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The role of the FOXP family of transcription factors in ASD

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Abstract. Autism spectrum disorders (ASD) is a neurodevelopmental disease with complex genetics; however, the genes that are responsible for this disease still remain mostly unknown. Here, we focus on the FOXP family of transcription factors as there is emerging evidence strongly linking these genes to ASD and other genes implicated in ASD. The FOXP family of genes includes three genes expressed in the central nervous system: *FOXP1*, *FOPX2*, and *FOXP4*. This unique group of transcription factors has known functions in brain development as well as the evolution of language. We will also discuss the other genes including transcriptional targets of FOXP genes that have been found to be associated with language and may be important in the pathophysiology of ASD. Finally, we will review the emerging animal models currently being used to study the function of the FOXP genes within the context of ASD symptomology. The combination of gene expression and animal behavior is critical for elucidating how genes such as the FOXP family members are key players within the framework of the developing brain.

Keywords: FOXP2, FOXP1, autism, genetics

1. Introduction

Autism is characterized by impaired language communication, qualitative impairment in social interactions, as well as restricted repetitive and stereotyped behaviors/interests [1]. The prevalence rate of autism in the general population is estimated to be 1% [2,3]. Moreover, there is a strong sex bias in the prevalence rates with ratios of 4:1, favoring males over females [4, 5]. A broader range of associated symptoms is also recognized, termed autism spectrum disorders (ASD). The heritability of ASD has been estimated to be ∼90%; making ASD the most heritable of the childhood onset neuropsychiatric disorders [6,7]. Autism is comorbid with a number of other diseases, including Rett, Fragile X, and Angelman Syndrome [8]. Numerous studies have searched for causal genetic factors for ASD, and the results point to a complex genetic architecture converging on signaling pathways involved in the development of the nervous system [9,10] that are also likely interacting with environmental and epigenetic phenomenon. The focus of this review will be to discuss the most recent genetic findings for ASD within the context of a family of transcription factors linked to language, brain development, and ASD.

2. The genetics of language

Identifying the genes involved in language is not only important for the understanding of disorders such as ASD, but it also provides a window into understanding the evolution of the human brain since spoken language is only present in humans. While other animals have developed methods of vocal communication, none have the ability to convey recursive ideas (i.e. ideas embedded within other ideas), although the idea of human-specific recursion is still being debated [11–14].

Similar to ASD, Specific Language Impairment (SLI) is a common developmental disorder with estimates of prevalence around 7% for school-aged children [15]. Moreover, twin studies have pointed to a genetic etiology for SLI with a complex genetic archi-

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tecture [16]. Patients with SLI are not cognitively impaired or socially withdrawn as in ASD, but they do have impairments in communication [17]. The gene encoding contactin associated protein-like 2 (*CNTNAP2*) has been associated with a specific endophenotype related to a core feature of SLI, nonword repetition defects [18]. CNTNAP2 is a member of the neurexin superfamily of proteins important in the development of cell adhesion and synaptic connections in the brain [19]. Both rare and common variations in *CNTNAP2* have been identified in patients with ASD [20–24]. Analysis of one patient cohort revealed an association between *CNTNAP2* variants and the age of the first spoken word, a language endophenotype [20]. Another group of patients with ASD and speech delay had multiple structural variations containing *CNTNAP2* [23]. CNTNAP2 has been shown to have an enriched expression in the human frontal pole [20,25]. In contrast, expression of CNTNAP2 in lower organisms is not focally enriched in any region of the cortex [25]. Furthermore, *CNTNAP2* knockout mice have decreased ultrasonic vocalizations [26]. These data support an important function for CNTNAP2 in cognition and language. Two additional SLI candidate genes have shown strong association to the disorder: the calcium-transporting ATPase 2C2 (*ATP2C2*) and c-MAF inducing protein (*CMIP*) genes [27]. However, neither of these genes has been linked to ASD. Thus, while there may be some convergence of ASD with other language disorders, the complex genetics of all of these disorders makes deciphering the signaling pathways even more challenging.

3. FOXP2

A key insight into identifying genes involved in language has come from investigations focusing on atypical phenotypic expression. Such investigations led to the discovery of a large multigenerational family known as the KE family, in which affected family members exhibit verbal dyspraxia, or deficits in coordinating the complex oral-facial motor movements associated with spoken language [28]. Moreover, affected individuals from the KE family also have below average IQ and syntactic impairments (i.e., an inability to use the proper rules associated with grammar) [28,29]. It was the cytogenetic investigation of this family plus an unrelated affected individual that uncovered the forkhead box P2 or *FOXP2* gene on chromosome 7 [30,31].

