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1. Abstract

Background: The syndrome Multiple Congenital Ocular Anomalies (MCOA) is a congenital eye disease in horses. Previous studies have shown that both the MCOA syndrome and the silver coat color in horses are caused by the same missense mutation in *PMEL17.* Horses homozygous for the *Silver* mutation (TT) are affected by multiple eye defects causing visual impairment or even blindness. Horses heterozygous for the *Silver* mutation (CT) usually have iridociliary cysts, occasionally extending into the peripheral retina. Clinical signs of visual impairment seem to be very rare in these horses. However, a recent study reported that Comtois horses carrying the *Silver* mutation had deeper anterior chambers of the eye compared to Wild-type horses. This could potentially cause refractive errors. The purpose of the present study was to study refraction of the eye in horses heterozygous for the *Silver* mutation to investigate if they have refractive errors. Ninety-four Icelandic horses were examined. All horses were genotyped for the missense mutation in *PMEL17*. Each of the 47 CT horses was matched by a Wild-type (CC) horse of the same age ± 1 year. A brief ophthalmic examination was performed and questionnaires regarding signs of visual impairment were filled out by the owners. Skiascopy was performed using a streak retinoscope (Heine Beta 200) and Trousseau racks without dilation of the pupils. Association between refraction and age, eye, genotype and sex was tested by linear mixed-effect model analysis. Pairwise interactions between each fixed factor (age, eye, genotype and sex) were included in the model.

Results: The mean refraction value ± SE was 0.00 ±0.12D for the right eye and 0.03 ±0.10D for the left eye in CT horses compared to 0.22 ±0.05D for both right and left eye in Wild-type horses. The interaction between age and genotype had a significant impact on the refractive state (P=0.001). A deviation towards myopia (nearsightedness) could be observed in CT horses older than 200 months (16.7 years). In the questionnaire, forty-one CT horse owners (87.2%) reported that their horse seemed to have no impaired vision at all.

Conclusions: The refractive state of young and adult CT horses were not significantly different from Wild-type horses. Although, CT horses older than 200 months (16.7 years) were more likely to develop myopia than Wild-type horses. This suggests that the *Silver* mutation exerts a slowly progressive effect on the optics of the eye.

2. Introduction

2.1 Background

Selective breeding of a certain phenotypic trait can result in unfavorable effects, as for example ocular anomalies in silver colored horses. Coat color-associated mutations are often linked to defects in sensory organs and nerves. Both melanocyte and neurocyte precursor cells migrate from the neural crest during embryogenesis and they are in this process regulated by the same genes. Melanocytes are essential for the function of sensory organs (Reissman & Ludwig, 2013).

The syndrome Multiple Congenital Ocular Anomalies (MCOA) is a congenital eye disorder. It was first described in Rocky Mountain Horses in 1999 (Ramsey *et al.*, 1999). Previous studies have shown that both the silver coat color and the MCOA syndrome in horses are caused by the same missense mutation in *PMEL17* (Brunberg *et al*., 2006; Andersson *et al*., 2013). The mutation, a change from cytosine (C) to thymine (T), causes a dominant dilution of the eumelanin (black and brown) pigment especially in the mane and tail (Brunberg *et al*., 2006). The silver coat color in horses can frequently be observed in Rocky Mountain horses and the French draft horse, Comtois (Ségard *et al.,* 2013). The silver coat color can also be found in for example Icelandic horses, American Miniature horses, Morgan horses and Shetland ponies (Komáromy *et al.,* 2011). The Icelandic horse is a popular breed in Sweden that carries the *Silver* mutation. There are currently about 1283 Silver colored Icelandic horses in Sweden registered in the web database program Worldfengur. In addition there are for example chestnut horses that may carry the *Silver* mutation (Worldfengur, 2016).

2.2 PMEL17

The gene *PMEL17* encodes the transmembrane glycoprotein PMEL17 also known as PMEL, SILV and gp100. PMEL17 is essential for the biogenesis of eumelanin in the melanosomes. PMEL17 forms fibrillary structures where melanin is deposited during melanogenesis (Berson *et al*., 2001; Raposo *et al*., 2001). Post translational and proteolytic processing of PMEL17 are required for a correct formation of the fibrillary matrix. It is not clear how these processes are regulated (Rochin *et al*., 2013; Kawaguchi *et al*., 2015). Different mutations in *PMEL17* are associated with hypopigmentation in horses, mice, chickens, zebrafish, dog and cattle (Brunberg *et al*., 2006; Martinez-Esparza *et al.*, 1999; Kerje *et al*., 2004; Schonthaler *et al*., 2005; Clark *et al*., 2006; Kuehn & Weikard, 2007). In horses, zebrafish and dogs the mutation is also reported to be associated with ocular anomalies (Andersson *et al*., 2013; Schonthaler *et al*. 2005; Clark *et al*. 2006). All these different mutations give us the opportunity to study the function of PMEL17 and its role in the melanogenesis. The *Silver* mutation in horses is located near the transmembrane domain of PMEL17 (Figure 1) (Brunberg *et al*., 2006). Mutations in or near the transmembrane domain result in a compact form of the fibrils and hypopigmentation (Watt *et al*., 2011). The number and the form of the melanocytes are thought to be regulated separately. A recent study in zebrafish showed that melanocytes have to be cylindrical to reach the apical retinal pigment epithelium (RPE) where they play an essential role for a normal function of the photoreceptors (Burgoyne *et*

al., 2015). A study from 2011 by Hellström *et al.* created a knock-out mouse line where *PMEL* had been inactivated. They showed that PMEL was important for eumelanin production in skin, choroid and retinal pigment epithelium. However, full-field electroretinogram (ERG) did not show any impaired function of the retina.

