doi:10.1111/j.1365-2052.2007.01619.x

Identification of a *tyrosinase* (*TYR*) exon 4 deletion in albino ferrets (*Mustela putorius furo*)

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Summary

Albinism is due to a lack of pigmentation in hair, skin and eye, and has been shown to occur in several animal species. Mutations of the *tyrosinase* (*TYR*) gene account for albinism in domestic cats, rabbits, cattle, mice and rats. In this study, we demonstrate that a *TYR* mutation accounts for albinism in the ferret (*Mustela putorius furo*). The coding sequence of the five exons of *TYR* was determined in genomic DNA from wild-type pigmented 'sable' coloured and albino ferrets. It was not possible to amplify *TYR* exon 4 in albino ferrets originating from different breeds. The deletion of exon 4 in albino ferrets was confirmed by Southern blot hybridization of genomic DNA from albino and pigmented ferrets. This is the first report of a deletion of a *TYR* exon in a non-human mammal.

Keywords albinism, carnivore, deletion, ferret, mammal, *Mustela putorius furo*, *TYR*, tyrosinase.

Complete albinism, which is the lack of the pigment melanin in skin, hair and eye, can be explained by mutations in the gene coding for the tyrosinase protein, which catalyses the first two steps in melanin synthesis. Mutations in the *tyrosinase* (*TYR*) gene are well known in man (Oetting & King 1994, 1999; Fukai *et al.* 1995; Oetting 2000) and mammals like rabbit (Aigner *et al.* 2000), cattle (Schmutz *et al.* 2004), mouse (Halaban *et al.* 2000), rat (Blaszczyk *et al.* 2005) and cat (Imes *et al.* 2006). In the ferret, albinism is inherited in an autosomal recessive manner (Grafodatskii *et al.* 1978), but the causative mutation has not been reported. Furthermore, no data are available for the incidence of albinism in the polecat (*Mustela putorius*), the archetype of the domesticated ferret.

In this study, we analysed the ferret *TYR* coding region from genomic DNA. Ferrets with pigmented 'sable' colour and complete albinism are illustrated in Fig. 1. The ferrets included in our study originated from separate pigmented and albino breeding lines. Pigmented ferrets had descended from animals obtained from Marshall BioResources (USA), whereas albino ferrets were originally obtained from private breeding colonies in Germany. No pedigrees were available on these animals. DNA was extracted from two pigmented ferrets and three albino ferrets (skin or liver samples) using standard procedures. *TYR* exons were amplified (Table S1) with primers based on the *TYR* sequence of the dog (Schmidt-Küntzel *et al.* 2005) representing the nearest relative of the ferret with already characterized *TYR* sequence. PCR products were separated in 1.5% TBE-agarose gels, purified and sequenced using standard procedures.

We were unable to amplify *TYR* exon 4 in three albino ferrets from two separate breeding colonies. In contrast, amplification of exon 3 was successful in the three albino animals. In two pigmented ferrets, both exons 3 and 4 were amplified. There were no differences in the sequences of *TYR* exons 1 to 3 and 5 between pigmented and albino ferrets from the different breeding lines (GenBank accession no. EF405957).

To confirm the absence of exon 4 in albino ferrets, Southern blot hybridization was performed. Hybridization probes for exons 4 and 5 were generated in the same manner as for sequencing reactions using primers in Table S1. For Southern blotting, genomic DNA from liver of one pigmented (wild type) and one albino ferret was digested with either EcoRI or HindIII. The digested samples were separated on a 0.8% TBE gel, blotted and hybridized with the DNA probes (Fig. 2). Hybridization with the exon 4 probe of both EcoRI and HindIII digested DNA resulted in bands from the pigmented but not the albino ferret (Fig. 2a). This pattern suggests a deletion of exon 4 in the albino ferret. One cleavage site for EcoRI was present within the sequence of the pigmented ferret, resulting in two fragments.

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Accepted for publication 29 April 2007

Figure 1 Coat-colour phenotypes in the domestic ferret (*Mustela putorius furo*). On the left a sable male ferret of wild-type colouration is depicted; on the right-hand side a female albino ferret exhibits complete albinism.