FOXP2 is a member of the forkhead family of transcription factors, and is highly homologous to two other forkhead transcription factors, *FOXP1* and *FOXP4* [32]. All three FOXP family members have several conserved domains: a polyglutamine tract, a zinc finger domain, a leucine zipper, and a forkhead DNA binding domain (Fig. 1A-C). Further analysis of the affected members of the KE family revealed a point mutation in the DNA binding domain of FOXP2 resulting in an amino acid change of arginine to histidine (R553H) [31]. Subsequent analysis of this particular mutation revealed that it disrupts the ability of FOXP2 to bind to DNA [33]. Affected individuals are heterozygous for this mutation, leading to the hypothesis that the mutant form of FOXP2 may act like a dominant negative, relocating the wildtype FOXP2 protein to outside of the nucleus where it can no longer act as a transcriptional regulator [33,34]. This type of interaction makes sense in light of evidence showing FOXP family members can either homodimerize (e.g. FOXP2-FOXP2) or heterodimerize (e.g. FOXP2-FOXP1) through their zinc finger domains [32]. In addition to the KE family, several other studies have described patients with mutations or truncations in FOXP2 displaying a similar verbal dyspraxia [35–40]. Together, these reports have firmly established the role of FOXP2 in language.

The expression of FOXP2 is not limited to the central nervous system; it is also expressed in the lungs, kidney, intestine, spleen, and skeletal muscle [41]. However, for the purpose of describing how FOXP2 is involved in ASD, we will limit our discussion to its expression in the brain. In humans, FOXP2 is highly expressed during mid-gestation (Fig. 2A) [42,43]. This is a critical time point in brain development during which neural specification is occurring and extensive configuration of the brain is taking place. Furthermore, the expression pattern of FOXP2 in humans declines postnatally and continues to decline to nearly undetectable levels in the adult human brain (Fig. 2A).

In contrast to the expression pattern of FOXP2 in humans, FoxP2 expression in both rodent and zebra finch brains remains at high levels from the embryonic period thru adulthood [42,44]. This dissimilarity in expression differences may point to a unique function of FOXP2 in the developing human brain, and relate to its role in a human-specific trait such as language. In humans, FOXP2 is primarily limited to expression in the striatum, specific thalamic nuclei, hippocampus, cerebellum, and layer six of the cortex [42,45,46]. Expression of FOXP2 in these regions of the brain, in particular the cortical-striatal expression, points to a potential role for FOXP2 in many neuropsychiatric disorders.

Studies examining genetic association between polymorphisms in *FOXP2* and autism have been inconclu-