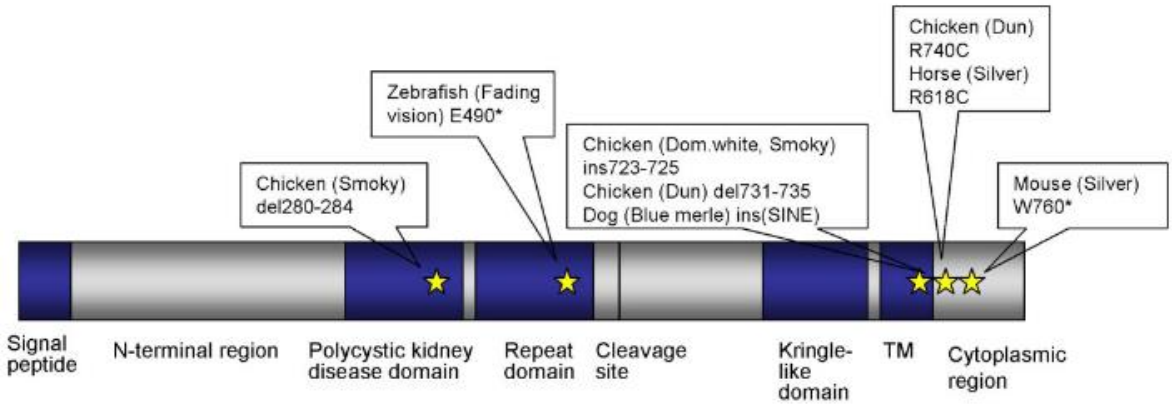

Figure 1. *The PMEL17 protein with domains and the location of known mutations. The Silver mutation in horses is at the same location as the Dun mutation in chicken, i.e. near the transmembrane domain (TM) of PMEL17 (Brunberg et al., 2006).*

2.3 Refraction of the eye and skiascopy

Refraction of light in the optic media of the eye is needed to focus an image on the photoreceptors in the retina. The anterior surface of the cornea followed by the lens have the highest refractive power of the eye (Gelatt, 1999). In an emmetropic (normally sighted) eye, parallel incident light rays are focused on the retina. In a hyperopic (farsighted) eye, the light is focused behind the retina, whereas they are focused in front of the retina in a myopic (near sighted) eye (Figure 2). Skiascopy also called retinoscopy is a method to determine the refractive power of the eye. This method is used both in veterinary and human medicine. A diverging light beam from a retinoscope is swept across the pupil of the patient. The movement of the reflex (or the shadow) is observed. If the reflex moves in the same direction (*with motion*) as the retinoscope, lenses of positive refractive power are put in front of the eye to reach the neutralization point where the pupil is completely filled by the light reflected from the fundus. If the reflex moves in the opposite direction (*against motion*) to the retinoscope lenses of negative refractive power are added to reach the neutralization point (Figure 3). The working distance is then subtracted (Davidson, 1997).

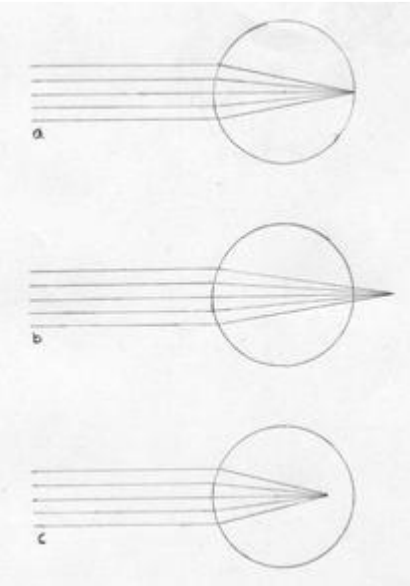

Figure 2. *Refraction of light beams in an emmetropic (normally sighted) eye (a), hyperopic (farsighted) eye (b) and a myopic (nearsighted) eye (c) (Löf, 2007).*

