Clinical Commentary

Hyperkalaemic periodic paralysis: Mother nature versus human nature

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In this issue, Diakakis *et al.* (2008) report an interesting case of a Criollo Argentino gelding (with no Quarter Horse breeding) presenting episodic muscle weakness associated with hyperkalaemia. The horse demonstrated similar symptoms in response to oral KCl challenge, the somewhat risky test for hyperkalaemic periodic paralysis (HYPP) prior to the availability of a genetic method (Cox 1985; Spier *et al.* 1990). The presentation of the horse suggests an ion channelopathy, although mutation analysis has not yet been done. Here, we compare this case to the better-known HYPP in Quarter Horses, Paints and Appaloosas, and review the human ion channelopathies. We also review the current regulatory environment for genetic mutations by breeding associations.

Sodium channel mutation terminology

Hyperkalaemic periodic paralysis in horses is caused by a single base-pair or point mutation of the sodium channel gene SCN4A which encodes the main (alpha) subunit of the muscle voltage-gated sodium channel, $Na_V1.4$. The alpha subunit consists of

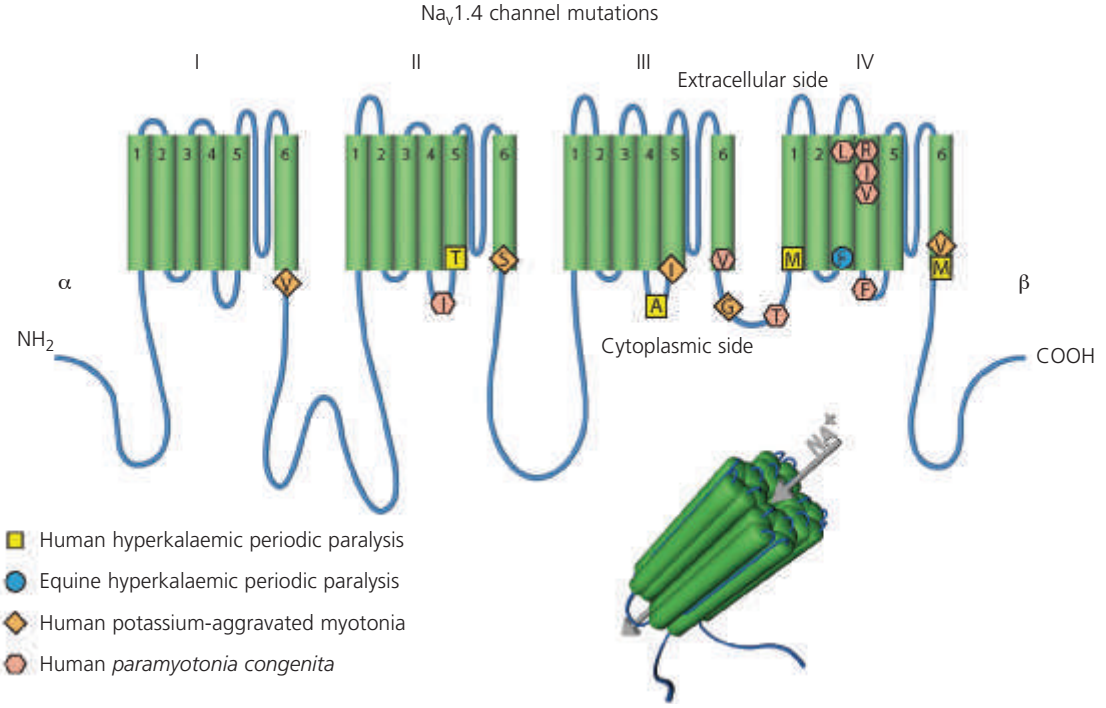

Fig 1: The alpha subunit of the sodium channel ($Na_V1.4$) is depicted. To date over 20 missense mutations (causing amino acid substitutions) resulting in altered muscle function have been discovered in man and one in horses. Reported mutations are shown (coloured symbols with conserved amino acid abbreviation).