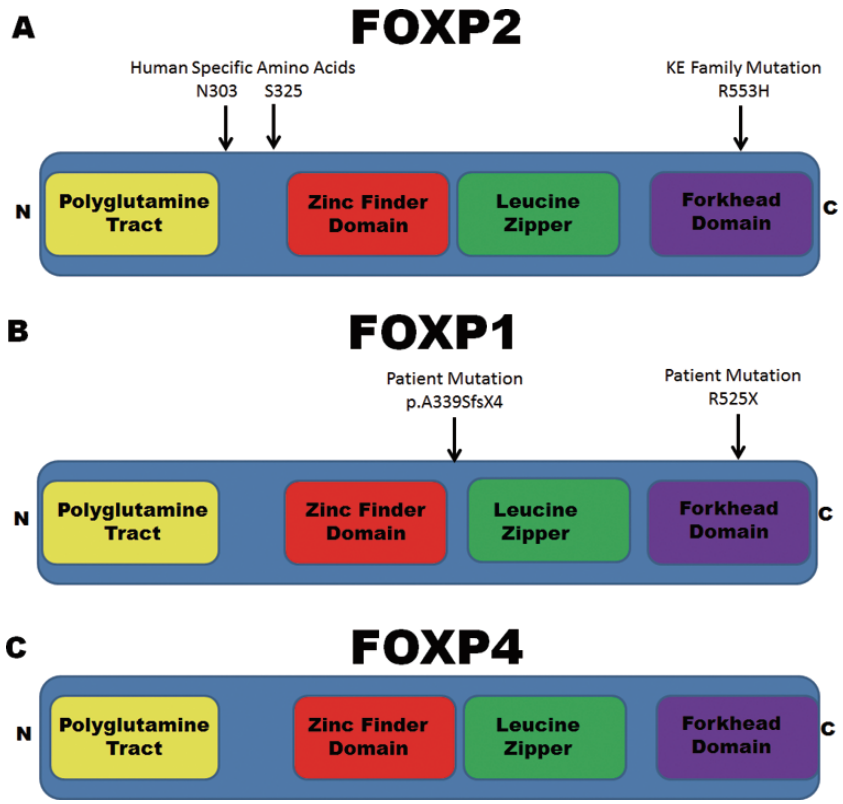

Fig. 1. Conserved domains of the FOXP family members. A) Schematic of FOPX2 showing the polyglutamine tract, a zinc finger domain, a leucine zipper, and a forkhead DNA binding domain including the mutation causing the disruption of FOXP2 in the KE family. B) Schematic of the FOXP1 mutation deletion in patient # 12817 from (O'Roak et al., 2011) and patient A from (Hamden et al., 2010) as well as the same functional domains. C) Schematic of FOXP4 highlighting its similarity with the other two brain expressed FOXP family members. (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0919)

sive. A number of initial studies reported no associations between polymorphisms in *FOXP2* and ASD [47–49]. Moreover, whole genome microarrays of ASD samples have not found an association of *FOXP2* with the disorder [50–56]. In contrast, at least four studies have uncovered polymorphisms in *FOXP2* associated with ASD [57–60], and potential imprinting of *FOXP2* has been uncovered in a group of patients with ASD [35]. Taken together these studies suggest the potential for direct disruptions to *FOXP2* leading to ASD. Nevertheless, these and many other studies are limited by sample size and fixed marker sets for genotyping. Future studies encompassing whole genome sequencing in large patient populations should more reliably address the direct role of *FOXP2* in ASD.

An alternative explanation for many of the contradictory findings correlating *FOXP2* variation with ASD may be due to the complex splicing of *FOXP2*. At least eight isoforms of *FOXP2* have been identified as well as almost a dozen additional splice isoforms (UCSC human genome browser hg19) [61,62]. Moreover, it has been suggested, based on Northern blot analysis that an isoform twice as large as any of the reported *FOXP2* isoforms may exist [62]. Nevertheless, stronger support for a role for FOXP2 in ASD can be found in the downstream signaling pathways regulated by FOXP2.

While the misregulation of the target genes controlled by FOXP2 is not fully characterized, several studies have uncovered FOXP2 regulation of ASD genes. Through the use of chromatin immunoprecipitation coupled to microarrays, a comparison of FOXP2 ChIP-chip targets in brain with a list of ASD candidate genes yields a number of overlapping genes [9,10,63]. This overlap includes: *A2BP1*, *DISC1*, *DPP6*, *ITGB3*, *MTF1*, *RPL10*, *RPS6KA2*, and *TDO2* (Table 1). In addition, an overlap of the differentially expressed genes regulated by human or chimpanzee FOXP2 with ASD genes also includes: *GRM8*, *DPYD*, *IGFPB3*, *MAOB*, and *VIP* [64]. Other, more directed, studies have detailed FOXP2 regulation of autism candidate genes, in particular *CNTNAP2* [18] and *MET* [65].