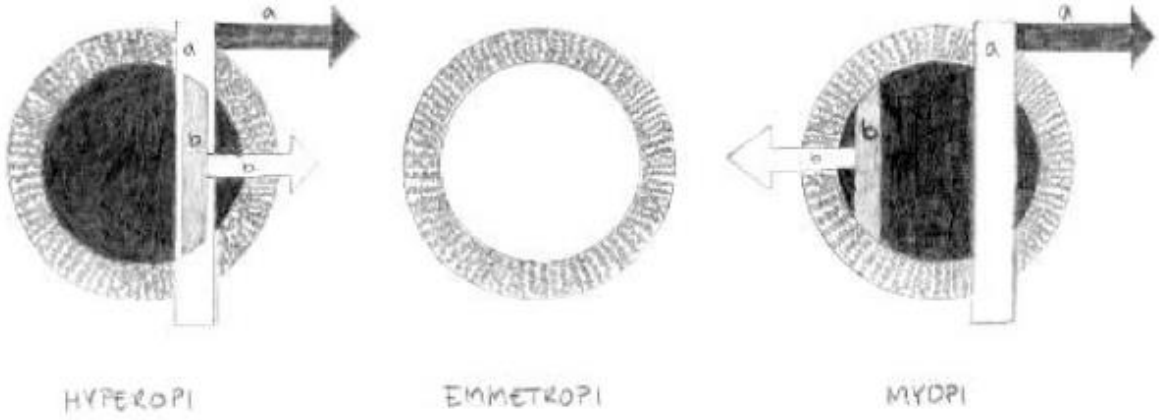

Figure 3. *The reflex moves in the same direction as the retinoscope in a hyperopic (farsighted) eye, the pupil is completely filled by the streak reflex in an emmetropic (normally sighted) eye and moves in the opposite direction of the retinoscope in a myopic (nearsighted) eye (Löf, 2007).*

2.4 MCOA

Clinically, Silver colored horses can be subdivided into two different groups according to the severity of the ocular defects; the Cyst phenotype and the MCOA phenotype. Horses with the Cyst phenotype are known to be heterozygous for the *Silver* mutation (CT) and they have less severe clinical signs including uveal cysts, usually in the temporal quadrant of the eye. The cysts are translucent and can range in diameter up to centimeter-size (Figure 4). Horses with the MCOA phenotype are known to be homozygous for the *Silver* mutation (TT). They have more severe

clinical signs including iridal stromal hypoplasia (underdevelopment of the stromal layer of iris), miotic pupils (small pupils that cannot be fully dilated), retinal dysplasia (abnormal organization of the cells in the retina), cataract (opacity of the lens), cornea globosa (enlarged cornea) and iridociliary cysts occasionally extending subretinally (Ramsey *et al.,* 1999; Andersson *et al.,* 2011a; Komaromy *et al,.* 2011; Ségard *et al.,* 2013). An incomplete dominant mode of inheritance has been suggested (Andersson *et al.,* 2011b).

Horses homozygous for the *Silver* mutation are affected by multiple eye defects causing visual impairment or even blindness (Ramsey *et al.* 1999; Andersson *et al*., 2011a; Komáromy *et al.,* 2011; Ségard *et al.,* 2013). It is still unknown if vision is impaired in CT horses. A recent study in Comtois and Rocky Mountain horses showed that homozygous horses had significantly deeper anterior chamber of the eye than heterozygous horses. It was also a significant difference in the depth of the chamber between homozygous and Wild-type horses as well as between heterozygous and Wild-type horses (Ségard *et al.,* 2013). An abnormal distance between the refractive media could potentially cause refractive errors. More information is needed to investigate if the MCOA syndrome affect the vision in horses heterozygous for the *Silver* mutation.

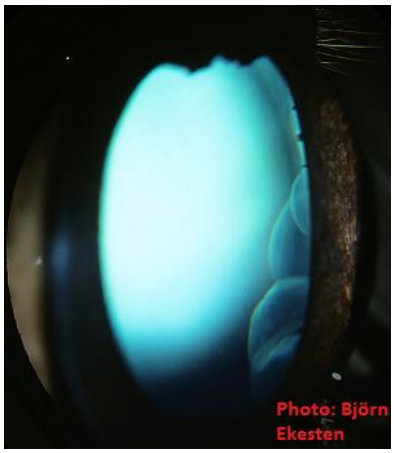

Figure 4. *Cystic structures in an Icelandic horse with the Silver mutation. The cysts are located temporally and arise from iris and the ciliary body.*

2.5 Aim of the study

The purpose of the present study was to study refraction of the eye in horses heterozygous for the *Silver* mutation to investigate if they have refractive errors.

3. Materials and methods

3.1 Animals

The study was approved and conducted according to national and local guidelines from the Swedish University of Agricultural Sciences (SLU). Hair samples were collected following owner's informed consent and according to ethical approvals (number C121/14). The CT horses were collected by posting information on social media and by contacting horse farms near Uppsala. The control horses were collected from the same stables as the CT horses. In total, 95 Icelandic horses were included in the study, 47 of them were heterozygous for the *Silver* mutation. Each of the 47 CT horses was matched by a control (Wild-type) horse of the same breed and age ± 1 year. Control horses were confirmed being Wild-type (CC) for both disease-alleles. Only one horse, a 22 year old Icelandic horse, was homozygous for the disease-causing allele. This horse was not included in the statistical analysis. The median age of both CT and control horses was 8 years with a range from 1 to 25 years in CT horses and from 1 to 24 years in control horses (Figure 5 and 6). Sex distribution was as follows: 20 males and 27 females in CT horses and 19 males and 28 females in controls.

