Grey area

By Geoff Orbell

Genetics of greying and melanocytic neoplasia in grey horses.

Greying in horses involves loss of pigmentation of the hair bulbs and shafts, not the epidermis, which usually retains pigmentation. It has been known for centuries to be a heritable trait with autosomal dominance and has been selected for in multiple breeds where the grey phenotype predominates. It has also been known for some time that faster-greying horses have a higher incidence of melanocytic neoplasms (up to 80% by 15 years old), most commonly in glabrous skin around the perianal/perineal regions and ventral tailbase, which can become malignant. Initially these are predominantly benign melanocytomas (90%) but 66% progress to malignant melanomas within two to three years (Hollis, 2024).

In 2008 a mutation at intron 6 in the Syntaxin 17 (*STX17*) gene on chromosome 25 was identified in grey horses and has become known as the 'Grey' gene (Rosengren et al., 2008). However, on its own this did not explain the variability in the speed of greying between individual horses or predict the development of melanomas. Both *STX17* and the neighbouring gene *NR4A3* are upregulated with expression of the grey gene; therefore the phenotypic effects of the mutation may be the result of a combined effect.

In 2024 the same research group identified that there were actually three different alleles for this locus with G1 carrying one copy (wild type) of the duplicated sequence, G2 carrying two copies and G3 carrying three copies, and that the speed and degree of greying and melanoma development were a dosage effect (Andersson, 2024; Rubin et al., 2024).

The G1 allele was most common in horses with pigmented coats and the G3 allele was the most prevalent allele in more rapid-greying grey or white horses and associated with a higher incidence of melanomas than the G2 allele, which was associated with slower greying and low risk of melanoma development.

An individual horse could therefore have a minimum of two copies (G1/G1) and would maintain a fully pigmented coat with minimal risk for melanoma development or could have a maximum of six copies (G3/G3), which would result in rapid greying and a high incidence of melanomas. Other allele combinations could be somewhere in between, but the presence of at least one G3 allele is most clinically relevant followed by the total number of copies of the mutation. This is difficult to confirm given the relatively low prevalence of the G2 allele in studied populations to date and the current commercial test cannot distinguish between a (G1/G3) and a (G2/G2), both of which would have four copies of the mutation. However, as the G2 allele is much less common than the G1 or G3 alleles, a (G1/G3) heterozygote is more likely than a G2 homozygote.

The Gray Copy Number commercial test is available through the University of California, Davis, and is being used to identify the future melanoma risk in grey horses or foals from grey parents and predominantly grey breeds.

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TABLE 1. **Relationship between genotype and phenotype at the grey locus in horses**

GENOTYPE	SPEED OF GREYING	INCIDENCE OF MELANOMA
G1/G1	non-grey	low
G1/G2	slow	low
G2/G2*	intermediate?	low?
G1/G3	fast	high
G2/G3*	fast	high
G3/G3	very fast	very high

REPRODUCED WITH AUTHOR'S PERMISSION (ANDERSSON L, 2024).

More information can be found on the UC Davis website: **https://vgl.ucdavis.edu/test/gray_copy_number**.

Melanocytic neoplasia

Melanocytic neoplasia development in grey horses is intimately associated with the *STX17* (Grey) gene. One 2012 study (Sundström et al., 2012) revealed that neoplastic melanocytes in grey horse melanomas had up to nine copies of the grey gene mutation with higher copy numbers in more aggressive tumours. The additional copies have accumulated somatically during tumour development, suggesting that the copy number expansion acts as a driver mutation. Each copy of the grey gene mutation provides binding sites for microphthalmia-associated transcription factor (MITF), which regulates gene expression in melanocytes. For G3 alleles, this would provide binding sites for three MITF molecules, which helps explain overstimulation in melanocytes leading to exhaustion of melanin production. This in turn leads to greying and enhanced proliferation of melanocyte differentiation from stem cells, leading to melanoma development.

However, there may also be polygenetic influences, as melanomas in grey horses also exhibit constitutive activation of the ERK pathway seen in human melanomas and both the *STX17* (Grey) gene and the neighbouring gene *NR4A3* are upregulated with expression of the grey allele. *STX17* itself is a SNARE protein involved with autophagy, which could be an important

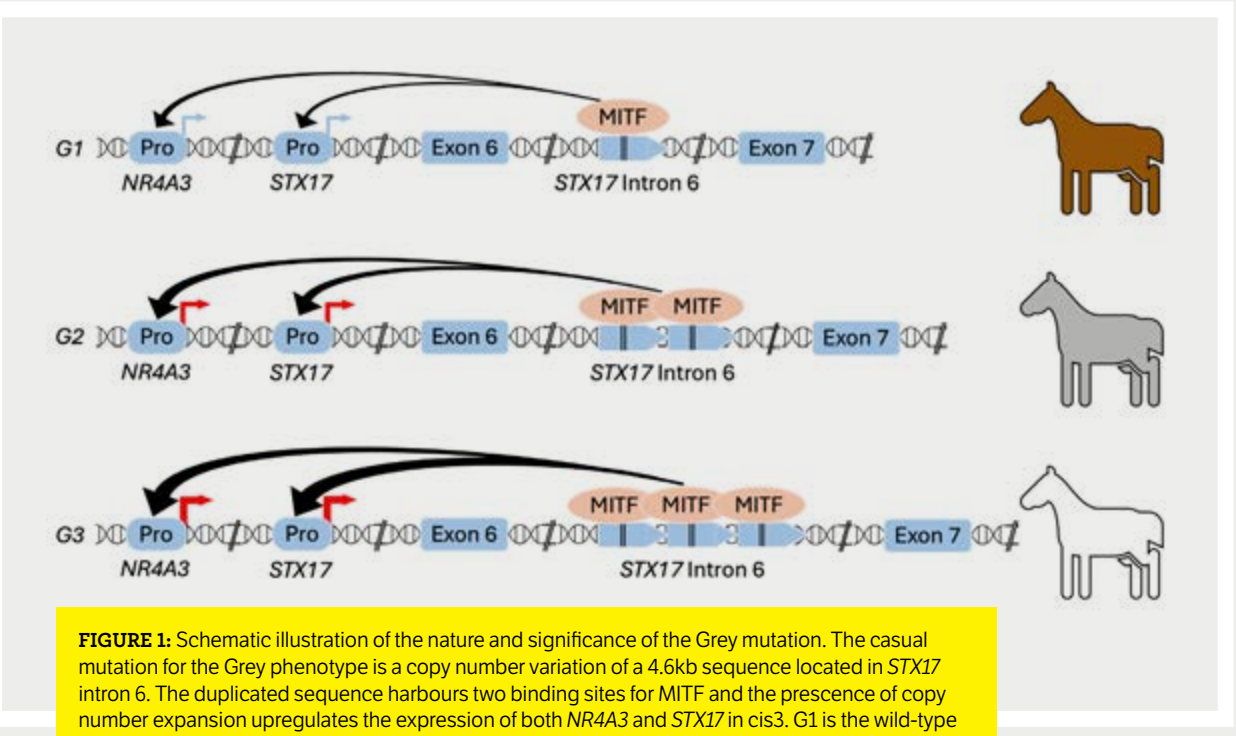

FIGURE 1: Schematic illustration of the nature and significance of the Grey mutation. The casual mutation for the Grey phenotype is a copy number variation of a 4.6kb sequence located in *STX17* intron 6. The duplicated sequence harbours two binding sites for MITF and the prescence of copy number expansion upregulates the expression of both *NR4A3* and *STX17* in cis3. G1 is the wild-type allele with a single copy of the 4.6kb sequence, G2 carries two copies and causes slow greying, and G3 carries three copies and causes fast greying. Pro promoter.
REPRODUCED WITH AUTHOR'S PERMISSION (RUBIN ET AL., 2024).

