

A Mutation in Hairless Dogs Implicates FOXI3 in Ectodermal Development

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Mexican hairless dogs (xoloitzcuintles) were considered sacred by the Aztecs, and statues of the dogs date back 3700 years. Hairless dogs were described as *Canis aegyptius* by Linné (1). The phenotype of hairless dogs is now classified as canine ectodermal dysplasia (CED) because these dogs have missing or abnormally shaped teeth in addition to a hair coat that is sparse or absent (Fig. 1). CED is inherited as a monogenic autosomal semidominant trait (2) linked to chromosome 17 (CFA 17) (3) in Mexican and Peruvian hairless dogs and in Chinese crested dogs. Heterozygous dogs are hairless, and homozygous mutants die during embryogenesis.

The spontaneous CED mutation offers an opportunity to gain further insights into the development of ectodermal appendages such as hair and teeth. Mutations in the *EDA*, *EDAR*, and *EDARADD* genes of the ectodysplasin signaling pathway produce a closely related phenotype called anhidrotic ectodermal dysplasia. Anhidrotic ectodermal dysplasia is characterized by the absence of hair, dentition abnormalities, and alterations in certain exocrine glands, the latter feature seen not at all or less severe in CED (4).

We used genomewide association mapping (5) to identify the causative genetic mutation (6). One single-nucleotide polymorphism (SNP) on CFA 17 showed strong association with the

hairless phenotype. Haplotype association testing revealed an 8-SNP, 160-kb haplotype with perfect phenotypic concordance. We fine-mapped the associated haplotype in a larger set of Chinese crested dogs along with Mexican and Peruvian hairless dogs. The mutant chromosomes of all hairless dogs from the three breeds shared identical marker alleles in a 102-kb interval between positions 41,045,331 and 41,147,100 on CFA 17 (6).

In this region of the dog genome (CanFam2.0), only two genes were predicted, including a dog-specific processed pseudogene with a truncated open reading frame. The other predicted gene, LOC483075, showed homology to the forkhead box (FOX) transcription factor gene family. In mammalian genomes, this family consists of about 50 members encoding several important regulators of development.

The dog genome assembly contains a gap in the region of the first exon of LOC483075, and we determined the complete sequence of this region (6). The resultant previously uncharacterized gene showed highest similarity to the mouse *Foxi3* gene (LOC232077), and we propose the symbol *FOXI3* for the dog gene (7). A human *FOXI3* gene with conserved open reading frame can be found in Celera's human genome assembly, whereas a putative sequencing error is present in the extremely GC-rich parts of the human reference assembly

(build 36.3). To examine whether *FOXI3* might be a reasonable functional candidate gene for CED, we analyzed its expression in mouse embryos. Whole-mount *in situ* hybridization experiments revealed highly specific expression of *Foxi3* transcripts in the epithelium of the hair and whisker placodes and developing teeth, consistent with a functional role for the gene in the development of these organs (6).

Sequence analysis of the *FOXI3* gene in hairless and coated Chinese crested dogs revealed a 7-bp duplication within exon 1 (Fig. 1) that produces a frameshift and a premature stop codon. We found perfect concordance of the duplication with the CED phenotype in 140 hairless dogs from the three studied breeds. The duplication was absent from 55 coated animals from the studied breeds and 32 coated dogs from 19 other breeds (6). Although it is unclear whether the mutant protein is actually expressed, with more than 95% of the normal *FOXI3* protein missing, it is unlikely that the mutant protein is functional. Any mutant protein produced will contain 218 altered amino acids and could potentially interfere with normal cellular function in a dominant-negative mechanism. However, we think the most likely explanation for the CED phenotype in hairless dogs is haploinsufficiency of *FOXI3* caused by the 7-bp duplication. According to this hypothesis, a complete lack of *FOXI3* in homozygous mutants causes the embryonic lethality.

We speculate that *FOXI3* is a downstream target of the ectodysplasin signaling pathway. This study illustrates how the extreme morphological diversity of dogs can be harnessed to gain new insights into developmental biology.

References and Notes

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Supporting Online Material

www.sciencemag.org/cgi/content/full/321/5895/1462/DC1

Materials and Methods

SOM Text

Figs. S1 to S5

References and Notes

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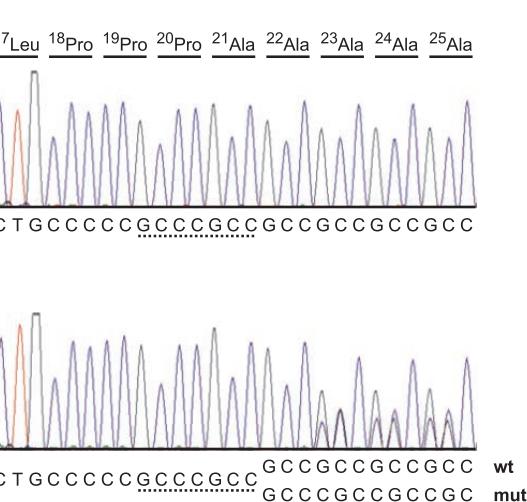


Fig. 1. Phenotype and *FOXI3* sequences of a (A) coated (wild-type, wt) or (B) hairless Chinese crested (mut) dog. Hairless dogs have a 7-bp duplication (underlined) in exon 1 of *FOXI3* (c.57_63dup7).