Mutations Associated with Coat Colours in Swedish Goats

– a focus on *MC1R* and *TYRP1* genes

Bojelo Dube

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Bojelo Dube

Supervisor: **Anna Maria Johansson, SLU, Department of Animal Breeding and Genetics**
Assistant supervisor: Sofia Mikko, Department of Animal Breeding and Genetics
Examiner: Martin Johnsson, SLU, Department of Animal Breeding and Genetics

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Swedish University of Agricultural Sciences
Faculty of Veterinary Medicine and Animal Science
Department of Animal Breeding and Genetics

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Abstract

Since the 1920s, the number of goat farmers and the goat population in Sweden has declined, hence the need to actively find ways to conserve various Swedish breeds. This study contributes to genetic diversity research by identifying the possible *TYRP1* and *MC1R* mutations that may contribute to coat colour in Swedish goat breeds. To conduct this study, sanger sequencing using the BigDye Direct Cycle kit was used and the sequences were analyzed and visualised for variations on Geneious Prime software. It was observed that the *TYRP1* gene, which was previously studied in other mutations found in other breeds, did not explain coat colour in Swedish goats. However, results indicate that two variants were found outside exon 8 and in the 3' downstream sequence, suggesting a possible linkage disequilibrium with another gene or various effects from the regulatory regions. An *MC1R* missense mutation, c.676A>G, causing an amino acid substitution p.K226E associated with red hair, was identified, revealing a high allele frequency for brown and white coloured goats. Previously reported mutations (p.F250V, p.C267W, p.61A) were not present, except in Goinge goats. The results highlight the genetic variation present in Swedish goats and recommends implementation of conservation plans for at-risk breeds to protect their genetic diversity.

Keywords: coat colour, mutation, *TYRP1, MC1R, genotype*

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Abbreviations

ASIP:	*Agouti Signaling Protein*
CNV:	Copy Number variants
DNA:	Deoxyribonucleic Acid
EDRNA:	*Endothelin Receptor type A*
KIT:	KIT Proto-Oncogene, Receptor Tyrosine Kinase
MC1R:	Melanocortin 1 Receptor
SNP:	Single Nucleotide Polymorphism
TYRP1:	Tyrosinase-Related Protein 1
αMSH:	Alpha-Melanocyte-Stimulating Hormone
NCBI	National Centre for Biotechnology Information
PCR	Polymerase Chain Reaction
NR3C2	Nuclear Receptor Subfamily 3 Group C Member 2
ARHGAP10	Rho GTPase Activating Protein 10
PRMT9	Protein Arginine Methyltransferase 9
TMEM184C	Transmembrane Protein 184C

1. Background

Introduction to Pigmentation Genetics

The study of pigmentation genetics has been carried out since the beginning of the 20th century with research on mice colours and has charted the progress in understanding genetic control of the phenotype. Searle (1968, cited in Sponenberg, 1994) explains that mammalian coat colour relies on the skin and hair melanin presence or absence which unravels the underlying genetic mechanisms behind white colouration in goats. In the same study he discusses that white spotting and pigment dilution is due to lack of melanocytes and reduced production of melanin by melanocytes respectively. Commonly in goats the wild type of colour from Bezoar-Middle East is viewed as the standard type (Sponenberg, 1994). Colours and patterns can be described or expressed by different alleles and loci some common colours include completely white, red/brown, grey and black with various shades of the colours. Goats also exhibit various facial and body patterns such as facial stripes, dorsal stripe, belt- like, patches of colour on the legs. According to Adelsteinsson, 1994, Adelsteinsson, (1970) hypothesised that colour pattern was due to Agouti locus allele with A^{wt} dominant to all the others and allele A^{a} the bottom recessive allele and there were intermediate codominant alleles to tan or white over black or brown coats.

Recently technological progress in equipment, molecular biology and genomic methods has made it easier for researchers to investigate the complex processes that underlie pigmentary phenotypes in several species such as horses, sheep, pigs and mice as well as goats. Livestock coat colour has been valuable to communities, primarily as a preference, has been linked to other production traits such as growth rate, fertility and milk yield and in some species like cattle, the coat colour and patterns are distinctive and are trademarks for specific breeds (Klungland et al., 2000).

In mammals, pigmentation is caused by the presence of either one of the two main melanin types from the enzymatic oxidation of the tyrosine amino acid (Fontanesi et al., 2013). These two types are eumelanins (black/brown pigments) and pheomelanin (yellow/red pigments). These melanins are produced by melanocytes only one at a time. When the pituitary gland releases alpha melanocyte stimulating hormone (αMSH), the melanocytes surface receptors for this hormone bind and trigger processes that activate adenylate cyclase, eumelanin is created. Melanocytes create pheomelanin in the absence of a signal that relies on αMSH and receptors. The action of αMSH receptors determines whether eumelanogenesis or pheomelanogensis occurs (Fontanesi et al., 2013). A network of tissue interactions and cellular signalling pathways directs the functions of the pigment cell at every level allowing pigment phenotype development and differentiation (Lamoureux et al., 2010) with approximately eighty genes affecting coat colour. Among the commonly studied genes in mammals are the Tyrosinase-related Protein (*TYRP1*), Melanogenesis associated Transcription Factor (*MITF*), and *KIT* ligand (*KITLG*) genes. These could directly or indirectly affect the production of two types of pigments, pheomelanin (yellow/red or white colour) and eumelanin (dark colour) in mammals. It is important to note that the two main loci that affect the level of eumelanin and pheomelanin levels are the *Extension* and *Agouti*. Playing an important role at the extension locus is the melanocortin 1 receptor *(MC1R*) gene and Agouti signalling protein (*ASIP*) which is encoded at the Agouti locus and acts as the antagonist to *MC1R*.

Introduction to Swedish goats

Traditionally, goat production in Sweden has not been very significant. While most small goat farmers in Sweden primarily keep goats as hobby farmers or to conserve certain breeds, some explore the retail potential of goat skins as an economic enterprise. It has been observed that in Sweden, reared goats are also

kept for their milk and meat. Over the years since the 1920s there has been a decline in the Swedish goat numbers as well as farmers with the number estimated to be about 20 000 goats belonging to about 100 farmers. (SVA 2024) Phenotypically the goats have different colours within breeds, and appearance not significant to a particular breed ranging from completely white, brown or black or a combination of these colours. The coat hair can also be short, long or curly. There are currently four local breeds: Swedish landrace, Göinget, Jämtget and Lappget.