Five Silver colored American Miniature horses and five controls were also examined and genotyped. However, this group of horses was too few to be included in the statistical analysis and they are therefore not presented in the results.

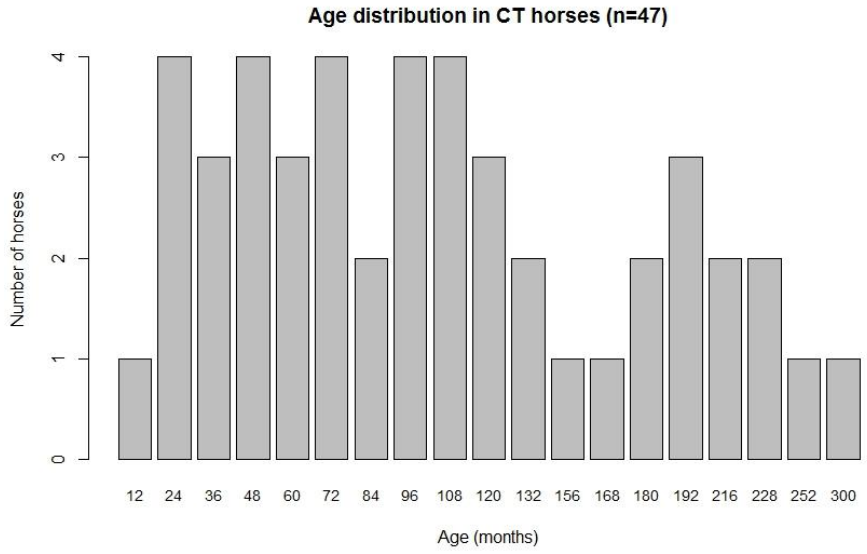

Figure 5. *The age distribution of the CT horses (n=47).*

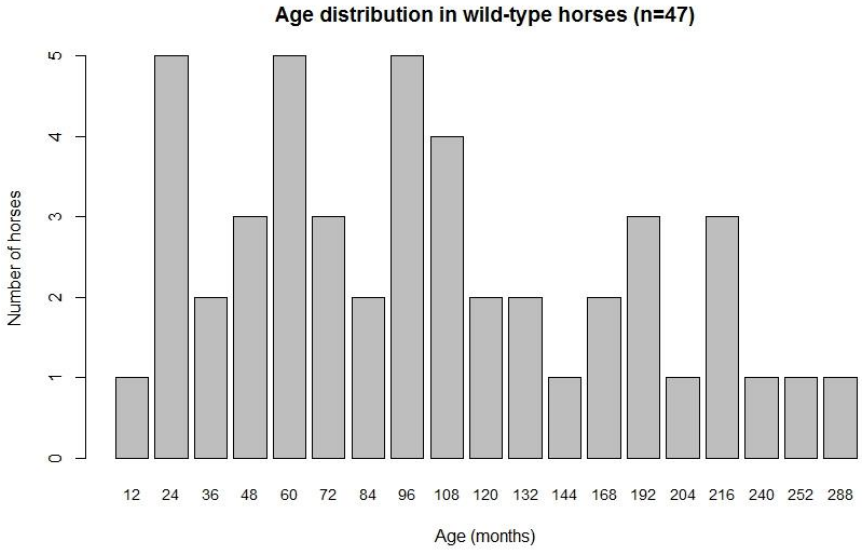

Figure 6. *The age distribution in controls (Wild-type) horses (n=47).*

3.2 Phenotypic assessment

Phenotypic data on all horses in the study were collected from a brief ophthalmic examination conducted by the author and owner reported questionnaires.

The ophthalmic examination was performed in the stable under dim-light conditions. The record used in this study can be found in Appendix 1. The examination included direct and indirect pupillary light reflexes, menace response and dazzle (light) reflex to control the function of the retina and the optic nerve. Palpebral reflex and corneal reflex were tested to control the function of the sensory innervation of the eye. The reflexes were all graded as normal, decreased or absent. If the reflex was decreased it was described as being either slow or incomplete. To detect opacities in the ocular media the fundus reflex from the retinoscope was inspected. Opacities were if present graded based on extension and location. The cornea was visually observed to find any signs of cornea globosa. Cysts or other anomalies were also noted.

Skiascopy was performed using a streak retinoscope (Heine Beta 200) and Trousseau racks without dilation of the pupils. Lenses with a 0.5 D difference were used to reach the neutralization point and the working distance was subtracted. The refractive power was measured both horizontally and vertically in both eyes. Astigmatism was defined as a difference of more than 0.5 D between the horizontal and vertical meridians.

The majority of the questionnaires were filled out by the owners during the eye examination of their horse and the rest were sent by post after the examination. In the questionnaire the owners were asked if their horses were difficult or easy to handle and to ride, easily frightened or not and if the horses showed any signs of impaired vision. Any known history of eye disorders was also

noted. The owners were asked to rank the answers on a scale from 1 to 10, where 1 represented good and 10 poor. The questionnaire also included questions about how well their horse performed within its area of use on a scale from 1 to 10, where 1 represented poor and 10 good. The questionnaire can be found in Appendix 2.

