

# A Molecular Genetic Perspective on Speech and Language

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## 2.1 INTRODUCTION

For decades there has been speculation about the potential contribution of inherited factors to human capacities for speech and language. Arguments for a genetic basis have drawn from an array of diverse fields and approaches, marshaling threads of evidence taken from formal linguistics, child development, twin studies, biological anthropology, comparative psychology, and so on. In recent years, through advances in molecular biology techniques, it has become possible to move beyond these indirect sources and let the genome speak for itself (Graham & Fisher, 2013). In particular, by studying neurodevelopmental disorders that disproportionately disturb speech and language skills, researchers started to identify individual genes that may be involved in the relevant neurobiological pathways.

Rather than being seriously rooted in biology (Fisher, 2006), much of the prior debate on genetic foundations of spoken language has treated genes as abstract entities that can mysteriously yet directly determine linguistic functions. Accounts that depend on “genes for grammar” and other such magic bullets are simply untenable in light of all that is known about molecular and cellular processes and how these processes are able to impact development and function of brain circuitry. The human genome comprises approximately 20,000 different protein-coding genes. Each such gene is a string of G, C, T, and A nucleotides, the specific order of which is used by the cellular machinery to thread together a specific polypeptide sequence of amino acid residues taken from 20 different types of amino acids that are available as building blocks. (Linguists might enjoy the fact that this is a discrete

combinatorial system with the potential to yield an infinite number of different amino acid strings.) The sequence of amino acids in a protein determines the way that it folds into a three-dimensional shape, and the protein’s shape determines the function that it will have in cells and in the body. In this way, the different DNA sequences of different genes are able to specify a plethora of distinct cellular proteins—enzymes, structural molecules, receptors, signaling factors, transporters, and others. Some of these proteins play roles in the ways that cells of the nervous system proliferate, migrate (move to their final position), differentiate, and form connections with each other during development; some might be important neurotransmitters or other factors that help strengthen or weaken synapses during learning. Overall, intricate webs of genes and proteins acting through complicated sequences of developmental events and via continual interactions with the environment lead to assembly of complex networks of functioning neural circuits, and it is the latter providing the behavioral and cognitive outputs of the system that we call the human brain.

Based on this, we should never expect simple direct connections between DNA and language, but this does not mean that we cannot track down genes that are most relevant to our human capacities. To the contrary, by pinpointing crucial genes (e.g., those for which mutations lead to language impairments) it is possible to gain entirely novel entry points into the critical neural pathways and use those to work toward mechanistic accounts that are properly built on biologically plausible foundations. In what follows, the promise and challenges of the approach are illustrated by focusing on *FOXP2*, a gene that is at the heart of this new paradigm.

## 2.2 THE DISCOVERY OF FOXP2

The starting point for the *FOXP2* story was the identification of an unusual family in which multiple close relatives suffered from similar disruptions of speech and language skills. This family, dubbed the KE family, spanned three generations and included 15 affected members as well as a similar number of unaffected relatives. Because the disorder was present in each successive generation and affected approximately half of the family members, it attracted the attention of geneticists who recognized that the pattern was consistent with dominant monogenic inheritance (Hurst, Baraitser, Auger, Graham, & Norell, 1990). In other words, it raised the remarkable possibility that this family's speech and language problems might be explained by a mutation affecting one copy of a single gene. Before any DNA investigations had even begun, commentators already began to speculate excitedly about the discovery of a "language gene" (see Fisher (2006) for a detailed account). At the same time, the affected members of the KE family became the focus of intensive neuropsychological studies to gain more insights into their profile of impairments.

According to these investigations, the most prominent aspect of the disorder is a problem mastering the coordinated movement sequences that underlie fluent speech (Vargha-Khadem et al., 1998). The affected people make articulation errors that are inconsistent (they can differ from one utterance to the next) and that become worse as the length and complexity of the utterance increases (Watkins, Dronkers, & Vargha-Khadem, 2002). These are characteristic features of a syndrome known as developmental verbal dyspraxia (DVD) or childhood apraxia of speech (CAS). The difficulties can be robustly captured by tests in which the participant is asked to repeat a series of pronounceable nonsense words of differing length and complexity (Liegeois, Morgan, Connelly, & Vargha-Khadem, 2011). CAS is generally thought of as a disorder of speech learning and production underpinned by neural deficits in the motor planning of sequences of orofacial movements. Intriguingly, the impairments in the affected KE family members are not confined to speech; they extend to the written domain, disturbing a wide range of linguistic skills, both expressive and receptive. To give some examples, affected relatives perform significantly worse than their unaffected siblings on lexical decision tasks, spoken and written tests of verbal fluency, nonsense word spelling, and processing of sentence- and word-level syntax (Watkins, Dronkers, et al., 2002). Given that these skills have developed in the context of a severe restriction in expressive skills, it is possible that such impairments are secondary consequences rather than primary deficits. In general, many

