# Modulation of *Fgf3* dosage in mouse and men mirrors evolution of mammalian dentition

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**A central challenge in evolutionary biology is understanding how genetic mutations underlie morphological changes. Because highly calcified enamel enables preservation of detailed dental features, studying tooth morphology enables this question to be addressed in both extinct and extant species. Previous studies have found that mutant mice can have severe abnormalities in tooth morphology, and several authors have explored the evolutionary implications of tooth number modifications in mutants. However, although they can potentially shed much light on evolutionary mechanisms, anomalies in tooth shape remain poorly studied. Here, we report that alterations in dosage of the *Fgf3* gene cause morphological changes in both genetically engineered mutant mice and in human patients. By comparing the dental morphologies in mice and humans carrying *Fgf3* mutations with primitive rodent and primate fossils, we determined that decreases in dosage of *Fgf3* lead to phenotypes that resemble the progressive reappearance of ancestral morphologies. We propose that modifications in the FGF signaling pathway have played an important role in evolution of mammalian dentition by giving rise to new cusps and interconnecting cusps by new crests. We anticipate that our multidisciplinary study will advance the detailed correlation of subtle dental modifications with genetic mutations in a variety of mammalian lineages.**

dental morphology | Muroidea | primates

The origin, diversification, and evolution of mammals are largely understood based on data from the dental fossil record. Dentitions of both fossil and extant mammals include a variety of key characters that reveal adaptive diversification through ≈200 million years of evolutionary history (1, 2). These dental characters include such features as the morphological arrangement of cusps (pointed elements) present on occlusal surfaces of the molar teeth. Studies of morphological changes in cusp number, position, and interconnections have informed taxonomy, phylogeny, and diet reconstruction.

For this reason, dental research provides a unique interface between evolutionary and developmental biology (evo-devo approach). By studying genetic regulation of cusp morphogenesis in extant model organisms, we can understand the mechanisms that may underlie morphological changes during evolution. Previous studies have found that mutant mice can have severe abnormalities in tooth morphology (3–6), and some authors have explored the evolutionary implications of tooth number modifications in mutants (7–9). However, to our knowledge, mutations in extant mouse or human samples that mimic morphologies of close ancestors have not been reported.

The mouse (*Mus musculus*) is the most widely used model for studies of mammalian development. Therefore, the superfamily that the mouse belongs to, the Muroidea (rats, mice, hamsters, and gerbils), makes up a crucial taxonomic group for evo-devo investigations. Over the last 45 million years (My), muroid rodents underwent a rapid adaptative radiation during which the dentition acquired huge morphological diversity. The first upper molar ($M^1$) of primitive (cricetine) muroid rodents has a crown made of six cusps linked by a longitudinal crest (10). The $M^1$ of mouse and other murine rodents displays at least eight cusps linked by transverse crests. This murine dental plan is presumed to have evolved from the cricetine through intermediary plans (10). However, the changes in genetic sequences that were responsible for the morphological modifications that occurred during the transitions between the plans have not been identified. Understanding these changes will build an important bridge between the fossil record and developmental genetics.

Because of its central role in tooth development (11–13), the Fibroblast Growth Factor (FGF) gene family is an attractive candidate for involvement in dental evolution. The FGF family is composed of at least 22 ligand-encoding genes, and its members are involved in development of many organs in addition to teeth (14). Recently, mutations in *Fgf3* have been demonstrated to be implicated in human and mouse microdontia (15, 16). We therefore set out to evaluate the role of *Fgf3* in the morphogenesis of human and mouse dentition, and to compare the effects of changes in *Fgf3* gene expression with modifications that occurred during mammalian evolution.