3.3 DNA isolation and genotyping

Genomic DNA was isolated from hair samples by the standard hair-preparation method as previously described (Jäderkvist *et al.* 2014). All horses in the study were genotyped for the missense mutation in *PMEL17* previously shown to be associated with the silver coat color and the MCOA syndrome (Brunberg *et al*. 2006; Andersson *et al*., 2013). The genotyping was performed by using custom designed TaqMan SNP Genotyping Assays (Applied Biosystems StepOnePlus™ Instrument by Life Technologies) for SNP g.73665304 on chromosome 6 (Andersson *et al*., 2013). Horses were classified according to the genotype into three groups; Wild-type (CC), heterozygous (CT) and homozygous (TT) for the *PMEL17* missense mutation.

3.4 Statistical analysis

All statistical analyses were performed in the software environment R-3.1.2 (R Development Core Team, 2014) and significance was defined as $P \leq 0.05$. Associations between refraction and age, sex, genotype and eye were tested by linear mixed-effect model analysis using the function lmer and the packages lme4 and car. There were two observations per horse, one for each eye (right and left) and the model used in lme4 was:

lmer(refraction~(age + sex + genotype + eye)^2+(1|horse), data=data, subset=(breed==1)

The dependent variable *refraction* is the horizontal refraction value determined by sciascopy. Lenses of a 0.5 D difference were used and the refraction value was defined as a continuous numeric variable. The fixed factors in the model; *age* (age of the horse at the examination in months as full years), *sex* (male or female), *genotype* (CT or CC) and *eye* (right or left), were defined as factor variables. Pairwise interactions between each fixed factor were included in the model. The identity of the horse was used as a random factor. A Wald chi-square test was used in the post hoc test with Anova type 3 in the package car to estimate p-values.

A Mann-Whitney-Wilcoxon test was performed to compare the median score value from the owner questionnaire between the two groups, CT and Wild-type. The traits *handling*, *riding*, *signs of impaired vision* and *easily frightened* were used.

4. Results

The mean refraction value ± SE was 0.00 ±0.12D for the right eye and 0.03 ±0.10D for the left eye in heterozygotes compared to 0.22 ±0.05D for both right and left eye in Wild-type horses. The refractive state of the majority of both CT and CC horses was close to 0 D or slightly hyperopic (farsighted) (Table 1), 43 CT horses (91.5%) were within the range ≥ -0.5D to ≤ +0.5D. No horses in the study were astigmatic. Two CT horses, 18 and 21 years old, had severe refraction errors on one or both eyes (Figure 7). The interaction between age and genotype had a significant impact on the refractive state (P=0.001). A deviation towards myopia could be observed in CT horses

older than 200 months (16.7 years) (Figure 7). No other interaction tested was statistically significant.

Table 1. *The refraction state of all 94 horses for right and left eye*

Right eye	**Number of CT horses**	**Percent**	**Number of CC horses**	**Percent**
Emmetropic (normal sighted) 0 D	23	48.9%	21	44.7%
Hyperopic (farsighted)				
Slightly (+0,5-1 D)	19	40.4%	23	48.9%
Moderate (+1,5-2,5 D)	0	0%	0	0%
Severe > +3 D	0	0%	0	0%
Myopic (nearsighted)				
Slightly (-0,5-1 D)	2	4.3%	3	6.4%
Moderate (-1,5-2,5 D)	1	1.9%	0	0%
Severe (-3D or more)	2	4.3%	0	0%

Left eye	**Number of CT horses**	**Percent**	**Number of CC horses**	**Percent**
Emmetropic (normal sighted) 0 D	22	46.8%	20	42.6%
Hyperopic (farsighted)				
Slightly (+0,5-1 D)	19	40.4%	24	51.1%
Moderate (+1,5-2,5 D)	0	0%	0	0%
Severe > +3 D	0	0%	0	0%
Myopic (nearsighted)				
Slightly (-0,5-1 D)	3	6.4%	3	6.4%
Moderate (-1,5-2,5 D)	2	4.3%	0	0%
Severe (-3D or more)	1	1.9%	0	0%