members of this family (regardless of CAS diagnosis) have a lower than average nonverbal IQ, which complicates discussions over the selectivity of the phenotype. Nevertheless, because nonverbal cognitive difficulties do not appear to cosegregate with the inherited disorder, it is argued that this is primarily a disturbance of speech and language rather than some form of general intellectual disability (Watkins, Dronkers, et al., 2002). These issues are discussed in more depth elsewhere (Fisher, Lai, & Monaco, 2003).

Screening of different parts of the genome revealed that the KE family disorder was strongly linked to genetic markers on one particular section of chromosome 7 (Fisher, Vargha-Khadem, Watkins, Monaco, & Pembrey, 1998). These markers were passed on from the grandmother to all other affected family members, but not to any unaffected relatives; that is, there was perfect cosegregation with the disorder. The molecular mapping data thus provided experimental confirmation that the speech and language problems of the family had a genetic origin and localized the responsible gene to a particular region of chromosome 7, which was given the name *SPCH1* (Fisher et al., 1998). After intensive analyses of this *SPCH1* interval (Lai et al., 2000), and aided by clues from another case (discussed later), the researchers eventually pinpointed a causative mutation in a novel gene given the name *FOXP2* (Lai, Fisher, Hurst, Vargha-Khadem, & Monaco, 2001).

*FOXP2* encodes a transcription factor—a regulatory protein that is able to modulate the activities of other genes (Vernes et al., 2006). The protein does so by directly binding to the DNA of these target genes and affecting how efficiently they are transcribed into messenger RNA molecules (the templates that are used for building proteins). *FOXP2* belongs to one particular group of transcription factors defined by the presence of a special type of DNA-binding motif referred to as a forkhead-box (or FOX) domain (Benayoun, Caburet, & Veitia, 2011). All the affected people in the KE family carry the same single nucleotide change in *FOXP2*, a G-to-A transition in a crucial part of the gene (Lai et al., 2001). This missense mutation leads to alteration of the amino acid sequence of the encoded protein. The mutated protein carries a histidine (H) residue, instead of arginine (R), at a key point of the forkhead domain, that prevents it from binding to the usual target sequences and severely disrupts its function as a transcription factor (Vernes et al., 2006). (Because this amino acid substitution is at the 553rd residue from the start of the protein, it is denoted by the symbol R553H.) The mutation is in a heterozygous state in the affected KE family members, meaning that one gene copy is intact and functioning normally but the other is dysfunctional.

Thus, it was hypothesized that two functioning copies of *FOXP2* are necessary for development of proficient speech and language skills (Lai et al., 2001).

### 2.3 FOXP2 MUTATIONS IN SPEECH AND LANGUAGE DISORDERS

Clearly, studies of the KE pedigree were pivotal in enabling the first identification of a gene contributing to speech and language functions. This family represents the most well-characterized example in the literature with respect to both the neuropsychological profile of the associated disorder and the functional impacts of the etiological mutation (Fisher, 2007). However, contrary to the usual story popularized in media reports and many scientific commentaries, the KE family is not the sole documented case of *FOXP2* mutation. Over the years, distinct etiological disruptions of this gene have been reported in several different families and cases, ranging from point mutations (change of a single nucleotide of DNA) to gross rearrangements of chromosome 7 that disturb the entire *FOXP2* locus (Newbury & Monaco, 2010). In fact, the original *FOXP2* paper included not only the KE family mutation but also an independent case of similar speech/language problems with a chromosome 7 rearrangement disturbing the locus (case CS, which is described later) (Lai et al., 2001), something that is often overlooked by commentators.

The predominant isoform of the *FOXP2* protein is 715 amino acids long and encoded by 2,145 nucleotides of DNA (split between several different coding exons); a disruptive mutation could potentially occur anywhere within this coding sequence. For rare dominant causal variants with large effect size, such as the R553H mutation found in the KE family, it is likely that the sequence change will be “private,” meaning that it is exclusive to just one family or case. Thus, when screening *FOXP2* in new cohorts of people with speech and language problems, it is necessary to thoroughly search for any variants across the entire known coding sequence rather than simply testing for presence/absence of a previously reported mutation. When such screening efforts have been performed in cohorts of people diagnosed with speech disorders, a number of novel *FOXP2* point mutations have been uncovered. For example, Laffin and colleagues (2012) sequenced *FOXP2* in 24 probands with a strict diagnosis of CAS and found that one case carried a heterozygous missense mutation yielding an amino acid substitution (asparagine-to-histidine at position 597, i.e., N597H) just beyond the end of the FOX domain. In a previous screening study of 49 children with clinical reports of CAS, MacDermot and colleagues (2005)

