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Case report

A missense mutation in the skeletal muscle chloride channel 1 (CLCN1) as candidate causal mutation for congenital myotonia in a New Forest pony

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Abstract

A 7-month-old New Forest foal presented for episodes of recumbency and stiffness with myotonic discharges on electromyography. The observed phenotype resembled congenital myotonia caused by *CLCN1* mutations in goats and humans. Mutation of the *CLCN1* gene was considered as possible cause and mutation analysis was performed. The affected foal was homozygous for a missense mutation (c.1775A>C, p.D592A) located in a well conserved domain of the CLCN1 gene. The mutation showed a recessive mode of inheritance within the reported pony family. Therefore, this *CLCN1* polymorphism is considered to be a possible cause of congenital myotonia.

Keywords: Muscle spasm; Myotonic discharges; Electromyography; Becker; Thomsen; CLCN1; Horse

1. Introduction

Myotonias are acquired or inherited neuromuscular disorders characterized by the slow relaxation of muscles after voluntary contraction or electrical stimulation. Genetic forms of congenital myotonia are characterized by impaired function of chloride, sodium or potassium ion transport channels in the skeletal muscle membrane [1–3]. It has been previously shown that sarcolemmal chloride conduction is required to preserve membrane potential and functional chloride channels are essential for resting potential stabilization. Different mutations in genes that code for ion channels are involved in several hereditary muscular diseases [1,2,4,5]. Human congenital myotonia is caused by recessive or dominant mutations of *CLCN1* causing Becker's (OMIM 255700) or Thomsen's disease (OMIM 160800). The muscle chloride channel CLCN1 regulates the electrical excitability of the skeletal muscle membrane [1,2,6,7].

The association between myotonia and chloride conduction was first described in myotonic mice [8] and the first mutation in the *CLCN1* gene shown to cause myotonia was found in domestic animals. The "fainting" goats which developed severe acute muscle stiffness and became immobile during vigorous movements or when startled showed a missense mutation of the *CLCN1* gene (p.A885P) [4]. In 1962 the first case of suspected equine myotonia was reported [7]. As the phenotype of the New Forest pony reported here strongly resembled the clinical signs and

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biochemical findings of both human and goat myotonia, we hypothesized that mutations in the *CLCN1* gene might also be responsible for the clinical signs observed in this animal.

In this case study we report a missense mutation in the equine *CLCN1* gene associated with a recessive inherited myotonia disease phenotype.

2. Case report

A 7-month-old New Forest pony stallion was presented to the Equine Clinic of Utrecht University. During the first 4–6 weeks of life the foal had developed normally. Main abnormalities observed by the owner were recurrent episodes of recumbency and difficulty rising to its feet as a result of muscle stiffness. The frequency of these episodes of recumbency had increased in the months prior to presentation. At the time of clinical examination the foal was found to be well developed with above average muscle mass when compared to other foals of the same breed. When stimulated (for example by opening of the stable door or when getting up) the foal seemed hyperreactive and temporary protrusion of the third eyelid occurred due to retraction of the eye uni- or bilaterally (Fig. 1A). During vocalization the whinnying ended in a high pitched squeaking, or a deep growling noise. The pelvic limbs were extremely straight (minimal flexion of the tarsi) (Fig. 1B), and the foal walked reluctantly with a stiff gait. When walking, the pelvic limbs did not always follow the track of the thoracic limbs and the foal easily lost its balance as a result of stiffness and occasionally lost its footing. General examination revealed no other abnormalities than retraction of the eye and protrusion of the third eyelid for approximately 30 s following inspection of the conjunctiva. Neurological examination revealed normal mentation, no cranial nerve deficits and occasionally a wide-based stance. Postural reactions were impossible to perform and picking up the limbs was hampered by the muscle rigidity.

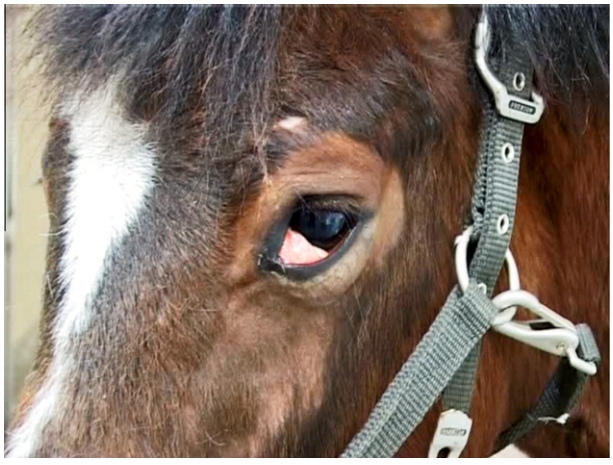

Fig. 1A. Retraction of the eyebulb due to the myotonia. Note the retracted eyebulb due to the myotonia.