Importance of coat colour genetics

Agricultural adaptation to future challenges is imperative and this means conservation of local breeds by promoting sustainable use and by conducting research to add more information to the existing databases. The phenotypic and genomic information of the Swedish goat population can lead to the recognition of the breeds as well as preservation of Swedish cultural background and community heritage. It is important to study this trait because previous research has shown that coat colour genetic mutations have pleiotropic effects with a few leading to negative effects such as skin, eye, ear disorders and disturbed reproduction (Linderholm et al., 2013), behavioural traits in some species: horses (Brunberg et al., 2013), sheep (Pascual- Alonso et al., 2014) and this can lead to improved welfare and production. Coat colour as a trait is also relevant to owners who include it in their selection goals according to their market needs.

Objective

It is important to explore Swedish goats' genetic diversity, particularly by examining certain traits, therefore this study aims to investigate mutations associated with coat colour in *MC1R* and *TYRP1* genes. These two genes have

been well documented for their influence on coat colour and were consequently chosen for this study because of their involvement in the most prevalent colours within the breeds.

2. Literature Review:

Genetic Mutations Associated with the Trait:

MC1R

In a study by Fontanesi et al., 2009, the results showed that the *MC1R* gene can influence eumelanin and pheomelanin phenotypes in goats of six different breeds, showing different coat colours. Five single nucleotide polymorphisms (SNPs) were identified, including one nonsense mutation (p.Q225X), three missense mutations (p.A81V, p.F250V, and p.C267W), and one silent mutation. The p.Q225X mutation could result in a shorter and potentially functionally altered *MC1R* protein. These mutations in the *MC1R* gene were associated with black and red coat colour however not in all breeds which shows that other factors may be important in coat colour determination. Of the sequenced at least one (1) white goat was homozygous for the p.Q225X mutation and one (1) other for the other allele, where else the others tested were heterozygous and this determination shows epistatic effects with the Agouti locus. Of the 93 Girgentana goats with red spots, 3 were heterozygous and 90 were homozygous for the nonsense mutation. All black Murciano-Granadina goats were either homozygous or heterozygous for the mutated allele of the c.801C>G SNP, which causes a cysteine to tryptophan change in a well conserved position.

Chen et al., 2019, in a study of expression of *MC1R* gene in Liaoning Cashmere goats with different coat colours, through q-PCR showed that this gene expression level was higher in the black coat-coloured goats than the white and that the protein was more abundant in them and *MC1R* was found in the inner root sheath (IRS) and the hair shaft cells (HS). In GWAS analysis of genomic architecture of pigmentation of 391 black and 138 brown Murciano-Granadina goats, sequences revealed a single G peak at position 748 in nine black and 10 brown goats, while only three brown goats showed a double GT peak at this location Guan et al.,

2021). In the same study it was confirmed that coat colour of Murciano-Granadina goats is explained by *MC1R* c.801C>G genotype (all brown goats are CC and all black goats are either GG or GC), which is the same as the results by Fontanesi et al.,(2009). In addition to the previously identified mutations as above, another interesting *MC1R* mutation found in Mongolian goats is a rare missense mutation c.770T>A, which may be linked to the black coat colour, even though effects could not be positively determined due to the presence of only six complete black goats which were heterozygous in this mutation (Ganbold et al., 2019).

ASIP

The agouti signalling protein, known as the antagonist of the melanocyte-stimulating hormone receptor *(MC1R)* is located at the agouti locus. This gene has also been identified to regulate pigmentation in mice (Bennett et al., 2003), sheep (Norris et al., 2007), cattle, as well as goats. When sequencing goats, *ASIP* gene three missense mutations were detected: p.A96G, p.C126G, and p.V128G in exon 4 (Fontanesi et al, 2009b, Badaoui et al., 2011).

However, according to the same study by Fontanesi et al, (2009b) the allele distribution and genotype frequencies, of these mutations are not associated or not completely associated with coat colour in the investigated goat breeds. For example, mutation leading to an amino acid substitution at position 96 (p.A96G) in Maltese goats was found to be heterozygous and the genotype frequency was low indicating that the black features may be due to another gene. The p.C126G mutation does not differentiate coat colour among the investigated breeds, and it removes the highly conserved cysteine residue and may affect the stabilisation of the structure of the protein which may suggest that other genes play a role in the coding for coat colour. The p.V128G is suggested to play a role in the dark coat colour associated with Murciano-Granadina goats. Some breeds like Girgentana and Saanen goats showed at least two additional copies of the *ASIP* gene compared to the reference sample which suggests that copy number variation may be associated with their white colour

TYRP1

Two non-synonymous variants (p.I478T and p.G496D) were identified and may impact *TYRP1* protein function with ^{496}Asp allele dominant over the wildtype allele based on genotype distribution in copper necked goats (Becker et al., 2015). Hence brown coat colour is not due to a single recessive loss of function allele and this was further verified by targeted matings between black wildtype-homozygous ^{496}Gly Nera Verzasca goats and a copper necked buck homozygous ^{496}Asp which produced offsprings heterozygous ^{496}Gly/^{496}Asp and all the kids showed a uniform solid brown colour (Dietrich et al., 2015). According to the same study this could be due to c.1487G>A variant causing the dominance and this same mechanism can be observed in pigs and it affects the transmembrane of *TYRP1* enzyme.

EDNRA

In a GWAS analysis, a significant association signal with EDNRA was revealed on chromosome 17 in Boer goats imported to Switzerland with 3 different colour groups: spotted, solid and traditional coloured. Whole genome sequencing showed a 1 Mb duplication on chromosome 17 associated with Boer goat coat colour and it contained part of the *NR3C2* gene and complete *ARHGAP10, PRMT9, TMEM184C,* and EDNRA genes. A missense mutation was identified in *EDNRA* (p. Tyr129His) within the duplicated region and further genotyping revealed that increasing CNV copy number correlated with decreased pigmentation in Boer goats (Menzi., 2016).

KIT

Through visual inspection of short read alignments two distinct copy number variants (CNVs) were identified in the Pak Angora -white and Barbari-white spotted goats (Henkel, 2019). This was a triplication of approximately 100 kb downstream of *KIT* in the Pak Angora, while in the Barbari breed, a duplication of the same region was detected including a complex rearrangement involving the

insertion of a genome segment from the 5'-flanking sequence of the *RASSF6* gene, along with a deletion in its central part. Both CNVs started approximately 63 kb downstream of *KIT*, covering a region of approximately 100 kb without known coding DNA.