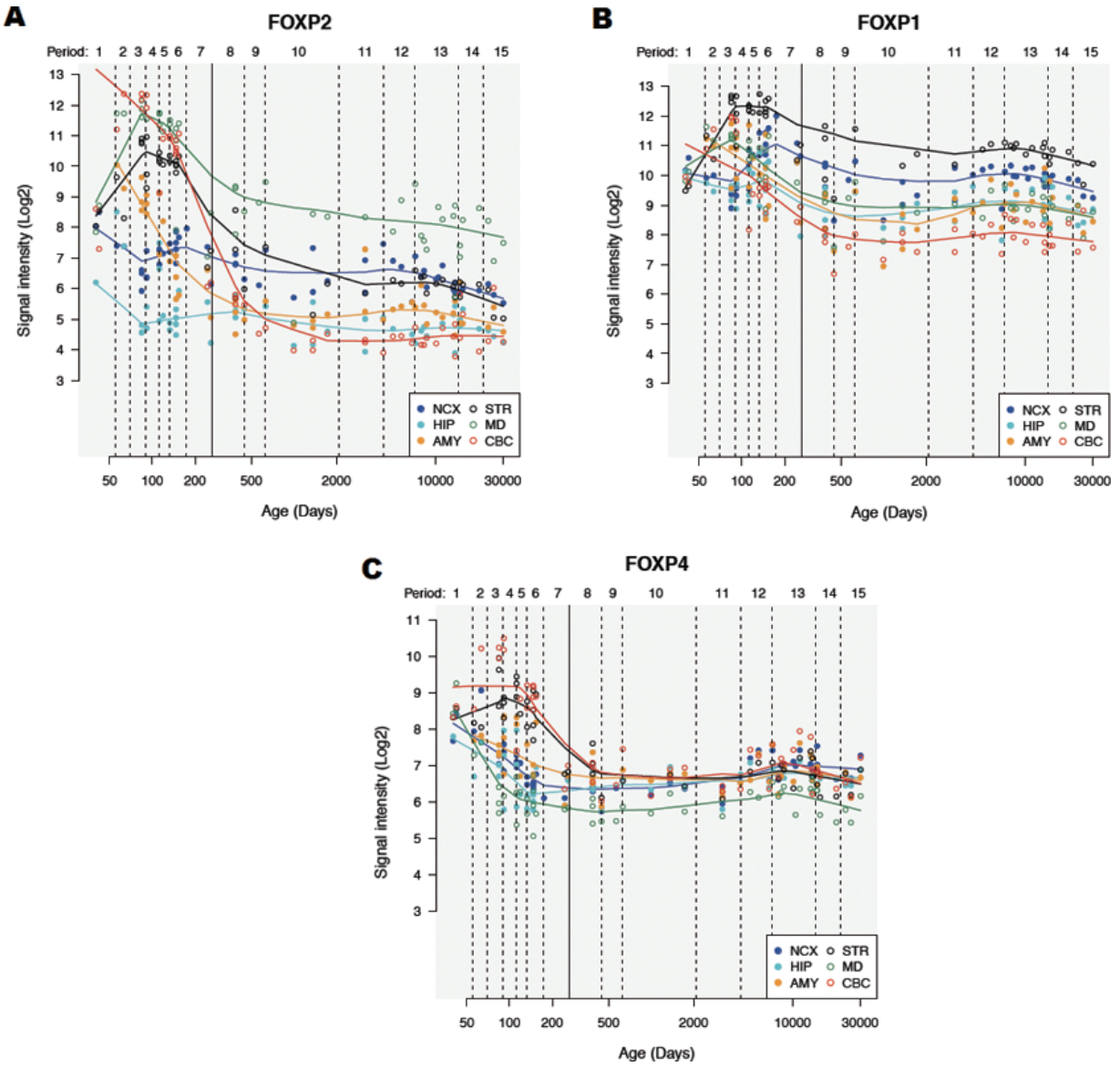

Fig. 2. Expression of FOXP family members in the developing human brain. Expression of A) FOXP2, B) FOXP1, and C) FOXP4 throughout human brain development. The solid line indicates birth. NCX = neocortex, HIP = hippocampus, AMY = amygdala, STR = striatum, MD = mediodorsal nucleus of the thalamus, and CBC = cerebellum. Data are derived from Kang et al. using the Human Brain Transcriptome database (http://hbatlas.org/). (Colours are visible in the online version of the article; http://dx.doi.org/10.3233/DMA-2012-0919)

In situ hybridization of both *FOXP2* and *CNTNAP2* in the developing human brain shows an inverse pattern of mRNA expression [18]. Moreover, forced expression of FOXP2 in human neuronal cells leads to a concomitant reduction of *CNTNAP2* [18]. Recent findings show FOXP2 is able to bind to the *CNTNAP2* gene in human neuronal cells [18], and in one individual with ASD and *CNTNAP2* variation, the FOXP2 binding site were deleted [23]. Collectively, these data suggest that FOXP2 directly represses CNTNAP2 expression. This regulation may be critical for human language and consequently be aberrant in both ASD and SLI.