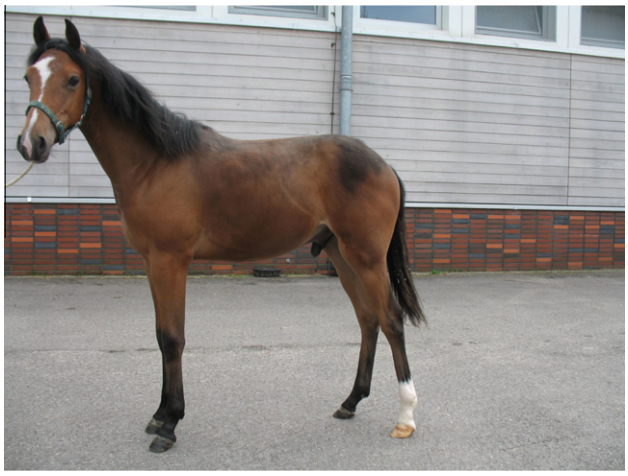

Fig. 1B. Posture of the patient at rest. Note the straight angle of the tarsus.

Manual flexion of the proximal metacarpo/metatarso-phalangeal joints was impossible. Passive movements of the neck and trunk were very limited and the foal was easily pulled off balance. Although the foal showed signs of weakness, ataxia was absent and the rigidity seemed to be the major problem.

Blood biochemical analysis showed a plasma sodium [Na] concentration of 129 mmol/l [ref. 135–150 mmol/l], potassium [K] of 2.8 mmol/l [ref. 3.0–5.9 mmol/l], magnesium of 0.77 mmol/l [ref. 0.80–1.20 mmol/l], chloride [Cl] of 99 mmol/l [ref. 96–107 mmol/l], total calcium [Ca] of 3.97 mmol/l [ref. 2.4–3.3 mmol/l] and phosphate of 1.48 mmol/l [[Pi]; ref. 0.8–1.8 mmol/l]. Blood obtained immediately after an episode of generalized stiffness revealed a [Na] of 138 mmol/l, a [K] of 4.1 mmol/l, a [Cl] of 95 mmol/l and a plasma glucose concentration of 4.1 mmol/l (ref. 3.9–5.6 mmol/l). The fractional excretion of all electrolytes in urine was within normal limits and the acid–base balance of the blood was normal. Muscle enzyme activities were not measured. DNA testing for the mutation of the skeletal muscle sodium channel gene (*SCN4A*) causing hyperkalemic periodic paralysis in Quarter horses was negative. Electromyographic (EMG) needle examination [9,10] using Viking Quest commercial EMG equipment showed prolonged insertional activity caused by spontaneous waxing and waning discharges after needle insertion and as a result of muscle contraction in all measured muscles including the subclavian and descending pectoral muscle. The results of EMG examination were consistent with myotonia [2,5,11,12] (Fig. 2).

Routine morphological (Gomori trichrome, H&E) and histochemical analysis (succinic dehydrogenase, cytochrome oxidase and m-ATPase) of biopsy samples from the M. semimembranosus, M. gluteus medius, M. vastus lateralis and M. triceps brachii caput longum were performed. There was no evidence of muscle damage, rhabdomyolysis or central nuclei but extreme heterogeneity in fiber diameters was noticed in all four muscle specimens examined. In the caput longum of the M. triceps brachii