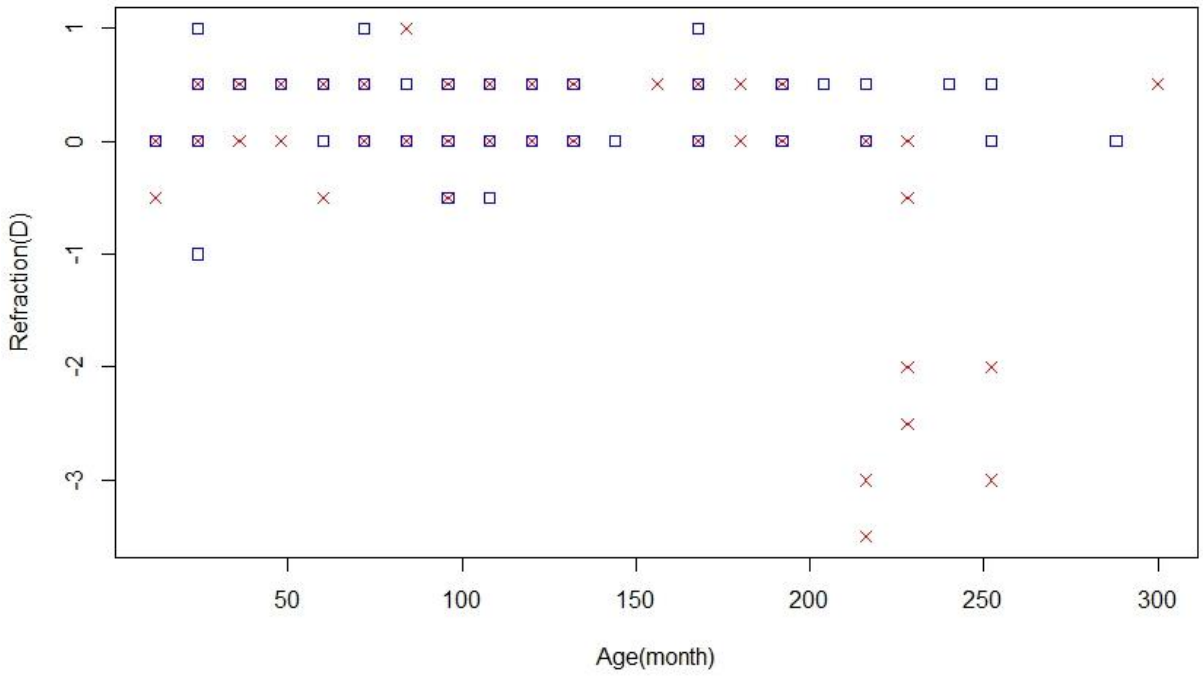

Figure 7. *The refractive values according to age in all 94 Icelandic horses. Blue squares indicate Wild-type horses and red crosses CT horses. (Notice that some horses of the same age have the same refractive value.)*

Forty-one CT horse owners (87.2%) reported in the questionnaire that their horse did not seem to have impaired vision at all (1/10). Four CT horse owners reported very mild problems attributed to visual impairment (2/10). Two CT horses had moderately impaired vision (5/10) according to the owners. One of those horses, an 18 year old horse was myopic. The horizontal refractive state was -3.5 D for the right eye and -3 D for the left eye. The other horse was only slightly hyperopic.

There was a significant difference in the median score between CT and CC horses for the trait *signs of impaired vision* (*P*=0.05). No significant differences were found for any of the other traits (*handling*, *riding* and *easily frightened*) (Table 2).

Table 2. *Median and mean score values from the owner questionnaire for all 94 horses*

Trait[a]	CT (median)	CT (mean)	CC (median)	CC (mean)	*P* [b]
Handling	1.0 (1-8)	1.9 (0.25)	1.0 (1-8)	2.0 (0.21)	0.30
Riding[c]	2.0 (1-8)	2.8 (0.34)	2.0 (1-8)	2.2 (0.28)	0.32
Signs of impaired vision	1.0 (1-5)	1.3 (0.12)	1.0 (1-2)	1.0 (0.02)	0.05
Easily frightened	1.0 (1-8)	2.3 (0.29)	2.0 (1-9)	2.8 (0.32)	0.26

[a] Score from 1 (good) to 10 (poor)

[b] Mann-Whitney-Wilcoxon test was performed to compare the median values for the 47 CT and 47 CC horses

[c] Only horses used for riding (35 CT and 34 CC horses) were included

Range of the median value and SE of the mean value in brackets

Only one horse in the study, a 21 years old CT horse, had immature cataract in one eye affecting about 25 % of the area of the lens in the coronal plane. Another horse, an 8 year old CT horse, had mild cornea globosa on the left eye. The oldest horse in the study, a 25 year old CT horse, had moderate miotic pupils on both eyes. All horses in the study had normal reflexes.

The homozygous (TT) horse had moderate miotic pupils, diffusely spread immature cataracts and mild cornea globosa of both eyes. The horizontal refraction was -3 D for right eye and -1.5 D for left eye. The horse was not suspected to have impaired vision according to the owner (2/10). However, the owner reported that the horse was quite difficult to handle (6/10) and to catch in the paddock.

5. Discussion

This study was performed to offer insight into how the MCOA syndrome affect the vision in horses heterozygous for the *Silver* mutation. A recent study showed that horses with the *Silver* mutation, both homozygotes and heterozygotes, had deeper anterior chambers of the eye compared to Wild-type horses (Ségard *et al.*, 2013). This could potentially cause refractive errors. Our results showed that the refractive state of young and adult CT horses were not significantly different from Wild-type horses. Although, CT horses older than 200 months (16.7 years) were more likely to develop myopia than Wild-type horses.

