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Human Genetics: The Evolving Story of *FOXP2*

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***FOXP2* mutations cause a speech and language disorder, raising interest in potential roles of this gene in human evolution. A new study re-evaluates genomic variation at the human *FOXP2* locus but finds no evidence of recent adaptive evolution.**

One longstanding challenge in biology is to understand how changes in the human genome contributed to the evolution of our species. With advances in molecular methods, this question can be investigated by comparing DNA sequences from *Homo sapiens* to those of other great apes, and even to archaic hominins, such as Neandertals, as well as by searching for signs of Darwinian selection in the genomic variation of living human populations [1]. In 2001, a study reported the first case of a gene mutated in a developmental speech and language disorder [2]. The culprit, a gene called *FOXP2*, attracted the attention of researchers across multiple disciplines [3]. Given its link to acquisition of spoken language skills, one of the most distinctive capabilities of *Homo sapiens*, this gene was seen as an obvious candidate for evolutionary study. This led to two independent reports in 2002, which established that, despite high sequence conservation in primates, the human FOXP2 protein differed from its chimpanzee counterpart at two amino-acid sites [4,5]. Moreover, when the studies examined nucleotide variation in the relevant part of the genomic locus in living populations, patterns were compatible with recent positive selection having acted on *FOXP2* [4,5]. *FOXP2* became a poster-child for genes that may have played a role in the emergence of modern humans. Now, more than 15 years later, Atkinson and colleagues [6] perform a thorough investigation of modern human *FOXP2* variation, taking advantage of genome sequences available from diverse populations across the world; these new analyses provide no support for recent selection, overturning prior conclusions.

FOXP2 was originally discovered through intensive studies of a large family in which fifteen relatives, across three generations, had problems sequencing the rapid co-ordinated movements that facilitate fluent speech, accompanied by impaired language production and comprehension [2]. All affected members carried a heterozygous point mutation in *FOXP2*, disturbing the function of the

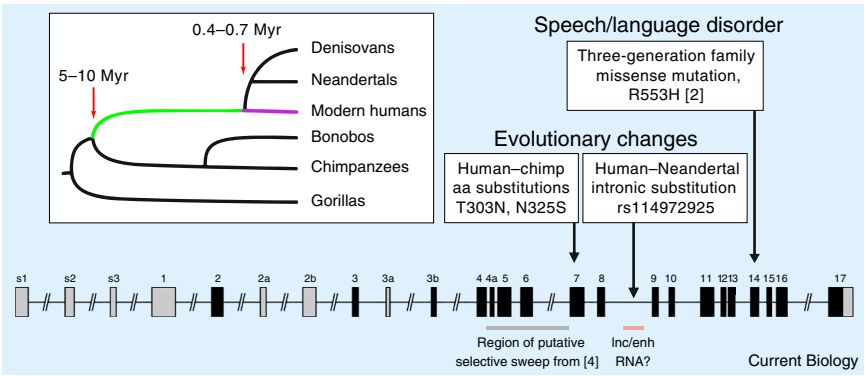

Figure 1. Changing views of *FOXP2* and human evolution.
The human *FOXP2* locus spans more than 600 kilobases of genomic DNA. Boxes represent exons (present in processed mRNA), lines represent introns (removed from mRNA by splicing), and black shading indicates exons that code for protein. In 2001 an exon 14 missense mutation (p.R553H) was found in members of a three-generation family affected by a rare speech disorder [2]. Evolutionary changes at the *FOXP2* locus that have been hypothesized as relevant for human origins are highlighted, including: two amino-acid (aa) substitutions in exon 7; the putative selective sweep originally proposed by [4]; an intronic derived polymorphism between exons 8 and 9; and a conserved element in that intron which may represent a long noncoding (lnc) or enhancer (enh) RNA. Note that the evolutionary changes [4–6,17] and known disorder-causing mutations [2,7] are entirely distinct from each other. Top left shows an evolutionary tree of hominins and closest primate relatives (adapted from [1]), with the lineage leading to archaic hominins in green, and the lineage leading exclusively to modern humans in purple.

encoded protein, a transcription factor that regulates other genes [2,3]. Following this first report, dozens of cases of distinct independent mutations have been identified [7], confirming that inactivation of one *FOXP2* copy can profoundly derail speech development while general cognitive skills are less affected. Over the past decade and a half, researchers have gained some tantalizing clues about FOXP2 function. For example, neuroimaging of affected individuals has revealed subtle alterations in structure or function of distributed brain circuits, especially those involving the cortex, basal ganglia and cerebellum, overlapping with regions of high FOXP2 expression [3,8].

Curiously, *FOXP2* is ancient history; the gene is found in similar form in rodents, birds, reptiles and fish among others [3]. Mice with the same mutations that cause speech disorder display altered sequencing of ultrasonic vocalisations [9]. Electrophysiological studies of the brains of these mice have revealed disrupted plasticity of cortico-basal ganglia circuits [10,11]. Knockdown of the avian *FOXP2* orthologue in a key basal ganglia nucleus in brains of zebra finches affects the variability of the songs that they learn [3,12]. Although expressed in other tissues, such as lung, heart and even bone [13], human and animal studies suggest that *FOXP2* supports development and function of a subset of neuronal circuits, including those relevant to motor skills and vocal behaviours; and that such circuits may be especially sensitive to gene dosage [3, 8–12].