Coat colour mutations associations with other traits

Heat stress

The coat serves as a primary protective barrier and its characteristics such as colour, depth and length influence the adaptation to various environments. Many goat breeds generally are resilient and well adapted to extreme climates due to their genetic and physiological characteristics, however some breeds such as the Saanen are susceptible to heat stress (Lima et al., 2022). The Swedish local goats have been studied before to also show that they acclimatise to the heat challenge (Hartman et al., 2020). Different coat colours affect the thermoregulatory response, with breeds with light and white coats reflecting 50-60% of direct solar radiation therefore lower heat absorption however the darker colours absorb more heat. This suggests that dark coat-coloured goats adapt to the cold environment better as they warm up faster, while lighter coloured goats have more advantage in warmer environments (Arenas Baez et al., 2023).

In addition, white-coated animals had lower physiological responses and cortisol values which are associated with skin temperature when compared to the black and brown-coated goats (Venkatesh et al., 2023). In the same study, black and brown goats had a *MC1R* genetic variation c.332C>G along with haplotypes CCGG, TCGG, and CCTC that were associated with reduced levels of cortisol, skin and rectal temperature in white goats. Further research is needed to evaluate this colour trait with regards to other fur characteristics.

Productivity

Studies have shown that the colour of the coat has an impact on reproductive characteristics and certain productive features, due to a preference for specific colour patterns in breeding based on perceived reproductive advantages and market value (Mia et al., 2018).

Light- coloured bucks exhibit higher quality semen traits such as progressive motility, concentration and viability which demonstrates resilience to heat stress compared to the darker coated bucks. Additionally, according to (El-Sherbiny et al., 2023) this could be due to the noted increased heat sensitivity of the dark coat bucks hence this can affect the reproductive performance patterns and testicular functions negatively.

In the study of association of coat colour and various productive and reproductive traits in Black Bengal breed, black goats have been shown to outperform the other colours when it comes to birth weight, age at puberty, daily weight gain, milk production, lactation length, age at first birth, and litter size (Mia et al., 2018). The study suggests that certain genetic variations and environmental adaptations that determine coat color could also play roles in metabolic pathways that impact production characteristics. Nevertheless, there are some contradicting studies indicating that this might not be true for all breeds for example coat colour of the Tunisian local goats was studied not to have a significant effect on body weight. (Mabrouk et al., 2010).

Animal Behaviour

There are common behavioural perceptions among animal owners about animals of certain colours. For example, there is a recurring belief that chestnut horses are likely to display negative behaviours. In a study investigating if horses (Icelandic

silver horses) carrying a mutation p.R618C in PMEL show stronger fear reactions than horses without the mutation, it was observed that silver horses were noted to be more cautious when entering the arena even before it became associated with a fear stimulus (Brunberg et al. 2013). Finn et al., (2006), asserts that some significant differences in bay and chestnut horses do exist, but however, "no evidence was found to support that chestnut horses are more likely than bay horses to display behaviours often associated with training difficulties".

In a separate study investigating the correlation of coat colour and behaviour in Australian Labrador retrievers, the only behaviour trait significantly associated with coat colour was familiar dog aggression with yellow Labradors, which showed a higher score for familiar dog aggression compared to both black and chocolate Labradors.

Furthermore, the influence of *TYRP1* genotypes on dog trainability was found to depend on the allele dosage meaning that dogs with a homozygous brown allele were less trainable than the ones without the allele. *MC1R* variant dogs with homozygous or heterozygous dominant E alleles exhibited a higher score of familiar aggression (van Rooy et al., 2019). This suggests that the presence of specific alleles of the *TYRP1* gene, rather than the actual coat colour, influences trainability, just as black heterozygous dogs for the brown allele showed reduced trainability (van Rooy et al., 2019). This case and similar studies suggest that it would be important to investigate similar behavioural patterns in other animal species such as Swedish goats.

3. Materials & Methods

Animal sampling and DNA extraction

The biological material collected for this study consisted of DNA extracted from nasal swabs and blood samples of 97 animals, using methods detailed by (Johansson et al., 2023). The selection of goats was done based on diverse coat colours and their relativity to each other, as well as their representation across breeds, herds and location. The goats included originated from 8 different farms and geographical locations spread across Sweden representing the recognised breeds as follows: 79 Swedish landrace (svensk lantras), 3 mixed breed jämtget-svensk lantrasget, 5 Jämtget, 6 Lappget, and 4 Göingeget. The goats' samples were from farmers who had provided pictures as well as descriptions. Goat coat colour was visually observed, described based on the provided pictures. Given the high variability of the coat colour patterns four major groups of colours were created and included individuals which displayed the colours as follows:

Black: solid black, black-white variegated mostly dark, black grey/ white

Brown: light brown, brown-white, brown/grey/black, nougat brown-variegated, brown-red, dark brown/white, brown-black, brown, beige, dark brown, white brown

Grey: Silver grey, dark grey, grey, grey-brown, white-grey variegated, white /silver grey, white with small grey spots

White: white

Table 1 below shows colour variation among the different goat breeds

Table 1. The distribution of coat colour groups according to the breeds

BREED	White	Brown	Black	Grey	Wild Type coloured	Grand Total

Svensk lantrasget	25	34	11	8	1	79
Lappget	2	1	1	2	-	6
Jämtget	-	3	2		-	5
Göingeget	-	-	2	2	-	4
Mixed-race jämtget-svensk lantrasget	-	1	1	1	-	3
Grand Total	**27**	**39**	**17**	**13**	**1**	**97**

Primer preparation

Two primer pair sequences designed to cover the whole coding region of *MC1R* gene were obtained from Guan et al., 2021. For amplification and sequencing of *TYRP1* exon 8 one pair of designed primers was obtained from Henkel et al., 2021. The details of both primers are as in Appendix table A1. Primer pairs for *MC1R* have been confirmed to amplify the target area in a previous trial study carried out on four Swedish landrace goats DNA. For the *TYRP1* primer pair only, a touchdown PCR was performed in a thermal cycler. For this procedure the AmpliTaq Gold Kit® from Applied Biosystem was used. The master mix was prepared for each tube as: 1µl-10x PCR Buffer I, 1µl-25 $MgCl_2$,0.2µl -dNTP mix (10 mM), 0.2µl-10µM forward Primer fwd,0.2µl-10µM reverse Primer, 0.05µl-Enzyme,6.35µl-MQ-H2O thoroughly mixed and finally the template DNA was added to the tubes. The thermal cycler was set to the following conditions:

Table 2. Touchdown PCR Thermal cycler conditions

No. of Cycles	Action	Time	°C
1x	Enzyme activation & denaturation	10 min	95°
20x	DNA denaturation	15s	95°
	Primer annealing	30s	60°
	Extension	1min	72°
20x	DNA denaturation	15s	95°
	Primer annealing	30s	55°
	Extension	1 min	72°
1x	Final extension	5min	72°
1x	Hold	infinity	15°

products were subjected to quality checks through 1% agarose gel electrophoresis to verify that *TYRP1* primers had amplified the target region and the product was approximately the right size,413bp.