## Results and Discussion

***Fgf3* Controls Dental Morphogenesis in Mice.** During molar tooth development, *Fgf3* is expressed in the primary enamel knot and in the dental mesenchyme at the cap stage (11, 17). Detailed examination of the dentition in *Fgf3* mutant mice revealed morphological anomalies of molar teeth (Fig. 1). However, the normal feeding habits of homozygous null mutants ($Fgf3^{-/-}$ mice) indicate that the molars are functional. Unlike most mouse mutants that display significant morphological variability (4–6), *Fgf3* mutants had highly similar phenotypes in all studied specimens. In heterozygote ($Fgf3^{+/-}$) mice, the $M^1$ mesio-lingual

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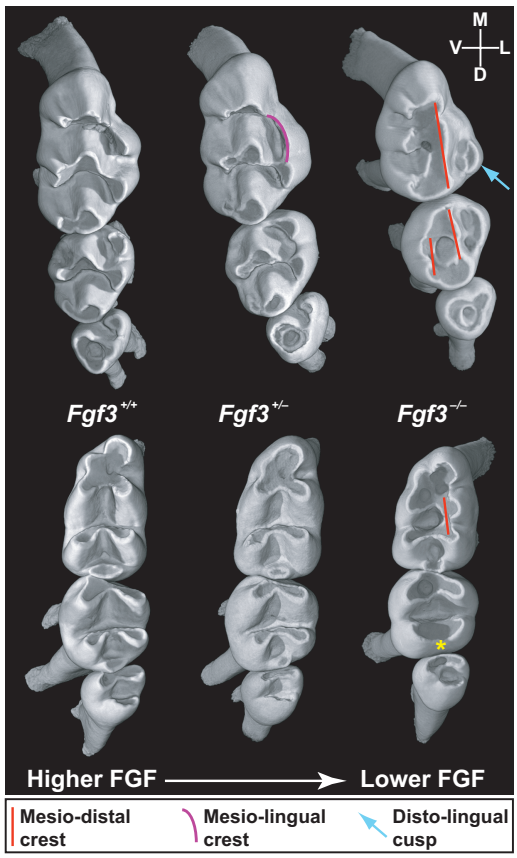


**Fig. 1.** Dental morphology of *Fgf3* mutant mice. Upper tooth rows on top and lower tooth rows on bottom. Purple arc indicates the mesio-lingual crest present in first upper molar of *Fgf3* heterozygous mutants. Red lines indicate the mesio-distal crests occurring in upper and lower teeth of *Fgf3* homozygous mutants. Yellow asterisk indicates loss of the hypoconid in second lower molar. Blue arrow indicates the single lingual cusp in first upper molar of *Fgf3* homozygous mutants. M, mesial; D, distal; V, vestibular; L, lingual.

cusp was hooked and connected to the enterostyle (purple arc, Fig. 1). This morphological difference between $Fgf3^{+/-}$ and wild-type (WT) mice was associated with a size difference, such that the $M^1$ of $Fgf3^{+/-}$ mice was smaller than in the WT (Fig. 2). The other molars of $Fgf3^{+/-}$ mice displayed almost the same morphology and size as in the WT (Figs. 1 and 2).

$Fgf3^{-/-}$ mice displayed smaller molars that had several morphological anomalies compared to WT and $Fgf3^{+/-}$ molars (Figs. 1, 2). The $M^1$ exhibited an abnormal mesio-distal connection (Fig. 1, red lines) and a single lingual cusp (Fig. 1, blue arrow). The second upper molar ($M^2$) also displayed mesio-distal crests and a single lingual cusp that stretched mesio-distally (Fig. 1, red lines). The third upper molar ($M^3$) was reduced, with an anterostyle connected to the cusp circle. In the mandible, the most important morphological modifications were the occurrence of a mesio-distal crest on the first lower molar ($M_1$) and the loss of the distal cusp on the second lower molar ($M_2$). These morphological modifications led to abnormal occlusion between upper and lower molars, which caused the wear of the enamel previously reported for lower molars (15) and also observed here for the $M^1$. These results show that *Fgf3* expression plays an important role in mouse dental development by controlling the size of teeth and regulating the number, position, and interrelation of cusps in molar teeth.