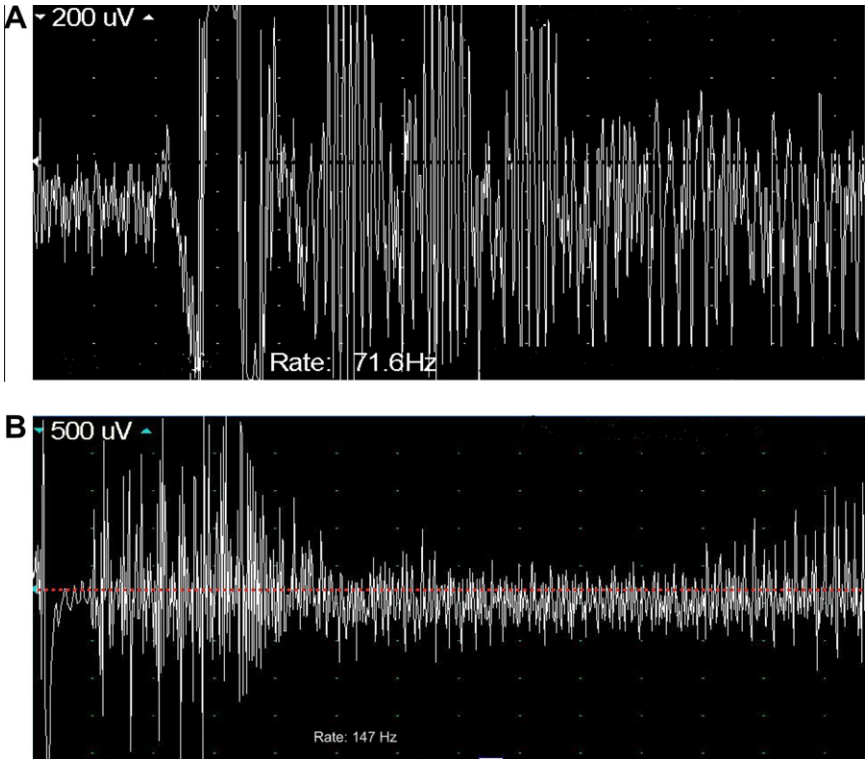

Fig. 2. (A) Myotonic discharges in the M. pectoralis descendens. (B) Myotonic discharges in the M. vastus lateralis. Dotted lines indicate the divisions, one division is 200 μV and 100 ms in 2A and 500 μV and 200 ms in 2B. The firing rate of the discharges is 72 Hz in 2A and 147 Hz in 2B.

