The effect on performance in sports in descendents of CLCN1 gene mutation carrier New Forest pony stallions

Authors: D.M. Dickhoff; I.D. Wijnberg

Abstract

Aim of the study: To determine if ponies descending from a CLCN1 gene mutation carrier stallion perform better in sports, compared to ponies that do not descent from a CLCN1 gene mutation carrier stallion.
Study design: Data analysis of 11.414 New Forest ponies, in which the relationship between the descent of the ponies and their sport performance are analyzed.
Methods: Ponies were divided in jumping, dressage and eventing categories. They were listed categorically from the lowest category to the highest and descendents from mutation carrier stallions were marked. Statistical analysis with logistic regression between the sport categories and within the categories has been performed using SPSS version 19. Significance was set at $p < 0.05$.
Results: Ponies descending from a mutation carrying stallion are significantly better performing in jumping. The odds of finding a descent in the highest jumping category is 7.6 compared to the lowest. In dressage, descendents from a gene mutation carrier stallion are performing significantly better, with an odds of 4.1 for performing in the highest category. In eventing, the odds of finding a descendent from a mutation carrying stallion in the highest category is 2.9 compared to the lowest.
Conclusion and clinical relevance: Ponies that are descendants of a mutation carrying stallion are performing significantly better in jumping, dressage and eventing. This conclusion might lead to breeding programs which includes stallions who carry this mutation, aiming to breed better performing ponies in equine sports, which is in contrast of the aim of the Studbook to eradicate the mutation.

Introduction

Recently, a mutation in de CLCN1 gene leading to congenital myotonia in New Forest ponies was discovered[1]. Myotonia is defined as: "the delayed relaxation of skeletal muscles after a voluntary contraction or a contraction induced by an electric or mechanical stimulus"[2]. Defects of ion transport channels in de skeletal muscle membrane can lead to myotonia. Mostly, it is a defect of sodium or chloride channels, but the potassium and calcium channels can also be involved[3]. CLCN1 is a skeletal muscle chloride channel. A mutation in the CLCN1 gene can lead to a defect in the muscle chloride channel, which regulates the electrical excitability of the skeletal muscle membrane. A reduction of the muscle chloride conductance causes membrane potential to rise, resulting in membrane hyperexcitability and hence myotonia[3]. The CLCN1 gene mutation had also been found in other species, which are listed in table 1.

Table 1: *Congenital myotonia in other species and their inheritance*

Species affected with inherited myotonia	**Inheritance**	**Defective channel**
Human[2]	Autosomal dominant (Thomsen's disease)	Chloride
Human[2]	Recessive (Becker's disease)	Chloride
Dog[3] (e.g. Miniature Schauzers[4], American Cattle dogs[5])	Autosomal recessive	Chloride
Goat (Tennessee goats[6])	Autosomal dominant	Chloride
Cat[3] (Any race[7])	Unknown	Unknown
Horse (New Forest ponies[1])	Autosomal recessive	Chloride

The severity of muscle channelopathies depends both on the degree of channel impairment caused by the mutation, and on the number of the mutant channels are engaged in the pathophysiological process. Homozygosity for dominant mutations increases severity of

muscle channelopathies[8]. This explains why congenital myotonia can occur in varying degrees of severity. In horses, the inheritance is autosomal recessive, which means that clinical signs are most apparent when ponies are homozygous for the CLCN1 gene mutation., leading to severely disabled individuals unable to perform[1]. Humane patients that are heterozygous for the mutation, carry the CLCN1 gene mutation but can be non-symptomatic carriers[9]. The effect of a heterozygous mutation of the CLCN1 gene in horses has not been described yet however overt clinical signs are absent. The chances of a foal inheriting the mutation are listed in table 2.

