

# *Itpr3* Is Responsible for the Mouse Tufted (*tf*) Locus

HILLARY T. ELLIS, MICHAEL G. TORDOFF, AND M. ROCKWELL PARKER

From the Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104.

Address correspondence to Michael G. Tordoff at the address above, or e-mail: [tordoff@monell.org](mailto:tordoff@monell.org).

The *tf* (tufted) locus is responsible for a classic phenotype of hair loss and regrowth in mice. It is a characteristic of the BTBR strain. Here, we use a combination of positional cloning methods and complementation mapping to identify *Itpr3*, the inositol triphosphate receptor type 3, as the gene responsible for the *tf* locus.

**Key words:** BTBR mouse, hair, inosine triphosphate receptor type 3

When a tufted mouse is 4 weeks old, lines of hair loss begin to spread in waves from its snout to its tail. The first wave takes about a week, the second about 18 days, and subsequent waves take a month or longer (Figure 1). The tufted (*tf*) locus was recognized as a spontaneous mutation in the year 1956 (Lyon, 1956) and early work located it near the kinky/fused (*Fi*) and brachyury (*T*) loci (Dobrovolskaia-Zavadskaia, 1927), which are components of the “t complex” on chromosome 17. This intricate region has been studied extensively (Russell, 1985; Silver, 1985) and for many years before the advent of polymerase-chain-reaction-based genotyping, the tufted pattern of hair loss was used as a visible marker to identify carriers of the lethal *TT* locus (Silver, 1985). However, the identity of *tf* was unknown.

The BTBR mouse is a common laboratory strain carrying the *tf* locus. The BTBR strain was originated by Lyon, who crossed mice carrying the *tf* mutation with stock, maintained by Dobrovolskaia-Zavadskaia, that carried the *T* locus (Dobrovolskaia-Zavadskaia, 1928). Festing (1968) reported that the strain was then developed successively by Bennett and Artzt, but Artzt (personal communication) and Wahlsten et al. (2003) reported it was developed by Dunn, who originally selected it for large litter size, may have crossed it with a 129 mouse strain, and transferred it to The Jackson Laboratory around the year 1969. The strain has been maintained at The Jackson Laboratory for more than 40 generations by sibling mating (see also Clew et al., 2005).

The BTBR strain is of interest for studies of taste perception because it appears to be unable to detect tastes mediated by G-protein coupled receptors (Ellis et al., 2011). To

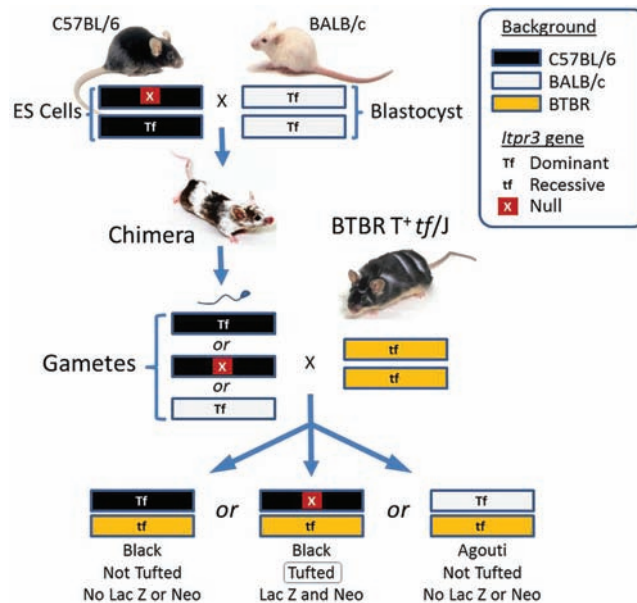


**Figure 1.** A mature adult BTBR  $T^+ tf/J$  mouse showing the characteristic waves of hair loss, which start from the dorsal surface of the head and migrate ventrally.

positionally clone the underlying genes, we produced BTBR  $T^+ tf/J \times$  NZW/LacJ  $F_2$  hybrids, phenotyped them with a series of taste preference tests, and conducted a genome scan. The preference for saccharin and other taste compounds was strongly linked to chromosome 17 (Ellis et al., 2011). To isolate this linkage, we generated a congenic line using marker-assisted selection to repeatedly backcross BTBR  $\cdot$  NZW mice to the BTBR strain. After 11 backcrosses, the congenic interval supporting the phenotypes involved a 0.8-Mb region on chromosome 17 containing 21 known and predicted genes (Tordoff et al., 2012).

We noticed that the tufted hair pattern was present in mice that inherited the BTBR  $\cdot$  BTBR haplotype but not the BTBR  $\cdot$  NZW haplotype in the congenic interval, implying that one of the 21 genes was responsible for the *tf* phenotype.

The strongest candidate gene to mediate taste deficits was *Itpr3*, the inositol triphosphate receptor type 3, which is a component of the taste transduction cascade (Nakashima and Ninomiya, 1999; Hisatsune et al., 2007). To evaluate the contribution of *Itpr3*, we produced *Itpr3*-knockout mice (Figure 2). We purchased C57BL/6 embryonic stem (ES) cell clones from the North American Conditional Mouse Mutagenesis project (NorCOMM; ES cell line MFGC N01293P1\_W239C5). The clones involve deletion of 300 bp spanning Exon 2 of *Itpr3* (Chr 17: 27194249-272194548) and included both Lac and Neo as part of the construct



**Figure 2.** The BTBR form of *Itp3* is responsible for the tufted (*tf*) phenotype. Using standard technologies, a construct conferring a knockout of *Itp3* on a C57BL/6 background was inserted into embryonic stem (ES) cells, incorporated by homologous recombination. The ES cells were injected into BALB/c blastocysts, producing chimeric mice. These were mated with inbred BTBR mice. The chimeric mice could have gametes of three haplotypes and so there were three types of offspring, each heterozygous and each containing one BTBR allele, which carries *tf* as a recessive locus. Only the mice with a nonfunctional copy of *Itp3* displayed the tufted phenotype.

