

# Brief Review of Equine Genomics: Prospects toward Exercise and Sports Science

## Abstract

Since horse's domestication, physical and physiological capacities have been targeted for selection, leading to the notable functional adaptations to exercise and training of the actual phenotypes registered in equestrian disciplines. During the past ten years, experiments using next generation sequencing attempted to identify specific loci in the equine genome associated with traits of interest (e.g. speed, stamina, strength). Some of these investigations are reviewed in this study within a comprehensive and comparative approach.

**Keywords:** DNA-sequencing; Genomics; Exercise physiology; Sports science

## Review Article

Volume 1 Issue 5 - 2017

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**Received:** September 27, 2017 | **Published:** October 12, 2017

## Introduction

The modern horse presents remarkable physical and physiological capabilities that distinguish them from the other mammals. Natural selection and more recently, artificial selection for speed and stamina provided to the modern horses unique adaptations to exercise and training. The actual phenotypes show high aerobic and anaerobic capacities (up to 200 ml/kg.min<sup>-1</sup>) [1,2] increased muscle mass to body weight ratio [2,3] and efficient locomotion [4,5], however whether these structure and functional changes are related to specific genetic variants remains unclear.

The equine genome sequencing [6] provided scientific basis to future analyses of genetic variations underlying superior phenotypes. Genetic variation known as single nucleotide polymorphisms (SNPs) have been described in humans [7,8] and recently in horses undergoing successful performance careers in athleticism [9]. Variants of MSTN gene encoding myostatin were related to best race distance among elite flat racing Thoroughbred [10,11]. Myostatin down regulates both myoblasts proliferation and differentiation and, therefore acts in the inhibition of muscle growth [12].

Further studies reported additional candidate genes for flat racing performance in Thoroughbred (THB) [9,11,13], with raising evidence for loci involved in glycolysis [9,14], oxidative phosphorylation [13] and fast twitch fibers contractile function [15,16]. PDK4 SNP (gene locus involved in the activity of pyruvate dehydrogenase complex) was not different between elite quarter horses (QH) and non-elite QHs engaged in short distances race (401 m). However, the frequency for the A allele was higher for the QHs than Arabian horses [14], in agreement with the inverse coupling between muscle fiber type and muscle bioenergetics (e.g slow twitch fibers release less energy through glycolytic pathway) [17].

Regatieri et al. [14] also observed a new polymorphism for MCT1 gene, which was found only in Arabian horses (18/53), although it was not related to increased endurance performance. MCT1 is involved in the synthesis of monocarboxylate, a Tran's

membrane lactate transporter, highly expressed in horses exposed to long-term endurance training [18]. Lactate mediated muscle fatigue is widely described during intense rides of short duration, however it is unlikely a factor for prolonged exercises of moderate to low intensities [19].

The aim of this article is to review scientific articles concerning the role of genetic variations in equine exercise physiology, focused on candidate genes for superior performance based on gene sequencing using next generation sequencing (NGS) techniques. A correlation with human genes and athletic performance was also attempted.

## Literature Review

### Origin and phylogeny

Equidae is a family within the odd toed ungulate Order Perissodactyla, composed by a single genus called equus [20]. The genus equus originated in North America and probably spread into the Old World through the Bering land bridge. There was also a migration route, despite its minor flux, to the South America in the Late Pliocene. The earliest fossil (Equus simplicidens), found in the North America was morphologically primitive and is likely the ancestor of all specimens of current domestic horse breeds [21].

The evolutionary history of the equus started 58 million years ago, from the hyracotherium (also known as Hypparion or Eohippos) and unfolded through a branched phylogenetic tree [22]. During the early Miocene, high rates of morphological changes occurred in the teeth of the family Equinae, underlying the shift from browsers (Parahyppus) to grazers (Merychippus) [23]. This new habit of pasture might be considered an essential feature for the adaptation and spread of equus population in the steppes of the Old World. The well-established trend toward increase in body size has also followed the evolutionary history of the equus

from the early hyracotherium to the modern domestic horse, although the rate of increase in body mass followed a non-linear pattern. During the first half of horse evolution (57 to 25 million years ago), there was probably no change in body mass, which was followed by a remarkable diversification in body size during the early-middle Miocene [24]. Furthermore, some important postural adaptations also have occurred during the Miocene in the primitive specimens of *Parahippus* and *Merychippus*. The capability of standing for long periods (23 hours per day) is a feature currently observed in the modern horse and according to evolutionary studies of fossil horses, it was developed during the middle Miocene (18 to 12 million years ago) [25].