Against this backdrop of conservation across vertebrates, how has *FOXP2* changed in human evolution (Figure 1)? Initial studies identified amino-acid substitutions in exon 7 that distinguish human FOXP2 from the chimpanzee protein and suggested that they were associated with a selective sweep within the last 100–200 thousand years [4,5]. However, when analysis of archaic hominin DNA became feasible, researchers found that the protein-coding sequence of *FOXP2* in Neandertals matched that of *Homo sapiens* [14]. This result suggested that, rather than being subject to recent selection, the two amino-acid substitutions were more likely already fixed in the common ancestor of Neandertals and modern humans, which lived at least 400 thousand years ago. Follow-up studies attempted to reconcile dating inconsistencies [15], for example by positing multiple successive evolutionary events at the locus, occurring before and after the Neandertal–human split [16]. One report suggested that the target of the putative recent sweep may have been within an intronic regulatory element [17].

While the original screen for selection investigated only part of *FOXP2* in 20 humans from different continents [4] (Figure 1), for their new study, Atkinson and colleagues [6] re-assessed the entire locus, using high-coverage genomes from next-generation sequencing of global human populations, including the Human Genome Diversity Panel (HGDP) and the 1000 Genomes phase 1 dataset. As in prior studies [4,5], selection was evaluated with Tajima's D, a statistic that uses patterns of diversity in DNA sequences to detect deviations from neutral (random) processes of evolution. The authors compared D estimates for *FOXP2* to a null distribution of expected values from the remainder of the genome (i.e. sequences with predominantly neutral population histories). When using a pooled HGDP dataset in which three-quarters of individuals were non-African, roughly matching the composition of the sample studied in [4], *FOXP2* gave significantly negative D values. These values lay in the bottom 5% of the distribution, traditionally viewed as evidence of positive selection [6]. However, when the HGDP dataset was split into African and Eurasian individuals, D values for *FOXP2* were within the normal range expected for the ancestry of each population, based on genomic background data [6]. The authors found that D values are not only skewed by changing population composition, but are also sensitive to genomic 'window' size, i.e. how much flanking DNA sequence is used [6]. Alternative methods consistently failed to detect a recent selective sweep [6].

What of the amino acids in exon 7 that distinguish the human and chimpanzee FOXP2 proteins? We already knew from archaic genome sequencing [14] that the substitutions are considerably older than the <200k year estimates of the first evolutionary studies [4,5]. Were they subject to more ancient selection, in common ancestors of Neandertals and modern humans? Atkinson and colleagues [6] assessed variation of *FOXP2* coding sequences in HGDP and in 10 chimpanzees, comparing rates of

within- and between-species variation for synonymous and non-synonymous substitutions [6]. The results were inconclusive. Interestingly, when inserted into transgenic mouse models, the exon-7 amino-acid substitutions have been shown to impact on plasticity of a subset of brain circuits in ways that differ from loss-of-function alleles, offering additional evidence of a potential evolutionary significance of such changes [18].

In searching for alternative targets of recent selection at *FOXP2*, previous work [17] had highlighted an intronic substitution that distinguishes most present-day humans from Neandertals and that alters a transcription-factor binding site in a putative regulatory element (Figure 1). As well as refuting a recent selective sweep in this part of the locus, Atkinson and colleagues [6] argue that the existing allelic variation observed in southern Africa is incompatible with this derived site playing a role in human origins [6]. They report that the site is part of a conserved intronic region that is transcribed in brain tissues (although not as part of the FOXP2 transcript) and that may represent an independent long noncoding RNA or enhancer RNA. Based on an elevated rate of derived polymorphisms in present-day humans, the authors suggest that this element experienced loss-of-function on our lineage. Importantly, it remains unclear whether the element actually helps regulate *FOXP2* activity in any species. Neural expression patterns of *FOXP2* are complex, dynamic and likely to be modulated by multiple elements [3]. Degradation of one conserved intronic region should not be misinterpreted as a loss-of-function of the gene locus in which it is embedded.

One final complication of the evolutionary story of *FOXP2* goes unaddressed in the study of Atkinson and colleagues [6]. Ancient DNA analyses have revealed that our ancestors encountered other archaic hominins outside of Africa and interbred with them, resulting in stretches of Neandertal and Denisovan DNA introgressed in the genomes of some extant human populations [19]. *FOXP2* is situated within a region that is significantly depleted of such archaic introgression, representing one of the largest deserts of admixture in the human genome [19]. However, the biological meaning of this finding is not yet understood.

Atkinson and colleagues [6] demonstrate convincingly that evidence of selection must be assessed carefully in humans, as conclusions can be inadvertently affected by cohort composition or genomic window size. But the lack of recent selection at *FOXP2* does not nullify its relevance for key aspects of our biology. Even without recent selection in humans, the unusual effects of *FOXP2* dysfunction mean that it is still a promising window into the neurobiology of speech and language [3]. And while the capacity to acquire spoken language may be unique to our species, the underlying genomic architecture is undeniably complex, involving interacting networks of a myriad different genetic factors [20]. We should not expect our origins to be explained in terms of just a single gene. *FOXP2* was only ever going to be one piece of an extremely elaborate puzzle.

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