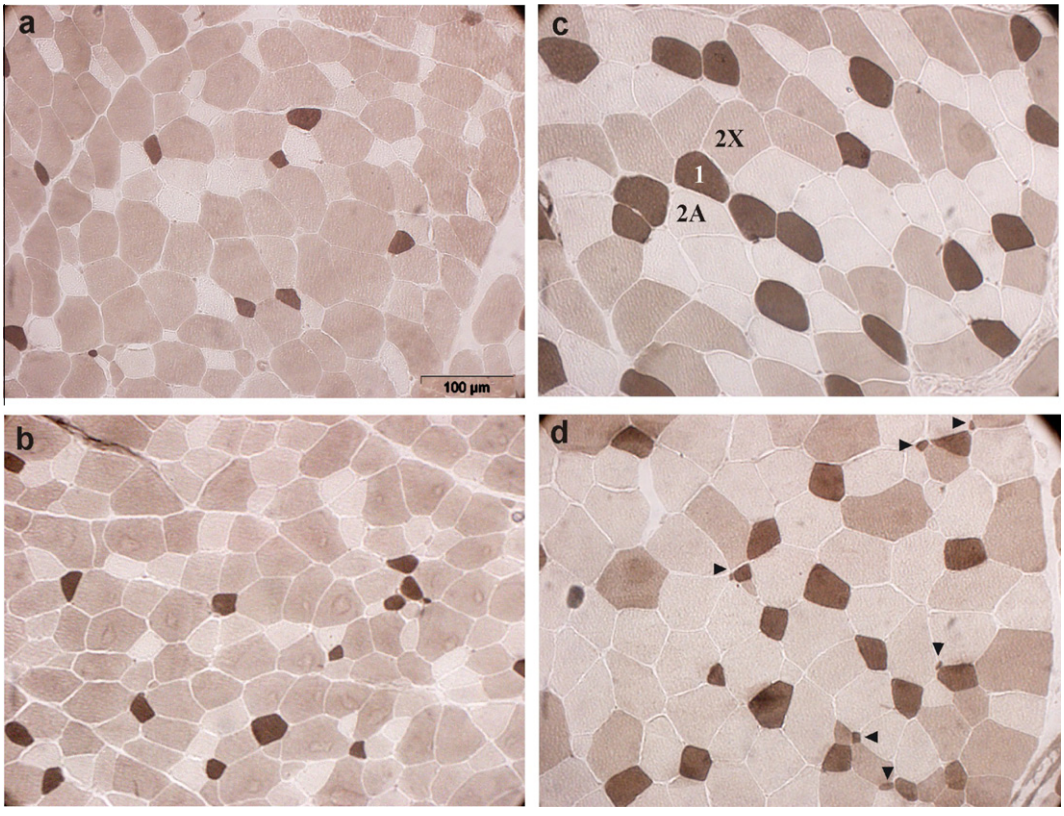

Fig. 3. Histochemical staining of muscle sections from patient affected by myotonia. Panel a. M. semimembranosus; panel b: M. gluteus medius; panel c: M. vastus lateralis; panel d: M. triceps brachii Caput longum. Bar: 100 μm. Muscle samples were frozen in cold isopentane. Serial sections (10 μm) were cut in a cryostat and stained for myofibrillar adenosine triphosphatase (m-ATPase) after acid (pH 4.55) pre-incubation. Slow fibers (type 1) are dark while types 2A and 2× are light. Images were acquired with a bright field microscope (Olympus Vanox AH-3, Japan), equipped with video camera and analyzed with an image analysis software (Olympus DP-software-70).

two distinct type 1 fiber populations were observed. The first consisted of fibers with a diameter of 50.36 ± 9.3 μm, whilst the second population (Fig. 3) consisted of fibers with a very small diameter, 20.21 + 8.8 μm.

We hypothesized that a mutation in the *CLCN1* gene may have been responsible for the clinical signs observed in the present case [7]. The analysed pedigree data suggested a monogenic autosomal recessive inheritance (Fig. 4A). Parents of the affected foal did not show any clinical signs. EDTA blood samples of the affected foal, its dam, its sire, other related ($n = 22$) and unrelated ($n = 21$) New Forest ponies, and 56 horses of various breeds were examined (Supplementary Table 2). Genomic DNA was isolated using standard methods. PCR primer pairs were designed for the amplification of *CLCN1* exons with flanking introns (Table 1) as described previously [13]. The equine *CLCN1* gene has 23 exons (NCBI Gene ID: 100050692), the total predicted transcript length is 2970 bp (Genbank acc. XM_001915636) encoding a protein of 989 aa (Genbank acc. XP_001915671). Initially, all exons and several introns of the equine *CLCN1* gene were amplified using standard protocols applied to a total of four DNA samples from the affected foal, its dam, its sire (Fig. 4A) and one unrelated control horse. The purified PCR products were directly sequenced from both directions using the ABI BigDye v3.1 kit and an ABI 3730 capillary sequencer (LifeTechnologies, Rotkreuz, Switzerland). Sequence data were analysed using Sequencher 4.9 (GeneCodes, Ann Arbor, MI, USA).

A total of three polymorphisms were identified, two single nucleotide substitutions in *CLCN1* exon 15 (c.1593A>G and c.1775A>C) and a three base pair insertion (c.2652_2653insCTT) in exon 23. No other mutations in exonic or splice site regions of the *CLCN1* gene were identified. The c.1593A>G transition did not result in amino acid exchange. The three family members (the foal and its parents) were homozygous for the mutant allele 1593G and the unrelated control horse was homozygous for the wildtype allele 1593A. This mutation was therefore not associated with the disease. A similar genotype distribution was observed for the in-frame insertion (c.2652_2653insCTT) leading to an additional leucine residue after codon 884 which affects a less conserved protein region. In conclusion, these two sequence variants are highly unlikely to be related to the proper function of the encoded protein.

Interestingly, the c.1775A>C transversion led to an amino acid replacement where aspartic acid is replaced by alanine (p.D592A). The affected pony was homozygous C/C for mutant allele1775C, the dam and the sire were heterozygous A/C and the control horse was homozygous A/A for the wildtype allele 1775A (Fig. 4B).

To verify the association of the c.1775A>C mutation with the clinical disease presentation an additional 42 New Forest ponies and 56 horses of 13 different breeds were screened using the methods described above (Supplementary Table 2). None of the investigated animals was