### Domestication and spread of *de equus*

The domestication of the horse is believed to interact historically with human cultures in the Eurasian steppes. There are archeological evidences that horses' domestication and the Kurgan Culture spread (Indo-European origins) had shared the same place of birth in the west Eurasia [26,27]. In addition to the archeological findings, strong support was provided in a genomic DNA study with 322 non-breed horses, sampled in 12 different locations, in order to represent the wide variety of demographic scenarios. Authors proposed a domestication origin in the west central Eurasian (modern territories of Ukraine and Kazakhstan), which is geographically different from the origin of wild progenitor of domestic horses (*E. ferus*), positioned in the east Eurasia [28]. Several questions emerged from such remarkable difference: why had horses originated in a certain place and been domesticated in another? Was there a priori migration flux of humans from east to west, which started the domestication process? Has the societies of west Eurasia developed the proper knowledge of domestication and equitation?

The genetic structuring of the horse domestication was carried out with such notable particularities, now currently investigated through DNA mitochondrial (m) models. Despite the concept that reduction in genetic variability is likely to occur due to domestication [29], the evolutionary process in horses seems to take the opposite way. The modern horse shows greater DNA m diversity than a single wild horse population, such as Alaskan and Przewalski's horse. The comparative DNAm analyses suggests that several horse population from different geographic origins with minimal within population genetic diversity were recruited during domestication [30]. When domestication spread out of central west Eurasia, high levels of introgression from wild local horse claims might justify this high genomic diversity of horses during domestication [28].

### Genes targeted for selection in horses

Genes undergoing recent selection have been studied in species, which are nowadays widely distributed and derived from a non-cosmopolitan ancestral population. In these species, reduction in population size and patterns of variability often underlies new habits of adapted phenotypes. These changes in the genetic "shape" may be investigated through population-level DNA data, in order to identify genetic basis of adaptation and selection [29].

Several approaches are now available to identify genes undergoing recent selection, whereas it requires previous knowledge of gene function and linkage mapping. The simplest method to map these genes is the identification of outlier loci, based on empirical distribution of some chosen features of the data [31]. It is generally assumed that loci targeted for selection are localized in the extreme tail of an empirical distribution (genome-wide distribution from the summary statistic) [29]. Gu et al. [13] identified 18 loci in the tail end of the distribution in a study employing hitchhiking mapping approach in several horse breeds. Seventeen loci were found on the negative extreme of the distribution (expected heterozygosity < -3.5), which undergone positive selection. The opposite extreme (positive end) shows the effect of balancing selection [32].

In order to identify genomic regions targeted for positive selection in Thoroughbred horses, several breeds were compared using FST and H0 approaches. Clusters of outlier loci with high values of FST (> 0.25) and low H0 were observed only in Thoroughbreds, which reveal evidence of population differentiation. Plotting inter-population FST and THB H0 across each chromosome of the most strongly selected loci, three loci were identified in Ewens-Watterson test: NVHEQ079 and TKY316 ( $p < 0.05$ ); TKY222 ( $p < 0.01$ ).

Based on H. Sapiens gene ontology (GO) database, 369 genes with functions on 35 GO Biological Process and 21 Molecular Process may distinguish THB from non-THB horses. According to the author, his main physiological pathways related to these genes were fatty acid metabolism, oxidative phosphorylation and actin cytoskeleton [32].

Further population-based studies of selection in horses using microsatellites and Di statistic (designed to detect significance that are near or at fixation within a population) supported the use of these techniques to identify novel variants and selection induced changes in equine genome [33]. The comparison of three different breeds (THB, QH and Paint Horse - PH) was carried out in order to identify regions of putative selection in horses engaged in different optimal race distance. The highest di values was observed on ECA 18 for QH and PH, which presented 780.7 kb haplotypes composed of 21 SNPs identified in 50%, 91.3% and 100% of THB, QH and PH respectively [33]. MSTN gene (center of haplotypes found) was sequenced in a cohort with (QHs) and without the whole amount of haplotypes (THB) and authors found an SNP in intron 1 of MSTN only in QHs, which had been previously described as a predictor of sprinting ability in Thoroughbreds [10].

