Two cysteine substitutions in the MC1R generate the blue variant of the arctic fox (Alopex lagopus) and prevent expression of the white winter coat

Dag Inge Våge a,*, Eva Fuglei b, Kristin Snipstad a, Janne Beheim a, Veslemøy Malm Landsem c, Helge Klungland c

a Norwegian University of Life Sciences (UMB), Department of Animal and Aquacultural Sciences, P.O. Box 5003, N-1432 Ås, Norway
b Norwegian Polar Institute, The Polar Environmental Centre, N-9296 Tromsø, Norway
c Department of Laboratory Medicine, Children and Women’s Health, Faculty of Medicine, NTNU, Morfologibygget, St. Olavs Hospital, N-7006 Trondheim, Norway

Received 6 August 2004; accepted 6 November 2004
Available online 27 June 2005

Abstract

We have characterized two mutations in the MC1R gene of the blue variant of the arctic fox (Alopex lagopus) that both incorporate a novel cysteine residue into the receptor. A family study in farmed arctic foxes verified that the dominant expression of the blue color phenotype cosegregates completely with the allele harboring these two mutations. Additionally to the altered pigment synthesis, the blue fox allele suppresses the seasonal change in coat color found in the native arctic fox. Consequently, these findings suggest that the MC1R/agouti regulatory system is involved in the seasonal changes of coat color found in arctic fox.

© 2005 Elsevier Inc. All rights reserved.

Keywords: Pigmentation; MC1R; Blue fox; Winter coat; Arctic fox; Polar fox; Alopex lagopus

1. Introduction

At high latitudes, mammals have to face seasonal changes in temperature, light, food availability, and colors in their surroundings. According to the species, there are various physiological adaptations to these environmental changes like hibernation, storage of energy via fat deposition and modification in pelage. The suprachiasmatic nucleus (SCN) of the hypothalamus is the principal component of the mammalian biological clock. The pacemaker of the SCN oscillates with near 24 h period, and coordinates a broad spectrum of physiological, endocrine and behavioral circadian rhythms. Consistent with its role in circadian timing, several experiments have provided evidence that SCN is the locus of the brains endogenous calendar, enabling organisms to anticipate seasonal changes (for review see [8]). Mechanisms of how the circadian rhythm of SCN is translated into seasonal variation and how seasonal variation in coat color is functionally regulated are not well understood.

The arctic fox (Alopex lagopus) is a circumpolar inhabitant of the Arctic, and occurs naturally in two color morphs, white and blue. In the white morph the production of pigment is dramatically changed with season. The summer pelage is characterized by a dark dorsal and a light ventral pattern, while the white winter coat is produced as an adaptation to snow and icy environments. A seasonal pelage shift occurs with molting of the highly insulative white winter fur in May to a shorter brown/grey summer fur. The initial growth of the winter fur occurs in September, and continues until early December. The winter fur is 200% thicker than the summer fur [21]. The exact mechanism of the down regulation in
pigment synthesis connected with the change to winter fur is not known. Blue fox is a color variant of arctic fox, showing a uniform dark grey/blue coat color during the summer. The dominant blue coat color is inherited like a simple Mendelian trait caused by a single gene. In the winter season, the blue fox develops a coat color that is lighter than the summer coat but have far more colored compared to the winter white native arctic fox (Fig. 1). At Svalbard, as in several other natural habitats of the arctic fox, 3–5% of the population is of the blue variant.

Dominant acting mutations in the \(MC1R\) causing a general eumelanizing effect are well known from several mammalian species including mouse, cattle, red fox and sheep. These have been functionally tested in cell assays, and have been found to produce a constitutively active receptor causing eumelanization mediated through the \(cAMP\) pathway \([11,14,19,25,26]\). Except for the \(silver\) fox, these mutations are almost completely epistatic to the \(agouti\) gene, even in the heterozygous state.