Five Silver colored American Miniature horses and five controls were also examined and genotyped. Unfortunately, this group of horses was too few to be included in the statistical analysis. The two different breeds in our study, the Icelandic horse and American Miniature horse, were analyzed separately because previous studies have shown that the refractive state may vary by breed both in horses and dogs (Rull-Cotrina *et al.* 2013; Bracun *et al.* 2014; Murphy *et al.* 1992). Bracun *et al.* (2014) investigated the refractive state in 333 horses and ponies in UK. They found significant differences in the refractive state between Thoroughbred crosses and Warmbloods as well as between Thoroughbred and Shires. A degree project by Östberg from 2007 also found

differences in the refractive state between different breeds. In total, 93 horses of five different breeds (Icelandic horses, Ponies, Thoroughbreds, Warmbloods and Standardbreds) were included in the study. The Icelandic horses were found to be more hyperopic and Thoroughbreds more myopic compared to the other breeds in the study.

The horses in the present study were examined without dilation of the pupil because previous studies have shown that dilation of the pupil is not needed before retinoscopy (Rull-Cotrina *et al* 2013; McMullen *et al,* 2014; Löf, 2007; Östberg 2007).

Since horses have horizontal pupils the horizontal refraction value was considered to be the most accurate value to be used in the statistical analysis. However, there were only small differences between the horizontal and vertical values and none of the horses in the present study had any signs of astigmatism.

The interaction between age and genotype had a significant effect on the refractive state (*P*=0.001). A deviation towards myopia could be observed in CT horses older than 200 months (16.7 years). However, the oldest horse in the study, a 25 year old CT horse, was slightly hyperopic. This horse had miotic pupils which may have affected the result. Our findings suggest that the *Silver* mutation exerts a slowly progressive effect on the optics of the eye. Horses with the MCOA syndrome could potentially develop a refractive error as previous studies have shown that these horses have a deeper anterior chamber of the eye compared to Wild-type horses (Ségard *et al*., 2013). Theoretically that should induce a deviation towards myopia. The MCOA syndrome is thought to be non-progressive (Ramsey *et al*. 1999). The study by Ségard *et al*. from 2013, where 59 Comtois and 16 Rocky Mountain horses were included, reported no differences in the observation of ocular abnormalities between young, adult and old horses. However, a progression of the number of cystic lesions with age were noted. The horses in their study had a median age of 3 years with a range from 10 days to 18 years. A study by Plummer and Ramsey from 2011, where 53 American Miniature horses were included, showed no correlation between age and presence of ocular abnormalities. The median age was 5.3 years with a range from 1.5 to 219 months (18.25 years). The median age of the horses was lower in the previous studies (Ségard *et al.,* 2013; Plummer & Ramsey, 2011) compared to our study. It would be interesting to investigate this further in a larger population of Silver colored horses with additional old horses included.

Other potential causes for myopia in the older horses in the present study are age-related causes. However, none of the old Wild-type horses had any myopic refractive errors. Human beings are reported to become myopic when they get older. A long-term study showed that young people were more hyperopic and when they got older they were more myopic (Lee *et al*, 2002). To our knowledge, there are few studies reported on how the refractive state changes with age in horses. A study from 2013 by Rull-Cotrina *et al.*, where 135 Spanish Thoroughbred and Crossbred horses were included, did not find any correlation between the refractive state and age. Löf investigated the refractive state in 116 Standardbred trotters and found a deviation towards myopia with increasing age (Löf, 2007). Hence, it is likely that both humans and horses become more myopic with age.

Eye (left or right) did not have an impact on the refractive state. This was as expected since the MCOA syndrome is reported to affect the eyes bilaterally (Ramsey *et al*. 1999). The previous study by Ségard *et al*. (2013) did not find any significant difference in depth of the anterior chamber between right and left eye.

Sex was not found to affect the refractive state. This was as expected and in accordance with previous studies (Rull-Cotrina *et al.,* 2013; Bracun *et al.* 2014).

The proportion of CT horses with refraction ±0.5D was similar to the proportion of horses reported to have normal vision according to their owners. A significant difference was found in the median score value between CT and CC horses for the trait *signs of impaired vision* (*P*=0.05). Probably this trait was significant just because of a few higher score values in the group of CT horses. The two different groups, CT and CC horses, had the same median value and the mean value was almost the same. No significant difference was found in the median score value between CT and CC horses for the trait *easily frightened*. Brunberg *et al*. (2013) performed a fear reaction test in Icelandic horses by moving a plastic bag towards the horses. Nine Silver horses (heterozygous for the *Silver* mutation), nine chestnut (Wild-type) and nine black or brown horses were included in the study. They found that the Silver colored horses were more fearful to enter the test arena than Wild-type horses. However, few horses were included in the study and the Silver colored horses showed no signs of altered behavior compared to Wild-type horses in the rest of the test.

The trait *performance* was not used in the statistical analysis because many different factors affected how well the horses performed. In addition, the youngest and the oldest horses in the study were not used for any activity. We have to take into account that each trait (*performance*, *handling*, *riding*, *signs of impaired vision* and *easily frightened*) was scored according to the owners in the questionnaire. No definitions were provided to the owners of the traits they were asked to judge and the horses were not grouped by age in the statistical analysis.

6. Conclusions

The refractive state in young and adult CT horses were not significantly different from Wild-type horses, Although, CT horses older than 200 months (16.7 years) were more likely to develop myopia than Wild-type horses. This suggests that the *Silver* mutation exerts a slowly progressive effect on the optics of the eye. Further research is needed to evaluate this information.