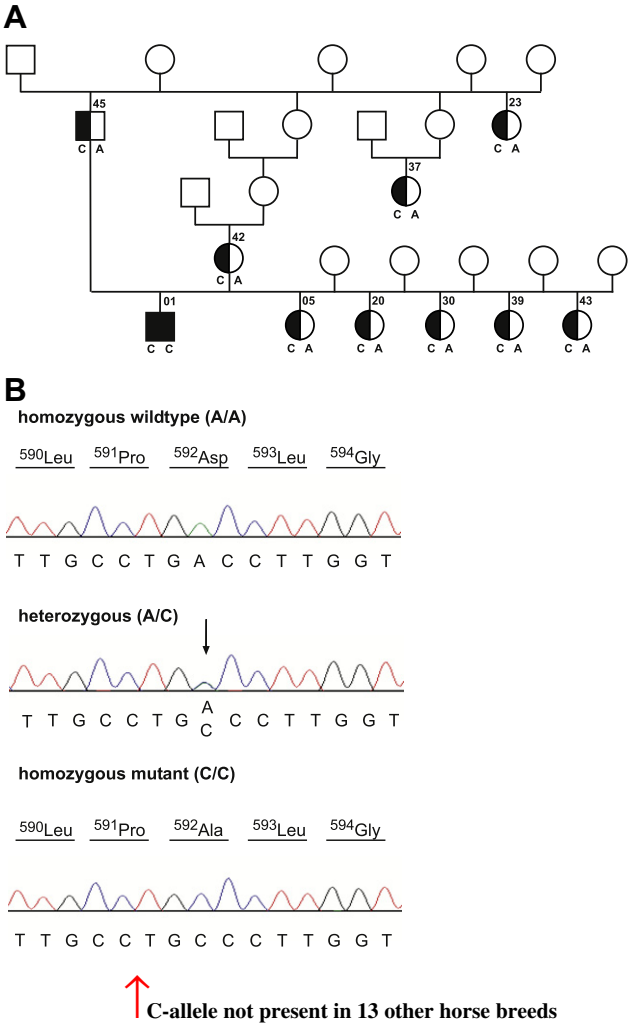

Fig. 4. Pedigree and sequence analysis of genomic DNA of the missense mutation c.1775A>C in exon 15 of the equine *CLCN1* gene of the case's family presented in this study. (A) Pedigree of the case's family presented in this study. DNA samples were available from numbered horses only. The myotonic pony is shown in solid black box, the arrow indicated the patient. The half black boxes are indicating the relatives heterozygous for the mutation. (B) Sequence analysis of genomic DNA of the missense mutation c.1775A>C in exon 15 of the equine *CLCN1* gene. Sequence chromatograms show the wildtype (1775AA; at the top), the carrier (1775A/C; in the middle) and the mutated (1775CC; at the bottom) individuals, respectively.

homozygous for the mutant allele 1775C. Of the genotyped New Forest ponies eight mares (including the mother) and one stallion (the sire of the affected foal) were heterozygous (A/C) for the mutant 1775C allele while all other horses and ponies were homozygous A/A (Supplementary Table 2, Fig. 4A).

To test whether the observed missense mutation affects *CLCN1* splicing we amplified the entire *CLCN1* cDNA of the affected foal. Total RNA extracted from four different skeletal muscles of the affected pony was subjected to a reverse transcription-PCR (RT-PCR) to analyse the *CLCN1* transcript. The entire coding sequence of 2970 bp of the *CLCN1* gene was amplified using five overlapping

Table 1
PCR primer pairs designed for the amplification of *CLCN1* exons with flanking introns.