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approximately 2000 amino acids, and this missense mutation altered the 1416th amino acid, substituting a highly conserved phenylalanine (F) residue to leucine (L) on the cytoplasmic side of the channel (Rudolph *et al.* 1992; Hanna *et al.* 1996). The amino acid number of the affected phenylalanine in horses is 1416, which is homologous to the phenylalanine at position 1412 in the rat muscle sodium channel gene and position 1419 in the human gene. The equine HYPP mutation can therefore be referred to as 'equine $Na_v1.4$-F1416L', homologous to 'rat $Na_v1.4$-F1412L' or 'human $Na_v1.4$-F1419L'. More than 20 voltage-gated sodium channel mutations have been identified in human families with HYPP and related diseases such as *paramyotonia congenita* and potassium-aggravated myotonia (reviewed below) (Ptácek *et al.* 1991; Lehmann-Horn and Jurkat-Rott 1999; Vicart *et al.* 2005; Catterall *et al.* 2005).

The sodium channel forms a pore through the plasma membrane, allowing intracellular movement of sodium upon depolarisation (activation) of the muscle (**Fig 1**). The alpha subunit contains the ion-conducting pore that allows for transport of sodium into the myofibre causing the upstroke of the action potential. $Na_V1.4$ normally allows rapid propagation of the muscle action potential along the surface membrane and radially into the myofibre via the transverse (t) tubules, where depolarisation triggers Ca^{2+} release from the sarcoplasmic reticulum.

The horse in the case report (Diakakis *et al.* 2008) probably has a mutation in the $Na_V1.4$, but could have a mutation involving another muscle membrane channel, such as the potassium channel or voltage-gated dihydropyridine-sensitive calcium channels that initiate intracellular Ca^{2+} release and muscle contraction.

The $Na_V1.4$ protein consists of approximately 2000 amino acids with characteristic intracellular and extracellular domains as well as 4 conserved membrane spanning domains (I–IV), each composed of 6 transmembrane subunits (1–6). The $Na_V1.4$ protein forms channels that control the flow of sodium ions into muscle cells. Many different mutations have been identified in the sodium channel alpha subunit (SCN4A) gene that result in 3 different inherited channelopathies, including HYPP, *paramyotonia congenita* and potassium-aggravated myotonia. Many of the mutations are located near the intracellular region of the protein and cause failure of inactivation of the sodium channel. This failure of inactivation causes membrane depolarisation, resulting in membrane irritability, myotonia or paralysis (Cannon *et al.* 1995; Hanna *et al.* 1996; Lehmann-Horn and Jurkat-Rott 1999).

All 3 of these diseases in man are potassium-sensitive, meaning potassium challenge could produce clinical symptoms of myotonia or weakness as occurred in the case report by Diakakis *et al.* (2008).

Sodium channelopathies

Hyperkalaemic periodic paralysis

Hyperkalaemic periodic paralysis (HYPP) occurs in approximately 4% of registered Quarter Horses (Bowling *et al.* 1996) and more rarely in man. This disease is characterised by episodes of uncontrollable muscle twitching, pronounced muscle weakness and difficulty regulating blood potassium. HYPP is inherited as a dominant disorder, meaning while only one allele is required to express disease (affected heterozygotes are depicted as N/H in the genetic test). Due to intensive line breeding, homozygous (H/H) horses have been identified (2 copies of the affected gene), and these are more severely affected (Naylor 1999). Some breeders misrepresent the heterozygotes (N/H) to potential buyers as being asymptomatic, implying that are not clinically affected. While these horses appear 'normal' most of the time, their muscle function is altered and they can suffer severe episodes of paralysis and even death. The heterozygotes (N/H) have a statistical probability of producing clinically affected offspring 50% of the time when bred to genotypical normal horses (N/N).