Table 2: *Chances of inheritance of the CLCN1 gene mutation in a descendent*

	Mare free	Mare carrier
Stallion free	Offspring is free	50% carriers, 50% free
Stallion carrier	50% carriers, 50% free	25% free, 50% carriers, 25% clinical signs

Many other genetic muscle defects that can be apparent in horses, dogs, humans or other species. For example, in humans and in the Quarter Horse is Hyperkalemic Periodic Paralysis (HYPP) a well-known ion channel congenital defect. A permanent defect in ion transport across the skeletal muscle cell membrane is apparent, resulting in increased sodium permeability, reduced polarization, and changes in muscle excitability[10]. The clinical signs are characterized by intermittent episodes of muscular tremor, weakness, and collapse.[11] The presence of these episodes makes horses with HYPP unsuitable for equine sports. In humans, muscle weakness during an episode of HYPP is most apparent[8]. Episodes can be triggered by stress, fasting, rest after exercise and eating food high in potassium. Though it is possible to take that in account, it is hard to escape certain conditions at any time in life.
In humans, congenital myotonia can be apparent in two genetic forms: autosomal dominant (Thomsen's disease) and autosomal recessive (Becker's disease). Both derive from a chloride channel mutation on the 7th chromosome, but the differences between the two types of myotonia are probably caused by different mutations of this gene[12]. In contrast to HYPP, there are cases reviewed were in humans who performed sports, still could continue with their sporting activities very well after discovering the disease[13,14,15]. Medical treatment was used to prevent cramps and muscle stiffness, by the use of carbamazepine[11] or mexiletine[13]. Muscle stiffness may be enhanced by inactivity, and is often relieved by exercise. Exercising daily with preventative medication may relieve muscle stiffness and is therefore the best therapeutic option in humans, who suffer clinically from congenital myotonia.[5]

Homozygote ponies suffering from congenital myotonia develop hypertrophic muscles and are stiff during movement[1,4]. Also, the pelvic limbs are extremely straight and horses can be imbalanced[1]. They may experience difficulty rising to their feed after recurrent episodes of lateral recumbency. Horses seem hyperreactive, protrusion of the nictitating membrane occurs after stimulation as well as abnormal vocalisation[1].

The mutation of the CLCN1 gene is probably introduced in the New Forest pony population by one stallion, starting at the beginning of the 1990's when this stallion was approved by the studbook as a breeding stallion[16]. It was a very popular stallion, so many descendents were bred and some of them became a breeding stallion themselves. Descendents competed in sports, in which many of them performed very well[4]. This suggested that, though the CLCN1 gene mutation was introduced into the population, the descendents could still be good competitors in equestrian games. The aim for the study was therefore to determine if ponies descending from a CLCN1 gene mutation carrier stallion perform better in sports, compared to ponies that do not descent from a CLCN1 gene mutation carrier stallion.

Materials and methods

Collecting data: After the gene mutation was discovered in 2011, all breeding stallions have been tested as a carrier of this gene mutation. Nine stallions tested positive as a carrier. An

overview of the mutation-carrying stallions was presented by the Dutch New Forest Pony studbook.

The Royal Dutch Equestrian Sports federation (KNHS) presented an overview of all adult New Forest ponies that participated in equestrian games since 1990, their performance and descent. This list contained 11.414 individual ponies, performing jumping, dressage and/or eventing and how many gain points they reached in the highest achieved level. 6455 ponies participated in dressage, from which 3226 were mares, 143 stallions and 3086 geldings. In jumping, 4349 ponies participated: 2079 mares, 107 stallions and 2163 geldings. 610 ponies participated in eventing, from which 261 were mares, 12 stallions and 337 geldings. The average age of the competing ponies was unknown, ponies are allowed to compete in equine sports when they are at least 4 years of age.

The higher the category, the lesser the amount of participants. In the highest categories, the number of participants were very few, therefore these groups are pooled together. In jumping, the >ZZ category contains not only ponies in the ZZ category, but also 4 ponies that are performing on a higher international level. In dressage, the >Z2 category contains 4 ZZ-L ponies and 1 ZZ-Z pony. In eventing, the >Z category contains also 1 ZZ pony, 4 CClP-1 ponies and 1 CClP-2 pony.

