

Whole-genome sequencing of alpaca revealed variants in *KIT* gene potentially associated with the white coat phenotype

The association between variants on *ASIP*, *KIT* and *MC1R* genes and fleece color has been described for alpaca (Chandramohan et al., 2015; Feeley et al., 2016; Jones et al., 2019; Pallotti et al., 2020; Tan et al., 2022). However, the genetics behind the white coat phenotype in this species is still unknown. In this regard, variants on *KIT* were associated in alpaca with gray- and blue-eyed white phenotypes (Jones et al., 2019; Tan et al., 2022) owing to its important role in melanogenesis (Roskoski, 2005). Moreover, previous segregation analysis suggested that the inheritance of white color in this species was due to a single gene which is completely dominant over black and brown pigmentation (Valbonesi et al., 2011).

In the present study, the segregation of *KITLG*, *KIT* and *MITF* genes, known to be involved in the white coat phenotype in different domestic species (Linderholm & Larson, 2013), was analyzed to find variants associated with white coat in alpaca. Six Peruvian alpacas belonging to two test-cross families were studied: white huacaya male × pigmented suri female and white suri male × pigmented huacaya female which gave birth to one white suri and one pigmented huacaya, respectively (Table 1). The animals were raised at the experimental station of the INIA (the Peruvian National Institute for Agronomic Innovation) located in Quimsachata, Peru. Sampling of the animals and genomic analysis were performed as reported in Pallotti et al. (2023). Briefly, genomic DNA extracted from skin biopsies

underwent whole-genome sequencing on an Illumina NovaSeq 6000 System, with a 150 × 2 bp mode and an average sequencing depth of 30×. After quality control and alignment to the VicPac3 reference genome, the variants were called using the standard joint-call GATK (McKenna et al., 2010) pipeline. All of the variants were annotated using SNPeff (Cingolani et al., 2012) and filtered according to the segregation of the white coat phenotype.

No segregating variants for white coat color were identified in *KITLG* and *MITF*. On the contrary, two different *KIT* variants segregated in white animals (Table 1): two white alpacas, belonging to family 1, were heterozygous (*G/A*) for the c.35G>A (p.Arg12His) variant, while one white alpaca, belonging to family 2, was heterozygotes (*G/C*) for the c.982G>C (p.Val328Leu) variant. Conversely, all of the pigmented animals showed a homozygous *G/G* genotype. The protein functional domains predicted using INTERPROSCAN (Quevillon et al., 2005) suggested that the two mutations were in the N-terminal region of the signal peptide and in the extracellular region, which are essential for the binding of the appropriate ligand and consequent activation of *KIT* (Roskoski, 2005). In addition, such results may suggest a dominant inheritance for alpaca white color as proposed by previous segregation studies (Valbonesi et al., 2011).

Although these results must be validated on larger sample, our findings refine the current understanding

TABLE 1 Segregation and genotypes of *KIT* c.35G>A (p.Arg12His) and c.982G>C (p.Val328Leu) variants in alpacas families.

Family	Phenotype	c.35G>A (p.Arg12His)	c.982G>C (p.Val328Leu)
1	White huacaya (sire)	<i>G/A</i>	<i>G/G</i>
	Pigmented suri (dam)	<i>G/G</i>	<i>G/G</i>
	White suri (kid)	<i>G/A</i>	<i>G/G</i>
2	White suri (sire)	<i>G/G</i>	<i>G/C</i>
	Pigmented huacaya (dam)	<i>G/G</i>	<i>G/G</i>
	Pigmented huacaya (kid)	<i>G/G</i>	<i>G/G</i>

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of the association between gene variants and white color in alpaca and suggest new potential *KIT* variants as a starting point for further studies on the genetics of white color in alpaca.

AUTHOR CONTRIBUTIONS

Stefano Pallotti: Conceptualization; data curation; formal analysis; writing – original draft; writing – review and editing. **Marco Antonini:** Writing – review and editing. **Valerio Napolioni:** Conceptualization; data curation; formal analysis; supervision; writing – original draft; writing – review and editing. **Carlo Renieri:** Conceptualization; writing – original draft; writing – review and editing.

CONFLICT OF INTEREST STATEMENT





The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The FASTQ files from the de novo WGS of six alpacas used in the current study are available in the NCBI SRA (Sequence Read Archive) repository under the project no. PRJNA1020284.

ETHICS STATEMENT

Alpaca skin biopsies were obtained in 2008 according to the guidelines of the Animal Ethics Committee of the University of Camerino. All experimental protocols were approved by the aforementioned committee.

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