Geneticists use the terms 'incomplete penetrance' and 'variable expressivity' to explain the often variable phenotypic effects of a particular genetic defect. Penetrance is a populational term and refers to the proportion of individuals with a particular mutation that exhibit signs and symptoms of a genetic disorder. If some mutants do not develop features of the disorder, the condition is said to have incomplete penetrance. Equine HYPP exhibits incomplete penetrance, which probably results from a combination of genetic and environmental modifiers. Genetic modifiers are other genetic traits in the horse genome that may make the disease milder or more severe, while environmental modifiers include effects of diet and exercise regimes. Incomplete penetrance makes it challenging for some breeders to understand the implications of not eliminating this disease from their breeding programmes. The term 'variable expressivity' refers to the range of expression among symptomatic individuals with the same mutation. The clinical signs of equine HYPP vary considerably - from mild muscle tremors to life-threatening heart and respiratory paralysis.

Although the features are highly variable, all horses with HYPP have the same mutation in the same gene. As with incomplete penetrance, variable expressivity is probably caused by a combination of genetic and environmental factors, as well as other genes regulating muscle function and expression of mutant channels (Zhou *et al.* 1994).

Hyperkalaemic periodic paralysis in horses was first recognised in the early 1980s in descendants of the immensely popular halter horse stallion, Impressive. This prolific sire, born in 1969, has 366,000 offspring registered with the American Quarter Horse Association (AQHA) (personal communication, Gary Griffith, AQHA Registrar, May 2008), and these offspring dominate the halter horse industry. Current estimates indicate that 4% of the Quarter Horse breed (over 5 million Quarter Horses are registered worldwide) may be affected. Unfortunately, the gene frequency has not decreased substantially in the past 15 years since genetic testing has been available to breeders, and controversy continues among horse breeders whose stock carry this gene (Spier 2006). Affected horses appear to have been preferentially selected as breeding stock due to their pronounced muscle development and there

is evidence of selection of HYPP-affected horses as superior halter horses by show judges (Naylor 1994).

Prior to the development of the DNA test, diagnosis was based on clinical symptoms of altered muscle function associated with hyperkalaemia or provocative challenge with potassium challenge. The DNA test was developed following sequencing of the $Na_V1.4$ gene (SCN4A) in a large pedigree of horses affected with the disease that were descendants of Impressive. As this stallion was so popular and prolific, HYPP also occurs in other stock horse breeds including the American Paint Horse, Appaloosa, Palomino, Pony of the Americas and even grade (unregistered) horses.

Although the same mutation has been identified in more than 65,000 HYPP-affected horses (data from Veterinary Genetics Laboratory, University of California, Davis, May 2008), there is a possibility that other mutations of the muscle sodium channel gene (or other genes) exist undetected in some horses as in the case in the report by Diakakis (2008). These horses would have the clinical syndrome of HYPP but would be declared 'normal' on the presently available commercial DNA test for HYPP.

HYPP and related diseases in man

Hyperkalaemic periodic paralysis

Hyperkalaemic periodic paralysis and 2 similar channelopathies, *paramyotonia congenita* and potassium-aggravated myotonia, are all caused by point mutations in the alpha-subunit of a sodium channel ($Na_V1.4$) expressed selectively in skeletal muscle. There are 9 different sodium channels expressed in the brain, peripheral nerves, cardiac or skeletal muscle. Sodium channels are critical for the generation and propagation of action potentials in neurons, heart and muscle. In human HYPP, the altered channel precipitates episodes of extreme muscle weakness, usually beginning in the teens or 20s, and depending on the type and severity of HYPP, will increase or stabilise until age 40 or 50 where attacks usually decline and can altogether stop. Factors that can trigger attacks include rest after exercise, potassium-rich foods, stress, fatigue, certain pollutants (e.g. cigarette smoke) and periods of fasting. Muscle strength improves between attacks, although affected people with certain mutations may have permanent muscle weakness (Lehmann-Horn *et al.* 2002; Catterall *et al.* 2005).