The sequence of the categories for jumping are – from low to high: B (beginners level: 1721 participants) – L (low level: 1223) – M (intermediate level: 778) – Z (heavy level: 478) – >ZZ (extra heavy level: 149), for dressage B (beginners level: 2305 participants) – L1 (low level 1: 1756) – L2 (low level 2: 538) – M1 (intermediate level 1: 907) – M2 (intermediate level 2: 319) – Z1 (heavy level 1: 329) - >Z2 (heavy level 2: 301) and for eventing B (beginners level: 343 participants) – L (low level: 143) – M (intermediate level: 41) - >Z (heavy level: 53).

These two data bases were used to analyse the performance level of the descendents of mutations carrier stallions compared to the performance level of descendents of non-mutation carrier stallions.

Data analysis: An overview was made to evaluate the results of the ponies in correlation to the presence of the mutation. It was determined how many descendents of mutation carrying stallions (DMCS) are present in the top 25% and top 10% of all ponies in all categories, and it was determined how many DMCS are present in each category: jumping, dressage and eventing. The categories were arranged from low to high. After determining the amount of DMCS that competed in a category, a percentage has been calculated compared to the total amount of participants in that category. Also, a percentage has been calculated for the amount of DMCS in the top 25% and top 10% within a category, compared to the total amount of participants in the top 25% and top 10%.

Statistical analysis: Logistic regression analysis and odds ratio's were calculated using the statistical program SPSS version 19. Significance was set at $p<0.05$. All higher categories were compared to the lowest sport level (beginners level (B)). Within the category, the odds ratio's between the total participants and the top 25% and top 10% were calculated. Results are listed in tables, significance is indicated with *.

Results

Jumping

The higher, the category, the higher the odd's ratio's. The odds of finding a descendent from a mutation-carrying stallion are 7.6 times greater in the highest category (>ZZ) then in the lowest category (B). The odds of finding a descendent from a mutation-carrying stallion are 3.3 times greater in the category Z then in category B. For the categories M and L, the odds are 2.7 respectively 1.7, compared to category B (table 3.1 and table 3.2).

Table 3.1: *Percentage of DMCS compared to the total amount of participants per category, and in the top 25% and top 10% of each category in jumping.*

Category	Participants	Potential mutants	%	Mutants top 25%	%	Mutants top 10%	%
B	1721	92	**5,34%**	35	**8,14%**	15	**8,72%**
L	1223	105	**8,59%**	47	**15,36%**	16	**13,11%**
M	778	103	**13,24%**	40	**20,51%**	19	**24,35%**
Z	478	75	**15,69%**	24	**20,00%**	10	**20,83%**
ZZ	149	46	**30,87%**	18	**48,65%**	7	**46,67%**

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category. DMCS: descendents of mutation carrying stallions

Table 3.2: *Odds ratio's for being a descendent of a carrier stallion for the CLCN1 gene mutation per category in jumping*

Category	Odds ratio
L	1.663*
M	2.703*
Z	3.295*
>ZZ	7.612*

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category

The odds of finding a DMCS are 1.5 times greater in the top 25% of the ponies, compared to the 100% participants in category B. In the L category, the odds of finding a DMCS are 1.9 times greater in the top 25% of the ponies, compared to all the participants in this category. The odds of finding a DMCS are 1.7 times greater in the top 25% of the ponies, compared to the 100% participants in category M. For the top 10%, the odds are 2.1 in the M category. No significant odds ratio's were found in the Z category. In the category ZZ or higher, the odds of finding a DMCS are 2.1 times greater in the top 25% of the ponies, compared to the 100% participants in this categories (table 3.3)

Table 3.3: *Odds ratio's for being a descendent of a carrier stallion for the CLCN1 gene mutation the top 25% and top 10% within each category in jumping*

Category	OR top 25%	OR top 10%
B	1.569*	1.692
L	1.932*	1.607
M	1.691*	2.110*
Z	1.343	1.414
>ZZ	2.121*	1.959

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category. OR: Odds ratio.

