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Short communication

Analysing the diversity of the caprine melanocortin 1 receptor (*MC1R*) in goats with distinct geographic origins

Koray Kırıkçı[a,1,2], Antonia Noce[a,1], Ali Zidi[a,1], Juan Manuel Serradilla[b], Juan Carrizosa[c], Baltasar Urrutia[c], Fabio Pilla[d], Mariasilvia D'Andrea[d], Juan Capote[e], Iosif Bizelis[f], Valentin Balteanu[g], Taina Figueiredo Cardoso[a,h], Shahin Eghbalsaied[i], Agueda Pons[j], Luz Ángela Álvarez[k], Michele Pazzola[l], Giuseppe Massimo Vacca[l], Gabriela Obexer-Ruff[m], Marcel Amills[a,*]

[a] *Department of Animal Genetics, Center for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Campus de la Universitat Autònoma de Barcelona, Bellaterra, 08193 Spain*
[b] *Departamento de Producción Animal, Campus de Rabanales, Universidad de Córdoba, 14071, Córdoba, Spain*
[c] *Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA). Estación Sericícola. La Alberca, Murcia, Spain*
[d] *Dipartimento di Agricoltura, Ambiente e Alimenti, Università degli Studi del Molise, Campobasso, Italy*
[e] *Instituto Canario de Investigaciones Agrarias, La Laguna 38108, Tenerife, Spain*
[f] *Department of Animal Science, Agricultural University of Athens, Athens 11855, Greece*
[g] *Institute of Life Sciences, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca 400372, Romania*
[h] *CAPES Foundation, Ministry of Education of Brazil, Brasilia, DF, Zip Code 70.040-020, Brazil*
[i] *Transgenesis Center of Excellence, Isfahan (Khorasgan) Branch, Islamic Azad University, Isfahan, Iran*
[j] *Unitat de Races Autòctones, Servei de Millora Agrària, (SEMILLA-SAU), Son Ferriol, 07198, Spain*
[k] *Universidad Nacional de Colombia, Sede Palmira, Valle del Cauca, Colombia*
[l] *Dipartimento di Medicina Veterinaria,Università degli Studi di Sassari, 07100, Sassari, Italy*
[m] *Swissuniversities, Department of Research & Development, Effingerstr. 15, 3001, Berne, Switzerland*

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ABSTRACT

In humans, the variability of the melanocortin 1 receptor (*MC1R*) gene has been associated with geography, being mainly determined by the amount of exposure to sunlight. Studies performed in pigs have also evidenced the existence of a geographic component in the distribution of *MC1R* haplotypes, probably as a consequence of an ancient split between Asian and European wild boars. Herewith, we have partially resequenced the caprine *MC1R* coding region in 58 goats from distinct geographic locations *i.e.* Colombia, Italy, Spain, France, Greece, Romania, Iran and Africa. The resulting dataset was merged with 39 previously published caprine *MC1R* sequences and a median joining network was built. This phylogenetic analysis did not yield any evidence of a relationship between geography and the clustering of caprine *MC1R* sequences, a result that was confirmed by performing a Mantel test with a previously published dataset of nine goat breeds (N = 319) with available *MC1R* genotypes. The majority of caprine *MC1R* variation was non-synonymous (c.676A > G, c.748G > T, c.764G > A and c.801C > G) and predicted to have functional effects. An analysis of goat *MC1R* sequences with the PAML 4 software provided evidence that two SNPs (c.764G > A and c.801C > G) might evolve under positive selection. The apparent lack of any link between caprine *MC1R* variation and geography might be explained by a complex array of factors including artificial selection for pigmentation phenotypes and recent divergence amongst goat breeds.

1. Introduction

Selection for coat color was probably implemented in ancient times as a consequence of religious beliefs and cultural preferences of livestock breeders (Zeder, 1994). For instance, the Book of Numbers establishes that red heifers need to be used in the purification rituals of people that have been in contact with a corpse, and black sheep were slaughtered in Chinese supplication ceremonies for rain

* Corresponding author.
E-mail address: marcel.amills@uab.cat (M. Amills).
[1] Koray Kırıkçı, Antonia Noce and Ali Zidi have contributed equally to this work.
[2] Present address: Department of Animal Science, Faculty of Agriculture, Ahi Evran University, Krsehir, 40000 Turkey.