Primer name	Forward primer sequence	Reverse primer sequence	bp	°C
PCR				
CLCN1exon1	GGCTGTTCCTCAGGTTCTCA	TCTCCAAGTGCACACTCAGC	604	60
CLCN1exon2	AGAACTTGGCGTCCTTGATG	TAGGTGTGCATGTGGAGAGG	482	60
CLCN1exon3	CATCTTCTCACGTTGCCTTG	CCTGGACCAGATTCACACCT	509	60
CLCN1exon4 + 5	GTGGCATCTTGTGAGGTGAA	GGTCCACGTCTGCTCCTAGA	717	60
CLCN1exon6	AATGTTCCTCCATCGAATGC	AGCACAAGAAGGCAATGTGG	463	60
CLCN1exon7	TGGCTTACCTGCCTTCCATA	GAACAAGATCAGGCAAGTGGA	389	60
CLCN1exon8	GCCTGATCCTGACGTTCTGT	CGTCTGCTCCACGACCTTAT	531	60
CLCN1exon9 + 10	GGTTGGAGCAAGGAAGGAAT	CAGAGCATTCTGTCCACCAA	706	60
CLCN1exon11 + 12	TTGCAGGAGTTCAGTGTGGT	AGGCTGTGGTCTCCAAGCTA	694	60
CLCN1exon13 + 14	GCTCCTCTCCTTGTTCTGGA	GCTGGAATGAAGATGTCATGG	748	60
CLCN1exon15 + 16	CAATGTCACTCCTGTCACATCC	TGGAACTCTTGTTCAGGTCACT	738	60
CLCN1exon17 + 18	CTGCTGAGTTGGCCAGAGG	TATGGAGACTAAACAAATAGGAACC	846	60
CLCN1exon19 + 20	GAGGTGAGGCACCTAGGAAG	CCTGCCTAGACTCGCATCTT	619	60
CLCN1exon21 + 22	GCGGATCTGAGGTTGAAGG	CCAGGACTGTGGCCATAATAG	545	60
CLCN1exon23	GATGTGTGTGAGGAGGCCTTA	GGTTAGAACCACCTGCGTGT	770	60
RT-PCR primers				
CLCN1_cds_1	GAGCTGTATTGTGGGGAGGA	AGGCCTTGAGGGTGAGGTAT	693	60
CLCN1_cds_2	CTGGGTCACCTTCCCACTTA	GGATACAGCAGGAGGTGCTT	693	60
CLCN1_cds_3	AATGGATTTCCCCTTTGACC	AAATTTGCTGAGCTGGTTCC	790	60
CLCN1_cds_4	CAGCCCTCCCTCTATGACAG	CGTTGAATCCTGGGTTGTTT	657	60
CLCN1_cds_5	CCAAGACCCTCCATCTTTCA	GGCCTCTTGACAGTCTGCAT	717	60

bp = product length; °C = annealing temperature.

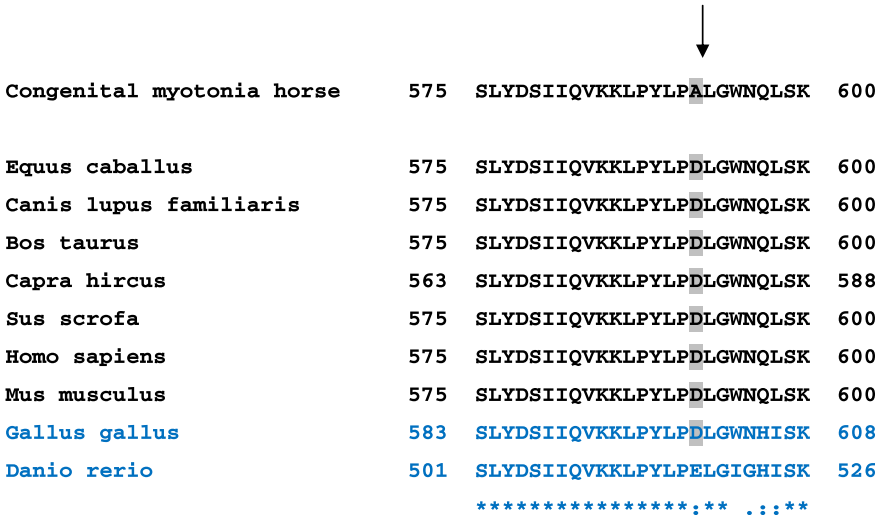

Fig. 5. Multiple sequence alignment of the CLCN1 protein in the region of the mutation demonstrates the conservation of aspartic acid residue 164 (D592) across species. The observed mutation in the myotonia affected horse is indicated by an arrow and the respective position is highlighted in gray. Identical residues are indicated by asterisks beneath the alignment. Genbank accession numbers of reference sequences: Equus caballus XP_001915671.1, Canis lupus familiaris NP_001003124, Bos taurus DAA30294, Capra hircus AAC48666, Sus scrofa XP_003134618, Homo sapiens CAA80996, Mus musculus NP_038519, Gallus gallus XP_425521, Danio rerio XP_695866. The position of the mutation associated with the congenital myotonia phenotype is indicated. The alignment was calculated with ClustalW (http://www.ebi.ac.uk). Identical residues are indicated by asterisks beneath the alignment, while colons and dots represent very similar and similar amino acids, respectively.

PCR products (Table 1) and subsequently re-sequenced from both directions as described above. No alterations of the transcript could be observed apart from the polymorphisms detected at the genomic level.