The gene encoding *MET* proto-oncogene has been strongly associated with ASD [51,66–68]. In particular, a polymorphism in the promoter of *MET* is associated with ASD, and results in reduced transcription of *MET* in cell lines [69]. Furthermore, MET expression is reduced in the temporal cortex of patients with this par-

ticular mutation [70]. The expression pattern of *MET* in the developing human brain is quite striking; it is primarily expressed in the cortex and it is enriched in the developing temporal lobe [65]. This pattern suggests a potential role in pathways important for language. While several studies have characterized the expression pattern and function of *Met* in the mouse [71–74], there is little known about the regulation of human *MET*. Due to the enrichment of MET in the temporal lobe and the previous identification of other ASD genes downstream of FOXP2, the hypothesis of whether MET might also be regulated by FOXP2 was tested. Examination of *MET* and *FOXP2* in the developing human fetal brain revealed inversely correlated expression patterns [65]. Forced expression of FOXP2 in human fetal neural progenitor cells resulted in a concomitant reduction of both MET mRNA and protein. Lastly, electrophoretic mobility shift assays (EMSAs) and ChIP demonstrated direct binding of FOXP2 to the *MET* promoter. Together, these results confirm that FOXP2 directly binds to the *MET* gene and downregulates its expression in differentiating human neurons [65]. Interestingly, FOXP2 is also expressed in the GI tract [41]. It is possible that dysregulation of *MET* by FOXP2 in the GI tract contributes to the GI manifestations experienced by a subset of ASD patients.

Table 1
Targets of FOXP2 implicated in ASD

Gene	Reference
A2BP1	4,6
CADPS2	1
CDH8	6
CNTNAP2	5
DISC1	4
DPP6	4
DPYD	2
FRMPD4	6
GNAS	6
GRM8	2
IGFBP3	2
ITGB3	4
MAOB	2
MCPH1	6
MET	3
MTF1	4
NTRK3	6
RPL10	4
RPS6KA2	4
SYN1	6
TDO2	4
UBE3A	6
VIP	2
ASD genes are from the SFARI gene database (https://gene.sfari.org/)	
Enard et al. (2009). *Cell*	1
Konopka et al. (2009). *Nature*	2
Mukamel et al. (2011). *J. Neurosci*	3
Spiteri et al. (2007). *Amer J. of Human Genetics*	4
Vernes et al. (2008). *N Engl J Med*	5
Vernes et al. (2011). *PLoS Genet*	6

4. FOXP1 and FOXP4

In the developing brain, the expression patterns of FoxP1 and FoxP4 have both distinct and overlapping patterns of expression with FoxP2 (Fig. 2B-C) [42,44, 75–77]. In the cortex, FoxP2 is found primarily in the deepest layer of the cortex, layer 6, whereas FoxP1 is found in layers 2–5. In contrast, FoxP4 expression has been found throughout all layers of the cortex [42,44, 76–78]. Furthermore, FoxP1, is expressed in both the striatum and thalamus, analogous to FoxP2, although unlike FoxP2, FoxP1 expression is low in the human cerebellum (Fig. 2B) and absent in the rodent cerebellum [44]. In contrast, FoxP4 is present in the cerebellum and hypothesized to be important for dendritic arborization of Purkinje cells [79]. FoxP4 is also expressed in the striatum but its expression declines from a late embryonic to early postnatal stage [76,80]. In human brain, only the cerebellum and striatum have high expression of FOXP4 (Fig. 2C). In the early postnatal hippocampus, FoxP4 expression is found in the hilar region and from CA3 to CA1 [44,76,77]. In contrast, FoxP1 is expressed mainly in CA1 with no FoxP2 expression in the hippocampus throughout development [44,76,77]. Interestingly, while the expression of human FOXP2 is low in the hippocampus (Fig. 2A), rodents may have no expression [44,77]. These expression patterns are critical, as not only can FoxP1 and FoxP4 homodimerize similar to FoxP2, but they can also heterodimerize with FoxP2 and each other [32].

A direct relationship between *FOXP1* and ASD has been recently uncovered. Several studies have found mutations, deletions, or copy number variations of FOXP1 in individuals with ASD [81–84]. Individuals with variation in *FOXP1* are reported to have: 1) language acquisition delays with the onset of the first word being 3.5 years of age [83,84], 2) a working vocabulary of less than 100 words by seven years of age [83], as well as 3) a below average IQ [82,83]. Furthermore, MRI scans from the brains of these individuals' show abnormally enlarged ventricles and also muscle tissue deformities [82]. Moreover, FOXP1 can also repress expression of *CNTNAP2* suggesting coordinated transcriptional regulation of ASD genes by FOXP family members [84]. Together, these data suggest that coor-

dinated downstream signaling of FOXP1 and FOXP2 may be important for language and the signaling pathways involved in ASD.