Potassium-aggravated myotonia

Potassium-aggravated myotonia (PAM) is a rare inherited disorder of skeletal muscle. Beginning in childhood or adolescence, people with this condition experience episodes of sustained muscle contractions with delayed relaxation (myotonia). These reportedly painless muscle cramps can be generalised throughout the body and occur following exercise and/or by eating potassium-rich foods. Potassium-aggravated myotonia ranges in severity from mild episodes of muscle stiffness to severe, disabling disease with frequent attacks. Unlike HYPP, potassium-aggravated myotonia is not associated with episodes of muscle weakness.

Mutations in $Na_V1.4$ that cause PAM increase the movement of sodium ions into skeletal muscle cells. The influx of extra sodium ions triggers prolonged muscle contractions, which are the hallmark of myotonia. Potassium-aggravated myotonia is inherited as an autosomal dominant, similar to HYPP and *paramyotonia congenita* (Vicart *et al.* 2005).

Paramyotonia congenita

Paramyotonia congenita (PC) is another rare neuromuscular disorder characterised by 'paradoxical' (para) myotonia. This disease has been termed paradoxical because it becomes worse with exercise whereas patients with 'classical' myotonia, or *myotonia congenita*, improve with exercise when muscles are warmed up properly, and also because the myotonia is induced by cold temperatures. Twenty-three different mutations of SCN4A have been found in patients with PC. Five of the 23 are potassium sensitive. Patients with PC typically present before 10 years of age and the disease has 100% penetrance (all patients will exhibit symptoms). Electromyography may be used to distinguish between PC and *myotonia congenita*. Clinicians can provoke episodes or myotonia and weakness/paralysis in patients in order to determine whether the patient has PC, HYPP or PAM, but now mutation studies assist in the distinction. Genomic sequencing of the SCN4A gene is the definitive diagnostic determinant, however; in one study of 26 patients with PC, 17 (71%) had a mutation in SCN4A and for 6 (29%) the specific mutation could not be identified (Miller *et al.* 2004).

Regulation of genetic mutations by breed associations

Horses affected with HYPP can be treated with potassium-wasting diuretics such as acetazolamide, with some reduction of symptoms. Attempts to keep diet low in potassium-containing feeds and avoiding stress may also reduce symptoms, but the degree that diet and medical treatment helps varies from horse to horse. Due to the unpredictable nature of episodes, horses with HYPP should only be ridden by experienced riders, if anyone, because episodes of paralysis can occur following exercise and a rider has to be alert to recognise an impending episode, which may cause the horse to fall.

Some equine breed registries have instituted rules to attempt to reduce this widespread disease. In 1996, AQHA officially recognised HYPP as a genetic defect or undesirable trait. To increase public awareness of this disease, mandatory testing for HYPP, with results designated on the Registration Certificate, began for foals descending from Impressive born after 1st January 1998. In response to requests from the membership, in 2004 the AQHA Stud Book and Registration Committee ruled that foals born in 2007 and later testing homozygous (H/H) would not be eligible for registration. Discussion of banning heterozygotes (N/H) is pending. Breeders opposed to restrictions argue that the disease can be

TABLE 1: Genetic diseases in the horse for which a mutation is known

Disease	Breed affected	Licensed laboratories
HYPP: hyperkalaemic periodic paralysis	Quarter Horse, American Paint Horse, Appaloosa, Pony of the Americas	Veterinary Genetics Laboratory (VGL) www.vgl.ucdavis.edu
HERDA or HC: hereditary equine regional dermal asthenia or *Hyperelastosis cutis*	Quarter Horse	VGL, Cornell University www.diagcenter.vet.cornell.edu
SCID: severe combined immunodeficiency	Arabian	VetGen (www.vetgen.com)
OLWS: Overo lethal white foal syndrome or ileocolonic aganglionosis	American Paint Horse, Quarter Horse	VGL
PSSM: polysaccharide storage myopathy	Quarter Horse, Warmblood	University of Minnesota Veterinary Diagnostic Laboratory (VDL) (www.cvm.umn.edu/umec/lab/home.html)
GBED: glycogen branching enzyme deficiency	Quarter Horse	VGL, VetGen
JEB: junctional epidermolysis bullosa	Belgian	VGL
MH: malignant hyperthermia	Quarter Horse	VGL, University of Minnesota VDL

controlled through diet and medication and that these horses are highly successful in the show ring. This rationale argues that the benefits of the mutation in the show ring outweigh the health risks to the horse and riders.