7. Future perspectives

It would be interesting to further investigate whether MCOA is a slowly progressive disease in a larger population of horses with additional old horses included. The best way would be to perform a long-term follow-up study of individual horses. Icelandic horses are ideal for this type of study as they are common in Sweden and usually gets older than many other breeds. To study this in additional breeds and to include homozygous horses would also be interesting. Previous studies have shown that the refractive state may vary by breed Rull-Cotrina *et al.* 2013; Bracun *et al.* 2014; Östberg, 2007). Additional functional studies are also important to get more information about the disease. For example additional studies of PMEL17 and how the melanogenesis is

regulated. To study the *Dun* mutation in chickens would also be helpful since this mutation is caused by the same missense mutation as the *Silver* mutation in horses.

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9. Study contributions

I planned the study together with my supervisors, searched for Silver colored horses, collected hair samples and designed the owner questionnaire, owner's consent and medical record. I isolated DNA, genotyped and examined all the horses, analyzed the data and drafted the thesis. I also performed all statistical analysis in R with advice from Ulf Olsson and Mikael Andersson Franko.

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11. Appendix 1

Medical record ophthalmic examination MCOA 2016

Horse ID:

Name:

ID number:

Sex:

Breed:

Color:

Date of birth:

Owner:

Date:

Anamnesis:

Reflexes: (normal/decreased (slow or incomplete) /absent)

Right eye:

Direct pupillary light reflex:

Indirect pupillary light reflex:

Menace response:

Palpebral reflex:

Corneal reflex:

Dazzle reflex:

Left eye:

Direct pupillary light reflex:

Indirect pupillary light reflex:

Menace response:

Palpebral reflex:

Corneal reflex:

Dazzle reflex:

Ophthalmic examination

Right eye:

Cornea: shape, if any signs of cornea globosa (with the light from the ophthalmoscope)

Other visual anomalies including cysts (with the light from the ophthalmoscope):

Left eye:

Cornea: shape, if any signs of cornea globosa (with the light from the ophthalmoscope)

Other visual anomalies including cysts (with the light from the ophthalmoscope):

Skiascopy right eye:

Horizontally:

Vertically:

Astigmatism (yes/no):

Fundus reflex (neutralization point):

Cataract: absent, + (slightly), ++ (moderate) or +++ (abundant)

Skiascopy left eye:

Horizontally:

Vertically:

Astigmatism (yes/no):

Fundus reflex (neutralization point):

Cataract: absent, + (slightly), ++ (moderate) or +++ (abundant)

Definition of the cataract

Location: Nuclear or cortical

Extension:

Absent: No visible cataract or only sporadically streaks. Normal fundus reflex.

Slightly: cataract with a distribution of less than 25 % of the area of the lens in the coronal plane.

Moderate: cataract with a distribution of about 25-50 % of the area of the lens in the coronal plane.

Abundant: cataract with a distribution of more than 50 % of the area of the lens in the coronal plane.

12. Appendix 2

Owner questionnaire for the MCOA study at SLU 2016

Name of the horse:

ID number:

Date of birth:

Breed:

Coat color:

Owner:

Address:

Telephone number:

E-mail:

1. What is the main use of your horse?

2. How well does your horse perform on a scale from 1 to 10, where 1 represents poor and 10 good.

1 2 3 4 5 6 7 8 9 10

Performs very poorly performs very good

Comments:

3. Is the horse difficult to handle?
   Indicate on a scale from 1 to 10, where 1 represents that the horse is very easy to handle and 10 very difficult to handle.

   1 2 3 4 5 6 7 8 9 10

Very easy to handle very difficult to handle

   Comments:

4. Only horses used for riding: Is the horse difficult to ride?
   Indicate on a scale from 1 to 10, where 1 represents that the horse is very easy to ride and 10 that the horse is very difficult to ride.

   1 2 3 4 5 6 7 8 9 10

Very easy to ride very difficult to ride

   Comments:

5. Does your horse show any signs of impaired vision? Indicate on a scale from 1 to 10, where 1 represents that the horse shows no signs of impaired vision at all and 10 that the horse shows clear signs of impaired vision.

1 2 3 4 5 6 7 8 9 10

No impaired vision at all clear signs of impaired vision

Comments:

6. If your horse seems to have impaired vision, indicate on which eye, left, right or both.

7. If your horse seems to have impaired vision, indicate whether the vision has worsened during the past two years?

8. Is there any known history of eye disorders? If yes, please specify what disorder and if the horse was treated.

9. Does your horse show any signs of adverse behavior? For example is hard to catch in the paddock. If yes, please specify which adverse behavior.

10. How easily frightened is your horse? Indicate on a scale from 1 to 10, where 1 represents that the horse is not easily frightened at all and 10 that the horse is very easily frightened.

    1 2 3 4 5 6 7 8 9 10

Not easily frightened at all very easily frightened

    Comments:

11. Has your horse ever been injured? If yes, please specify how many times, what kind of injury and how it occurred.

12. Additional valuable information about your horse: