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ESTIMATED FREQUENCIES OF MYOSTATIN MUTATIONS (DOUBLE MUSCLING) IN AUSTRALIAN TROPICALLY ADAPTED BEEF BREEDS

K.L. Moore[1], D.J. Johnston[1] and T. Grant[2]

[1] Animal Genetics Breeding Unit*, Armidale, NSW, 2351 Australia
[2] Queensland Department of Primary Industries, Toowoomba, QLD, 4350 Australia

SUMMARY

Myostatin mutations have been identified in many beef breeds and have been associated with increased carcase yields, reduced fat, increased calving difficulty, delayed puberty and reduced female fertility. This study aimed to estimate the allele frequencies of myostatin mutations in Australian tropically adapted beef breeds. Nine myostatin variants (viz. C313Y, D182N, E226X, E291X, F94L, NT419, NT821, Q204X and S105C) were assessed in Brahman (n=2,554), Droughtmaster (n=2,188), and Santa Gertrudis (n=904) cattle that were part of the Repronomics research project. Results showed the myostatin variants NT821 and F94L were segregating in the Droughtmaster and Santa Gertrudis breeds but not in the Brahman. In total, 17.4% and 26% of Droughtmaster and Santa Gertrudis animals carried at least one copy of a myostatin allele for either variant. The frequency of the NT821 allele was 0.08 and 0.12, and the F94L allele was 0.01 and 0.02, respectively, for Droughtmaster and Santa Gertrudis animals. Hardy-Weinberg equilibrium was observed for both NT821 and F94L in both breeds. However, there were fewer than expected two copy NT821 animals for both breeds. With no variants detected in Brahman, the myostatin variants likely originated from the *Bos Taurus* influence of the Droughtmaster and Santa Gertrudis breeds. The allele frequencies of NT821 in the Droughtmaster and Santa Gertrudis breeds, along with the literature reports of increased calving difficulties associated with NT821, indicate that breeders should monitor and manage the presence of the NT821 myostatin variant. Further research should be undertaken to quantify the size of the effect that having one and two copies of the mutation has across all economically important traits.

INTRODUCTION

The myostatin gene influences muscle production and is responsible for 'double muscling' in beef cattle (Fiems 2012; McPherron and Lee 1997). Research has identified many variants of the gene including C313Y, D182N, E226X, E291X, F94L, NT419, NT821, Q204X and S105C. The mutation variants are either deletions or substitutions, but all result in making the myostatin gene inactive (Dunner *et al*. 2003). In an extensive review, Fiems (2012) reported double-muscled animals had increased carcase yields with reduced fat but have also been linked to increased calving difficulty and reduced female fertility. However, limited studies have considered myostatin mutations in tropically adapted beef breeds. Vankan *et al*. (2010) reported that the F94L mutation was present in Australian Droughtmaster animals but not Brahman, and the NT821 mutation has been identified in Australian Santa Gertrudis animals (O'Rourke *et al*. 2012). Neither study estimated the breed allele frequencies of these myostatin variants. This study aimed to estimate the allele frequencies of myostatin mutations in Australian Brahman, Droughtmaster and Santa Gertrudis breeds.

MATERIALS AND METHODS

Animal Data. Myostatin genotypes were obtained for Brahman (n=2,554), Droughtmaster

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(n=2,188), and Santa Gertrudis (n=904) animals. Animals were genotyped on Neogen's GGP_TropBeef chip which included nine myostatin variants: C313Y, D182N, E226X, E291X, F94L, NT419, NT821, Q204X and S105C. Animals were born between 2015 and 2023 and were part of the Repronomics project, an extensive reference population targeting female reproduction traits and collecting economically important phenotypes from birth to slaughter (Johnston *et al.* 2017). DNA samples were collected on all project animals born and the dataset included calves that died at or shortly after birth. Brahman, Droughtmaster and Santa Gertrudis sires were selected to represent the wider breed, especially if the sire was influential and had limited female reproduction information available. There were 104, 83 and 41 sires represented in the dataset, respectively, for Brahman, Droughtmaster and Santa Gertrudis. Sires were mated to cows of the same breed, except that at the start of the Repronomics project some Droughtmaster and Santa Gertrudis sires at the Brian Pastures herd were mated to base Tropical Composite cows from the previous Beef CRC genetics project (Barwick *et al.* 2009). Myostatin genotypes were unavailable for the base cows at the start of the project, but approximately 50% of the sires were genotyped for myostatin. Female calves produced during the project were retained in the cow herd and were the dams of future generations. Cows were only culled if they failed to wean a calf.

Statistical analyses. Allele frequencies were calculated based on the number of animals with 0, 1 or 2 copies of the myostatin alleles. A chi-square test was used to test for Hardy-Weinberg equilibrium based on the allele frequencies calculated from the current study and tested against a critical value of 3.84, based on 1 degree of freedom and P<0.05.

RESULTS AND DISCUSSION

No myostatin variants were shown to be segregating in the Brahman, but both the NT821 and F94L myostatin variants were present in the Droughtmaster and Santa Gertrudis (Table 1). Of the Droughtmaster and Santa Gertrudis animals, 17.4% and 26% carried at least one myostatin allele from either variant (Table 2) with similar allele frequencies across males and females (results not shown). Myostatin variants C313Y, D182N, E226X, E291X, NT419 and S105C were shown not to be segregating in any breed, with all genotyped animals having no copies of these myostatin variants. However, one Droughtmaster sire was a heterozygote for Q204X, with 40 out of 66 genotyped progeny also being heterozygous for Q204X. No other animals in the dataset were found to have a copy of the Q204X mutation. Ryan *et al.* (2023) identified that the Q204X mutation was present in 27% of Irish Charolais and 8% of Limousins.

Table 1. Frequency of myostatin alleles for the NT821 and F94L myostatin variant in Brahman, Droughtmaster and Santa Gertrudis animals

	NT821					F94L				
Breed	**0**	**1**	**2**	**N animals**	**Freq(p)**	**0**	**1**	**2**	**N animals**	**Freq(p)**
Brahman	2,554	0	0	2,554	0.000	2,554	0	0	2,554	0.000
Droughtmaster	1,852	331	5	2,188	0.078	2,138	48	0	2,186	0.011
Santa Gertrudis	697	199	8	904	0.119	872	32	0	904	0.018

The estimated gene frequency of the NT821 mutation was 0.08 for Droughtmaster and 0.12 for Santa Gertrudis animals (Table 1). Animals with at least one NT821 allele were sired by 50 and 32 different sires, respectively, for Droughtmaster and Santa Gertrudis. Hardy-Weinberg equilibrium was tested, and for Droughtmaster (X^2=5.91) and Santa Gertrudis (X^2=2.23), the NT821 myostatin variant was not in Hardy-Weinberg equilibrium (critical value=3.84, P<0.05, df=1) for Droughtmaster, while the Santa Gertrudis subset did not depart significantly from Hardy-Weinberg equilibrium. For Droughtmaster, 13 homozygous NT821 animals were expected, and just five homozygotes were observed. A similar pattern was observed for Santa Gertrudis, with 13 homozygous NT821 animals expected and eight homozygotes observed. It is not yet understood why fewer homozygotes were observed; further research is required, but it may be due to embryo loss, myostatin resulting in a higher number of calf deaths due to dystocia (and calves were not found to obtain a DNA sample) or myostatin being linked to poor reproductive success (Repronomics cows that fail to wean a calf are culled).

Table 2. Number and (frequency) of myostatin genotypes for combinations of NT821 and F94L myostatin variants in Droughtmaster and Santa Gertrudis animals

		Droughtmaster					**Santa Gertrudis**		
		F94L					**F94L**		
		0	**1**	**2**			**0**	**1**	**2**
NT821	**0**	1,805 (0.826)	46 (0.021)	0 (0.000)	**NT821**	**0**	669 (0.740)	28 (0.031)	0 (0.000)
	1	328 (0.150)	2 (0.001)	0 (0.000)		**1**	195 (0.216)	4 (0.004)	0 (0.000)
	2	5 (0.002)	0 (0.000)	0 (0.000)		**2**	8 (0.009)	0 (0.000)	0 (0.000)

The frequency of the F94L allele was 0.01 for Droughtmaster and 0.02 for Santa Gertrudis animals (Table 1). Animals with at least one F94L allele were sired by 21 and 13 different sires, respectively, for Droughtmaster and Santa Gertrudis. Hardy-Weinberg equilibrium was tested, and for both Droughtmaster (X^2=0.26) and Santa Gertrudis (X^2=0.29), the F94L myostatin variant was in Hardy-Weinberg equilibrium (critical value=3.84, $P<0.05$, df=1).

The NT821 and F94L variants have been identified in many beef breeds (Dunner *et al*. 2003: Ryan *et al*. 2023), but in particular, the NT821 variant has been associated with Belgian Blue and F94L with Limousin breeds. A sizeable Irish study showed that 99% of purebred Limousins had at least one copy of F94L, and 99% of purebred Belgian Blue had at least one copy of NT821 (Ryan *et al*. 2023). The Irish study considered 12 *Bos Taurus* breeds and reported that although both variants were associated with calving difficulty, the impact was smaller for F94L and greatest for NT821. The effect of one and two copies of the NT821 mutation was an increase in calving difficulty scores of 0.13 and 0.37 for calves with myostatin and an increase of 0.05 and 1.30 for cows with myostatin, respectively. The same study also reported that F94L and NT821 resulted in heavier, more muscled and leaner carcases. Calves with one and two copies of the F94L mutation yielded 7.6kg and 20.6kg heavier carcases than zero copy animals, respectively, and 0.5 and 1.6 scores higher for conformation, respectively. One copy of F94L resulted in carcases 0.26 scores leaner than zero copy animals. However, two copies of F94L animals were shown to have fat scores 1.21 higher than zero copy animals, which is against the reported trend of leaner carcases. Calves with one and two copies of the NT821 mutation yielded 16.6kg and 23.3kg heavier carcases than zero copy animals, respectively, and 1.3 and 2.6 scores higher for conformation, respectively. Calves with one and two copies of the NT821 mutation were leaner than zero-copy animals by 0.98 and 3.85 fat scores. Although an association with reduced female reproduction has been reported (Fiems 2012), no studies have reported the impact of one or two copies of the mutation. These effects reported in the literature highlight the need for further research to fully understand the impact of these myostatin variants in Australian tropical beef breeds.

Droughtmaster and Santa Gertrudis were developed as composites with Brahman and *Bos Taurus* origins. In developing both composites, the predominant *Bos Taurus* breed was Shorthorn. Other breeds may also contribute to the genetic makeup of Droughtmaster and Santa Gertrudis from the grading-up process used in some herds to build numbers. Given that no myostatin variants were detected in the Brahman population of this study, it is probable that the NT821 and F94L variants detected originated from the *Bos Taurus* content of the composites. Both variants were detected in the Irish purebred Shorthorn populations, with 1% and 7% of Irish Shorthorns having one or more copies of the F94L and NT821 variants, respectively (Ryan et al., 2023). In the present study, Droughtmaster and Santa Gertrudis had 2% and 3% of animals with one or more copies of the F94L variant, respectively. The number of Droughtmaster and Santa Gertrudis animals with one or more copies of NT821 was much higher than that observed in Irish Shorthorns (and the other Irish breeds considered, apart from Belgian Blue), with 15% and 23% of animals having one or more copies of NT821, respectively. Selection for more muscled animals is likely to lead to increases in the frequency of NT821 over time, in the absence of considering the myostatin status of animals.

CONCLUSIONS

This study showed that none of the myostatin variants considered in this study were segregating in the Brahman population, but NT821 and F94L were present in the Droughtmaster and Santa Gertrudis populations. F94L was present at lower frequencies, and literature reports that it increases muscling but has minimal effect on calving difficulty. NT821 was segregating at higher frequencies and has been reported to increase muscling and calving difficulties. The increase in meat yield may be advantageous but needs to be balanced with the risk of calving difficulties and reduced female reproduction observed in other studies. As most research has been undertaken on *Bos Taurus* breeds, further research is required to investigate the impact of these myostatin mutations on the tropically adapted beef breeds in Australia. Droughtmaster and Santa Gertrudis breeders should monitor and manage the presence of the NT821 myostatin variant. To inform the potential impact on future breeding programs and genetic evaluation, further research will be undertaken to quantify the size of the effect that having one and two copies of the mutation has across all economically important traits.

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