identified another type of causal variant, a nonsense mutation that inserts a stop codon halfway through the gene (arginine-to-stop at position 328, i.e., R328X) that is predicted to yield a severely truncated *FOXP2* protein. This variant was in the heterozygous state, like the other etiological *FOXP2* mutations. It was found in three family members, the proband, his sister who also had a CAS diagnosis, and his mother who had a history of speech problems. A small number of other potential mutations of interest were identified by the MacDermot study, including a Q17L substitution (glutamine-to-leucine at position 17, near the start of the protein), but in those cases the causal significance was unclear because they did not cosegregate with the disorder in affected siblings (MacDermot et al., 2005; Vernes et al., 2006). Most recently, an individual with CAS was identified carrying an intragenic deletion of two nucleotides in the *FOXP2* locus predicted to yield an abnormal truncated protein (Turner et al., 2013). Specifically, the loss of the two nucleotides yields a shift in the reading frame of the coding sequence at position 415 of the protein; after this point, five novel amino acids are incorporated immediately followed by a premature stop codon. Just as for the R328X mutation, the resulting mutant protein completely lacks the FOX domain.

So far, two types of gross chromosomal rearrangements have been reported to affect *FOXP2*: translocations (Feuk et al., 2006; Kosho et al., 2008; Lai et al., 2001; Shriberg et al., 2006) and deletions (Feuk et al., 2006; Lennon et al., 2007; Palka et al., 2012; Rice et al., 2012; Zeesman et al., 2006; Zilina et al., 2012). In the translocation cases, part of chromosome 7 is exchanged with part of another chromosome; because the chromosome 7 breakpoint in these cases lies directly within (or close to) the *FOXP2* locus, this is expected to interfere with the activity of the disrupted copy (Feuk et al., 2006; Kosho et al., 2008; Lai et al., 2001; Shriberg et al., 2006). The first example of a *FOXP2* translocation was found in a child known as CS, as reported in the same paper that uncovered the KE family mutation (Lai et al., 2001). Most reported *FOXP2* translocations are *de novo*—the rearrangement is present in the case but not found in parents or siblings. However, Shriberg and colleagues identified a family (TB) in which a mother and daughter both carried the same translocation directly disrupting *FOXP2* and reported that the associated speech problems (Shriberg et al., 2006), language impairments, and cognitive profiles (Tomblin et al., 2009) were notably consistent with those previously observed for people carrying the missense mutation in the KE family (Watkins, Dronkers, et al., 2002).

In the reported large-scale deletion cases, one copy of *FOXP2* is completely lost from the genome, often together with other flanking genes (Feuk et al., 2006;

Lennon et al., 2007; Palka et al., 2012; Rice et al., 2012; Zeesman et al., 2006; Zilina et al., 2012). Investigations of the phenotypes observed in these cases again support the idea that damage to one copy of *FOXP2* is sufficient to derail speech and language development, although the larger deletions that encompass multiple other genes are often noted to include additional problems. As with the translocations, although most cases are *de novo*, there is at least one report of an inherited rearrangement: a mother and a son carrying the same deletion of *FOXP2* (as well as neighboring genes *MDFIC* and *PPP1R3A*) and both diagnosed with CAS (Rice et al., 2012). Interestingly, there are no reports of any human with disruption of both copies of *FOXP2*, presumably because a total absence of the gene would be lethal (Fisher & Scharff, 2009).

## 2.4 FUNCTIONS OF FOXP2: THE VIEW FROM THE BENCH

The identification of a particular gene underlying a trait is often portrayed as the endpoint of a scientific study. In reality, this kind of discovery is more akin to a new beginning because it opens up entirely novel avenues for investigating the basis of the trait from the perspective of the gene in question. Thus, the identification of *FOXP2* may have been something of a paradigm shift for the language sciences because it facilitated a series of innovative molecular investigations into the neurobiological pathways and evolutionary history of spoken language using this gene as a unique entry point (Fisher & Scharff, 2009). Such work has called on a diverse array of experimental strategies and model systems, ranging from neuronal cells investigated at a laboratory bench, to genetic manipulations in animals, to studies of humans (Graham & Fisher, 2013).

