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BRIEF REPORT

The *KIT*:c.376G>A variant in German and Swiss alpacas (*Vicugna pacos*) with different coat colors

Classic grey is a pattern that is overlaid on the base color rather than a color, with areas of lighter shades on the face, neck, and legs. This trait is supposed to be controlled by two alleles at a single, incompletely dominant locus and has been associated with the *KIT proto-oncogene, receptor tyrosine kinase* (*KIT*) gene (OMIA 000209–30 538). The homozygous mutant *KIT* genotype is hypothesized to be embryonic lethal while the homozygous wild type genotype is known to display a normal base coat color (Munyard, 2011). Recent research revealed that the classic grey phenotype is caused by a single nucleotide substitution (c.376G>A) in the third exon of the *KIT* gene, leading to an amino acid substitution at position 126 (p.126Gly>Arg) (Jones et al., 2019). A solid white coat and blue irides characterize the blue-eyed white (BEW) phenotype (Jackling et al., 2014). It is non-lethal, but in general undesirable because it is associated with deafness (Gauly et al., 2005). Breeding records suggest that the BEW phenotype in alpacas is caused by a combination of the alleles involved in classic grey and in the tuxedo or piebald pattern (Munyard, 2011). So far, the genotypes in BEW alpacas reported for *KIT* c.376 (AG) have been consistent with this hypothesis (Jones et al., 2019).

In this study, the *KIT*:c.376G>A variant was genotyped in 246 alpacas with different coat colors from Germany and Switzerland (for genotyping method see Appendix S1). Collection of blood samples in Switzerland was approved by the Cantonal Committee for Animal Experiments (Canton of Bern; permit 71/19) and in Germany by the Veterinary Department of the Regional Council of Giessen (19 c 20 15 h 02 Gi 19/1 KTV 22/2020).

The genotype *AA* was not detected in any of the analyzed samples, confirming its presumed lethality (Jones et al., 2019; Munyard, 2011). All animals that were phenotyped as classic grey were heterozygous (*AG*). In accordance with previous studies (Jones et al., 2019; Munyard, 2011), we observed some so-called 'cryptic' grey cases among fawn, brown, black, and patterned alpacas (Figure 1a–c, animals at bottom pictures; Table S1). A completely unexpected finding was the determination of the *GG* genotype in one out of 10 BEW alpacas. This BEW alpaca (Figure 1d, upper picture) was also reported to be deaf. Repeated genotyping and Sanger sequencing of a second sample from the same animal confirmed the result.

The absence of the *KIT* c.376 A allele in an alpaca with proven BEW phenotype opens new theories on the underlying molecular genetic factors. One possibility is that, instead of the *KIT* c.376 A allele itself, a very close (linked) unknown variant is contributing to the BEW phenotype. In that case the BEW alpaca with *GG* found in this study could carry a rare recombination of the *KIT* c.376 G allele and this unknown other allele. However, the *KIT* c.376 A allele was never considered the only causal variant for BEW because the vast majority of alpacas with this allele present the classic grey genotype. Therefore, at least a second allele seems to be involved. It is also possible that the BEW phenotype has an oligo- or polygenetic basis with more than two variants contributing to it. Such an additive mode of inheritance of the BEW phenotype might also explain why only a proportion of BEW alpacas is bilaterally or unilaterally deaf (Gauly et al., 2005; Jost et al., 2020). At least one variant contributing to BEW may also be located outside of the *KIT* region in another gene known to be causal for pigment-associated deafness, e.g. *MITF* or *EDNRB* (Strain, 2015). In any case, the detection of (more) causal variants for the BEW phenotype will help to avoid breeding deaf alpacas.

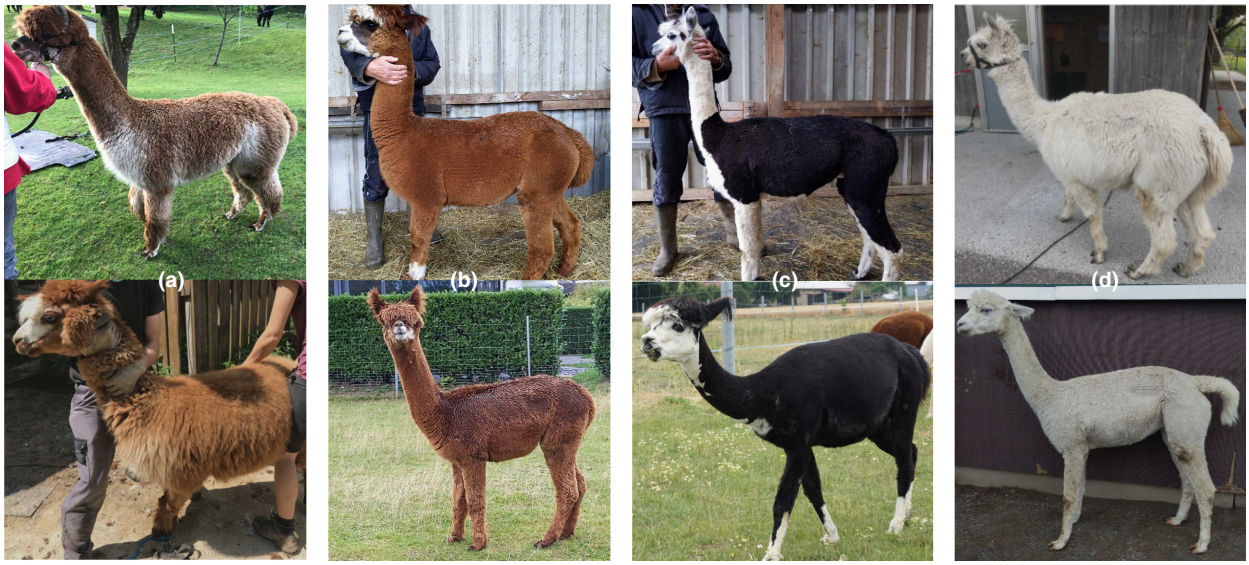

FIGURE 1 Pairs of alpacas with same coat color phenotype, but different *KIT*:c.376G>A genotypes (top: *GG*, bottom: *AG*). (a) Medium fawn, (b) medium brown, (c) black tuxedo, (d) blue-eyed white (BEW)

KEYWORDS

blue-eyed white, classic grey, cryptic grey, deafness, lethality

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

The data supporting the findings of this study can be found in Table S1 and Appendix S1.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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