

## FOXP2's Involvement in Speech Defect

### Introduction

Developmental speech and language defects occur in approximately 7% of children who are otherwise normal (no defects in hearing, intelligence, etc.) (Vargha-Khadem *et al*, 1998). Comparing this disorder to two common inherited diseases—Huntington's disease (one in 10,000 Americans) and to Cystic Fibrosis (one in every 3,900 live births in America)—speech disorders occur frequently in the human population (Huntington's Disease; Facts About CF). Few speech disorders, however, are inherited in a simple Mendelian way; most have polygenic inheritance (Hurst *et al*, 1990). Among these genetic speech defects, the KE family was discovered, consisting of three-generations where half of the members are affected by a developmental verbal dyspraxia. The most prominent symptom are verbal dyspraxia where there are severe impairments in the selection and sequencing of fine orofacial movements and orofacial dyspraxias that causes a relative immobility in the lower face, impairing speech movements (Lai *et al*, 2001; Vargha-Khadem *et al*, 1998).

The discovery of the KE family has allowed researchers to isolate the gene, FOXP2. This gene was the first to be found to be involved in speech and language. A recent study by Takahashi *et al* (2003) found that FOXP2 is highly enriched in the striatum of the basal ganglia in the brain. The striatum is involved in the process of procedural memory; therefore, orofacial dyspraxias in patients with FOXP2 mutations may be related to a dysfunction in the striosomal system in the striatum. Further investigation of FOXP2 may provide an understanding for the building process of language processing.

## Results

### *Mode of Inheritance*

This speech and language disorder seems to be transmitted as a simple Mendelian autosomal dominant trait. All of the affected family members in the KE family have the same type of speech and language defect, though they vary in the degrees of severity (Hurst *et al*, 1990). Through analysis of the pedigree, approximately half of this three-generation family is affected with the speech defect, both male and female alike (fig 1). In addition, male II-6 only passed on the trait to one of his three daughters (Fisher *et al*, 1998). When a peak multipoint mapping of the region was performed, the result was that the gene locus co-segregated with the speech and language disorder. The locus is flanked by *D7S2459* and *D7S643* (fig 2), which was found to have a LOD score of 6.62 at recombination frequency ( $\theta$ ) 0 (Fisher *et al*, 1998; Lai *et al*, 2000). Both the inheritance patterns and LOD score hinder the possibility of X-linkage and supports a disorder with full penetrance at a single locus, transmitted as an autosomal dominant trait.

### *Map Location*

The gene involved in speech and language, *FOXP2*, was later found to be located within the locus designated as *SPCHI* through an analysis of the affected members in the KE family and individual CS.

In 1998, Fisher *et al* made a genome-wide search for linkage of the region that co-segregates with the speech and language disorder, using fluorescence-based genotyping of microsatellite markers that were spaced evenly throughout the genome. They found strong evidence of linkage for markers on the long arm of chromosome 7. By analyzing the marker haplotypes, the locus of the gene (*SPCHI*) was localized to the 27.4-cM region between the

markers *D7S527* and *D7S530* (fig 2). Performing a multipoint map indicated that the 3 LOD unit confidence interval for *SPCHI* spans between *D7S2459* and *D7S643*, a 5.6-cM region in chromosomal band 7q31, where the peak LOD score was 6.62 (fig 3).

By using data from the BAC/PAC sequence contigs Lai *et al* (2000) developed four novel polymorphic markers from the intervals *D7S2459*—*D7S692* and *CFTR*—*D7S643* to refine the proximal and distal recombination breakpoints of *SPCHI*. By analyzing the recombination breakpoint in individual III-12, the proximal end of *SPCHI* was refined to 013A (fig 2). In addition, the distal end of the gene locus was refined to 330B (fig 2) by determining that the recombination breakpoint in individual III-3 mapped between 363B-084A and 330B.