Laboratory experiments using genetically modified human cells are important for establishing whether putative etiological mutations impact gene function (Deriziotis & Fisher, 2013). As noted, several different point mutations of *FOXP2* have been found in people with CAS; some cosegregate with disorders in a family, like the R553H substitution and the R328X truncation, whereas others are found in just a single proband, such as the Q17L (MacDermot et al., 2005) and N597H substitutions (Laffin et al., 2012). Vernes and colleagues (2006) studied the functional significance of R553H, R328X, and Q17L variants by expressing the mutated proteins in cultured human cell lines, assessing properties such as protein stability, intracellular localization (normal *FOXP2* protein is located in the nucleus of the cell), DNA-binding capacity, and ability to repress target genes. R553H and R328X showed obvious disruptions in most or all of

these assays, strongly supporting their causal roles, whereas Q17L did not show any functional differences from the normal protein in this system, so its etiological relevance remains uncertain (Vernes et al., 2006). At the time of writing this book, no functional analyses of the N597H substitution had yet been reported.

Crucially, even though they involve rather basic model systems (as compared with neural circuits or living brains), cell-based analyses can go well beyond simply validating disruptive effects of mutations. By applying state-of-the-art genomic and proteomic techniques, researchers can use cellular models to gain new insights into neurogenetic mechanisms, which can have direct relevance to human biology (Deriziotis & Fisher, 2013). The *FOXP2* literature provides particularly apt illustrations of this principle in action. Because *FOXP2* encodes a transcription factor working to regulate the expression of other genes, it can be thought of as a hub in a network of molecules, a number of which might also be related to speech and language development. Thus, over the years, several studies have used cellular models to screen parts, or all, of the genome, searching for target genes regulated by *FOXP2* (Konopka et al., 2009; Vernes et al., 2007, 2008).

In 2008, a study of human neuron-like cells grown in the laboratory found that the *FOXP2* protein binds directly to a regulatory sequence within a gene called *contactin-associated protein-like-2*, or *CNTNAP2* (Vernes et al., 2008). The researchers went on to show that when they artificially increased expression of *FOXP2* in cultured cells, this caused a significant reduction in *CNTNAP2* mRNA levels, a finding that was further supported by analyses of developing cortical tissue from human fetuses, in which there was an inverse correlation between expression levels of the two genes. To test for connections between *CNTNAP2* and language development, the team assessed sets of common DNA variations (single-nucleotide polymorphisms [SNPs]) from different parts of the gene in a cohort of 184 families with typical forms of specific language impairment (SLI) previously collected by the UK SLI consortium. They identified a cluster of SNPs in one section of the gene (around exons 13–15) that showed association with measures of performance on language tasks, most notably the nonsense word repetition test; children who carried a particular set of risk variants scored significantly lower than others (Vernes et al., 2008). Intriguingly, in a prior study screening *CNTNAP2* in children with autism, the same risk variants had been associated with delayed language, as indexed by “age at first word” (Alarcon et al., 2008). Because the Vernes et al. (2008) study explicitly excluded any children diagnosed with autism, the convergent findings suggest that the *CNTNAP2* risk variants might be implicated in language-related problems across distinct clinical boundaries of neurodevelopmental disorders.

In a later study, the same variants were shown to be consistently associated with assessments of early language acquisition (at 2 years of age) in 1,149 children from the general population, suggesting that the effects extend beyond disorder into normal variation (Whitehouse, Bishop, Ang, Pennell, & Fisher, 2011).

*CNTNAP2* is a member of the neurexin superfamily that encodes a transmembrane protein that has been implicated in multiple fundamental processes in the developing and mature nervous system (Rodenas-Cuadrado, Ho, & Vernes, 2013). It helps to cluster potassium channels at nodes of Ranvier in myelinated axons, and it has also been linked to mechanisms of neuronal migration, dendritic arborization, and spine formation during development (Anderson et al., 2012). Diverse *CNTNAP2* variants (rare mutations and common polymorphisms) have been associated with a range of neurodevelopmental disorders, including not only SLI and autism but also epilepsy, schizophrenia, Tourette syndrome, and intellectual disability (Rodenas-Cuadrado et al., 2013).

After the identification of the *CNTNAP2* connection, additional functional reports have further demonstrated the value of tracing *FOXP2* networks for understanding language-related disorders. *FOXP2* has been shown to regulate *uPAR* and *SRPX2*, genes potentially implicated in a form of rolandic epilepsy that also involves speech apraxia (Roll et al., 2010). (However, see Lesca et al. (2013) for evidence that casts doubt on the role of *uPAR*/*SRPX2* in this disorder, instead implicating a different gene, *GRIN2A*). Intriguingly, *SRPX2* regulation by *FOXP2* is thought to be an important mediator of synaptogenesis (Sia, Clem, & Huganir, 2013). Other *FOXP2* targets of particular clinical relevance include the receptor tyrosine kinase *MET*, proposed as a candidate for autism (Mukamel et al., 2011), and *DISC1*, a gene that was originally implicated in schizophrenia (Walker et al., 2012).