**Decreases in Dosage of *Fgf3* Mimic Changes during Evolution of Molar Teeth in Muroid Rodents.** The evolution of muroid molars has been extensively studied based on the abundant fossil record. These studies mainly focused on the $M^1$, which is widely considered as the most informative tooth for phylogenetic relationships. Over the last 45 My, muroid rodents showed a rapid adaptive radiation and their dentition underwent considerable morphological diversification. The $M^1$ primitive morphology encompasses six cusps linked by a longitudinal crest (Fig. 3, *Democricetodon*), whereas the derived $M^1$ of the mouse and most murine rodents has eight cusps without a longitudinal crest. The murine dental plan is thought to derive from the cricetine plan via several major morphological changes (10): (*i*) appearance of a new cusp on the centro-lingual side of the tooth; (*ii*) disappearance of the cricetine longitudinal crest; and (*iii*) development of a new mesio-lingual crest. An example of a specimen exhibiting these changes is the fossil genus *Potwarmus* (Fig. 3), considered as a stem Murinae (18). These modifications occurred in species displaying an intermediary dental plan between the cricetine and the murine plans (10). The final modification that led to the derived murine dental plan occurred when the mesio-lingual crest was replaced by a mesio-lingual cusp, as in *Mus* (Fig. 3). This sequence of modifications is mapped onto the phylogenetic tree of studied species in Fig. S1.

In the $Fgf3^{+/-}$ $M^1$, the mesio-lingual cusp was replaced by a mesio-lingual crest (Fig. 3), which is akin to the modification that occurred from *Potwarmus* to *Mus*. A further decrease in FGF dosage in the homozygous $Fgf3^{-/-}$ mice led to the loss of the mesio-lingual cusp and the occurrence of a mesio-distal crest (Fig. 3). The modifications that result as FGF levels decrease were the reverse of the morphological modifications that occurred during evolution of the $M^1$, as seen from comparison of *Democricetodon* to *Potwarmus* (Fig. 3). Thus, progressive decreases in *Fgf3* dosage in heterozygous and homozygous mutants mimic the changes seen during mammalian evolution, in which a crest arises before arrival of a new cusp. This series of events has been reported often in the fossil record (10, 19, 20). Moreover, as loss of function of a single gene (*Fgf3*) causes changes in the same characters that were altered during evolution, our results provide further evidence that the characters associated with the emergence of the murine dental plan may be nonindependent, as previously reported for other dental features (3).

**Humans Carrying *FGF3* Mutations Have Similar Dental Morphology to Primitive Primates.** It has recently been reported that humans carrying two null *FGF3* genes have microdontia (16), but tooth shape in these patients was not previously examined. Our detailed examination of tooth casts from *FGF3* null patients revealed a number of major morphological anomalies in the molar teeth. Most important among these was that the first and second upper molars were characterized by a reduction in size associated with the loss of the hypocone (disto-lingual cusp, Fig. 4). As a result, they displayed only three cusps arranged as a triangle. This atypical morphology is highly uncommon in the extant Anthropoidea (new world monkeys, old world monkeys, gibbons, and great apes), most of which display at least four cusps on their molars. However, *Bahinia*, a 40-My-old Asian primate, had molars with no hypocone (21), and absence of the hypocone is considered to be a significant ancestral trait among primates (22). Importantly, *Bahinia* is considered to be one of the oldest anthropoids, and thus represents the morphotype of primitive anthropoid primates (21). Thus, the absence of the hypocone in molars of humans carrying *FGF3* mutations results in a tooth whose main cusps, both in number and arrangement, resemble the presumed ancestral anthropoid molars (Fig. 4). As decreased FGF dosage causes the reappearance of an ancestral dental character in humans, the acquisition of the hypocone in anthropoid primates may be linked to modifications in FGF dosage.