Besides investigating the KE family, Lai *et al* (2000) also analyzed individual CS, who has a *de novo* balanced reciprocal translocation t(5;7)(q22;q31.2). The breakpoint mapped in the *D7S2459*—*D7S643* interval. Using fluorescence *in-situ* hybridization and BAC clones, the breakpoint was mapped to the single clone *NH0563O05*, which is now designated as *FOXP2* (fig 4) (Lai *et al*, 2001).

### *Identification of the Gene*

In 2001, Lai *et al* screened the KE family for mutations in the *FOXP2* gene. They found a G-to-A nucleotide substitution in exon 14 of the affected family members, which was also found to co-segregate perfectly with the speech and language defect (fig 5). Using a restriction enzyme based assay with *MaeII* (the nucleotide transition destroys a *MaeII* restriction site), 364 independent chromosomes from a control group of normal Caucasians were screened and found absent in the mutation. Thus, this test reassures that the mutation is not a naturally occurring polymorphism. The mutation is predicted to occur in the most highly conserved part of the

forkhead DNA-binding domain of FOXP2, which is adjacent to a histidine residue that makes direct contact with the target DNA. Many studies have shown that an inactivation or loss of the forkhead domain caused by mutation in the FOX genes can lead to human disease (Lai *et al*, 2001). Studies on FOXC1 duplications causing anterior-chamber defects of the eye provided evidence that the correct dosage of the forkhead transcription factors gene is crucial in embryogenesis (Lai *et al*, 2001).

#### *Type of Protein Encoded*

By analyzing the carboxy-terminal portion of the predicted protein sequence, a segment of 84 amino acids were found to be similar to the characteristic DNA-binding domain of the forkhead/winged helix (FOX) family of transcription factors (Lai *et al* 2001). In addition, a nuclear localization signal was found, which suggests that it can be a transcriptional regulator (Kaufmann and Knöchel, 1996). Thus, FOXP2 is required to initiate or regulate transcription since it participates in forming the transcription-initiation complex. Consequently, it is a key player in directing cell specialization and pattern formation during embryo development.

#### **Discussion**

The carboxy-terminal portion of FOXP2's protein sequence allowed it to be classified as being part of the forkhead/winged family of transcription factors (Lai *et al*, 2001). This forkhead family and similar transcription factors are present within many different species, ranging from yeast to humans (Kaufmann and Knöchel, 1996). As a transcription factor, it is needed to initiate or regulate the transcription of DNA by its contribution in the formation of the transcription-initiation complex.

The forkhead domain of these transcription factors is evolutionarily conserved and has the characteristic structure consisting of three amphipathic alpha-helices followed by two large loops called “wings” (Lai *et al*, 2001; Kaufmann and Knöchel, 1996). The third alpha-helix is presented to the major groove of the target DNA, and it is the most highly conserved region of the forkhead domain (Lai *et al*, 2001). This DNA-binding domain interacts with a specific DNA sequence, while another domain in the protein interacts with the nearby promoter, either stimulating or inhibiting DNA transcription (Lodish *et al*, 2000). Since it also contains a nuclear localization sequence and is present in the nucleus, FOXP2 is as a result, a key player in directing cell specialization and pattern formation during development; a key regulator in embryogenesis.

The mutation in FOXP2 that occurs in the KE family occurs in the third alpha-helix found adjacent to a histidine residue that makes direct contact with the target. This G-to-A nucleotide transition (fig 5) in exon 14 is predicted to cause an arginine-to-histidine substitution in the forkhead DNA-binding domain of the gene, which abolishes the DNA-binding of FOXP2, and subsequently disrupts transcription activation properties (Lai *et al*, 2001; Kaufmann and Knöchel, 1996). Because of the loss or inactivation of the forkhead domain, haplo-insufficiency occurs during embryological development. This haplo-insufficiency in the brain during the key stages of embryogenesis leads to the abnormal development of neural structures important for speech and language (Lai *et al*, 2001).

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