It is not only the downstream targets of *FOXP2* that may be informative for making links to human phenotypes. Transcription factors never act alone; they work together with other interacting proteins to regulate their targets. *FOXP1* is the most similar gene in the genome to *FOXP2*. In some cells in the central nervous system, these two genes are coexpressed (Teramitsu, Kudo, London, Geschwind, & White, 2004), and the resulting proteins have the capacity to directly interact with each other, acting together to regulate targets in a coordinated manner (Li, Weidenfeld, & Morrisey, 2004). Rare causative mutations of *FOXP1* have been implicated in a small number of cases of autism and/or intellectual disability, accompanied by notably severe speech and language problems (Bacon & Rappold, 2012). Moreover, it has been shown that *FOXP1* actively represses the *CNTNAP2* gene, and an autism screening study that sequenced all human protein-coding genes identified an affected child who carried disruptive mutations in both *FOXP1* and

*CNTNAP2*, “hits” in two different parts of the same functional pathway (O’Roak et al., 2011). Efforts are underway to identify and characterize all the other key protein interactors in this pathway (Deriziotis & Fisher, 2013).

## 2.5 INSIGHTS FROM ANIMAL MODELS

The human capacity for acquiring complex spoken language appears to be unique in the natural world (Fisher & Marcus, 2006). At first glance this may seem to preclude any chance of biologically meaningful genetic studies in animal models. However, the majority of human genes did not appear spontaneously in our species (Varki & Altheide, 2005). So, after human studies have identified a gene implicated in speech and language, an obvious next step is to examine the broader evolutionary history of the gene and assess whether its function(s) in nonspeaking species can be informative for understanding its contributions to human brain development (Fisher & Marcus, 2006).

*FOXP2* has a particularly deep evolutionary history, with versions of the gene described in many different vertebrate species, including monkeys (Takahashi et al., 2008), ferrets (Iwai et al., 2013), mice (Ferland, Cherry, Preware, Morrisey, & Walsh, 2003; Lai, Gerrelli, Monaco, Fisher, & Copp, 2003), rats (Takahashi, Liu, Hirokawa, & Takahashi, 2003), bats (Li, Wang, Rossiter, Jones, & Zhang, 2007), birds (Haesler et al., 2004; Teramitsu et al., 2004), reptiles (Haesler et al., 2004), and fish (Bonkowsky et al., 2008). Researchers have investigated neural expression patterns for most of these species, determining where and when the gene is transcribed and/or translated in developing and mature brain tissue. These studies found striking similarities in distantly related vertebrates, with concordant expression in neuronal subpopulations of cortex, thalamus, basal ganglia, and cerebellum. Thus, it seems likely that activities of *FOXP2* in the human brain are built on evolutionarily ancient functions in the vertebrate central nervous system (Fisher & Marcus, 2006).

As is apparent from the previous paragraph, there have been a large number of studies characterizing the corresponding versions of this gene found in different species. A proper discussion of the many findings from this research area is beyond the scope of this chapter. The interested reader is referred to recent reviews (French & Fisher, 2014; Wohlgemuth, Adam, & Scharff, 2014). Here, a sample of the work is provided, focusing on two of the most extensively studied model systems: mice and (briefly) birds (Fisher & Scharff, 2009). Much progress has already been made in uncovering relevant neural mechanisms via work with these two complementary models, and there is promise of more insights as the field develops.

The laboratory mouse is widely used in the field of neurogenetics, in large part due to the availability of a comprehensive toolkit for genetic manipulations (French & Fisher, 2014). Mice carry their own version of the *FOXP2* gene, which has the symbol *Foxp2*. The most recent common ancestor of humans and mice lived more than 75 million years ago but, despite this lengthy time since divergence, the sequence of the human *FOXP2* protein differs very little from that of its mouse counterpart (Enard et al., 2002). In a sequence of more than 700 amino acid residues, there is one small change in the length of a stretch of glutamines and three sites where one amino acid is substituted for another. In contrast to the substitutions that cause disorder, these evolutionary substitutions occur outside known domains of the protein and are predicted to have only subtle effects on function (see “*FOXP2* in Human Evolution” section for further commentary). In addition to very high conservation of protein sequence, the neural expression patterns are remarkably consistent; for example, in both humans and mice the gene is particularly highly expressed in deep layers in the cortex, medium spiny neurons in the striatum, and Purkinje cells in the cerebellum (Ferland et al., 2003; Lai et al., 2003).