### Heritability of aerobic capacity

Aerobic capacity is nevertheless a complex trait in athletic horses, as well as in other mammals and results from the sum of inherent capabilities and the ability to adapt to training stimulus [34]. Aerobic capacity is currently estimated through the measurement of maximal oxygen uptake [35], which might reach the value of 180 ml/kg/min in trained Thoroughbred horses [36]. In addition to the intrinsic capacity of an organism to transfer energy through aerobic metabolism in the untrained

state, the ability to adapt to physical training is a trait believed to be influenced by certain genetic features [7]. Furthermore, it is also a concept underlying the a priori hypothesis that superior exercise performance is in part, genetically determined.

Adaptation to aerobic training, heritability patterns and the response to divergent selection were investigated in a founder population of rats exposed to 8 weeks of treadmill running. Total distance covered showed a wide range of variation (from -110 to +430 meter gained) after training. Moreover, first offspring of high responders to aerobic training were able to cover 161 m more than founders, while offspring of low responders showed no difference with founders on their endurance capacity. Thereby, heritability was estimated at 0.43, which means that genetic contribution to the ability to adapt to aerobic training is about 43% [37].

## Genes of Interest

### MSTN gene

MSTN gene, composed of three exons and two introns encodes Myostatin, a member of transforming growth factor  $\beta$  super family, which down regulates muscle hyperplasia and hypertrophy in mammals. Myostatin (previously referred to as GDF-8) expression appears to be localized to myotome compartment in embryos of mouse (10.5 days post-coitum) and is likely to be continuously expressed in several skeletal muscles of adults [12]. The biological function of myostatin was described in mouse through comparison of wild animals with mutant types, produced after disruption of MSTN gene by homologous targeting in embryonic stem cells. Homozygous mutant animals were about 30% larger and showed total muscle cell 86% higher than their heterozygous and wild types littermates [12]. Natural mutations of MSTN gene correlated to increased muscle mass were also identified in cattle [38], sheep [39], dogs [40], fishes [41], humans [42] and horses [43].

The very high muscle mass to body weight ratio observed in horses (55%), compared to other species [3], is probably a trait targeted for artificial selection, especially in Thoroughbred horses [32]. Considering the important contribution of muscle power to flat race ability, the association between MSTN sequence variant and racing performance was investigated in a series of population-basis case control study by separating thoroughbreds ( $n=148$ ) on the basis of retrospective racecourse performance. Genotypes of two single nucleotides polymorphisms attempted (g.6649373737C>T and g.66494218A>C) were not more common among winning thoroughbreds than non-winning thoroughbred horses, however the comparison of horses winning short race (<8 furlongs) with horses winning long distance race (>8 f) revealed significant association [10]. The hypothesis that MSTN SNPs are correlated to best race distance in Thoroughbred horses was also raised in a study using a whole genome association approach. One hundred and eighty-nine elite thoroughbred horses (at least one win), were grouped following the optimal racing distance (<7 f, 8-10 f and >10 f), which were then correlated to sequence variation in specific locus of MSTN gene. Two markers on ECA 18 were identified (BIEC2-417274 and BIEC2-417495) when sprinters (<7 f) were compared to middle-distance horses (8 - 10 f). It was noted that MSTN gene is located between the two significant SNPs

at 68490208 - 66495180 bp [11]. Sequence variants described on specific locus of MSTN gene seems to be correlated with different morphological types [43], which may increase the chance for superior performance, especially for sprinter Thoroughbreds, however whether these genomic markers are linked with physiological capabilities such as repeated sprint ability, peak power output and maximal accumulated oxygen deficit (MAOD) remains unknown. In humans an initial effort has been done in order to identify genetic variations of known muscle mass and muscle strength cohorts [44]. Despite the day-to-day variations on indexes of strength, studies with athletes present significant potential to clarify subjects about muscle development in response to training of certain genotypes, due to a greater background of physical tests concerning maximal voluntary contraction [45]. A comparative study on several mammalian species suggested that human MSTN gene have been targeted for natural selection. The five polymorphic sites are remarkably conserved in humans. This high level of conservation indicates that mutations in humans may present functional implications [46].