DNA Amplification, Sequencing, and Sequence Analysis

The genotype was determined using the methodology outlined in (Johansson et al., 2023). Each DNA sample was aliquoted to a concentration of 4 ng/µl. The following procedures were done for all the three primer pairs respectively. A polymerase chain reaction using the BigDye Direct sequencing kit was run and this contained the designed primers mixture at 0.8 µM of each primer aliquoted at 1.5µL per sample, along with 5.0 µL of BigDye Direct PCR Master Mix, and 2.5 µL of deionized water and 1.0 µL of DNA. For this reaction, enzyme activation and denaturation phase at 96°C for 5 minutes, followed by 35 amplification cycles DNA denaturation at 94°C for 30 seconds, primer annealing 62°C for 45 seconds, and extension at 68°C for 45 seconds. A final extension step at 72°C for 2 minutes concluded the PCR.

The cycle sequencing was performed using 2.0 µL of BigDye Direct Sequencing Master Mix and 1.0 µL of BigDye Direct M13 forward or reverse primer tail respectively together with 10 µL from the first PCR reaction making a solution of 13µL. After spinning the plates briefly, the reactions were run in the thermal cycler (Applied Biosystems™ ProFlex™ PCR System) at 37°C for 15 minutes, 80°C for 2 minutes, with an initial denaturation phase at 96°C for 1 minute, followed by 25 cycles at 96°C for 10 seconds, 50°C for 5 seconds, and 60°C for 4 minutes and cooled down to 4°C. The products were then purified using the BigDye XTerminator Purification Kit for each well, using a 45µL SAM™

solution and 10μLXTerminator®. The reaction plates were vortexed for 20 minutes at 2500 rpm and spun in the centrifuge 1000x g for 2 minutes. Lastly, a capillary electrophoresis was conducted in the Applied Biosystems® 3500xL Genetic Analyzer to sequence the DNA.

Data Analysis

The sequence data collected from the Genetic Analyzer was first edited by trimming low-quality regions and then assembled and aligned to enable the comparison of the sequences. Variants detection was also performed by comparing the sequences with the goat *MC1R* gene Accession: FM212940.1 and for *TYRP1* Accession: NC_030815 respectively as reference sequences obtained from the NCBI genome browser. The amino acid coordinates for the variants were identified based on where they occur in the reference protein sequence. These sequences were visualised and analysed using Geneious Prime software.

4. Results

This chapter presents the findings of the investigations aimed at identifying variants in *TYRP1* and *MC1R* that could potentially influence goat coat pigmentation.

TYRP1 Variants

As seen in Table 3 below, The the sample sequences were inspected for variations and two positions were identified in the non-coding region specifically c.*46A>C and one 32 bases away in the 3' downstream sequence, c.*78T>C. In the Lappget breed goats of this study, none carried any of the two variants. The Jämtget and the Blandras jämtget-svensk lantrasget displayed the unmutated genotype and heterozygous for both variants. The Svensk lantrasget breed had the highest number of animals sequenced and showed the greatest genetic diversity with 48 homozygous AA and TT at both positions, 20 heterozygous AC and TC while only 6 were homozygous for both the mutant genotype. The variant genotypes were present in homozygous and heterozygous state for both positions as well unmutant in the Goingeget. The distribution of the genotypes is as seen on Table 2 below. The previously studied nonsynonymous mutations in *TYRP1* p.G496D p.I478T (Becker et al., 2014) genotypes were not found in the Swedish goats.

Table 3. *TYRP1* genotype distribution by colour and breed

	c.*46A>C			c.*78T>C		
Colour Group	**AA**	**AC**	**CC**	**TT**	**TC**	**CC**
Brown	21	10	2	21	10	2
White	17	11	0	17	11	0
Grey	8	3	1	8	3	1
Black	9	3	2	9	3	2
W type	1	0	0	1	0	0
Total	**56**	**27**	**5**	**56**	**27**	**5**
Breeds						
Swedish landrace	46	21	4	46	21	4
Goinget	2	1	1	2	1	1
Jämt goat	1	4	0	1	4	0
Lapp goat	6	0	0	6	0	0
Blandras	1	1	0	1	1	0
Total	**56**	**27**	**5**	**56**	**27**	**5**

As the goats had various patterns it was also interesting to look at them, dorsal striped (fourteen), grey/black spots on face or body (eight), variegated (four), striped face (8) and white belt like around belly (four) as shown in Appendix table A3. A combination of the genotype variants at the two loci was used to form haplotypes. For instance, the AATT haplotype was more common with a dorsal stripe pattern, while the ACTC haplotype is linked to badger-like or two stripes on the face. Overall the variegated pattern and white belt-like marking less frequent in the patterned population.

Out of the 97 samples selected for testing, 63 were successfully genotyped, 28 showed sequencing errors, or either the reverse or forward sequence was suitable for use, as shown in Appendix Table A10 for further details.

MC1R Variants

This study aimed to investigate mutations related to coat color in the MC1R gene among different goat color groups in Swedish breeds. The analysis focused on variants that showed genotype distributions across both color groups and breeds. The following table (Table 4) illustrates the genotype distributions. Brown and white goats exhibit higher frequencies of the variant GG genotype in c.676A>G, while the grey group has the lowest frequency. The allele frequencies indicated a higher prevalence of the G allele in c.676A>G, with a frequency of 0.62, compared with the wild-type A allele (Table A8). The brown group exhibited a relatively balanced distribution of genotypes, with the highest frequency for the AG genotype. The white group also displayed a similar distribution pattern, while the gray and black groups exhibited lower diversity in genotype frequencies. Three other SNPS c.748T>G, C.801C>G and c.183C>T, were predominantly wildtype genotypes TT, CC respectively across all colours and breeds with minimal variation observed in the black and grey Göinge breed. The Swedish Landrace breed demonstrated the highest genetic homogeneity, particularly for c.748T>G p.F250V and C.801C>G p.C267W., which may be attributed tothethe larger sample size in this group. In contrast, the Goinget breed exhibited greater genetic diversity than all the other breeds in the study. The Jämt goat breed displays a high frequency of the AA genotype for c.676A>G and TT genotype for

c.748T>G p.F250V, indicating limited genetic variation. The Lapp goat breed showed a relatively balanced distribution of the AA and AG genotypes for c.676A>G.