Researchers have generated several different mouse models for studying *Foxp2* functions, including animals in which the gene is completely knocked out (Shu et al., 2005) and others that carry known etiological mutations that cause speech problems in humans (Groszer et al., 2008). If both copies of *Foxp2* are damaged (e.g., when mutations are in the homozygous state), then the mice cannot survive; they live for only 3 or 4 weeks after birth, during which time they develop at a substantially slower rate than normal siblings, show significant delays in maturation of the cerebellum, and have severe general problems with their motor system (Groszer et al., 2008; Shu et al., 2005). Thus, a total absence of functional *Foxp2* protein is lethal, which is consistent with the lack of any reports of humans carrying homozygous mutations in the gene. The cause of death in homozygous animals is unknown but may relate to one of the various non-neural sites in the body where *Foxp2* is expressed; for example, it is switched on in subtypes of cells in the lungs and cardiovascular system (Li et al., 2004). As a brief aside, transcription factors and other regulatory molecules are typically expressed in a range of tissues and cell types in different organs of the body. They exert distinct effects at different sites, depending on the sets of cofactors that they interact with, which is another example where biology takes advantage of the power of combinatorial systems and is a reminder of why specific “language genes” are unlikely to exist (Fisher, 2006).

Despite the associated lethality, investigations of mice that completely lack functional *Foxp2* have revealed some fundamental roles of the gene in early development

and patterning of the central nervous system (French & Fisher, 2014). The results from such studies are helping to inform hypotheses about the contributions of the human gene to development and patterning of neural circuits in our species. For example, one report used *Foxp2* mouse models to uncover networks of direct and indirect target genes during embryonic brain development (Vernes et al., 2011). The researchers found that there was an overrepresentation of genes implicated in biological processes like neurite outgrowth and axon guidance, consistent with prior findings from human cells (Spiteri et al., 2007; Vernes et al., 2007). They went on to validate this putative functional role in striatal precursor cells taken from the mouse embryos, finding that an absence of functional *Foxp2* led to reduced branching and shorter neurites in these cells (Vernes et al., 2011). Other studies of embryonic mouse cortex using different techniques (genetic manipulations in utero) have confirmed roles for *Foxp2* in neurite outgrowth (Clovis, Enard, Marinaro, Huttner, & De Pietri Tonelli, 2012) and also suggest potential functional impacts on other developmental processes such as neurogenesis (Tsui, Vessey, Tomita, Kaplan, & Miller, 2013) and neuronal migration (Clovis et al., 2012).

In stark contrast to the severe consequences of damage to both copies of *Foxp2*, mice that carry disruptions in the heterozygous state (i.e., only one copy is mutated or knocked out) live long healthy lives, usually without any obvious adverse outcome (Groszer et al., 2008). Such findings are concordant with descriptions of humans with heterozygous *FOXP2* mutations, who typically do not have associated medical problems or gross general developmental impairments (Laffin et al., 2012; Lai et al., 2001; Lennon et al., 2007; MacDermot et al., 2005; Rice et al., 2012; Shriberg et al., 2006; Turner et al., 2013).

Several studies of cognition, behavior, and electrophysiology in the heterozygous mouse models have built on prior observations that corticobasal ganglia and corticocerebellar circuits are key conserved sites of expression. Groszer et al. (2008) investigated heterozygous mice carrying the same mutation as the KE family and reported delays in learning to run on accelerating rotarods and voluntary running wheel systems against a background of normal motor behaviors. In slices taken from the brains of these mice, they also observed altered synaptic plasticity in corticostriatal and corticocerebellar circuits, most notably a lack of long-term depression for glutamatergic synapses on medium spiny neurons of the striatum (Groszer et al., 2008). A follow-up study used *in vivo* electrophysiology to record directly from medium spiny neurons in live behaving mice while the animals learned to run on accelerating rotarods (French et al., 2012). In mice that were heterozygous for the KE family mutation, compared with normal littermates, these neurons had significantly elevated basal firing

rates as well as striking abnormalities in both their modulation and their temporal coordination during motor skill learning. The discovery of disturbed striatal plasticity during learning of a complex motor task in mice is intriguing because neuroimaging studies of humans with the same mutation have independently suggested striatal dysfunction as a potential core feature of their disorder (Liegeois et al., 2003, 2011; Vargha-Khadem et al., 1998; Watkins, Dronkers, et al., 2002). Another behavioral study of this mouse model demonstrated reduced performance on a learning task in which the animals had to associate auditory signals with motor outputs (Kurt, Fisher, & Ehret, 2012). This last investigation also compared the learning dynamics with those of another mouse line that carried a different etiological mutation of *Foxp2*, reporting that the degree of impairment seemed to be affected by the type of mutation (Kurt et al., 2012).

