Comment on "Human-Specific Gain of Function in a Developmental Enhancer"

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Comment on "Human-Specific Gain of Function in a Developmental Enhancer"

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Prabhakar *et al.* (Reports, 5 September 2008, p. 1346) argued that the conserved noncoding sequence *HACNS1* has undergone positive selection and contributed to human adaptation. However, the pattern of substitution in *HACNS1* is more consistent with the neutral process of biased gene conversion (BGC). The reported human-specific gain of function is likely due to the accumulation of deleterious mutations driven by BGC, not positive selection.

The characterization of functional noncoding regulatory elements positively selected during human evolution is of major importance for understanding the genetic basis of human-specific adaptations. One possible approach to identify such elements entails searching for genomic regions that are highly conserved across nonhuman vertebrates but strongly divergent, that is, rapidly evolving, in humans (*1*, *2*). These regions, called HACNSs (human-accelerated conserved noncoding sequences) or HARs (human-accelerated regions), are good candidates for being regulatory elements under positive selection.

Prabhakar *et al.* (*3*) reported the detailed analysis of the first of these candidates, the 546–base pair (bp) long *HACNS1*. *HACNS1* has accumulated 16 human-specific changes since the human/chimpanzee divergence, which represents a substitution rate four times as high as would be expected given the average neutral substitution rate in the human genome. Thirteen of the 16 changes are clustered in a small region (81 bp) of *HACNS1*. Using a mouse model, the authors demonstrated that human *HACNS1* could drive the expression of a reporter gene in the mesenchyme of the early-developing forelimb and later-developing hindlimb in embryos. This pattern of expression was very different from the one observed when the chimpanzee or macaque *HACNS1* sequences were assayed. Prabhakar *et al.* further showed through directed mutagenesis that the 13 changes in the 81-bp region are responsible for the difference in enhancer activity between human *HACNS1* and its orthologs in apes. Accelerated sequence evolution is a hallmark of positive selection. The authors therefore concluded that these 13 substitutions have been driven by positive selection. They suggest that these changes may have contributed to the evolution of human-specific digit and limb patterning (*3*).

Positive selection, however, is not the only possible explanation for accelerated sequence evolution. Biased gene conversion (BGC) is a neutral process associated with meiotic recombination, which favors the fixation of AT → GC mutations (*4*). Given that recombination often occurs in hotspots (< 2 kb), BGC can create strong substitution hotspots, thereby leading to spurious signatures of positive selection (*5*–*7*). BGC was identified through its effect on neutral sites (*6*–*9*), but it can also drive the fixation of weakly deleterious mutations in functional elements (*5*, *10*). Noteworthy features of BGC are that its prevalence is particularly high in subtelomeric regions (*7*) and that it is much more strongly associated with the rate of crossovers in male than in female germlines (*6*, *7*, *9*). We have previously shown that among the HARs that were proposed as candidates for positive selection, many show the hallmarks of BGC: There is an excess of HARs in regions of high recombination rate and the pattern of substitution in HARs is strongly biased toward GC (*5*). This is precisely the pattern observed in *HACNS1*. First, this element is located in a subtelomeric region of chromosome 2, where the rate of male crossover is particularly elevated [2.77 cM/Mb, compared with 0.98 cM/Mb on average for autosomes; regions with a male crossover rate higher than 2.77 cM/Mb represent only 7% of the genome (*11*)]. Second, among the 16 substitutions in *HACNS1* there are 14 AT → GC substitutions, 2 GC → CG substitutions, but not a single GC → AT substitution. Functional elements (coding or noncoding) in mammalian genome are not particularly GC-rich, so there is a priori no reason why selection should systematically favor AT → GC over GC → AT mutations. Conversely, this pattern of substitution is exactly the one expected under the BGC model.

Prabhakar *et al.* (*3*) reject neutral hypotheses for three reasons: (i) the substitution rate in *HACNS1* is four times the local neutral rate; (ii) the authors claim that under the neutral BGC hypothesis "one would expect an increase in the overall substitution rate across the entire region of increased AT → GC substitution" but that the human-specific substitution rate is only elevated in a narrow 81-bp region of *HACNS1* and is close to the local average outside this region; and (iii) their experiments demonstrate that these human-specific substitutions have a substantial functional impact.

The first point is not an argument against the BGC model: As with selection, strong BGC episodes can result in substitution hotspots (*5*–*7*, *12*). The second point is a misinterpretation of the cited article by Galtier and Duret (*5*). In this paper, we indicate that a BGC-driven substitution hotspot must be GC-biased. However, the reverse proposal is not true: Weak BGC can lead to an excess of AT → GC substitutions without strongly affecting the substitution rate, as shown theoretically and empirically (*7*). Recombination hotspots vary in strength, are evolutionarily unstable, and tend to move rapidly (*13*). The pattern presented by Prabhakar *et al.* [figure 4 in (*3*)] suggests that the evolution of the 81-bp segment has been driven by a strong episode of BGC due to an intense and/or long-lived recombination hotspot, in a region otherwise affected by weaker recombination hotspots. This is in agreement with current knowledge about the spatiotemporal distribution of recombination in humans (*14*).

Finally, the third point is the most important argument raised by the authors: The strong functional changes associated with the human-specific substitutions in HACNS1 imply that its rapid evolution was driven by adaptation. This logical implication, however, does not necessarily hold; not only advantageous substitutions have a phenotypic effect. We know that BGC can overcome natural selection and drive the fixation of weakly deleterious AT → GC mutation (*5*, *10*). The fact that *HACNS1* is under very strong purifying selective pressure in nonhuman vertebrates indicates that, in most species, mutations in this element have some deleterious effect. Hence, the most likely interpretation of the observed substitution pattern is that the human-specific changes correspond to weakly deleterious mutations, driven to fixation by BGC. In other words, the strong functional shift in human *HACNS1* enhancer activity probably results from the accumulation of numerous weakly deleterious substitutions.

In conclusion, we contend that the substitution pattern in *HACNS1* does not support the hypothesis of positive selection. Although we cannot formally exclude that *HACNS1* somehow contributed to human adaptation, the most parsimonious interpretation is that the evolution of *HACNS1* merely reflects the maladaptive consequences of recombination hotspots—the Achilles' heel of our genome.

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