5. Animal models of FOXP genes

Animal models have become an important component of studying the pathophysiology of neurodevelopmental diseases such as ASD [85]. Because of the complex genetic heterogeneity and environmental interactions that contribute to ASD, well-defined animal models that can display core symptoms of the disorder are crucial for research into causes and treatments. Rodents make excellent models for ASD due to their complex behavioral repertoire, which includes: vocal communication, sociability, and intelligence [86–88]. There are numerous mutant mice strains used in ASD research (for a review see [89–92]) including Foxp2 and some of its target genes. In addition, FoxP2 expression and function has also been examined in the songbird [42,93].

Due to the role of FOXP2 in language, a number of studies have investigated the role of Foxp2 in rodent vocalizations. The most widely used method for eliciting vocalizations in young rodents is through maternal separation in which infant rodent pups emit ultrasonic vocalizations (USVs) in response to separation from their mother [94–96]. Mice with mutations similar to that of the KE family point mutation [97,98] show motor defects, reduced USVs, reduced long term depression (LTD; a measure for a reduction in synaptic efficiency), abnormalities of the cerebellum, and impairments with auditory-motor association learning [34, 97,98]. Furthermore, it has been shown that Foxp2 is not required for the innate vocalizations in very young pups [99]. These authors posit that the defects in USVs observed later in development are likely due to generalized issues with development and motor function [99]. As the molecules and pathways underlying USVs are unknown, it is still uncertain what the structure, or disruption to the structure of USVs, might mean. There is little evidence to suggest that rodent vocalizations are learned, similar to song in birds or language in humans, although the properties of rodent calls are known to be influenced by variables such as genetic background, age, gender, motivation, and environmental factors [100–104]. Finally, gene expression studies have been carried out in both Foxp2 knockout mice [105] and Foxp2 humanized mice [106], and Foxp2 genome wide binding studies have also been conducted in mice [105]. A number of ASD genes have been identified as potential Foxp2 targets in these studies (Table 1). These data are an important first step in elucidating the Foxp2 target genes mediating the phenotypes of these genetically modified animals. Further studies will need to be undertaken to place these target genes within the context of specific behaviors such as USVs or LTD and within the larger framework of ASD biology.

FoxP2 is also expressed in the brains of songbirds and its expression changes during song acquisition in the zebra finch [42,107–109]. This plasticity in FoxP2 expression suggests that this gene actively participates in the signaling cascades involved in vocal learning and/or production. Likewise, reduction of FoxP2 in songbirds leads to a disruption of learned song specifically in the precision and quality of the song [110]. Additional work has demonstrated FoxP2 reduction also leads to a decrease in spine density in an area of the zebra finch brain that has a comparable function to the mammalian striatum [111]. This result mirrors what is seen in the striatum of mice heterozygous for *Foxp2* [98,106]. These studies also demonstrate that FoxP2 may be playing a role in the learning and/or the shaping of the overall complexity of vocalizations.

The results from both rodent and songbird research highlight the importance of FoxP2 in learned motor skills and place an emphasis on the role of FOXP2 in the more complex aspects of speech and language rather than simply a motor function. These data also support the use of FoxP2 animal model systems for the study of brain development and targeted therapeutics in ASD. In addition, there is also evidence suggesting that Foxp1 may also be involved in CNS organization of motor behavior. A recent study has found that mice with a conditional knockout of *Foxp1* in motor neurons have profound impairments in limb coordination during motor behaviors [112]. The functions and targets of Foxp1 or Foxp4 in the developing mammalian brain have yet to be determined.

6. Future directions

Examination of FOXP2 in multiple systems is uncovering the downstream functions of this gene that may be important for ASD including target genes, cellular phenotypes, and modulation of animal behavior. These studies are critical for placing the FOXP2 target genes into context within the framework of the developing brain. Moreover, it will be important to more

fully understand the role of both FOXP1 and FOXP4, as well as their target genes, in the nervous system. Developing a complete appreciation for the function of FOXP-regulated signaling pathways in ASD will require integration of genetic, developmental, molecular, and behavioral research. This will provide a more complete picture of FOXP function in ASD and assist in elucidating potential targets for therapeutic interventions.

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