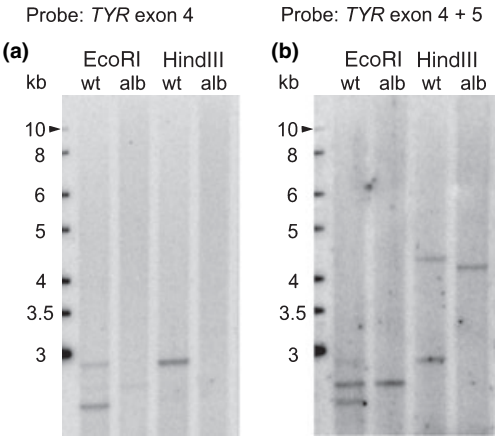

Figure 2 Southern blot analysis shows a deletion of exon 4 *TYR* in the albino ferret. (a) *TYR* exon 4 and (b) *TYR* exons 4 and 5 probes were used for hybridization. A 1-kb ladder indicates the relative sizes of the hybridizing bands. Note the absence of a band in albino DNA when hybridized with exon 4 but a signal was present when hybridized with exon 5 probe.

In addition, a second blot (Fig. 2b) was hybridized with probes pooled for exons 4 and 5. Again, signals for hybridization with exon 4 probe were only visible in the DNA of pigmented but not albino ferrets. Weak hybridization in Fig. 2b was caused by a lower concentration of the exon 4 probe than used in Fig. 2a. Exon 5 hybridization occurred with both pigmented and albino ferret DNA. EcoRI digestion shows the same signal pattern in pigmented and albino ferrets for the exon 5 probe. The albino ferrets had a smaller fragment than the wild-type ferret on the HindIII-digested DNA. We speculate that the deletion of exon 4 includes the loss of a HindIII restriction site and that the fragment that hybridizes to the exon 5 probe is smaller because of the deletion of this restriction site (Fig. S1).

In order to determine the size of the deletion, long-range PCR was attempted. Yet, our efforts were not successful probably due to the fact that this part of the gene is too large for long-range PCR (about 50 kb in canines).

The mammalian *tyrosinase* gene is composed of five exons, with exon 4 spanning codons 394–456. This region contains a copper-binding site (CuB) that, together with other domains, plays an important role in tyrosinase catalytic activity. Most of the missense mutations in humans accounting for albinism have been found in or flanking the two copper-binding domains (Oetting 2000), suggesting a loss of protein function if exon 4 was deleted. For *TYR* RNA, various splice variations characterized in humans (Fryer *et al.* 2001) and mice (Ruppert *et al.* 1988) are known to produce an inactive enzyme and are thought to be degraded rather than processed. Yet, a splice variation that skips exon 4 has not been characterized in man or mouse.

Gross deletions in *TYR* have been reported in humans, including a complete deletion of the *TYR* gene (Schnur *et al.* 1996), a deletion of exon 1 (Coupry *et al.* 2001) and deletions of exons 4 and 5 (Ray *et al.* 2005), all of which result in oculocutanous albinism. A deletion of exons 4 and 5 of both *TYR* alleles in one human resulted in complete albinism (C. Ray, personal communication), further supporting the loss of protein function resulting from the deletion of exon 4 in the ferret. In other mammals, complete albinism results from missense or frame-shift mutations. Additionally, most sequence alterations flank or are located within the copper-binding region CuB. In the white New Zealand rabbit, complete albinism is due to a missense mutation at residue 373 located in the CuB region, one of the two clusters with a high incidence of mutations (Aigner *et al.* 2000). Likewise, a missense mutation in residue 299, which flanks CuB, is present in the albino Wistar rat (Blaszczyk *et al.* 2005). In the mouse, the common albino mutation in BALB/c occurs at C85S (Halaban *et al.* 2000). In cattle, a frame-shift mutation caused by an insertion in codon 316 is associated with complete albinism, with absence of pigmentation in hair and skin and pink eyes (Schmutz *et al.* 2004). The domestic albino cat lacks codon 325 causing a premature stop codon (Imes *et al.* 2006). These mutations in cat and cattle are located 5′-end of the CuB region. In the albino ferret a gross deletion is evident that has not been described in other mammals up to now. Taken together, we propose that deletion of *TYR* exon 4 accounts for complete albinism in the domestic albino ferret.

Acknowledgements

We thank Y. Klenk for technical assistance in Southern blot hybridization. This study was supported by the International Graduate School of Neuroscience and the SFB 509/A11 of the Deutsche Forschungsgemeinschaft.

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Supplementary Material

The following supplementary material is available for this article online from http://www.blackwell-synergy.com/doi/full/10.1111/j.1365-2052.2007.01619.x

Table S1 PCR primers and conditions for the amplification of the ferret *TYR* gene from genomic DNA.

Figure S1 A hypothetical model for the hybridization pattern of *TYR* exons 4 and 5 after HindIII digestion.

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