It is interesting to note that these mouse studies uncovered effects on auditory-motor associations and motor skill learning that are not confined to the orofacial system. It remains unresolved whether such effects might be detectable in humans with *FOXP2* dysfunction (Peter et al., 2011), or if this instead points to a refinement of gene function in the human lineage. Studies of impacts of rodent *Foxp2* on vocal behaviors have yielded somewhat conflicting data (Fisher & Scharff, 2009; French & Fisher, 2014). What has been consistently established is that mouse pups that totally lack functional *Foxp2* have greatly reduced vocal output (Gaub, Groszer, Fisher, & Ehret, 2010; Groszer et al., 2008; Shu et al., 2005). Normally, when a young mouse pup is isolated from its mother and/or the nest, it produces ultrasonic calls that elicit its retrieval. When *Foxp2* is completely missing, pups produce few (if any) isolation calls; however, they do emit ultrasonic calls with complex properties when put in situations of greater stress. Although some researchers interpret these findings as evidence of specific roles of *Foxp2* in pup vocalization (Shu et al., 2005), others have pointed out that pups that lack this gene have very severe general motor problems and global developmental delay, making it impossible to draw conclusions about selective effects (Gaub et al., 2010; Groszer et al., 2008). For heterozygous mouse pups, which carry one damaged and one normal copy of *Foxp2*, there is debate regarding whether there are differences in amounts of vocalization (Groszer et al., 2008; Shu et al., 2005), and in-depth studies of properties of the vocalizations that are produced failed to find significant differences in normal littermates (Gaub et al., 2010). A study of rats reported that amounts of *Foxp2* protein are higher in brains of male pups, and that this correlates with production of a higher number of isolation calls as compared with female pups (Bowers, Perez-Pouchoulen, Edwards, & McCarthy, 2013). The researchers went on

to assess sex differences of *FOXP2* protein levels in Brodmann Area 44 of the human brain by using post-mortem tissue from a small number of 3- to 5-year-old children (five boys and five girls). One caveat is that although the male versus female samples were age-matched, they differed greatly in ethnic background, introducing a major confound. Bowers and colleagues (2013) observed higher amounts of *FOXP2* protein in the human females and interpreted this as evidence that elevated protein levels “are associated with the more communicative sex.” Given the very small number of data points (particularly from humans) and the fact that there are never going to be simplistic mappings from genes and proteins to communication skills (Fisher, 2006), this wide-reaching conclusion may be premature (French & Fisher, 2014).

At the time of writing this chapter, reports of impacts of rodent *Foxp2* on vocalization skills have focused exclusively on pup calls without describing, for example, effects on the ultrasonic “songs” of adolescent males (Fisher & Scharff, 2009). Nevertheless, although rodent vocalizations can provide a useful readout for studying the bases of social behaviors, it is thought that mice have very restricted abilities for using auditory experience to shape their vocal output (Hammerschmidt et al., 2012). Auditory-guided vocal learning is an important skill that underlies our abilities for acquiring speech, and mice are unlikely to provide an appropriate animal model for investigating this particular trait. Luckily, by looking further afield in the animal kingdom, it has been possible to find alternative model systems. Perhaps the most informative of these has been the zebra finch, a songbird that has provided entry points into both the neurobiology and neurogenetics of vocal learning.

A young male zebra finch learns its song during a critical developmental period by matching it to a template that it hears from an adult tutor (Bolhuis, Okanoya, & Scharff, 2010). Zebra finches have their own version of *FOXP2*, known as *FoxP2*. Intriguingly, expression levels of *FoxP2* in a key site of the songbird brain are correlated with changes in vocal plasticity (Haesler et al., 2004; Teramitsu, Poopatanapong, Torrisi, & White, 2010; Thompson et al., 2013). This key site is Area X, a striatal nucleus that is an essential part of a neural circuit known to mediate vocal learning. The zebra finch studies have gone beyond simply observing correlations by adopting cutting-edge molecular genetic tools to selectively reduce (“knock down”) levels of *FoxP2* expression in the living songbird brain. In a landmark paper, Haesler and colleagues (2007) reported that such *FoxP2* knockdown in Area X (but not surrounding areas) during the developmental period of song acquisition led to incomplete and inaccurate imitation of tutor song. Further studies of knockdown birds indicate that *FoxP2* loss yields reduced density for dendritic spines of spiny

neurons (Schulz, Haesler, Scharff, & Rochefort, 2010) and interferes with dopamine modulation of activity propagation in a corticostriatal pathway involved in song variability (Murugan, Harward, Scharff, & Mooney, 2013). As with the mouse models, neural plasticity in striatal circuitry emerges as a common theme associated with this gene (Murugan et al., 2013; Schulz et al., 2010). There is insufficient space available in this chapter to give a full account of all the relevant songbird studies; for further information on this burgeoning area of work, the interested reader is referred to reviews by Bolhuis et al. (2010), Scharff and Petri (2011), and Wohlgenuth et al. (2014).

## 2.6 FOXP2 IN HUMAN EVOLUTION

As shown, *FOXP2* has a deep evolutionary history with conserved functions in neural plasticity of a subset of vertebrate brain circuits. However, given its known links to human speech and language, it is reasonable to ask whether the gene has changed in any interesting ways during the evolution of our species (Fisher & Marcus, 2006). Of the three amino acid substitutions that distinguish the human and mouse version of the protein, two occurred on the human lineage after splitting from the chimpanzee at some point within the past 5 or 6 million years (Enard et al., 2002). These evolutionary changes occur outside the known domains of the *FOXP2* protein and are predicted to have only subtle (if any) effects on function. Moreover, they are entirely distinct from the known mutations that have been implicated in human speech and language disorder. Nevertheless, several investigations support the idea that the differences between the human and chimpanzee proteins have some functional significance. For example, one investigation compared human neuron-like cells expressing each version of the protein and reported quantitative differences in the regulation of some of the downstream targets of this transcription factor (Konopka et al., 2009). To study their effects on a living brain, Enard and colleagues (2009) inserted the human-specific amino acids into the mouse *Foxp2* locus. Remarkably, they observed effects on neurite outgrowth and plasticity that were in the opposite direction to those seen for loss-of-function mutations of this gene (Enard et al., 2009; Groszer et al., 2008; Vernes et al., 2011), and that seemed to be specific for corticobasal ganglia circuitry (Reimers-Kipping, Hevers, Paabo, & Enard, 2011).

The timing of these amino acid substitutions has been a matter of some debate. Initial studies estimated that they had arisen within the past 200,000 years, concordant with evidence that the *FOXP2* locus had been subject to Darwinian selection during the origin of modern humans (Enard et al., 2002). However, subsequent work has

indicated that the supposedly human-specific substitutions are also found in Neanderthal samples, indicating an earlier origin and predating the human–Neanderthal split several hundred thousand years ago (Krause et al., 2007). Further investigations revealed noncoding changes (i.e., those that do not affect amino acid sequences) that occurred on the human lineage after the split from Neanderthals, and that might have affected the way that the expression of *FOXP2* is regulated. It is possible that these later changes may impact on functions of the gene and could explain the evidence of relatively recent Darwinian selection at the locus (Ptak et al., 2009). Thus, human *FOXP2* may have been subject to multiple selective events during human evolution, which might have involved modifications of its functions in neural circuitry. Obviously, we cannot go back in time to formally test whether such modifications were really relevant for the emergence of language. Regardless, based on its deeper evolutionary history, it is unlikely that *FOXP2* was a sole trigger for appearance of this complex suite of skills, but instead it represents one piece of a complex puzzle involving other factors (Fisher & Ridley, 2013).

## 2.7 CONCLUSIONS

*FOXP2* is not the only gene to have been implicated in speech and language, although it provides perhaps the clearest links to the underlying biology. For example, studies of common forms of SLI have suggested several other candidates, like *CNTNAP2* (introduced above), *ATP2C2*, and *CMIP* (Newbury & Monaco, 2010; Newbury et al., 2009). As genomic technologies continue to advance at an astonishing rate, we can expect more and more of the critical molecules to be uncovered (Deriziotis & Fisher, 2013). This chapter has sought to provide an illustration of the new vistas that open up after the identification of a language-related gene, emphasizing that such a discovery is just a starting point for functional investigations. One emerging approach in the language sciences that has not yet achieved its full potential is that of neuroimaging genetics, that is, testing for associations between genetic variants and variability in structure and/or function of language-related circuits of the human brain. Investigations of structural and functional consequences of rare mutations associated with disorder have been informative (Liegeois et al., 2003, 2011; Vargha-Khadem et al., 1998; Watkins, Vargha-Khadem et al., 2002). However, the effects of common variations in genes of interest have been more difficult to decipher (Hoogman et al., 2014), and reports have generally been underpowered for detecting biologically meaningful effects (Bishop, 2013; Graham & Fisher, 2013). It is likely

that large-scale sophisticated studies involving genetic information, structural and function neuroimaging data, and performance on cognitive measures will yield exciting new insights. Overall, these developments in bridging genes, neurons, circuits, brains, and cognition are bringing us closer to understanding the basis of our most mysterious human capacities.

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