Dressage

In the L1, M1 and Z1 categories, Odds ratio's were less then 1 which indicated that DMCS are performing significantly worse then ponies who are non DMCS (table 4.1 and table 4.2). But when odds ratio's are calculated within each category for the top 25% and top 10%, DMCS seemed to perform significantly better than non DMCS, especially in the highest category (table 4.3). The odds of finding a DMCS are 1.8 times greater in the top 10% of the ponies, compared to the 100% participants in category B. In the L1 category, the odds of finding a DMCS are 1.5 times greater in the top 25% of the ponies, compared to the 100% participants in category L1. There were no significant odds ratio's found in the L2 category. The odds of finding a DMCS are 1.7 times greater in the top 25% of the ponies, compared to the 100% participants in category M1. No significant odds ratio's were found in the M2

category. In the category Z2 or higher, the odds of finding a DMCS are 4.1 times greater in the top 10% of the ponies, compared to the 100% participants in these categories.

Table 4.1: *Percentage of DMCS compared to the total amount of participants per category, and in the top 25% and top 10% of each category in dressage*

Category	Participants	Potential mutants	%	Mutants top 25%	%	Mutants top 10%	%
B	2305	181	**7,85%**	52	**9,03%**	31	**13,42%**
L1	1756	96	**5,47%**	36	**8,20%**	16	**9,09%**
L2	538	37	**6,88%**	8	**5,93%**	5	**9,26%**
M1	907	50	**5,51%**	21	**9,25%**	9	**9,89%**
M2	319	21	**6,58%**	5	**6,25%**	1	**3,13%**
Z1	329	9	**2,73%**	4	**4,88%**	3	**9,09%**
>Z2	301	17	**5,68%**	8	**10,63%**	6	**20%**

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category. DMCS: descendents of mutation carrying stallions

Table 4.2: *Odds ratio's per category in dressage*

Category	Odds ratio
L1	0.679*
L2	0.867
M1	0.685*
M2	0.827
Z1	0.330*
>Z2	0.085

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category.

Table 4.3: *Odds ratio's for the top 25% and top 10% within each category in dressage*

Category	OR top 25%	OR top 10%
B	1.165	1.819*
L1	1.545*	1.729
L2	0.853	1.382
M1	1.747*	1.881
M2	0.946	0.458
Z1	0.281	0.513
>Z2	1.960	4.103*

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category. OR: Odds Ratio

Eventing

The odds of finding a descendent from a mutation-carrying stallion are 2.9 times greater in the highest category (>Z) then in the lowest category (B) (table 5.1 and 5.2). Within eventing categories, no significant odds ratio's were found (table 5.3).

Table 5.1: *Percentage of DMCS compared to the total amount of participants per category, and in the top 25% and top 10% of each category in eventing*

Category	Participants	Potential mutants	%	Mutants top 25%	%	Mutants top 10%	%
B	343	28	**8,16%**	9	**10,46%**	5	**14,71%**
L	173	11	**6,36%**	4	**9,30%**	1	**5,88%**
M	41	4	**9,76%**	2	**20%**	0	-
>Z	53	11	**20,75%**	3	**22.64%**	2	**37.74%**

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category, ZZ: extra heavy category. DMCS: descendents of mutation carrying stallions

Table 5.2: *Odds ratio's per category in eventing*

Category	Odds ratio
L	0,764
M	1,216
>Z	2,946*

Legends: L: light category, M: Middle category, >Z: Heavy or extra heavy category

Table 5.3: *Odds ratio's for the top 25% and top 10% within each category in eventing*

Category	OR top 25%	OR top 10%
B	1.315	1.940
L	1.510	0.920
M	2.312	-
Z	0.740	0.925

Legends: B: Beginner category, L: light category, M: Middle category, Z: Heavy Category,OR: Odds Ration

Discussion

The most important findings of the study presented above are the following: DMCS perform significantly better in jumping compared to ponies that are not descending from CLCN1 mutation carrying stallions. In eventing, there is a significant difference between the highest and lowest level of performing, in favour of the DMCS. In dressage, within the highest category (Z2) the odds of finding DMCS in the top 10% is also signifcantly high. These findings that in the highest categories the odds ratio's for being a carrier are higher that in the lowest category, suggest that being a DMCS is advantageous for a successful sport career, regardless the type of sport a pony is involved in. This hypothesis is also illustrated by the fact that significantly more ponies were carrier in the higher categories that in the lower category, at least for jumping and eventing.

The explanation for this fact might be due to the hypertonic muscles in the hindquarters of the pony[17], which can result in a more powerful take-off when the horse jumps an obstacle. This might be due to the enhanced contractility, e.g. prolonged contraction. In equine patients with congenital myotonia, typical waxing and weaning EMG patterns are discovered[4]. In humans with non-dystrophic myotonia, measurement with ultrasound gave elevated echo intensities, which was inversely correlated to the corresponding ranges-of-motion[18]. Differences were seen in thickness, ranges-of-motion and force of four skeletal muscles between non-dystrophic myotonia patients and the control group. In human patients with myotonia, muscles were hypertrophic (+0.5-0.9SD), echo intensities were elevated (+1.3-2.2SD) and range-of-motions were decreased (-0.27-0.43SD)[18]. Elevated echo intensities are often seen in myotonic patients, due to the increase of connective tissue and fat[19]. Hypertrophic muscles are due to the increase of protein synthesis in the muscle cells, which lead to thicker muscle fibers and therefore a thicker muscle. This might lead to a greater muscle force. The decreased range-of-motion that was seen in human patients with myotonia[18], might be due to muscle stiffness, which has also been seen in equine patients with myotonia[1, 17]. To what extend muscle stiffness is present in carrier horses in unknown at this moment, but apparently, in none of the types of sport the carrier status was performance limiting.

In each type of sport, it appeared that more DMCS ponies were overrepresented in the top 25% best performing ponies than non DMCS ponies. However it appeared that in the upper 10% best performing ponies no further increase in representation of the DCMS ponies was noticed. This fact might be explained by other influences, such as the riders quality. Some riders are very experienced, others are not and there is a chance that a rider therefore might be

contra productive for optimal performance for the pony during a contest. Also, the intensity and quality in training of the horse can also be different between individual horses and riders. Some of them might be better or more intense trained then others. The age of the competing horse in sport can also be a depending factor. When a horse is older when the training starts, it might be harder for this horse to get to the top level, comparing to younger horses. The ages of the 11.414 ponies are unknown, therefore no calculation in this study can be done to determine if age is a depending factor for the performance in sports.

All stallions that are used for breeding have been tested if they are carrying the mutation. However, the frequency of the presence of the mutation in the examined population is unknown. Inheritance of the mutation is also influenced by maternal genetic background, meaning if mares are DMCS themselves. A limitation of the study is that information about the background of the mares could not be acquired from the data set of the sports federation. Ideally, all New Forest ponies that are competing in sports should be tested if they are carrying the CLCN1-gene mutation. Because of logistic and financial reasons, only data files have been used.

The results that were achieved with this research might be useful to the studbook, owners from New Forest ponies and their breeding programmes. The stallion that was first identified being a carrier, was a highly successful performer and therefore a very popular breeding stallion. When the mutation of the CLCN1 gene was first discovered, the Dutch New Forest pony studbook planned on eradicating this mutation out of the population. When a pony is homozygous for the mutation, clinical signs can occur and severely affects the welfare of the pony. But when a pony is heterozygous for the mutation, clinical signs may not appear and their sports performance can be positively influenced, as is proven with the results above. Though sport performance might improve, the welfare of the descending ponies should always be kept in mind when breeding with CLCN1 gene mutation carriers, because homozygosity will be leading to severely disabled individuals unable to perform[1]. Breeding gives always certain insecurity and it is questionable if focussing on sports performance is the right thing to do, when animal welfare might be at risk.