The hypocone is known to have appeared independently

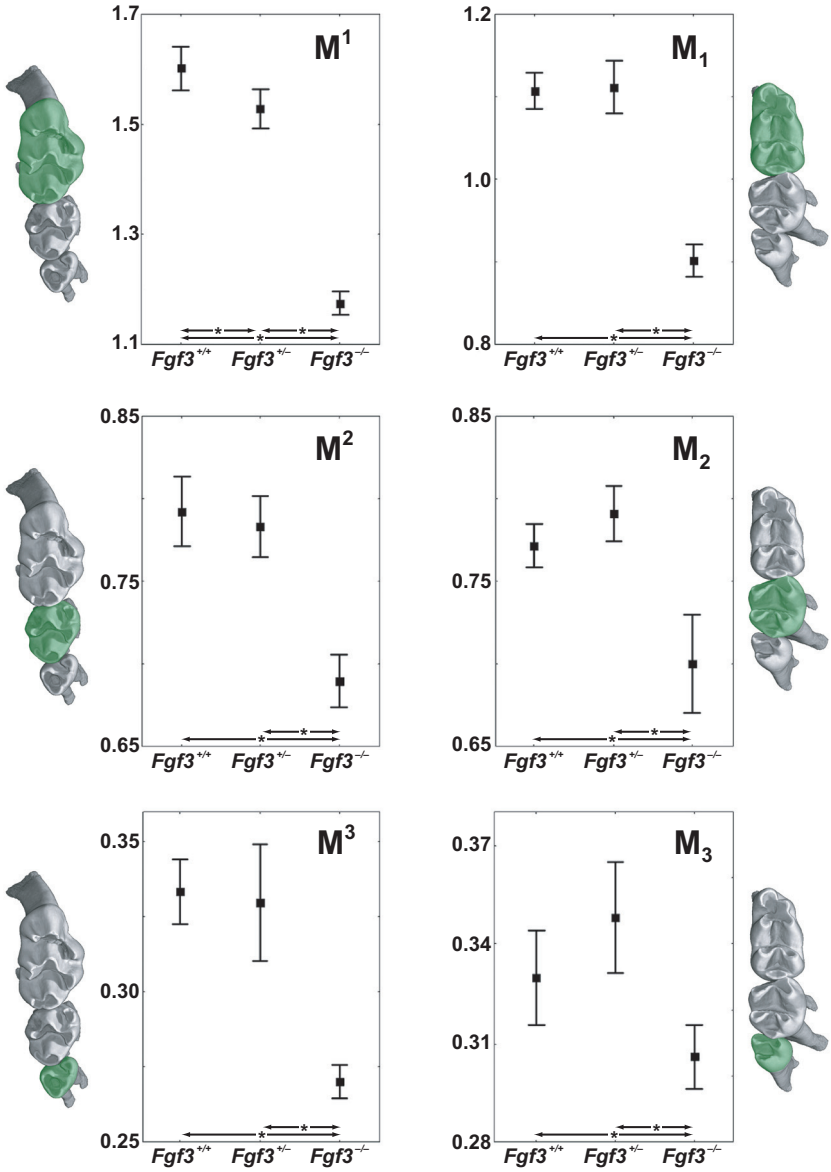


**Fig. 2.** Dental size of *Fgf3* mutant mice. The green tooth beside each diagram indicates the tooth studied, with the wild-type tooth row as the example. Bars indicate the mean value and the confidence interval. WT, wild-type mice. Asterisks indicate statistically significant differences at $P < 0.05$.

many times over the course of mammalian evolution, as well as over primate evolution, indicating that appearance of this cusp is a convergent trait. The emergence of this new cusp during several independent events suggests that it provided a functional masticatory advantage to herbivorous and omnivorous mammals. For this reason, the hypocone is considered to be a key innovation associated with the diversification of these groups (23). Here, we have provided a potential link between the appearance of the hypocone in primates and increases in FGF expression. From our studies, we cannot determine the relative role of FGF signaling in development of the hypocone in different mammals, and presumably changes in different signaling pathways could lead to formation of a hypocone as a convergent trait in different species.

**Concluding Remarks.** Together, our data from mutant mice and fossil muroids show that changes in FGF dosage lead to phenotypes that resemble the progressive reappearance of ancestral morphologies. In light of these atavisms, we propose that increases in FGF signaling were involved in the emergence of the murine dental plan. Our results from human samples also show that a decrease in FGF dosage led to dental morphotypes that resemble the ancestral molars, which lack the hypocone. In both mammals that we studied, acquisition of the derived dental morphology from the primitive one appears to be correlated with an increase in FGF dosage. The humans and mice carrying *Fgf3* mutations do not fully replicate the morphology of the ancestors, but this is to be expected as it is likely that a number of genes were involved in the evolution of mammalian molars.

