dimorphic expression in neural structures involved in song learning and production [104].

The first study to screen language-related disorders for potential *FOXP1* mutations sequenced the coding region of this gene in a panel of 49 verbal dyspraxia probands [105]. This was the same panel in which *FOXP2* mutations had previously been identified [61] but no pathological coding changes of *FOXP1* were detected [105]. However, following this, several cases of people with deletions or mutations of the *FOXP1* locus have been reported, associated with complex neurodevelopmental disorders involving multiple symptoms, which often include severe disruptions of speech and language.

A single child was identified with hypertonia and contractures of the hands and feet, blepharophimosis, intermittent muscle spasms and speech delay. This child carried a de novo deletion of 3p14.1 that encompassed four genes: *FOXP1*, *EIF4E3*, *PROK2* and *GPR27* [106]. Subsequently, another patient was identified with a deletion of 3p14.1, but in this case the deleted region only spanned the coding region of a single gene, *FOXP1* [107]. Despite only directly affecting a single gene locus, the patient again showed a complex phenotype that included gross motor delay, Chiari I malformation, epileptiform discharges and limited verbal output [107]. Given that *FOXP1* is known to play a key role in motor neuron development and connectivity [55, 56, 108], it might be expected that *FOXP1* mutations would yield abnormal motor development and related phenotypes. The challenge lies in determining if the observed speech problems in these patients are due to aberrant development of specific speech-related pathways in the central nervous system or simply a consequence of global motor defects.

Two studies searched for alterations to the *FOXP1* locus in patients with Intellectual Disability (ID) and ASD or speech delay [109, 110]. Screening of 80 ASD and 30 ID probands identified a single patient with a de novo deletion encompassing only the *FOXP1* coding region [109]. A second patient was identified with a de novo nonsense mutation altering the *FOXP1* protein to produce a shorter protein that lacked part of the DNA-binding domain [109]. The mutated protein, *FOXP1* R525X, was no longer able to regulate gene expression [109]. Both patients displayed global developmental delays coupled with severe language impairments, but no deficits in oromotor coordination were observed [109]. While both patients also displayed autistic features, only the patient carrying the nonsense mutation R525X met clinical criteria for an ASD diagnosis [109].

FOXP1 mutations were also found in an independent screen of patients with moderate ID, general developmental delay, reduced expressive and receptive vocabulary and general speech delay [110]. In a large genome-wide screen for CNVs in 1,523 patients, three cases were identified with deletions that only affected the *FOXP1* locus. However, it should be noted that a single deletion affecting four genes (*FOXP1*, *EIF3E3*, *PROK2* and *GPR27*) similar to that observed by Pariani et al. was also found when screening a panel of 4,104 control DNA samples. Horn and colleagues also identified 5 *FOXP1* missense mutations in patients with ID that were not observed in control panels [110]. Although no functional analysis was

performed to determine the effects of these mutations, it was predicted that the changes might contribute to the observed phenotype.

A recent study that sequenced the exomes (the entire coding region of an individual's genome) of sporadic autism patients demonstrated four parent child trios with potentially causative mutations [111]. One of the trios was of particular interest as the autistic proband carried a *de novo* mutation of the *FOXP1* gene. This mutation resulted in an early stop codon in the protein sequence that produces a severely truncated protein product that lacks the key functional domains of *FOXP1* [111]. However, this proband carried a deleterious mutation in another gene, *CNTNAP2*, that was inherited from his mother (who is not autistic) and was also passed on to the unaffected sibling. Thus, this *CNTNAP2* mutation did not segregate with the disorder and could not, on its own, be considered to be causative. Functional assays investigating the effects of both the *FOXP1* and *CNTNAP2* mutations gave some intriguing findings. As noted above, *CNTNAP2/Caspr2* had previously been implicated in autism and had been shown to be regulated by *FOXP2*—the most closely related protein to *FOXP1*. O’Roak et al. demonstrated that the presence of normal *FOXP1* is able to downregulate the expression of *CNTNAP2*, but that when the patient identified *FOXP1* mutant protein was introduced, *CNTNAP2* expression levels were no longer repressed; they were in fact massively increased compared to controls [111]. This could potentially represent a ‘two-hit disease model’ in which the *FOXP1* mutation not only has a direct phenotypic effect but also produces further effects by increasing the expression of the deleterious form of *CNTNAP2* [111].

Thus, the potential contributions of *FOXP1* to speech and language functions make a more complex story than that seen for *FOXP2*. It appears that *FOXP1* disruptions are not a major or specific cause of language disorder but that rare mutations of this gene yield susceptibility to complex disorders involving ASDs, ID, generalised developmental and motor delays, often accompanied by speech and language deficiencies. It has been suggested that these data reveal a more global impact on brain development resulting from *FOXP1* disruption than is observed from *FOXP2* mutations, despite the close homology and overlapping expression patterns [109]. Key to understanding the different effects of *FOXP1* and *FOXP2* mutations may lie in not only understanding the differences in the pathways they regulate but also understanding the specific neuronal subtypes where these genes are required. Given their close homology it has been suggested that these genes are able to functionally compensate to some degree for each other when genetic disruptions occur. However, this may not be equally true for all target genes or in all types of neurons. Furthermore, there are some regions of the brain where the expression of these genes are mutually exclusive, such as the hippocampus (*FOXP1* is present) or the amygdala and cerebellum (*FOXP2* is present). Thus, in order to understand the contributions of *FOXP1* and *FOXP2* to language development, it will be necessary to understand how the functions of these transcription factors overlap and which functions are specific to each family member (as well as when and where they are required).