The Appaloosa Horse Club (ApHC) recommends, but does not require, testing for HYPP as a requirement for registration in descendants of Impressive. To assist with the prevention of HYPP symptoms, the ApHC approved the conditional use of acetazolamide during competition for HYPP positive horses, provided that parentage has been verified and test results appear on the Certificate of Registration (Anon 2008a). To date, the American Paint Horse Association and Pony of the Americas Club has not taken any action regarding registration of horses with HYPP.

The question remains as to how much responsibility a breed registry should accept in regulating mutations once a disease is recognised and can be definitively diagnosed by DNA tests. Is the breed association's responsibility to simply inform the membership and public of heritable diseases, or must they also attempt to police their propagation? The recent development of equine genome maps and the complete sequencing of the horse genome has greatly accelerated the pace of genetic discovery. Presently, DNA tests exist for several equine diseases (**Table 1**). The mission statement of the AQHA states the registry's intent "to record and preserve the pedigrees… while maintaining the integrity of the breed" (Anon 2008b). While the market typically dictates the value of horses with undesirable traits, in the case of HYPP, it is notable that prior to restriction of registration, the frequency of H/H genotypes actually increased following availability genetic testing (Spier 2006), presumably due to continued success in the show ring. Equine breeding practices for Thoroughbred racehorses have recently come under scrutiny since the well-publicised death of the horse Eight Belles in the Kentucky Derby in May 2008. There is speculation that modern American Thoroughbreds may be more 'fragile' due to inbreeding, and The Jockey Club recently appointed a team comprised by veterinarians, owners and racing officials to review breeding practices, medications such as corticosteroids and track surfaces. Notably, both Barbaro and Eight Belles were descendants of Native Dancer, whose race career was also shortened by musculoskeletal injury (Weinbach 2008a,b).

When popular horses from a bloodline are bred, or inbred, to favour a particular expression of a trait such as speed or muscularity, genetic diseases are an unfortunate consequence. It may appear impractical to require testing for the numerous diseases that will be elucidated in the coming years and it is difficult to regulate breeding practices. Nonetheless, by creating an excess of homozygotic descendants, the domination of contemporary horse breeding by any particular bloodline, regardless of its positive attributes, will generate long-term, costly and sometimes inhumane problems such as genetic diseases. Because planning can prevent such problems, breed associations and veterinarians must continue to counsel and educate breeders and prospective buyers about genetic diseases and genetic testing in order to guard the long-term health and welfare of the horse.

One problem in human medicine we can anticipate in the future for veterinarians is the issue of patenting tests. As the number of genetic tests for horses expands, the horse industry may experience problems with exclusivity of testing, as is currently hotly debated within human molecular diagnostic testing. Tests can be patented, and those institutions holding the patents can opt for either 'exclusive licence' to a single testing lab, or 'nonexclusive' licences where any lab can license and offer the test. Exclusive licences are more lucrative to both the patent holder and the single reference lab, as exclusivity enables a functional monopoly on testing, resulting in higher costs to the consumer. However, this raises the spectre of 'single ownership' of genetic heritage, a particularly controversial topic in human genetics. For example, breast cancer genes are owned and tested by a single company, with

costs for testing considerably higher than other human genes. Ownership of animal genes is less ethically inflammatory, yet the proliferation of exclusive licences to different labs for each horse gene may force the horse-owner to send a sample to a dozen different labs for each different mutation. Fortunately, the HYPP test is offered by the University of Pittsburgh to all requesting laboratories as a nonexclusive licence, and this may set the stage for similar actions by other patent holders.

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