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because this color symbolizes water (Tao, 2007). Nonetheless, there were also practical reasons for selecting certain colors *e.g.* white wool is much easier to dye than the coloured one. In domestic animals, this process of selection contributed to generate a huge repertoire of pigmentation patterns that contrast strongly with the monochrome coat of their wild ancestors.

Pigmentation is a polygenic trait determined by a large number of loci (Sturm et al., 2001). The melanosomal protein complex is formed by tyrosinase, the enzyme that catalyses the synthesis of melanin, plus two other enzymes: tyrosinase-related proteins 1 and 2 (TYRP1 and TYRP2). Tyrosinase-related protein 2 catalyses the synthesis of 5,6-dihydroxyindole-2-carboxylic acid from DOPAchrome, which is converted to eumelanin by TYRP1 (Kondo and Hearing, 2011). Besides, both TYRP1 and TYRP2 contribute to the stabilization of tyrosinase (Sturm et al., 2001). Another key player in the determination of coat color is the melanocortin 1 receptor (*MC1R*) gene. Binding of this molecule, on the cell surface of melanocytes, by proopiomelanocortin (POMC) raises the levels of cAMP and activates tyrosinase, thus inducing the synthesis of black eumelanin (Sturm et al., 2001). The agouti-signaling protein (ASIP), that is also a ligand for MC1R, has the opposite effect i.e. by lowering the activation of tyrosinase promotes the synthesis of red/yellow pheomelanin (Makova and Norton, 2005; Parra, 2007).

In humans, *MC1R* diversity has been linked to geography and, more specifically, to the amount of sunlight exposure (Savage et al., 2008). This differential distribution is not only explained by drift and demographic factors, but also by natural selection. In this way, dark, eumelanin-rich photoprotective pigmentation is considered to be advantageous at tropical and equatorial latitudes because it is associated with a decreased rate of ultraviolet-induced folate degradation (Jablonski and Chaplin, 2010). In contrast, a light skin is favoured in geographic areas with reduced sunlight because it enhances the synthesis of vitamin D_3 (Jablonski and Chaplin, 2010).

Genetic diversity of livestock pigmentation genes has been less studied at an intercontinental scale than that of humans (Switonski et al., 2013). Remarkable differences in the distribution of *MC1R* haplotypes have been detected when comparing Chinese and European swine (Giuffra et al., 2000). Previous studies performed in goats characterized the diversity of the *MC1R* gene (Fontanesi et al., 2009; Nicoloso et al., 2012; Badaoui et al., 2014), but it was difficult to ascertain if it is associated with geography because only Italian and Spanish populations were sampled. In the current work, we aimed to investigate if there is a link between goat *MC1R* polymorphism and geography by analysing individuals from several locations covering a broad geographic range.

2. Materials and methods

2.1. Goat sampling

Blood samples were collected by jugular venipuncture from Colombian (N = 9), Italian (Sarda breed, N = 7), French (Saanen breed, N = 8), Iranian (Lori-Bakhtyari and Lori, N = 3), Greek (Youra breed, N = 2), Romanian (Carpathian breed, N = 7), Sahelian (N = 6) and Spanish (Majorcan breed, N = 8; Palmera breed, N = 8) goats (Supplementary Table S1). Sampling was performed by trained veterinarians in the context of sanitation campaigns and parentage controls not directly related with our research project. In all instances, veterinarians followed standard procedures and relevant international guidelines to ensure an appropriate animal care (ARRIVE guidelines, https://www.nc3rs.org.uk/arrive-guidelines; EU Directive 2010/63/EU for animal experiments). Genomic DNA was purified with the DNeasy Blood & Tissue Kit (Qiagen, Barcelona, Spain) and resuspended in ultrapure water.