3. Discussion

Myotonia is a primary skeletal muscle disease characterized by delayed relaxation of skeletal muscle after sudden forceful contraction, sometimes associated with clinical signs of weakness. In humans, congenital myotonia, type Thomsen [14] and generalized myotonia, type Becker [15] are inherited as an autosomal dominant and recessive trait, respectively [3]. Mutations of the *CLCN1* gene have been identified in adr/adr mice [8] and "fainting" goats [4]. Further, *CLCN1* animal models have been described in dogs. Independent autosomal *CLCN1* mutations, with a recessive mode of transmission, cause myotonic phenotypes in

Miniature Schnauzers [16] and in Australian Cattle Dogs [11]. The pony presented in this report demonstrated clinical signs and EMG changes strikingly similar to those observed in myotonic goats, supporting our assumption that the disease phenotype in these two domestic species might have been caused by independent *CLCN1* gene mutations [5,12]. Blood from the foal was examined for the presence of mutations in the *CLCN1* gene and found to be homozygous for the missense mutation c.1755A>C in exon 15. The associated *CLCN1* mutation was probably introduced by the grandfather of the affected foal, as all carriers identified so far could be traced back to this stallion (Fig. 4A). This putative founder was the paternal grandfather and the maternal grand-grandfather of the affected foal. Sequencing of unrelated controls of different breeds (Supplementary Table 2) showed that the *CLCN1* c.1775A>C polymorphism was not present in other horses. The fact that none of the examined horses unrelated to the New Forest pony breed was shown to be carrier of the disease associated allele, indicates that this might be a quite recent mutation, limited to a single breeding line which has not yet spread into the general population. It is possible that a spontaneous *CLCN1* germline mutation occurred in the assumed founder stallion.

The consequence of the c.1775A>C mutation was the substitution of aspartic acid for alanine in codon 592 of the C-terminal cytoplasmic domain of the equine CLCN1 protein. There was no indication that this mutation affects splicing of *CLCN1*. The affected amino acid position is highly conserved across mammalian orthologues (Fig. 5). The human CLCN1 protein is enclosed by two related protein domains, the voltage gated chloride channel and the CBS domain, respectively [17]. As CLCN1 functions as a homooligomer, probably consisting of four subunits [17], it is possible that the codon 592 mutation affects the proper formation of the functional ion channel. The potential impact of this myotonia associated amino acid substitution was evaluated by PolyPhen (http://genetics.bwh.harvard.edu/pph/). The substitution affected a strongly conserved residue and was predicted to be "probably damaging" with a PSIC score difference of 2.25, which implies that the substitution is very likely to affect protein function or structure. The deficiency of functional chloride channels is a very plausible explanation for the observed phenotype. The equine missense mutation seems to be perfectly associated with the condition even though it is located distant to the causative *CLCN1* mutation described in "fainting" goats [4,6,18].

The morphometric results resemble data reported for human patients affected by congenital myotonia: a selective atrophy of type I fibers has been observed in human skeletal muscles [19] together with muscle fiber hypertrophy [1]. However, breed and age differences do exist in muscle morphology [20] and muscle tissue of other New forest ponies was not available to strengthen these conclusions. Human congenital myotonia has also been reported to be result of a heterogeneous trait: apart from the association of congenital myotonia with type I fiber atrophy, generalized muscle fiber hypertrophy was observed in three quarters of patients affected by recessive myotonia, whereas the remainder had normal muscle fiber size [1,21]. A moderate hypertrophy of myotonic fibers has previously been reported in both myotonic goats, [22] and more recently in myotonic dogs [23]. Muscle enzyme activities were not available from this patient but are reported to vary from normal to moderately elevated [2,11] and therefore do not provide conclusive evidence.

In conclusion, this study reports the first case of congenital myotonia in horses for which the molecular genetic cause was analyzed and which was associated with a missense mutation of the equine *CLCN1* gene. It illustrates the importance of precise clinical recognition of putative inherited diseases and illustrates once again the value of the recently deciphered horse genome sequence in unraveling the molecular basis of such genetic anomalies. Our finding enables direct genetic testing and the eradication of this genetic disease from the New Forest pony breeding population. Furthermore, this study provides an additional genetically characterized large animal model for human myotonia.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.nmd.2011.10.001.