In the present study we have identified two substitution mutations in the melanocortin 1 receptor (\(MC1R\)) in the blue fox, that co segregate completely with the blue fox phenotype in crosses between blue fox and winter white arctic fox. In addition to causing a uniform dark summer pelage, the two mutations also prevent the development of a completely white winter pelage. These findings indicate that the \(agouti/MC1R\) regulatory system is involved in seasonal changes in coat color found in arctic fox.

2. Materials and methods

2.1. Animals

The family study with farmed blue foxes was done based on a cross between a heterozygous female blue fox and a white male in the research farm at the Norwegian University of Life Sciences (Fig. 1). Among ten offspring, two were blue foxes and the remaining eight were of the winter white phenotype. In addition to the family study, blood samples were drawn from seven wild arctic foxes (of the white morph) that were caught near Ny-Ålesund (78°55′N, 11°56′E), Svalbard, Norway. These animals were a part of a larger project at the Norwegian Polar Institute, Tromsø, Norway \([6,7]\). Verification of the mutations found in the blue fox was also done in a blue fox originating from Finland.

2.2. PCR amplification and sequencing

DNA was isolated from blood samples following standard protocols. The complete coding region of the \(MC1R\) gene was amplified with primers AL1 and AL2 (Table 1). All primers are designed based on the red fox \(MC1R\) sequence (X90844). The PCR reaction was carried out in a 20 \(\mu\)l reaction containing 20ng genomic DNA, 10 pmol of primers AL1 and AL2, 200 \(\mu\)M \(dNTP\), standard buffer conditions and 1.5 U Taq polymerase. DNA was denatured for 3 min at 94 °C, and PCR run for 35 cycles at 95 °C for 15 s, 60 °C for 15 s, and 72 °C for 30 s. Sequencing of the complete region was carried out with
Primers used in the study to amplify, sequence and genotype the arctic fox MC1R gene.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Position</th>
<th>Direction</th>
<th>Sequence of primer in 5'-3' direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>AL1</td>
<td>54 to 35</td>
<td>Forward</td>
<td>GAA CTG AAG GAC CCA CTT GAG</td>
</tr>
<tr>
<td>AL2</td>
<td>500 to 497</td>
<td>Reverse</td>
<td>TAC ACC ACC CAT TCT GGC CAT</td>
</tr>
<tr>
<td>AL3</td>
<td>192 to 211</td>
<td>Forward</td>
<td>CAA GAA CCG CAA CCT GTA CT</td>
</tr>
<tr>
<td>AL4</td>
<td>42 to 63</td>
<td>Reverse</td>
<td>TGC TGC GGA AGG TCG CAA TG</td>
</tr>
</tbody>
</table>

Positions are given relatively to the translational start A/G of the red fox MC1R gene (X90844).

2.3. RFLP analysis

Following the detection of two mutations in the MC1R gene of arctic fox, a RFLP reaction was developed to distinguish between the two variants. Shortly, primer AL1 and AL4 were used to amplify a 116 bp region containing the first of two linked mutations of interest localized to amino acid positions 5 and 280. PCR was carried out in an 20 μl reaction containing 10 pmol of each primer and 20 ng DNA, and was run for 35 cycles at 95 °C for 15 s, 60° for 15 s, and 72° for 15 s. The PCR fragment was cut with Apgal at 25 °C overnight. If the wildtype allele is present, the reaction will produce two fragments of 71 and 45 bp. If the blue fox allele is present, Apgal will not cut the fragment. The mutation localized to amino-acid position 280 was genotyped by DNA-sequencing (Applied Biosystems) in the present study.

3. Results and Discussion

By sequencing the complete coding region of the MC1R gene of the winter white arctic fox (AI786717), as well as the blue color variant (AI786718), two mutations causing amino acid substitutions were discovered. In position 5, the glycine residue is substituted by a cysteine in the blue fox, while a phenylalanine is replaced by a cysteine at position 280. These two mutations are localized to the initial extracellular region of the receptor and the external boundary of transmembrane domain 7, respectively. A family study based on a cross between a heterozygous female blue fox and a white male fox (Fig. 1) verified that the two mutations cosegregated with the dominant blue phenotype. The wild arctic foxes from Svalbard have a MC1R genotype identical to the Norwegian blue fox.