2.2. Amplification and sequencing protocols

By using primers FW1, 5′-CCT GCA CTC CCC CAT GTA C-′3 and REV1, 5′-TGC GGA AGG CAT AAA TGA GG-3′, we amplified a fragment of approximately 0.7 kb of the *MC1R* gene that in previous studies was shown to contain most of its polymorphism (Fontanesi et al., 2009; Badaoui et al., 2014). Polymerase chain reactions were performed in a final volume of 15 μL containing 1.5 μL of 10 x PCR buffer, 2.5 mM $MgCl_2$, 0.3 μM of each primer, 0.25 mM of each dNTP, 0.75 U Taq Gold DNA polymerase (Applied Biosystem, Foster City, CA) and 50 ng genomic DNA. This reaction mixture was heated to 95 °C for 10 min, followed by 35 cycles of 95 °C for 1 min, 62 °C for 1 min and 72 °C for 1 min. Subsequently, a final extension step at 72 °C for 10 min was carried out. Amplification products were purified with the ExoSAP-IT PCR Cleanup kit (Affymetrix, Santa Clara, CA) and sequenced in both directions with primers FW2, 5′-ACC TGC TGG TGA GCG TCA G-3′ and REV1. Sequencing reactions were prepared with the Big Dye Terminator Cycle Sequencing Kit v1.1 (Applied Biosystems) and electrophoresed in an ABI 3730 DNA Analyzer (Applied Biosystems). Chromatograms were edited with the SeqScape software v2.5 (Applied Biosystems), and all sequences were submitted to the GenBank database (accession codes: KT071610-KT071667).

2.3. Phylogenetic and positive selection analyses

We carried out phylogenetic and statistical analyses by using our *MC1R* dataset plus 39 goat *MC1R* sequences (Supplementary Table S1) retrieved from GenBank (Badaoui et al., 2014). A median-joining network was built with the Network 4.6 software (Bandelt et al., 1999) by using default parameters. The codeml program of the PAML 4 package (Yang, 2007) was employed to detect positive selection. Maximum likelihood estimates of the *w*-ratio (d_N/d_S), *i.e.* the rate of non-synonymous substitutions per non-synonymous site (d_N) divided by the rate of synonymous substitutions per synonymous site (d_S), were obtained for each codon of the MC1R-encoding region under analysis. We contrasted models 7 (neutral model), which assumes a β-distribution for the *w*-ratio ($0 \leq w \leq 1$), with model M8 (selection model), which takes into account an extra category of sites with $w_1 > 1$ (positive selection). The performance of a likelihood ratio test, where twice the difference in the log-likelihood values corresponding to models 7 and 8 is compared with a χ^2 with two degrees of freedom, was used to assess the statistical significance of positive selection. Bayes Empirical Bayes inference was employed to determine the posterior probability that a given codon evolves under positive selection. We also performed a Mantel test (Mantel, 1967) to investigate if there is any relationship between geography and *MC1R* variation. In this way, we used a published dataset (Badaoui et al., 2014) of 319 goats genotyped for the c.673C > T, c.676A > G, c.748G > T, c.764G > A *MC1R* single nucleotide polymorphisms (SNPs). These goats belonged to the following breeds: Cilentana Nera (N = 26), Garganica (N = 41), Grigia Molisana (N = 13), Girgentana (N = 19), Malagueña (N = 43), Murciano-Granadina (N = 81), Tinerfeña (N = 38), Majorera (N = 20) and Palmera (N = 38). The Mantel test estimates the linear correlation between two matrices of the same rank, *i.e.* matrices of geographic (measured in km) and genetic (F_{ST} coefficient) distances, in order to find out if both parameters are associated (Mantel, 1967). We computed F_{ST} coefficients amongst these 9 breeds with the Genepop on the Web software (http://genepop.curtin.edu.au, Rousset, 2008). The Mantel test was carried out with the *XLSTAT* statistical package (https://www.xlstat.com/en).