### PDK4 gene

Pyruvate dehydrogenase kinase isoforms (PDKs) down regulate the activity of pyruvate dehydrogenase complex (PDC), a key enzyme responsible for glucose oxidation by catalyzing the conversion of pyruvate to acetyl-CoA [47]. The up regulation of PDK4 isoforms might impair tricarboxylic acid cycle and then reduce potential energy output of pyruvate processing, thereby acting on the selection of energy source for cellular metabolism. There are evidences that PDK4 expression is involved in the switch from carbohydrate to fatty acid as the main energy substrate during prolonged hibernation [48]. The role of PDK4 on fatty acid metabolism might be an interesting issue for riding horses. In a study investigating energy expenditure and respiratory quotient of Arabian horses undergoing endurance ride, it was observed that fat is likely the main source of energy for muscle metabolism [49]. The effect of fat adaptation on bioenergetics indexes, such as lactate breakpoint have been observed in Arabian horses during endurance training [50] and is also an additional evidence for the metabolic specialization toward ATP production from fat oxidation.

PDK4 isoform have been previously referred as a candidate gene for athletic performance in horses [9]. Hills et al., observed that A:A and A:G genotypes (THB) showed higher performance than G:G genotypes (16.1 - 16.6 lb handicap advantage). Difference in allele frequency of PDK4 gene was also observed between Arabian and Quarter Horses [14] suggesting that it might be involved in the control of energetic pathways during exercise in horses. In humans, PDK4 expression is believed to be regulated by hypoxia [51] and muscle glycogen content prior to exercise [52].

## Next-Generation Sequencing

NGS is a technology developed to high-throughput biological data acquisition. Sanger sequencing was the first method, which enabled the precise collection of DNA data, by sequencing through a capillary-based, semi automated method. Firstly, an amplified DNA template is produced, using shotgun de novo or target sequencing (PCR simplification). Then the amplified template is stochastically fragmented and submitted to cycles



of de-naturation and reconstruction, while fluorescently labeled nucleotides (ddNTPs) are incorporated to the terminal portion of the fragments. The reactions are mediated by DNA-polymerase enzyme, primers containing known DNA sequence and free nucleotides. Thereby, the aim of reconstructing DNA templates with fluorophores is to tag each nucleotide for further identification. In sequencing assay, fragments travel into the gel filled capillary, while ddNTPs are identified according to the laser excitation response. In the last step, DNA sequence is assembled through bioinformatics methods [53].

Several platforms have been developed over the past 20 years based on Sanger biochemistry and led by industries and institutions such as Roche Applied Science<sup>TM</sup> (454 sequencing), Illumina<sup>TM</sup> (Solexa Technology), Applied Biosystems<sup>TM</sup> (SOLID), Harvard (Polonator), Helicos<sup>TM</sup> (Molecule Sequencer Technology). The main difference between these platforms, known as short reads NGS and the pioneer Sanger sequencing is that a sequence platform enables the collection of DNA data from millions of reaction, carried out in parallel, instead of the ordered ddNTPs reading [54]. Short reads sequencing gather two different approaches: sequencing by ligation (SBL) and sequencing by synthesis (SBS) and both enables fast and precise DNA-sequencing.

### Acknowledgement

P.B. reviewed all cited papers and wrote this article. W.R. reviewed this article.

### Conflict of Interest

None.

### References

- Jones JH, Longworth KE, Lindholm A, Conley KE, Karas R H, et al. (1989) Oxygen transport during exercise in large mammals. I. Adaptative variation in oxygen demand. *J Appl Physiol* 67(2): 862-870.
- Lovel DK, Rose RJ (1993) Changes in skeletal muscle composition in response to interval and high intensity training. *Equine Exercise Physiology* 3: 215-222.
- Gum HM (1987) Muscle, bone and fat proportions and the muscle distribution of thoroughbreds and other horses. *Equine Exercise Physiology* 2: 253-264.
- Lawson SEM, Marlin DJ (2010) Preliminary report into the function of the shoulder using a novel imaging and motion capture approach. *Equine Vet J Suppl* 42(38): 552-555.
- Harrison SM, Whitton RC, Kawcak CE, Stover SM, Pandy MG (2010) Relationship between muscle forces, joint loading and utilization of elastic strain energy in equine locomotion. *J Exp Biol* 213(Pt 23): 3998-4009.
- Wade CM, Stock KF, Jönsson L, Ricard A, Mark T (2016) Genome-Wide Analysis Reveals Selection for Important Traits in Domestic Horse Breeds. *Anim Front* 6(1): 45-52.
- Bouchard C (2012) Genomic predictors of trainability. *Exp Physiol* 97(3): 347-352.
- Sarzynski MA, Loos RJ, Lucia A, Pérusse L, Roth SM, et al. (2016) *Advances in Exercise, Fitness and Performance Genomics in 2015*. *Med Sci Sport Exerc* 48(10): 1906-1916.
- Hill EW, Gu J, Givney MBA, MacHugh DE (2010) Targets of selection in the Thoroughbred genome contain exercise-relevant gene SNPs associated with elite racecourse performance. *Anim Genet* 41(Suppl 2): 56-63.
- Hill EW, Gu J, Eivers SS, Fonseca RG, Givney MBA, et al. (2010) A sequence polymorphism in MSTN predicts sprinting ability and racing stamina in thoroughbred horses. *PLoS ONE* 5(1): e8645.
- Binns MM, Boehler DA, Lambert DH (2010) Identification of the myostatin locus (MSTN) as having a major effect on optimum racing distance in the Thoroughbred horse in the USA. *Anim Genet* 41(Suppl 2): 154-158.
- Pherron MAC, Lawler AM, Lee SJ (1997) Regulation of skeletal muscle mass in mice by a new TGF- $\beta$  superfamily member. *Nature* 387(6628): 83-90.
- Gu J, Hugh MDE, Givney MBA, Park SDE, Katz LM, et al. (2010) Association of sequence variants in CKM (creatine kinase, muscle) and COX4I2 (cytochrome c oxidase, subunit 4, isoform 2) genes with racing performance in Thoroughbred horses. *Equine Vet J Suppl* 42(Suppl 38): 569-575.
- Regatieri IC, Pereira GL, Neto TAR, Ferraz GC, Curi RA, et al. (2016) Polymorphisms in MCT1, CD147, PDK4 and DMRT3 genes in Arabian and Quarter Horses. *Journal of Equine Veterinary Science* 48: 161-165.
- Mata X, Vaiman A, Ducasse A, Diribarne M, Schibler L, et al. (2012) Genomic structure, polymorphism and expression of the horse  $\alpha$ -actinin-3 gene. *Gene* 491(1): 20-24.
- Thomas KC, Hamilton NA, North KN, Houweling PJ (2014) Sequence analysis of the equine ACTN3 gene in Australian horse breeds. *Gene* 538(1): 88-93.
- Rothe QE, Rivero JLL (2001) Co-ordinated expression of contractile and non-contractile features of control equine muscle fibre types characterised by immunostaining of myosin heavy chains. *Histochem Cell Biol* 116(4): 299-312.
- Koho NM, Hyypä S, Pösö AR (2006) Monocarboxylate transporters (MCT) as lactate carriers in equine muscle and red blood cells. *Equine Vet J* 38(Suppl 36): 354-358.
- Andany AM, Ojén LM, Calderón FR, Rodríguez AE (2014) Comprehensive review on lactate metabolism in human health. *Mitochondrion* 17: 76-100.
- Fadden MBJ Fossil (2005) Horses-Evidence for Evolution. *Science* 307(5716): 1728-1730.
- Bowling AT, Ruvinsky A (2000) *The genetics of the Horse*. CAB International, USA, p. 1-25.
- Fadden MBJ (1995) Fossil Horses: Systematics, Paleobiology and Evolution of the Family Equidae. *Terra Nov* 7(1): 102-103.
- Fadden BJ (1988) Fossil horses from "Eohippus" (Hyracotherium) to Equus, 2: rates of dental evolution revisited. *Biol J Linn Soc* 35(1): 37-48.
- Fadden MBJ (1986) Fossil Horses from "Eohippus" (Hyracotherium) to Equus: Scaling, Cope's Law and the Evolution of Body Size. *Paleontol Soc* 12(4): 355-369.

25. Hermanson JW, Macfadden BJ (1996) Evolutionary and functional morphology of the knee in fossil and extant horses (Equidae). *J Vertebr Paleontol* 16(2): 349-357.
26. Anthony DW, Bogucki P, Comsa E, Gimbutas M, Jovanović B, et al. (1986) The "Kurgan Culture," Indo-European Origins, and the Domestication of the Horse: A Reconsideration (and Comments and Replies). *Curr Anthropol* 27(4): 291-313.
27. Outram AK (2009) The earliest horse harnessing and milking. *Science* 323(5919): 1332-1335.
28. Warmuth V, Eriksson A, Bower MA, Barker G, Barrett E, et al. (2012) Reconstructing the origin and spread of horse domestication in the Eurasian steppe. *Proc Natl Acad Sci* 109(21): 8202-8206.
29. Thornton KR, Jensen JD, Becquet C, Andolfatto P (2007) Progress and prospects in mapping recent selection in the genome. *Nature* 1-9.
30. Jansen T, