Genetic Basis of Human Language Acquisition

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Abstract

Humans possess the unique ability to speak sophisticated languages through vocal learning. Studies have tried to understand the biological basis of human language acquisition. In this review, we discuss research focusing on a family with an inherited language disorder that leads to the discovery of a gene encoding a transcription factor known as forkhead box P2 (FoxP2). Studies on other model organisms, such as birds and mice, have provided insights into the unique role of FoxP2 in language acquisition and how the mutation in FoxP2 affects its function. Finally, we propose future directions to deepen our understanding of the mechanism of FoxP2 action, the pathogenesis of FoxP2 mutation-associated language disorder and potential treatments.

Introduction

Humans are the only known animal to have the ability to compose syntactic sentence structures with words to speak sophisticated languages. Language acquisition in humans is based on skills such as abstraction and the use of syntactic rules (1). All human babies can develop grammatical rules by simply listening to adults, regardless of the language. Songbirds and hummingbirds are also vocal learners who have the ability to imitate songs with great accuracy. However, they do not have the ability to generate endless sentence structures and combinations to convey a message as humans can (2). Dolphins, another mammal, can communicate with whistle-like sounds produced by vibrating connective tissue, similar to the way human vocal cords function, and through burst-pulsed sounds, though the nature and extent of that ability are not known (3). Non-human primates, such as chimpanzees and bonobos are able to learn words, but they lack the crucially non-recursive syntactic abilities that

distinguish human language (1). One of the most famous bonobos (a species of primate similar to chimpanzees), Kanzi learned to communicate through a lexigram board, pushing symbols that stand for words. Although Kazi relied heavily on the lexigram board, when given 660 spoken instructions, asking them to deal with familiar objects in novel ways, Kanzi responded correctly to 74 percent of the instructions. Nim Chimpsky, named after Noam Chomsky, was the chimpanzee subject of an extended study of animal language acquisition at Columbia University (4). The goal of the study on Nim was to challenge Noam Chomsky's theory of universal grammar. Nim was taught to communicate through sign language, where he made over 20,000 sequences, however, the trainers noticed that Nim was merely repeating signs done and it is believed that, in total, Nim knew about 125 signs (5). Although the result does not disprove the existence of universal grammar, the result does not directly support its existence either as many factors could contribute to a negative result, making the information difficult to interpret. The experiments described above indicate that despite sharing almost 99% percent of the same DNA as humans (6), non-human primates are still not able to speak languages as humans can, although some individuals can be trained to a certain extent. The similarities in genes that humans have with other primates suggest that humans have acquired language abilities through gaining novel functions of homologs shared with other animals rather than evolving completely novel genes. Such observations suggest that there is much room for further investigation on how genes are expressed and the protein products interact with each other in the human nervous system, which uniquely allows humans to speak languages.

Infants can acquire languages at an incredible rate by simply listening to adults, regardless of the type of language, although little is known about the mechanisms that underlie the acquisition process (7). However, the ability to learn any type of language through simple

exposure suggests the existence of a universal grammar, which may be determined by a corresponding neuronal circuit structure. Previous studies have uncovered distinct brain areas that are involved in different aspects of language processing. The neurological circuits in our brain that can process language are located in the cerebral cortex. The left cerebral hemisphere helps us speak and communicate. A French neurologist, Pierre Paul Broca, identified Broca's area located in the posterior region of the frontal lobe. Patients with a lesion (caused by strokes) in the Broca's area can understand language and have no motor deficits but can only speak with isolated words. Furthermore, Karl Wernicke identified the Wernicke's area. Patients with a lesion in the Wernicke's area could speak and form words but could not understand language (8), suggesting that Broca's area is involved in speaking and the Wernicke's area is involved in understanding language. The neurological circuits in our brain that can process language are formed during the process of development and determined by genes. In order to understand more about language, it will be essential to identify the genetic basis underlying such developmental processes.

How genes expressed in the nervous system control the development of such neuronal circuits remains to be elucidated. Studies have helped isolate the FoxP2 gene, which plays a crucial role in language capability as a mutation of this gene creates a language disorder, as manifested in the KE family. Further, studies on mice, songbirds, and hummingbirds suggest that FoxP2 is necessary for vocal and motor learning skills. These studies have suggested a role of FoxP2 in the development of neural circuits underlying language capability in humans and will be the focus of this review. Future directions that further expand our knowledge on the role of FoxP2 in language capability will be discussed in the end.

Discovery of the FoxP2 Gene in a Family with an Inherited Language Disorder

A severe language and speech disorder was found to affect about half of the 30 members of the four-generational KE family (9). Clinic presentation of the affected members' disorder transcends the generation of morphosyntactic rules to include impaired processing and expression of other areas of grammar, grossly defective articulation of speech sounds, and, further, a severe extralinguistic orofacial dyspraxia. The affected members of the family suffer from a grammar-specific disorder, initially providing support for the existence of "grammar genes." However, since the inherited disorder does not affect morphosyntax exclusively; rather, it affects intellectual, linguistic, and orofacial praxis functions generally, the family does not provide support for the existence of grammar-specific genes (10). The discovery of such a family helps us learn more about the genetic basis of the human faculties of speech and language through the neural and genetic correlates of their disorder (10).

A pedigree of the speech and language disorder of family KE suggests that the disease was transmitted as an autosomal-dominant monogenic trait (11) as half of the children were affected by the disorder. Through haplotypes, researchers narrowed down the mutation to the SPCH1 locus on chromosome 7 as all affected members possess the same combination of genotypes at polymorphic markers in the D7S5527-D7S530 (SPCH1) region of chromosome 7, suggesting that the mutation resides in the SPCH1 region located on chromosome 7. The isolation of SPCH1 offered the first insight into the molecular genetics of the developmental process that culminates in speech and language (12). However, due to both limited resolution in genetic mapping and sequencing capacity, it was nearly impossible to determine the precise gene responsible for the mutation.

An unrelated individual, CS, was later identified who also had a similar language disorder. Patient CS had a chromosomal translocation involving the SPCH1 interval (11). Findings showed that both the KE family and patient CS possessed phenotypes that had substantial impairment of expressive and receptive language abilities (11). Using a series of bacterial artificial chromosome clones, the translocation breakpoint of patient CS was mapped to within a single clone, NH0563O05, and did not reveal any additional associated genomic rearrangements in the vicinity of the translocation (11).

The NH0563O05 clone was mapped to the FoxP2 gene located in the SPCH1 area and prompted researchers to sequence the FoxP2 gene of the KE family members. Through the sequence of the gene, a point of mutation (R553H) residing in the forkhead domain was found to exist in the affected members of the KE family (11). Such discoveries suggest that FoxP2 plays an important role in the developmental process that culminates in speech and language (11) as a mutation of this single gene creates a severe language and speech disorder. The discovery of FoxP2 provided a genetic handle for the further investigation on the neurological basis of language and other associated intelligence phenotypes in model organisms, such as mice and birds.

Role of FoxP2 in Vocal Learning in Hummingbirds and Songbirds

The trait of vocal learning can be found in only three types of birds: parrots, hummingbirds, and songbirds. Songbirds and hummingbirds have developed the rare trait of vocal learning, this being the ability to acquire vocalizations through imitation rather than instinct (13). This ability is significantly similar to those of humans, as we develop languages through listening to adults. Thus observational studies on hummingbirds and songbirds have helped further understand the role that FoxP2 plays in vocal learning as they bear behavioral and

neural parallels which makes these birds a genuine model for investigating the genetic basis of speech and its pathologies (2).

A study that used lentivirus-mediated RNA interference (RNAi) to reduce FoxP2 levels in Area X, a basal ganglia structure necessary for song learning, helped further our understanding of FoxP2 and how it evolved. The RNAi approach is a short interfering hairpin RNA (shRNA) containing sense and antisense sequences of the target gene connected by a hairpin loop which is expressed from a viral vector (2). Two specific shRNAs (shFoxP2-f and shFoxP2-h) were designed and used in the study; both strongly reduced the levels of overexpressed FoxP2 protein *in vitro*, but did not change the levels of overexpressed protein levels of FoxP1 (the closest homolog to FoxP2). Furthermore, a control shRNA was designed to not target any zebra finch gene (shControl, and this shRNA did not affect the expression of either FoxP2 or FoxP1 *in vitro*. In the studies both shFoxP2-f and shFoxP2-h were used interchangeably for subsequent *in vivo* experiments as they both targeted FoxP2 with similar efficiency (2). The virus infects neurons and in turn, reduces the FoxP2 expressions in Area X which is believed to be responsible for vocalization. Results from the studies show that a knockdown of FoxP2 resulted in an incomplete and inaccurate imitation of tutor song, which was evident early during song ontogeny and persisted into adulthood. The acoustic structure and the duration of adult song syllables were abnormally variable, similar to word production of the KE family and patient CS. That the reduction of FoxP2 levels affected the outcome of both song learning and speech development supports the hypothesis that during evolution, ancestral genes and neural systems were adapted in the human brain and gave rise to the uniquely human capacity of language (2).

Hummingbirds share significantly similar structures in the forebrain with seven discrete regions that are involved in vocal learning and production as songbirds (13). Studies on

hummingbirds further supported the theory that vocal learning and associated brain structures evolved independently although there could have been strong constraints that influenced the evolution of forebrain vocal nuclei in hummingbirds (13). A study on hummingbirds used ZENK messenger RNA synthesis in the brain driven by neuronal depolarization, and its detection can be used to identify select regions that are activated by specific stimuli or behaviors, allowing the mapping of the vocal communication areas throughout the brains of hummingbirds and other birds without the disruption of natural behaviors. This mapping allows researchers to map freely ranged hummingbirds as singing behaviors are difficult to obtain under captivity, and under other methods, it is impossible to identify relevant brain areas (13). Three groups were used in this experiment and compared: the silent control group, hearing-only group, and hearing and vocalizing group. The silent controls were birds caught in the early morning before the start of the dawn chorus and were found with low ZENK expressions as they did not hear the dawn chorus. Furthermore, in the hearing only birds showed that hearing induced ZENK expression in seven brain areas that are conserved among avian species, while the hearing and vocalizing birds showed vocalizing-induced ZENK expression in eight discrete areas. The results support hypotheses such as that vocal learning may have evolved independently or the trait of vocal learning was lost through evolution and now can only be found in a few species. It remains to be determined whether the FoxP2 gene is specifically expressed in neurons in those brain areas and whether a deficiency in FoxP2 genes would disrupt proper circuit formation within those areas.

Role of FoxP2 in Mouse Vocalization and Intelligence

Human and mouse FoxP2 show similar expression patterns in the developing brain, with expression detected in the basal ganglia, thalamus, and inferior olive (14). Furthermore, the

human FoxP2 protein differs at only 2 amino acids compared with its mouse homologue (14), allowing precise alignment of the human and mouse FoxP2 homologues and generation of a mouse FoxP2 mutant mimicking the human mutant discovered in the KE family. Mice also communicate with their mothers when they are pups with whistle-like sounds. Due to the similarities of the FoxP2 gene in humans and mice, they were used in many studies to further understand the developmental processes of language found in FoxP2.

To further analyze FoxP2 function in speech learning, a study used homologous recombination to generate a knockin (KI) mouse for FoxP2 (R552H), corresponding to the human FoxP2 (R553H) mutation. The homozygous FoxP2 mice showed reduced weight, immature development of the cerebellum with incompletely folded folia, Purkinje cells with poor dendritic arbors, and less synaptophysin immunoreactivity, and achieved crisis stage for survival 3 weeks after birth (14). This shows that the mutant mice had gross neurological developmental abnormalities. However, the homozygous KI mouse is not appropriate to assess language functions and disorders, such as those of the KE family, as all members of the KE family are heterozygous. The heterozygous FoxP2 mice showed a similar increase in weight and cerebellum as the Wild-type. A standard behavioral analysis was performed on three littermates to further analyze the phenotype of the FoxP2 (R552H)-KI mice, including assessing righting reflex (where the mice were placed on their backs) and mid-air righting (where the mice were dropped from the air). The Homozygous FoxP2 (R552H)-KI pups were delayed in their ability to right themselves when placed on their backs or in midair, whereas most heterozygous FoxP2 (R552H) mice did not exhibit obvious delays. Furthermore, the righting reflex of homozygous and heterozygous mice was seven and three times more delayed in respect to the wild-type mice. Suggesting that some motor deficiencies are present in the heterozygous KI mice. In the

midair-righting assay, the homozygous mice showed a clear difference from wild-type mice while the heterozygous mice were not significantly different from the wild-type (14). The communication signals that mice pups usually produce are whistle-like sounds with frequencies between 30 kHz and 100 kHz. This sound plays an important communicative role in mother-offspring interactions because they elicit prompt responses from the dam concerning caregiving behaviors. The number of USVs (whistles) and sonic/USVs (clicks) of 8-day-old pups after separation from the mother were impaired in both the heterozygous and homozygous mice. The heterozygous FoxP2 (R552H)-KI pups showed modest impairment for USVs while the wild-type pups mainly produced whistle-type USVs. Both heterozygous and homozygous pups showed severe impairment in the number of USVs. Homozygous FoxP2 (R552H)-KI mice did not produce any USVs because of a severe loss or near cessation of activity (14). In the cerebellum of the homozygous FoxP2 (R552H)-KI mice the FoxP2 showed nuclear aggregation in some of the Purkinje cells and the dendritic shafts of the homozygous Purkinje cells were thin, with less elaborated calbindin-positive dendritic arbors and reduced synaptophysin reactivity compared with the wild-type Purkinje cells (14). Furthermore, synaptophysin reactivity in the dendritic arbors of the heterozygous cells was at a level between that in the homozygote and the wild type. In both the homozygous and heterozygous FoxP2 (R553H)-KI mice, the metabotropic GABA receptor (GABABR) was more poorly expressed in the dendrites although it was expressed in the migrating granule cells. At P19 and P45 the heterozygous dendrites had recovered and elaborated as well as wild-type dendrites, but the homozygous dendrites still remained at an immature level at P19 (14). Cytoplasmic polyQ aggregates induce endoplasmic reticulum (ER) stress in cells and thus, cytoplasmic and/or nuclear aggregates of ectopically expressed FoxP2 (R552H) induced the ER stress in cells (14). These results provide insights

into the common molecular mechanisms between the mouse USV and human speech learning as well as the relationship between the USV and motor neural systems.

Two human-specific amino acid substitutions in the FoxP2 gene can help further the understanding of how genetic changes may have adapted in the human nervous system to allow the unique acquisition of language and speech (15). These two human-specific amino acid substitutions were introduced into the endogenous FoxP2 gene of mice (15). The humanized FoxP2 mice and wild-type (WT) mice were placed in a T-maze with spatial cues (promoting place-based/declarative learning) and without spatial cues (promoting response-based/procedural learning). The humanized mice performed better than the WT in the T-maze with spatial cues while relatively the same without spatial cues. When transitioning from place-based to response-based learning the humanized FoxP2 mice exhibited enhanced abilities to make transitions. However, when transitioning from response-based learning to place-based their learning rates did not differ from those of the WT. Suggesting that it is specifically the transition from declarative learning to procedural learning that is enhanced by the introduction of the humanized form of FoxP2, therefore facilitating the transition from declarative to procedural learning that is proposed to occur during striatum-dependent habit learning (15). These findings suggest the possibility that the humanized FoxP2 phenotype reflects a different tuning of corticostriatal systems involved in declarative and procedural learning, a capacity potentially contributing to adapting the human brain for speech and language acquisition (15).

Future perspective

The KE family provided a genetic handle for researchers to further investigate FoxP2 through model animals, such as songbirds, hummingbirds, and mice. These model animals give

us insight into how FoxP2 plays a role in language acquisition and speech. However, there is much yet to be known about FoxP2 such as how it is presented in other mammals, its role in circuit formation in certain forebrain areas, and the biochemical behavior of the mutant.

Research on dolphins, another mammal possessing a unique capability of vocalization, could help further the understanding of FoxP2 and its role in vocalization. The dolphin FoxP2 sequence is highly similar to the human homolog, suggesting a conserved function. Furthermore, bottlenose dolphins have signature whistles, a whistle that is unique to a specific individual. These signature whistles are developed during a dolphin's first year and to obtain each individual whistle sound, dolphins undergo vocal production learning (16), similar to humans. However, relatively limited research has been done on the vocal learning of signature whistles in dolphins. Thus, a study on the vocalization of bottlenose dolphins through generating a genetically modified dolphin with either a knockdown of FoxP2 or introducing the equivalent of the human R553H mutant could help us understand whether FoxP2 plays a role while dolphins undergo vocal production learning of their signature whistles and strengthen our knowledge on FoxP2's role on vocal learning in humans.

Although we know that FoxP2 levels affected the outcome of song learning and the discrete forebrain areas of hummingbirds during vocal learning, it is unclear whether the FoxP2 gene is expressed in specific subpopulations of neurons located in those discrete areas of the hummingbird brain, and whether a lack of FoxP2 genes would disrupt proper circuit formation in those areas. Additional studies that compare the expression pattern of FoxP2 across different neuronal populations within the regions that were found to be active when hummingbirds were engaged in imitation and vocal learning is necessary. Such studies could be performed by precisely delivering the FoxP2 shRNA lentivirus to each of those regions and observing whether

knocking down FoxP2 in any of those regions would produce a strong phenotype or delivering a mutant FoxP2 gene to those regions to see if whether it disrupts the proper function of the endogenous FoxP2. These studies would establish a stronger link between FoxP2 and vocalization in songbirds and hummingbirds.

In the knockin mice study (14), it was discovered that heterozygous and homozygous FoxP2 (E552H)-KI showed nuclear aggregation in some of the Purkinje cells and immature dendrites. This discovery suggests that FoxP2 may have an intrinsic tendency to aggregate, which is exacerbated by the R553H mutation. In fact, there is a long stretch of glutamine in FoxP2, which is known to contribute to aggregation of the protein Huntingtin in patients of Huntington's disease. Purifying the nuclear aggregation in some of the Purkinje cells and testing its behavior could further the investigation of this idea. In addition, potential therapeutic strategies to treat neurodegenerative diseases associated with protein aggregation include: protein stabilization to prevent the conformational changes that enable aggregation, protein reduction to lower the concentration of the aggregation-prone protein and thereby slow aggregation, aggregate clearance or remodeling to reduce proteotoxicity, cellular proteostasis network adaptation to enhance proteome quality control, and reducing seeding and cell-to-cell spreading (17). These therapies may treat diseases such as Huntington disease and could treat the KE family and similar patients with the same severe language disorder if FoxP2 is proven to be prone to aggregation.

Further studies could help advance our understanding of the mechanism underlying the molecular basis of human language acquisition and identify therapies that could treat patients with genetic deficiencies.

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