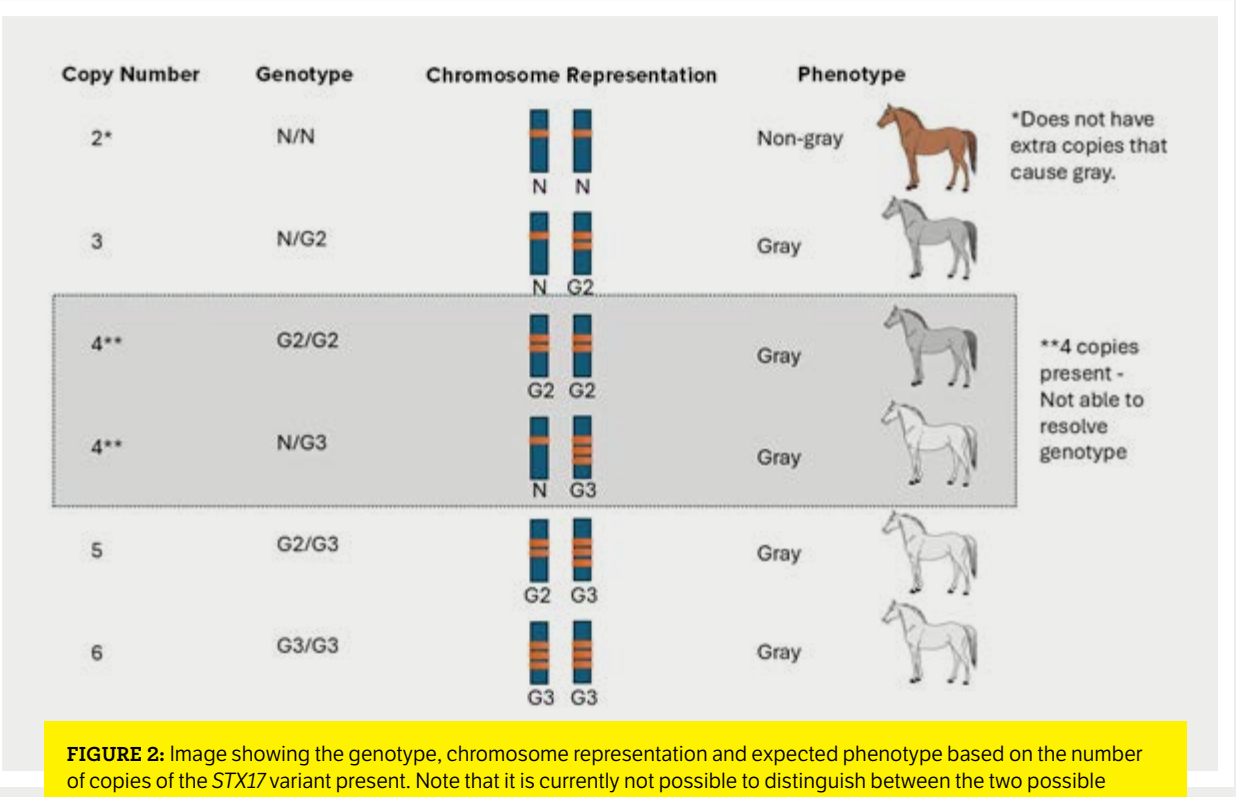

FIGURE 2: Image showing the genotype, chromosome representation and expected phenotype based on the number of copies of the *STX17* variant present. Note that it is currently not possible to distinguish between the two possible genotypes in horses that have four copies of Grey.
REPRODUCED WITH AUTHOR'S PERMISSION (UC DAVIS VETERINARY GENETICS LABORATORY [VGL], 2024) CAMPUS).

component of melanin homeostasis (Lee et al., 2022), whereas *NR4A3* has been associated with oncogenesis of some sarcomas in humans.

Grey horses that are phenotypically black at birth (homozygous a/a at the *Agouti* locus) are also at higher risk of developing malignant melanomas (Curik et al., 2013). Interestingly, melanomas in grey quarter horses are less common than in other breeds, which may relate to reduced incidence of the grey gene mutation (Teixeira et al., 2013). VS

Geoff Orbell is a veterinary pathologist at Awanui Veterinary.

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