Perspectives: Language Genetics and Animal Models

This chapter has aimed to present a snapshot of the current knowledge of genetics underlying both normal and disrupted language development. Much of this information was initially obtained from studies of individuals with language-related disorders, which provided the identity of several critical risk genes. However, once these genes or risk factors are identified, new questions arise. What are the normal functions of these genes? How are molecular pathways or neural circuitry in the brain affected when their sequence is altered? In order to begin answering these types of in-depth functional questions, it becomes necessary to move into model systems. Although human model systems are possible, in the form of post-mortem tissue samples or in vitro cell cultures, such models provide only limited options for investigating a trait as complex as language. In addition to the scarcity of human tissue samples, particularly for individuals with well-defined language disorders, the use of post-mortem tissue restricts the range of experimental techniques that can be used to assess gene function and there is no way to manipulate the genetic background of the tissue.

Immortalised human cell lines allow researchers to circumvent some of the above problems since they will grow in the laboratory, can be used for an array of live functional analyses and can be manipulated to alter the sequence and/or expression of particular genes. However, the conclusions that can be drawn from such studies are restricted by the artificial nature of these cells. Typically, these cell lines are derived from tumour biopsies and thus have been altered during the progression of the cancer, often displaying many differences with neurons, including multiple chromosomal abnormalities. They do not represent any particular neuronal subtype, rather they are classified as ‘neuron-like’ cells. Furthermore, the cells are a homogeneous population of a single cell type, existing in a monolayer or in suspension. They experience few of the interactions with other cell types or external signals that a normal neuron would have in the brain, essential for directing the complex molecular programmes that distinguish different cellular subpopulations. This is particularly relevant when investigating language as many cell types make up the distributed neural circuits that are thought to underlie human speech and language.

Animal models therefore provide researchers with an excellent tool to study the role of genes in the context of a functioning brain with evolutionary ties to our own. Animal models allow us to manipulate gene expression and observe the effects at multiple levels, from DNA and protein to functional or neurobiological analysis. And although we cannot directly assess language in an animal model, we can look at behaviours that are related to aspects of brain function necessary for language use, such as learning and memory.

Studies of FOXP2 homologues in animal models have provided much of the key information regarding the role of this gene. Studies in mouse models with mutated versions of *Foxp2* have identified molecular and functional networks regulated by *Foxp2* in the developing brain; demonstrated that *Foxp2* is important for neurite outgrowth, synaptic plasticity and motor-skill learning [77, 93]; and highlighted

evolutionary differences by observing the effect of having a humanised version of the protein present in the mouse brain [84, 87]. Chimpanzee cells and tissue have also been used in an attempt to understand how FOXP2 regulatory networks have evolved to contribute to language-related processes [112].

Studies in zebra finch have illustrated the importance of FoxP2 for learned vocalisations, since reduced levels of FoxP2 in the brain affect the ability of the songbirds to correctly imitate and learn song from a tutor [88]. Most recently, sophisticated bioinformatic analyses have also demonstrated a range of FoxP2-related gene networks that appear to be differentially regulated during singing in the zebra finch, suggesting some activity-dependent regulation of these networks [113].

The recent emergence of methods to measure brain activity in living animals will doubtlessly greatly enhance our understanding of the genetic underpinnings of speech and language. Already, studies have been able to measure brain activity in awake, behaving mice and demonstrated neurological differences in how normal brains behave during motor learning compared to Foxp2 mutant brains [114]. The rapidly developing field of optogenetics provides further ways in which the activity and connectivity of neural networks can be probed to determine how genes contribute to processing in language-related structures of the brain. Optogenetics involves introducing light sensitive molecules (opsins) into subsets of cells in the brain. When the cells are exposed to light of specific wavelengths, the neurons that carry these opsins can be either activated or silenced. In this way, it is possible to control and measure the activity through specific neural circuitry, and by combining this with mutant mouse models, it is possible to observe the role of specific genes on the functioning of these circuits [115]. In the future, optogenetics is likely to not only provide insight into the evolution and function of language-related networks in the brain but help us to understand the genetic mechanisms underlying their development.

In summary, to understand the genetic basis of speech and language pathways in the brain, it will be necessary to integrate information gained from clinical studies in human patients with the elegant genetic and behavioural manipulations that can be performed in animal models. Only in this way will it be possible to understand how the faculty for language evolved in the brain and the genetic, molecular and neural mechanisms underlying this most complex human trait.

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