Both the murine dental plan in rodents and the acquisition of the hypocone in mammals are highly convergent dental characters that developed independently in a number of mammalian lineages, suggesting that changes in signaling by *Fgf3* or a similarly simple molecular modification may be involved in a number of speciation events. We therefore propose that modifications in the dosage of FGF family members have played an important role in the evolution of mammalian dentition.

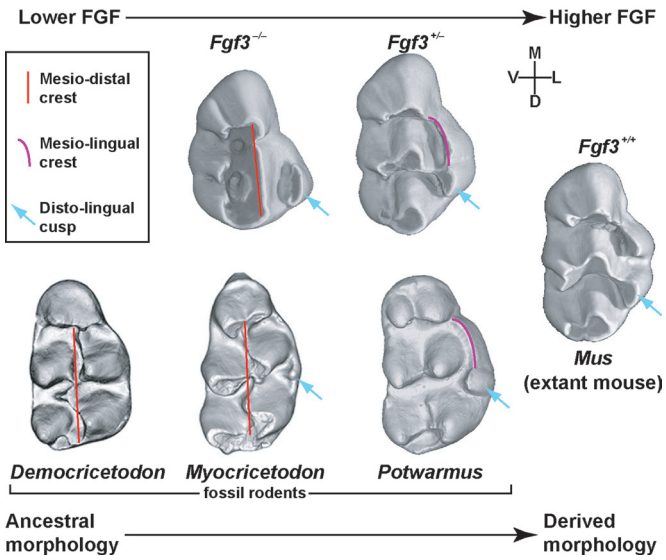


**Fig. 3.** Dental morphology of *Fgf3* mutant mice and fossil rodents. As *Fgf3* dosage is decreased, the mesio-lingual cusp is first transformed into a crest (*Fgf3*$^{+/-}$), and is then lost while a mesio-distal crest arises (*Fgf3*$^{-/-}$). *Democricetodon* illustrates the cricetine dental plan, which is modified during murid evolution by the occurrence of a supplementary disto-lingual cusp (*Myocricetodon* morphology), followed by the loss of the mesio-distal crest and the occurrence of a mesio-lingual crest (*Potwarmus* morphology), that is finally transformed into a mesio-lingual cusp (*Mus* morphology). The arrow indicating the relative levels of FGF signaling apply only to the allelic series of *Fgf3* mutant mice. As we do not know the ground state of *Fgf3* expression levels in muroid ancestors, it is impossible to speculate on levels of signaling in those species. M, mesial; D, distal; V, vestibular; L, lingual.

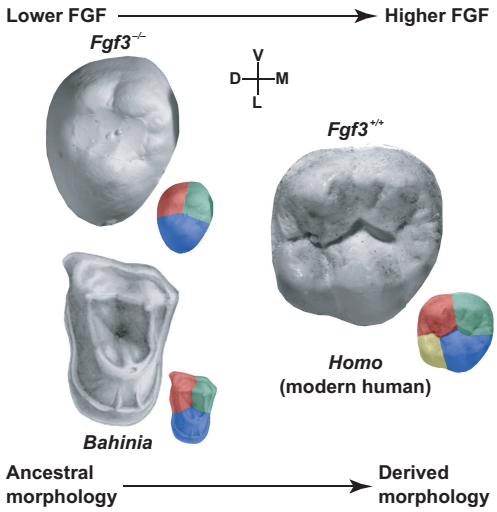


**Fig. 4.** Dental morphology of humans carrying *FGF3* mutations and ancestral primate morphology. The diagram beside each molar indicates the dental area represented by each main cusp. The hypocone is indicated in yellow. In humans carrying *FGF3* mutations, the hypocone is lost. *Bahinia* illustrates the primitive morphology of primate molars. During evolution, the hypocone was acquired, leading to the general pattern present in modern Anthropoids. As in Fig. 3, the arrow indicating the higher and lower levels of FGF signaling applies only to the patients carrying *FGF3* mutations and not to *Bahinia*, for which no information on FGF signaling is available. *Bahinia* drawing from Jaeger et al. (21). M, mesial; D, distal; V, vestibular; L, lingual.

According to the cascade model of tooth development (24), cuspal patterning is regulated by a balance of activators and inhibitors. Imbalances in this system may result in modifications of cusp number and/or location. We observed that as *Fgf3* dosage is decreased, teeth become progressively smaller and have fewer cusps, in both mice and humans. FGFs as a group are generally activators, and *Fgf3* in particular has been shown to induce primary enamel knots (7). Decreases in FGF signaling would be expected to modify the activator/inhibitor balance and thus disturb the normal formation of either primary or secondary enamel knots, which in turn would affect the final dental morphology.

Interestingly, *Fgf3* is expressed specifically at the primary enamel knot stage in epithelium and mesenchyme, but it is not expressed in secondary enamel knots (11). Thus, although the precise mechanism by which *Fgf3* affects cuspal patterning is not known, it is clear that the major effects of this gene occur at or immediately after formation of the primary enamel knot, rather than at later stages.

In our study, both humans and mice carried mutations that result in total loss of function of the *Fgf3* gene. Several previous studies have indicated that modifications of noncoding regions are correlated with interspecific morphological differences similar to those observed during evolution and have proposed that such noncoding changes are more common than coding changes (25, 26). Because members of the FGF family have pleiotropic functions in many aspects of development, it seems unlikely (but not impossible) that total loss-of-function mutations such as those studied here would occur during evolution. We propose that a likelier situation is that modifications of noncoding elements that regulate FGF expression specifically in developing teeth occurred. Comparative studies of both coding regions of FGF genes involved in dental development and noncoding regions that regulate these genes may lead to discovery of mutations linked with evolutionarily relevant morphological modifications.

## Methods

**Fossils.** Studied fossils come from various collections and fossil sites. *Democricetodon* sp. comes from the early Middle Miocene locality Blanquatères 1, France (27). *Myocricetodon parvus intermedius* comes from the Middle Miocene of Pataniak 6, Morocco (28). Both are conserved at the Institut des Sciences de l'Evolution of the Université Montpellier 2. *Potwarmus thailandicus* was discovered in the middle Middle Miocene of Li Mae Long, Thailand (29) and is conserved at the Collections de Paléontologie of the Université Claude Bernard Lyon I.

**3-D Data Acquisition of Rodent Teeth.** Tooth rows of mice were imaged using X-ray-synchrotron microtomography at the European Synchrotron Radiation Facility (ESRF), beamline ID19 and BM5, with monochromatic X-ray beams at energy of 25 keV for *Fgf3* mutants and wild-type mice. A cubic voxel of 5.06 $\mu$m was used. Isolated first upper molars of extinct taxa (*Democricetodon*, *Myocricetodon*, and *Potwarmus*) were digitized during experiments using X-ray synchrotron microtomography at the ESRF on the beamline ID19, with monochromatic X-ray beam at energy of 30 keV and moderate propagation phase contrast. A cubic voxel size of 2.8 $\mu$m was used. 3-D renderings were performed using VGStudiomax software.

**Statistical Tests.** Occlusal surface area of cheek teeth was measured from digitized pictures using Optimas software. Statistical tests were performed to compare tooth size. Analysis of variance (ANOVA) followed by Student *t* tests with Bonferroni correction were performed to compare tooth size among genotypes and determine statistically significant differences at $P = 0.05$ threshold.

**Mutant Mice.** *Fgf3* mutants have been described in ref. 30. Forty-five mice were studied: 20 *Fgf3*$^{-/-}$, 15 *Fgf3*$^{+/-}$, and 10 wild-type littermates. The *Fgf3* mutant allele is a complete null in which the entire *Fgf3* coding region has been deleted, and in situ hybridization showed no *Fgf3* expression in the mutants (30). *Fgf3* null mice weigh approximately 60% as much as their littermate controls. Mutant mice also have abnormal tails (30).

**Human Samples.** Dental casts of one *FGF3* null individual with a homozygous c.616delG (p.V206SfsX117) mutation were from Ankara University School of Medicine. This mutation causes a frameshift resulting in a completely altered and most likely nonfunctional protein, and phenotypic data and mutation analysis have been published (16). The control casts were obtained from the University of California San Francisco Craniofacial Clinic collection.

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