Previously reported MC1R mutations causing dominant eumelanizing effects are localized within or close to the 2nd and 3rd transmembrane (TM) domains of the 317 amino acid long receptor [3,13]. However, since cysteine has the capability of forming disulfide bridges with other cysteine residues, a conformational change of the receptor with an effect comparable to the TM2/TM3 – mutations might be foreseen. Such interactions could be formed either between the two new cysteine residues, or to cysteine residues localized to any of the extracellular regions of the receptor. Both alterations in the MC1R gene of the blue fox are found within relatively well conserved amino acid positions of the receptor (Fig. 2). The 5th position is conserved in all mammals characterized so far, except for mouse which has a glutamine substituting the glycine. In position 280, all species are identical except for pig which has a valine substituting the phenylalanine. Based on the present data, it is not possible to conclude whether one or both of these mutations are involved in the observed phenotypic change.

The darker dorsal and lighter ventral pattern found in the summer pelage of the arctic fox is comparable to different agouti alleles found in mouse, affecting the dorsum and ventrum independently. This phenomenon is explained by alternative promoter use in the agouti gene [4,15,20,23]. In this respect, the blue fox mutation act as a classical constitutively activated MC1R mutation in being epistatic to the agouti gene. Inter-species crossing between the winter white arctic fox and the standard silver variant (sild^E^*) of the red fox (Vulpes vulpes) provide firm evidence for a functional agouti protein in the arctic fox. The standard silver fox do not carry a functional agouti protein, and the red color found in the offspring (named Golden Island) must therefore be ascribed the functional “arctic” agouti protein [17,25].

Based on classical genetic studies [2] a hypothesis was put forward that a dominant agouti allele (A^w^) is responsible for gradually eliminating all eumelanin from the birth coat of the pups and from the colored summer coat of adults, thus replacing the summer color by the white winter coat. A functional explanation of this could be that the equilibrum between the agouti protein and α-MSH is shifted according to the photoperiods, with a higher concentration of α-MSH during summer, and a relatively higher level of agouti protein during the winter season. According to this model one should expect a yellow/red winter coat instead of the observed white one. However, a general reduction in melanocyte activity
combined with a rapid growth of the winter coat may dilute the pheomelanin in a level giving this white appearance.

α-MSH and UV radiation are well known as major physiological signals that modulate skin pigmentation. [1,2,22] Melanocytes react to these signals by proliferation, increased dendriticty and elevated pigment synthesis. Prolonged day length during summer increases the UV radiation and the local α-MSH production in the skin is increased. Although the fox skin is not directly subjected to sunlight, it has been proposed that the hair shaft may function as a fiber-optic system for guiding UV light to the hair follicle [9]. This has of course to be verified experimentally, but should not yet be exclude as a possible contribution to the eumelanization seen in the summer pelage. In addition to a local reaction in the skin, due to increased day length, SCN guided signals do most likely play an important role in forming a coordinated reaction to seasonal changes. Changes in circulating levels of α-MSH synthesized in the pituitary gland is one possible SCN-guided signal.

Based on our results we do assume that the two mutations found in the MC1R gene of the blue fox are acting as a classical dominant mutation, causing a constitutive active receptor. Such mutations are capable of activating the receptor in the absence of the α-MSH ligand [14]. These processes may explain both the general darker pelage of the blue fox, the lack of a light ventrum and a lesser responsiveness to shifts in the agouti/α-MSH equilibrium influencing the seasonal changes in coat color.

Acknowledgement

The Research Council of Norway and the Norwegian Fur Breeders Association provided financial support.

References

[18] Rieder S, Taourit S, Mariat D, Langlois B, Guerin G. Mutations in the agouti (ASA), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (Equus caballi). Mamm Genome 2001;12:405–5.