(see <http://www.knockoutmouse.org/martsearch/project/42456>). They were verified, isolated, and then injected into BALB/c blastocysts by the Gene Targeting Service and Transgenic and Chimeric Mouse Facility at the University of Pennsylvania. The first batch of clones resulted in 12 male and 6 female offspring, which had a chimeric range between 10% and 95% (judged by coat color; white = BALB/c; black = C57BL/6).

To expose the gene, we used complementation mapping (Mackay, 2004) in which the action of the recessive *tf* locus was visible only when unmasked by elimination of the dominant locus of the candidate gene. Mice that inherited wild-type BALB/c or C57BL/6J alleles did not display the tufted phenotype, which is to be expected because each contains a dominant *Tf* allele that masks the action of the *tf* allele inherited from the BTBR strain. However, mice with the BTBR background and a heterozygous *Itp3* null allele had the tufted phenotype (Figure 2). This unmasking of the phenotype confirms that the BTBR form of *Itp3* is responsible for *tf*, the tufted locus. Mice with the homozygous *Itp3* null alleles on either the BTBR or C57BL/6J backgrounds were also tufted, showing that the BTBR form of *Itp3* is nonfunctional.

Sequencing the BTBR form of this gene revealed a 12-bp deletion in Exon 23 (Chr 17: 27238069; National Center for Biotechnology Information Build 38.1), which codes for amino acids 983–986. This is probably unique to the BTBR strain; it is not present in the C57BL/6NJ

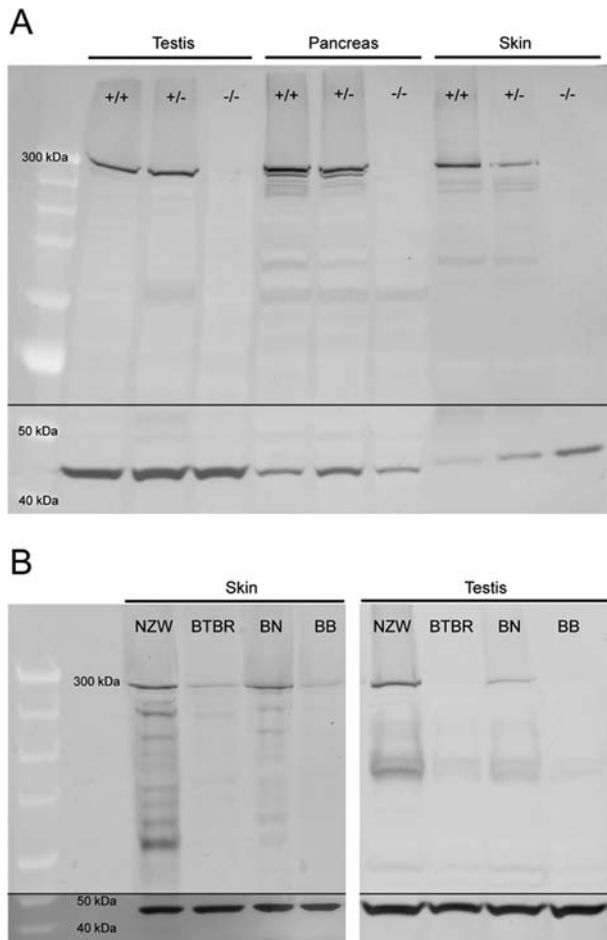
(reference sequence), C57BL/6J (our work), NZW/LacJ (our work), or any of the 17 strains sequenced by the Sanger Mouse Genomes Project (Wellcome Trust Sanger Institute, 2012). The deletion apparently causes almost complete loss of the ITPR3 protein in skin and other tissues (Figure 3; Supplementary Material online). How the loss of *Itp3* interferes with hair growth is less clear. Recent work suggests that a defect in the nuclear translocation of NFATc1 in the K6-positive bulge cells of telogen follicles may cause loose anchorage of club hairs in the early telogen–anagen transition of the hair cycle (Sato-Miyaoka et al., 2012). Whatever the mechanism, our work to investigate taste perception solves a 56-year-old mystery. The BTBR mouse has a defective *Itp3* gene to blame for its tufted hair—as well as its dysfunctional taste perception.

## Supplementary Material

Supplementary material can be found at <http://www.jhered.oxfordjournals.org/>

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**Figure 3.** Western blots showing expression of ITPR3 protein in mice of various genotypes: (A). ITPR3 protein is abundant in C57BL/6 mice that are wild-type (+/+) or heterozygous (+/-) knockouts at *Itp3* but the protein is absent in homozygous (-/-) *Itp3* knockouts. (B). ITPR3 protein is abundant in inbred NZW mice and mice with the BTBR/NZW (BN) haplotype in the congenic interval of chromosome 17, which includes *Itp3*, but the protein is absent or minimal in inbred BTBR mice and mice with the BTBR/BTBR (BB) haplotype in the congenic interval of chromosome 17. Mice with abundant ITPR3 have normal hair; those with absent or minimal ITPR3 have tufted hair. Bottom of each panel shows that all lanes were positive for the control protein,  $\beta$ -actin.

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