Letter to the Editor
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A *Tyrosinase* missense mutation causes albinism in the Wistar rat

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Summary

Tyrosinase serves as a key enzyme in the synthesis of melanin. In humans mutations in the *TYR* gene are associated with type 1 oculocutaneous albinism (OCA1) that leads to reduced or absent pigmentation of skin, hair and eye. Various mutations causing OCA in man, mouse, rabbit and cattle have been identified throughout the *Tyrosinase* gene including nonsense, missense, frameshift and splice site alterations. Here we report a missense substitution at codon R299H in exon 2 of the *Tyr* gene in the albino Wistar rat. As this very exchange has already been described in OCA patients, our findings reinforce the significance of this region for normal catalytic activity of tyrosinase protein.

Oculocutaneous albinism (OCA), a group of autosomal-recessive diseases in humans and animals, is characterized by reduced or absent melanin in skin, hair, and eyes. OCA1 in humans results from mutations in the *TYR* gene that codes for tyrosinase (EC 1.14.18.1), the key enzyme in pigment biosynthesis in mammals. Tyrosinase is the first enzyme in the melanin synthesis pathway converting tyrosine to DOPA and then to dopaquinone (see e.g. Cooksey et al., 1997). The enzyme contains 529 amino acids and binds the substrate via two copper binding sites (CuA and CuB) of which especially CuB is important for enzymatic activity (Olivares et al., 2002). In man, the *TYR* gene is about 65 kb in length and the coding region comprises 5 exons (Giebel et al., 1991). Although close to 150 different *TYR* mutations have been characterized in man (Oetting and King, 1999; see albinism database at URL: http://albinismdb.med.umn.edu/) and other species (e.g. mouse: Beermann et al., 2004; rabbit: Aigner et al., 2000; cattle:

Table 1. Primers and conditions for PCR amplification of the rat *Tyr* gene

PCR system	Primer sequence (5′ → 3′)	Location	Fragment length (bp)	Polymerase	Annealing T/MgCl (°C/mM)
1-1 UTR F	AAGCATTTGATGTAGGAAGGG	E 1		HSTaq[a]	55/1.5
1-1 R	AAACCCATGAAGTTTCCAGAG	E 1	433	HSTaq[a]	55/1.5
1-2 F	CATCCTTTTGTCCAATGCAC	E 1		HSTaq[a]	55/1.5
1-2 R	AAAATCAATGTCCCTCC	E 1	433	HSTaq[a]	55/1.5
1-3 F	GGTCGACACCCATGTTTAAG	E 1		HSTaq[a]	54/1.5
1-3 UTR R	GAAGATGTGGCTGCTGAAATA	E 1	393	HSTaq[a]	54/1.5
2 UTR F	CGCATTTTGCATAAATTGG	E 2		Taq[b]	54/4.5
2 UTR R	CAAAGCTTAGCATTGCAAAAC	E 2	366	Taq[b]	54/4.5
3 UTR F	TCAGAATCCCAATATCAAATG	E 3		Taq[b]	50/1.5
3 UTR R	CCACAATTTATAAGGTTTGGT	E 3	403	Taq[b]	51/1.5
4 UTR F	GGAGATGGTAACTTGTCAAAG	E 4		Taq[b]	50/1.5
4 UTR R	AGAGATTGCCCACATTAGAC	E 4	373	Taq[b]	50/1.5
5 UTR F	CAACCCAAGCATCTTACTAC	E 5		Taq[b]	50/1.5
5 UTR R	CAGACCTTTTAGTCCCACTC	E 5	356	Taq[b]	51/1.5

[a]HotStarTaq DNA Polymerase, Qiagen, Hilden, Germany; [b]Taq DNA Polymerase, Qiagen, Hilden, Germany.

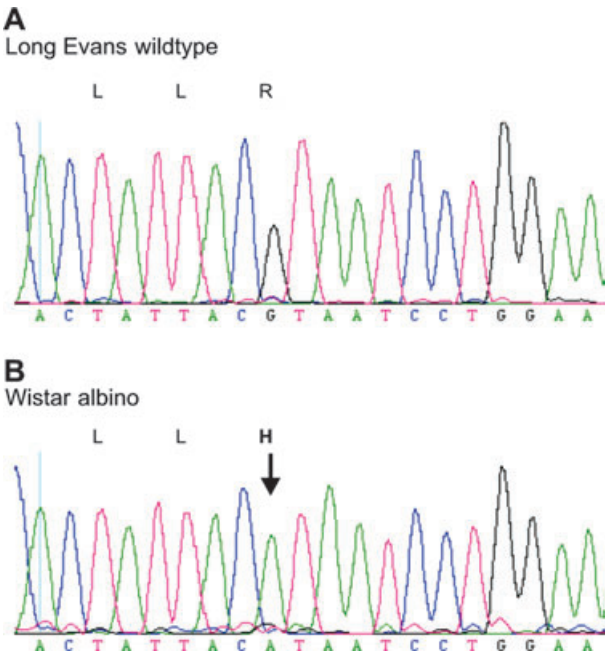

Figure 1. Arg299His substitution as caused by the mutation in exon 2 of the Wistar rat *Tyr* gene. Sequence derived from the pigmented Long Evans rat (A) and the albino Wistar rat (B) with the derived amino acids on top. Both strands were sequenced.

Schmutz et al., 2004) the molecular basis of the 'albino mutation' in the rat has not yet been characterized.

We examined the tyrosinase encoding region of the albino Wistar rat and compared it with the *Tyr* gene of the pigmented Long Evans rat. Pigmented Long Evans HsdBlu and albino Wistar Unilever HsdCpb were purchased from Harlan Winkelmann GmbH (Borchen, Germany). Rats were bred and raised in the animal facility of the Institute of Zoology and Neurobiology at the Ruhr-University (Bochum, Germany). Genomic DNA was extracted using the QIAamp DNA mini kit (according to manufacturer's protocol) from skin tissue that was collected during unrelated experiments. Primers were designed in intronic regions located close to coding regions of the *Tyr* gene during order to screen the whole coding region. Primers (Table 1) were designed based on the rat sequence of chromosome 1 (NW_047561) and the *Tyr* mRNA sequence (XM_238901). The coding parts of the *Tyr* gene were amplified by PCR, for conditions see Table 1.

Sequencing reactions were carried out by the dideoxy-chain termination method using BDT (Perkin-Elmer, Norwalk, CT, USA) according to the manufacturer's instructions. All sequencing reactions were run on an automated DNA sequencer (Applied Biosystems 377, Darmstadt, Germany) with the respective analysis software.

We identified a nucleotide exchange in exon 2 in the sequence of the albino Wistar rat by comparison with the *Tyr* gene of the pigmented Long Evans rat (Fig. 1) and the pigmented Norway rat (XM_238901). This mutation in homozygous state causes an amino acid alteration Arg299His. Arg299 is conserved in man, mouse, dog and rabbit. The 299His mutation has already been described in humans affected by OCA1A without any tyrosinase activity and consequential complete lack of pigmentation (Gershoni-Baruch et al., 1994). These findings emphasize the functional significance of this mutation leading to OCA in the Wistar rat.

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