Acknowlegdements: The authors thank the New Forest pony Studbook, and especially Mrs D. Frederiks and Mrs T. Scholtens for their help in achieving and organising the used archives

References

[1] Wijnberg ID et al.: "*A missense mutation in the skeletal muscle chloride channel 1 (CLCN1) as candidate causal mutation for congenital myotonia in a New Forest pony*" Neuromuscul Disord (2011), doi:10.1016/ j.nmd.2011.10.001

[2] Reed et al .(2010): "Equine Internal Medicine" 3th ed. St. Louis: Saunders Elsevier Inc. *508-509*

[3] McKerrell R.E.: "*Myotonia in man and animals: confusing comparisons*" Equine Vet J 1987; 19: 266-267

[4] Rhodes T.H. et al.: "*A missence mutation in canine ClC-1 causes recessive myotonia congenital in the dog"* FEBS Lett. 1999 Jul 30;456(1):54-8

[5] Finnigan D.F. et al.: "*A novel mutation of the CLCN1 gene associated with myotonia hereditaria in an Australion Cattle Dog"* J Vet Intern Med. 2007 May-Jun;21(3):458-63.

[6] Bryant S.H.: "*Myotonia in the goat."* Am N Y Acad Sci. 1979;317:314-25

[7] Gaschen F. et al.: "*Congenital disease of feline muscle and neuromuscular junction."* J Feline Med Surg. 2004 Dec;6(6):355-66

[8] Arzel-Hézode M. et al.: "*Homozygosity for dominant mutations increases severity of muscle channelopathies*." Muscle Nerve. 2010 Apr;41(4):470-7. doi: 10.1002/mus.21520.

[9] Sasaki R. et al: "Compound *heterozygous* mutations in the muscle chloride channel gene (*CLCN1*) *in a Japanese family with Thomsen's disease"* Rinsho Shinkeigaku. 2013;53(4):316-9.

[10] Naylor J.M.: "*Equine hyperkalemic periodic paralysis: review and implications"* Can Vet J. 1994 May; 35(5): 279-285

[11] Sloet van Oldruitenborgh-Oosterbaan MM.: "*HYPP: hyperkalemic periodic paralysis in the horse"* Tijdschr Diergeneeskd. 1999 Mar 15;124(6):176-81

[12] Kuhn E.: "*Myotonia congenital (Thomsen) and recessive generalized myotonia (Becker)"* Nervenarzt. 1993 Dec;64(12):766-9

[13]Chew K.T. et al.: "*Myotonia congenital in a young, active man: symptoms and physique tip the diagnosis"* Phys Sportsmed. 2004 Jul;32(7):18-21. doi: 10.3810/psm.2004.07.449.

[14] Weinberg J. et al: "*Occult presentation of myotonia congenita in a 15-year-old athlete"* Am J Sports Med. 1999 Jul-Aug;27(4):529-31

[15] Burnham R.: "*Unusual causes of stiffness in two hockey players*" Clin J Sport Med. 1997 Apr;7(2):137-40.

[16] http://www.kantjes-newforest.nl/kantje%27s_ronaldo_general_info.htm

[17] Schooley et al.: "*Atypical Myotonia Congenita in a Foal*" J Equine Sciences (2004) doi:10.1016/ j.jevs.2004.10.006

[18] Trip et al.: "*Muscle ultrasound measurements and functional muscle parameters in non-dystrophic myotonias suggest structural muscle changes*" Neuromuscul Disord 2009 Jul;19(7):462-7. doi: 10.1016/j.nmd.2009.06.36

[19] I.M.P. Arts et al: "*Spierechografie: een techniek in Beweging*" *Tijdschr Neurol Neurochir 009;110:114-21*