The allele frequencies shown in Appendix Table A7 indicated a higher prevalence of the G allele in c.676A>G, with a frequency of 0.62, compared to the A allele. The T allele was predominant in c.748G>T p.F250V and c.183C>T, with frequencies of 0.98 each. The C allele was predominant in c.801C>G p.C267W, with a frequency of 0.98. One previously published variant (Fontanesi et al., 2009), the nonsense mutation c.673C>T (p.Q225X) which creates a stop codon at position 225 in the protein sequence and affect the functions of *MC1R* by possibly causing a frame shift or deletion that leads to continuous production of pheomelanin, resulting in a red coat colour was not found in all the samples.

Table 4. *MC1R* Distribution of genotypes by colour and breed

Colour Group	**c.676A>G p.K226E**			**c.748T>G p.F250V**			**C.801C>G p.C267W**			**c.183C>T,**		
	AA	**AG**	**GG**	**TT**	**TG**	**GG**	**CC**	**CG**	**GG**	**CC**	**CT**	**TT**
Brown	8	15	12	36	-	-	36	-	-	39	-	-
White	1	10	13	25	-	-	25	-	-	23	-	-
Grey	3	6	2	11	-	1	11	-	1	11	1	2
Black	4	5	10	15	1	-	15	1	-	16	-	-
Total	**16**	**36**	**37**	**87**	**1**	**1**	**87**	**1**	**1**	**89**	**1**	**2**
Breeds												
Swedish landrace	7	30	37	74	-	-	74	-	-	74	-	-
Goinge goat	2	1	-	1	1	1	1	1	1	1	1	2
Jämt goat	4	-	-	4	-	-	4	-	-	5	-	-
Lapp goat	2	3	-	5	-	-	5	-	-	6	-	-

Blandras	1	2	-	3	-	-	3	-	-	3	-	-
Total	**16**	**36**	**37**	**87**	**1**	**1**	**87**	**1**	**1**	**89**	**1**	**2**

When looking at the patterns and the missense mutations, c.676A>G of *MC1R* as shown in Table 3 above the genotypes inherited together as AA could be linked to black and white markings and black face masks, while AG dorsal stripes 7 whereas the variant GG was more frequent with the dorsal striped goats (Appendix Table A5).

Out of 95 and 94 samples, 66 and 54 exhibited good quality sequences for the first part and second towards the end of coding region of *MC1R* respectively. This is a still substantial for the efforts. There were some sequences that were analysed even though they sequenced partially. Further details in Appendix Table A10.

5. Discussion

This study was undertaken to identify the genetic mutations responsible for different coat colours in Swedish goats, potentially caused by tyrosinase-related protein 1 and melanocortin receptor 1. Several *TYRP1* and *MC1R* gene variants have been identified in various breeds of goat, highlighting the significant genetic diversity. The discussion below considers past research on colour coat studies in relation to this study. In addition to highlighting observed phenomena, this chapter also highlights the strengths and limitations of the study before concluded with future concerns.

Mutations in *TYRP1*

The sequence visualisation and comparison of *TYRP1* gene samples comparison showed two novel SNPs both found outside the coding area in the 3'downstream sequence. The influence of other genetic factors specific to the Swedish goat population are suggested by the discovery of novel *TYRP1* polymorphisms, alongside the absence of certain known mutations, p.G496D and p.I478T. The presence of SNPs outside the coding area could be due to influence from with an unknown gene or regulatory region. For example (Cheli et al.,2010) reported that transcriptional regulation of *TYRP1* by MITF (Microphthalmia-associated transcription factor), binding to *TYRP1* promoter regions can enhance its expression.This also raises the possibility that even though the SNP is not in *TYRP1*, it might be inherited together with a variant in *TYRP1* due to its physical proximity to the chromosome or the strongest association with another causal variant outside the gene.

The revelation of nonprotein-coding mutations such as c.*46A>C and c.*78T>C is a well-documented occurrence in genetic research as supported in a study by Li et al., (2022) two novel mutations were found in tyrosinase gene -89T>G, located at the core of the initiator of the *TYR* promoter and c.47C>A. In their instance, one

change affects how the gene is turned on, while the other affects how the *TYR* protein is made; consequently, these variations may impair or lead to a complete loss of function of *TYRP1* in the identified mutations. In the goats investigated a short distance of 46bp and 78bp away from the coding area is found. This outcome is contrary to that of Becker et al. (2014), who in a GWAS found the strongest genetic association to the *TYRP1* brown locus approximately 2 Mb away from the *TYRP1* gene. Therefore, there could be a possibility of reference bias, where the reference sequence does not capture the genetic diversity of the population under study. In the future, whole genome sequencing and functional assays can confirm the biological relevance of these variants. The mutations c.*46A>C and another 32 bases T>C away in the 3' untranslated downstream sequence may not necessarily justify colour as they are present in different colours and across different breeds. These polymorphisms were also in the heterozygous and homozygous states across the samples, as shown in Table 3 above which shows genetic diversity but also that they may not directly influence colour. The frequency for wildtype alleles for both variants (Appendix table A7) is higher which indicates that it is conserved and has a role despite the environmental or genetic variations that happen over time.

The previously studied mutations, c.1433T>C (p. I478T and c.1487G>A (p. G496D) (Becker et al.,2014) known to change parts of the transmembrane domain of the *TYRP1* protein which might affect normal anchoring were not found. All the samples were homozygous TT and GG respectively. This indicates that these mutations were absent in our samples, suggesting that other genetic or environmental factors may have contributed to the observed phenotypic variations.

Turning now to the observed pattern summary, the results suggests that these haplotypes could play a crucial role in determining specific coat colour patterns, potentially through their effects on *TYRP1* function and expression as seen on Appendix table A3. However, these findings are somewhat limited by the small sample size in each group.

As previously pointed out in the results section of this report, 63 of the 97 samples were successfully genotyped. This is an above average success rate. Appendix Table A10 shows the sequencing details. Several factors could have affected this rate such human error when handling the samples as during sample preparation contamination, degraded DNA and PCR errors.

Mutations in *MC1R*

Based on the results of sequencing *MC1R,* an unexpected mutation was found, a missense c.676A>G causing an amino acid substitution p.K226E, also associated with the red head, according to Wu et al. (2006). The results indicated a notable variation in the distribution of the c.676A>G genotypes (AA, AG, and GG) across different coat colour groups: Brown, White, Grey, and Black, as well as in different breeds (Table 4 above). This highlights the genetic diversity present in the population and that the relationship between coat colour and genotype can be explored.

The *G* allele frequency in all the samples is higher (68%), compared to the *A* allele (38%), and considering the distribution of coat colours among genotypes. Based on the amino acid change p.K226E (Wu et al.,2006), inference can be made that the change to glutamic acid might have a functional impact on the *MC1R* that leads to variations in melanin production and different pigmentation pathway, favouring lighter pigmentation (white or brown coats). The balanced distribution of coat colours in AG individuals suggests that neither allele is completely dominant over the other, this could be due to having a neutral effect on protein function of this mutation (Ganbold et al., 2019). In this case the high frequency (Appendix Table A8) of homozygous GG maybe associated with the production of pheomelanin colours and will be consistent with the findings of Ganbold et al., (2019) and Wu et al. (2006). They suggested that the *G* allele linked to white colours might be due to loss-of-function. As these alleles are present across the colours and breeds studied it remains difficult to exclusively associate each the mutation with the coat colour as p.K226E mutation had a neutral effect on protein function with (Ganbold et al., 2019).

In relation to breeds it is interesting that the GG genotype is present only in the Swedish landrace however this could have been limited by the population size sampled in other breeds. The Jämt goat, Lapp goat and Goinge goat have limited genotype diversity, lacking the GG genotype entirely. This could be due to population bottlenecks and genetic drift leading to a loss of the G allele and reduced genetic pool as these goat populations are considered endangered (FAO,2024). This implies need for conservation strategies to be put in place.

Another finding is that mutations reported previously: missense mutations, p.F250V, p.C267W and silent mutation p.61A (Fontanesi et al.,2009, Wu et al.,2006, Ganbold et al.,2019) were not present except in the Goingeget as seen on Table 3. The mutation c.183C>T in one white with black ears Goingeget was displayed in a heterozygous state and two also white but with grey spots and black ears Goingeget were homozygous TT. Interestingly, the T allele was not reported in white goats in previous studies on saanen (white), girgentana, (white cream with usually small red spots on the face), (Fontanesi et al., 2009), and white Ganjam and Keonjhar goats (Venkatesh et al., 2023), but only at a lower frequency in white Mongolian goats (Ganbold et al.,2019).

This T allele is at low frequency 3% as shown in Appendix Table A7, in Swedish goats sampled. It is also present Goingeget which were few hence, a larger sample size investigation, particularly on the Goingeget, may clarify the effects of this mutation. the missense mutations p.F250V and p.C267W were found to be heterozygous and homozygous in two Goingegets in the similar manner although one sample was excluded due to poor sequences. These are shown in the figures 1a and 1b below. This is consistent with earlier findings in Maltese goats characterized by a white body, black cheeks and ears, which linked this mutation to the black coat colour, (Fontanesi et al., 2009). It is possible that the Goinge goat is different and has a lot of diversity from the other local breeds, as most mutations were noticed in the samples.

Figure 1a.

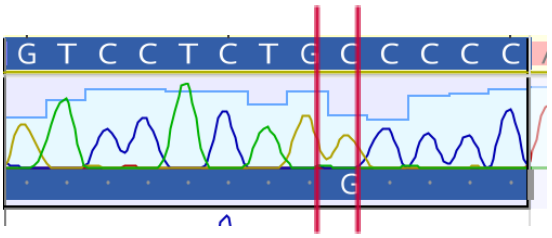

Figure 1b.

Figure 1a. shows the Goinge goat with a white body and black and grey spots on the ears and around eyes. This carried the homozygous mutations at c.748G>T, C.801C>G, c.183C>T and was at AA for c.676A>G. *Figure 1b.* shows the chromatogram shot of position 801.

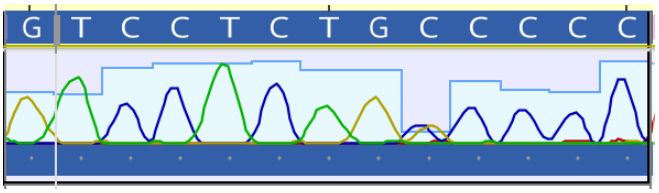

Figure 2a .

Figure 2b.

Figure 2a. shows the Goinge goat with a white body and black and grey spots on the ears and around eyes. This carried the heterozygous mutations at c.748G>T, C.801C>G, c.183C>T and was at AA for c.676A>G. *Figure 2b.* shows the chromatogram shot of position 801.

On further examination, the missense mutation p.A81V(c.242C>T) was not exhibited in all the samples except in four which displayed a high peak T and smaller peak of G which were excluded due to messy sequencing and missing opposite sequence to verify if indeed they are heterozygous. Of important notice is that all goats exhibited TT at position 748 which was different to the reference sequence Accession: FM212940.1 which had a GG (Camosciata_delle_Alpi, Swiss breed). This means the Swedish breeds are different and supports the studies that the Swedish goat gene pool has high genetic variability with a Nordic

genetic heritage; close to the Norwergian goats (Manunza et al., 2023). This affirms the relevance of this study as contributing to the knowledge as a step towards identifying the uniqueness of the native breeds for conservation and sustainable breeding programs.

To ascertain the influence of this variant c.676A>G of *MC1R* on patterns a thorough investigation can be done with a larger sample size to verify the result as seen.

good quality sequences for the first part and second towards the end of coding region of *MC1R* was 66 and 54 respectively which above average compared to the targeted sample Appendix Table A10. This is a still substantial for the efforts. The reason for this poor sequences is the same as discussed for the TYRP1 sequences.

Strengths and limitations of the study

The biggest challenge was obtaining failed sequences, which reduced the number of planned samples; however, for both genes, at least 50% had successful sequencing which could have been due to human error when handling samples. As there were fewer solid coat colours and distinct breed colours, defining coat colours was complicated; therefore, goats were grouped. Another challenge was that the picture was sometimes obscured, unclear, or affected by lighting, making accurate observation and documentation more difficult. Notwithstanding these limitations, this study is the first of its kind to be conducted on Swedish goats, and these findings form the basis for potential future studies and useful for developing future strategies to ensure sustainable genetic management.

Future concerns

The absence of previously identified and novel mutations did not clearly link the investigated brown, black, grey, or white phenotypes. This suggests that black or

red/yellow pigmentation may not be induced by the extension locus where the *MC1R* is located. Sponenberg et al., 1998 implied that most of the colour variation in goats is explained by most of the Agouti locus alleles. In view of this, future research investigating other genes, such as Agouti signalling protein, can be conducted to determine their ability to influence coat colour in goats.

They could examine biochemical mechanisms at play in both *MC1R* and *TYRP1*, or genes that may have epistatic or functional effects that might cover the action or interact to affect melanogenesis and gene expression.

6. Conclusion

This project set out to identify the genetic mutations responsible for different coat colours in Swedish goats, with a focus on TYRP1 and MC1R genes. For TYRP1, two novel SNPs c.*46A>C and c.*78T>C, were found to likely influence coat colour. They were found in either a homozygous or heterozygous state across all breeds, with the unmutated allele being more common in both. Identification of these mutations suggests potential regulatory influences on gene expression.

In the case of MC1R, three missense mutations p.K226E, p.F250V, and p.C267W and a silent mutation p.61A were investigated in Swedish goats; however, only p.K226E was found in different forms in all breeds and colours. The other two missense mutations were only found in two goats, suggesting that this may be different from the others, and that some factors and genetic interactions may be at play.

The challenge of this study was that the small sample sizes of Jämt, Goinge, and Lapp goat breeds might have affected the expression of diversity; hence, it is recommended to even out the numbers in the future. Future research could investigate other potential genes that influence coat colour through epistatic or functional interactions with TYRP1 and MC1R. Additionally, the biochemical mechanisms involved in melanogenesis, and gene expression should be explored for a more comprehensive understanding of the genetic factors influencing coat colour in Swedish goats.

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8. Popular Science Summary

TYRP1 (Tyrosinase-related protein 1) is essential for the melanin biosynthesis pathway and influences both the type and quantity of melanin produced. *TYRP1* functions in the conversion of dopaquinone to eumelanin, a key step in melanin production. The master regulator of pigment-type switching is the melanocortin-1 receptor, *MC1R.* This leads to the upregulation of many genes required for pigment production, such as *TYR* and *TYRP1*. *TYRP1* codes for brown phenotypes, whereas *MC1R* is expressed in black phenotypes. This study aimed to identify the genetic mutations responsible for different coat colours in Swedish goats, potentially caused by tyrosinase-related protein 1 and melanocortin 1 receptor.

Two new single nucleotide polymorphisms (SNPs) were identified outside the coding area of TYRP1. Known mutations (c.1433T>C and c.1487G>A) affecting the transmembrane domain of the protein were absent in the samples, suggesting that other factors might influence coat color. In addition, the SNPs were c.*46A>C and another 32 bases away T>C in the 3' untranslated region were found across different colors and breeds. A missense mutation (c.676A>G) leading to an amino acid change (p.K226E) showed variation across coat color and breed. The distribution of this mutation was not clearly associated with specific colors. However, the c.748G>T, c.801C>G, and c.183C>T variants were found only in white goats with black/grey spots.

Study Limitations: Challenges included failed sequences and difficulties in defining coat colors. However, this study lays the groundwork for future research on goat coat color genetics.

Future research should explore other genes, such as the Agouti signaling protein, and investigate the biochemical and functional mechanisms and gene interactions affecting melanogenesis

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10. Appendices

APPENDIX 1: Primer Details

Table A1. Designed Primers Used in the Amplification of the Coding Regions.

Primer	**Forward Sequence (5'-3')**	**Reverse Sequence (5'-3')**
MC1R Pair 1	AGCCATGAGTTGAGCAGGAC	ACCCAGATGGCTGCAATGAT
MC1R Pair 2	CTGTGGACCGCTACATCTCC	GAAGGCCTGTGTACCCTCAC
*TYRP1*_exon8	GCCTGGAACAATGCCTACAT	ATGGACATGCACAAGGAAGG

(Guan et al., 2021 and Henkel et al., 2021)

1b. Primer preparation:

Touchdown PCR protocol details

APPENDIX 2

1% Agarose Gel Electrophoresis Protocol
Materials

- 6g agarose in clean beaker
- 60 ml 1x TAE-buffer
- 6 µl GelRed
- 6x loading buffer

The agarose was mixed with 1x TAE-buffer, boiled until it melted and then cooled down to a temperature that the bottle can be held. 6 µl GelRed was added and mixed thoroughly, and the solution was poured into a tray and combs placed. After it solidified and combs removed, the gel was loaded with 1 well with 5µl ladder and 5µl sample mixed with 1µl 6x loading buffer per well. The gel was then run at 80V for 1 hour and the bands visualised on the UV table.

APPENDIX 3: *TYRP1* Genotype Summary

Table A2. *TYRP1* Genotype Summary.

Breed	No. of samples	Coat Colour	c.*46A>C	c.*78T>C
Lappget	1	Black White	AA	TT
Svensk lantrasget	6	Black White	AA	TT
Svensk lantrasget	1	Black White	CC	CC
Svensk lantrasget	2	Black White	AC	TC
Svensk lantrasget	1	Black	AA	TT
Blandras jämtget-svensk lantrasget	1	Brown White	AA	TT
Lappget	1	Brown White	AA	TT
Svensk lantrasget	2	Brown Black	AA	TT
Svensk lantrasget	3	Light Brown	AA	TT
Svensk lantrasget	8	Brown White	AA	TT
Svensk lantrasget	1	Brown Red	AA	TT
Svensk lantrasget	3	Beige	AA	TT
Svensk lantrasget	1	Brown	AA	TT
Svensk lantrasget	1	Dark brown	AA	TT
Lappget	2	Grey	AA	TT
Göingeget	1	Grey	AC	TC
Göingeget	1	Black	AA	TT
Svensk lantrasget	5	Grey	AA	TT
Jämtget	1	White	AA	TT
Lappget	2	White	AA	TT
Svensk lantrasget	14	White	AA	TT
Svensk lantrasget	1	Wild Coloured	AA	TT
Jämtget	3	Brown/Grey/Black	AC	TC
Svensk lantrasget	3	Brown	AC	TC
Svensk lantrasget	3	Brown White	AC	TC
Svensk lantrasget	2	Brown	CC	CC
Svensk lantrasget	11	White	AC	TC
Göingeget	1	Black	CC	CC
Svensk lantrasget	1	Grey	AC	TC
Svensk lantrasget	1	Grey	CC	CC
Blandras jämtget-svensk	1	Grey	AC	TC

lantrasget				
Jämtget	1	Black	AC	TC
Svensk lantrasget	1	Brown/Grey/Black	AC	TC
Göingeget	1	Grey	AA	TT

APPENDIX 4: *TYRP1* Pattern Summary

Table A3. *TYRP1* Pattern Summary.

Summary	AATT	ACTC	CCCC
Dorsal stripe	8	5	1
Grey/black spots on ears/body	2	5	1
Variegated	4	0	0
Badger like or two stripes on face	0	8	0
White belt like (White on belly)	0	1	3

APPENDIX 5: *MC1R* Genotype Summary

Table A4. *MC1R* Genotype Summary.

Breed	No. of samples	Coat Colour	c.676A> G p.K226E	c.748G> T p.F250V	c.801C> G p.C267W	c.183C>T
Jämtget	2	Brown/Grey/ Black	AA	TT	CC	CC
Jämtget	1	Black	AA	TT	CC	CC
Jämtget	1	Brown	AA	TT	CC	CC
Göingeget	1	Black	AG	TT	CC	CC
Göingeget	1	Grey	AA	GG	GG	TT
Göingeget	1	Black	AA	TG	CG	TC
Lappget	1	Black	AG	TT	CC	CC
Lappget	1	Grey	AA	TT	CC	CC
Lappget	1	White	AA	TT	CC	CC
Lappget	1	White	AG	TT	CC	CC
Lappget	1	Grey	AG	TT	CC	CC
Blandras jämtget-svensk lantrasget	1	Grey	AA	TT	CC	CC
Blandras jämtget-svensk lantrasget	1	Brown-White	AG	TT	CC	CC
Svensk lantrasget	1	Black	GG	TT	CC	CC
Svensk lantrasget	6	Black-white	GG	TT	CC	CC
Svensk lantrasget	2	Black-white	AG	TT	CC	CC
Svensk lantrasget	1	Brown	AA	TT	CC	CC
Svensk lantrasget	2	Brown-White	AA	TT	CC	CC
Svensk lantrasget	1	Grey	AA	TT	CC	CC
Svensk lantrasget	8	White	AG	TT	CC	CC
Svensk lantrasget	5	Grey	AG	TT	CC	CC
Svensk lantrasget	6	Brown-White	AG	TT	CC	CC
Svensk lantrasget	1	Brown/Grey/ Black	AG	TT	CC	CC
Svensk lantrasget	1	Black Brown	GG	TT	CC	CC
Svensk lantrasget	1	Black	GG	TT	CC	CC

Blandras jämtget-svensk lantrasget	1	Black	AG	TT	CC	
Svensk lantrasget	3	Beige	GG	TT	CC	CC
Svensk lantrasget	3	Light Brown	GG	TT	CC	CC
Svensk lantrasget	1	Brown	GG	TT	CC	CC
Svensk lantrasget	1	Brown Red	GG	TT	CC	CC
Svensk lantrasget	3	Brown White	GG	TT	CC	CC
Svensk lantrasget	1	Dark Brown	GG	TT	CC	CC
Svensk lantrasget	1	Grey	GG	TT	CC	CC
Svensk lantrasget	11	White	GG	TT	CC	CC
Svensk lantrasget	1	Grey	GG	TT	CC	CC
Svensk lantrasget	1	Brown Black	AA	TT	CC	CC
Svensk lantrasget	1	Dark Brown	AA	TT	CC	CC
Svensk lantrasget	1	White	AA	TT	CC	CC
Svensk lantrasget	2	Beige	AG	TT	CC	CC
Svensk lantrasget	2	Light Brown	AG	TT	CC	CC
Svensk lantrasget	2	Brown	AG	TT	CC	CC
Svensk lantrasget	1	Dark Brown	AG	TT	CC	CC
Göingeget	1	Grey				TT
Lappget	1	Brown				CC
Jämtget	1	Black				CC
Svensk lantrasget	1	Black-White				CC
Svensk lantrasget	1	Brown				CC
Svensk lantrasget	1	White				CC
Svensk lantrasget	1	Brown White				CC
Svensk lantrasget	2	White	GG	TT	CC	
Svensk lantrasget	1	Black-White	GG	TT	CC	
Svensk lantrasget	1	White	AG	TT	CC	

APPENDIX 6: *MC1R* Pattern Summary

Table A5. Pattern Summary.

Pattern	AATTCCCC	AAGGGGTT	AATGCGTC	AGTTCCCC	AGTTCC	GGTTCCCC
Black and white markings	3					
Black face mask	3					
Dorsal stripe	2			8		8
Grey small spots		1				
Black ears			1	1		
White grey variegated	1			2		
Stripes on the face	1			7	1	2
White belt like	1			2		1
Grey colour						2
Brown leg						1

APPENDIX 7: Genotype and Allele Frequency

Table A6. *TYRP1* Genotype and Allele Frequency.

SNPs	Genotype	No. Of Samples	Genotype Frequency	Allele	Allele Frequency
c.301A>C	AA	56	0,64	A	0,79
	AC	27	0,3	C	0,21
	CC	5	0,06		
c.333T>C	TT	56	0,64	T	0,79
	TC	27	0,3	C	0,21
	CC	5	0,06		

Table A7. *MC1R* Genotype and Allele Frequency.

SNPs	Genotype	No. Of Samples	Genotype Frequency	Allele	Allele Frequency
c.676A>G p.K226E	AA	16	0,179775281	A	0,38
	AG	36	0,404494382	G	0,62
	GG	37	0,4515730337		

c.748G>T p.F250V	TT	87	0,977528	T	0,98
	TG	1	0,011236	G	0,02
	GG	1	0,011236		
c.801C>G p.C267W	CC	87	0,977528	C	0,98
	GG	1	0,011236	G	0,02
	CG	1	0,011236		
c.183C>T	CC	89	0,967391	C	0,98
	TT	2	0,021739	T	0,02
	TC	1	0,01087		

APPENDIX 8: Genotype Disribution of Identified Haplotypes

Table A8. *TYRP1* Genotype Distribution of Identified Haplotypes of c.*46A>C and c.*78T>C.

Coat Colour Group	No. of animals	Haplotype Frequency		
		AATT	CCCC	ACTC
Black	14	0.64	0.14	0.21
Brown	33	0.64	0.06	0.30
Grey	12	0.67	0.08	0.25
White	28	0.61	0.00	0.39
Wild Coloured	1	1.00	0.00	0.00

Table A9. *MC1R* Genotype Distribution of Identified of SNPs

	No. of Animals	SNP	Genotypes		
			AA	GG	AG
Black	16	c.676A>G	0.13	0.56	0.31
Brown	36		0.22	0.36	0.42
Grey	12		0.33	0.17	0.50
White	25		0.08	0.52	0.40
			GG	TT	TC
Black	16	c.748G>T	0.06	0.94	
Brown	36			1.00	
Grey	12			0.92	0.08
White	25			1.00	

			CC	GG	CG
Black	16	c.801C>G	0.94	0.06	0.00
Brown	36		1.00	0.00	0.00
Grey	12		0.92	0.00	0.08
White	25		1.00	0.00	0.00
			CC	**TT**	**TC**
Black	16	c.183C>T	0.94	0.00	0.06
Brown	39		1.00	0.00	0.00
Grey	13		0.85	0.15	0.00
White	23		1.00	0.00	0.00

APPENDIX 9: Sequence Quality Status

Table A10. Sequence Quality Status for *MC1R* and *TYRP1*.

	Sequence Quality Status			
	Good	Partial (either Reverse only or Forward only)	Failed	TOTAL
MC1R -1 part	66	25	4	**95**
MC1R -2 part	54	35	5	**94**
TYRP1	63	28	6	**97**