References

[1] Lossin C, George Jr AL. Chapter 2 myotonia congenita. In: Rouleau Guy, Gaspar Claudia, editors. Advances in genetics. Academic Press; 2008. p. 25–55.
[2] F- Wu, Ryan A, Devaney J, Warnstedt M, Korade-Mirnics Z, Poser B, et al. Novel CLCN1 mutations with unique clinical and electrophysiological consequences. Brain 2002;125:2392–407.
[3] Kullmann DM. Neurological channelopathies. Annu Rev Neurosci 2010;33:151–72.
[4] Beck CL, Fahlke C, George Jr AL. Molecular basis for decreased muscle chloride conductance in the myotonic goat. Proc Natl Acad Sci USA 1996;93:11248–52.
[5] Cherian A, Baheti N, Kuruvilla A. Muscle channelopathies and electrophysiological approach. Ann Indian Acad Neurol 2008;11:20–7.
[6] Bryant SH, Conte-Camerino D. Chloride channel regulation in the skeletal muscle of normal and myotonic goats. Pflugers Archiv 1991;417:605–10.
[7] Steinberg S, Botelho S. Myotonia in a horse. Science 1962;137:979–80.
[8] Gronemeier M, Condie A, Prosser J, Steinmeyer K, Jentsch TJ, Jockusch H. Nonsense and missense mutations in the muscular chloride channel gene Clc- 1 of myotonic mice. J Biol Chem 1994;269:5963–7.

[9] Wijnberg ID, Van Der Kolk JH, Franssen H, Breukink HJ. Needle electromyography in the horse compared with its principles in man: a review. Equine Vet J 2003;35:9–17.
[10] Wijnberg ID, van der Kolk JH, Franssen H, Breukink HJ. Electromyographic changes of motor unit activity in horses with induced hypocalcemia and hypomagnesemia. Am J Vet Res 2002;63:849–56.
[11] Finnigan DF, Hanna WJB, Poma R, Bendall AJ. A novel mutation of the CLCN1 gene associated with myotonia hereditaria in an Australian cattle dog. J Vet Intern Med 2007;21:458–63.
[12] Arzel-Hézode M, Sternberg D, Tabti N, et al.. Homozygosity for dominant mutations increases severity of muscle channelopathies. Muscle Nerve 2010;41:470–7.
[13] Drögemüller C, Drögemüller M, Leeb T, et al.. Identification of a missense mutation in the bovine ATP2A1 gene in congenital pseudomyotonia of Chianina cattle: an animal model of human Brody disease. Genomics 2008;92:474–7.
[14] George Jr AL, Crackower MA, Abdalla JA, Hudson AJ, Ebers GC. Molecular basis of Thomsen's disease (autosomal dominant myotonia congenita). Nat Genet 1993;3:305–10.
[15] Koch MC, Ricker K, Otto M, et al.. Linkage data suggesting allelic heterogeneity for paramyotonia congenita and hyperkalemic periodic paralysis on chromosome 17. Hum Genet 1991;88:71–4.
[16] Rhodes TH, Vite CH, Giger U, Patterson DF, Fahlke C, George Jr AL. A missense mutation in canine ClC-1 causes recessive myotonia congenita in the dog. FEBS Lett 1999;456:54–8.
[17] Steinmeyer K, Lorenz C, Pusch M, Koch MC, Jentsch TJ. Multimeric structure of CIC-1 chloride channel revealed by mutations in dominant myotonia congenita (Thomsen). EMBO J 1994;13:737–43.
[18] Camerino DC, Pierno S, De Luca A, Bryant SH. Antimyotonic effects of tocainide enantiomers on skeletal muscle fibers of congenitally myotonic goats. Neuromuscul Disord 2000;10:160–4.
[19] Brooke MH, Engel WK. The histographic analysis of human muscle biopsies with regard to fiber types. 3. Myotonias, myasthenia gravis, and hypokalemic periodic paralysis. Neurology 1969;19:469–77.
[20] Rivero JLL, Galisteo AM, Agüera E, Miró F. Skeletal muscle histochemistry in male and female Andalusian and Arabian horses of different ages. Res Vet Sci 1993;54:160–9.
[21] Berchtold MW, Brinkmeier H, Müntener M. Calcium ion in skeletal muscle: its crucial role for muscle function, plasticity, and disease. Physiol Rev 2000;80:1215–65.
[22] Atkinson JB, Swift LL, Lequire VS. Myotonia congenita. A histochemical and ultrastructural study in the goat: comparison with abnormalities found in human myotonia dystrophica. Am J Pathol 1981;102:324–35.
[23] Vite CH, Cozzi F, Rich M, Klide AK, Volk SW, Lombardo R. Myotonic myopathy in a miniature Schnauzer: case report and data suggesting abnormal chloride conductance across the muscle membrane. J Vet Intern Med 1998;12:394–7.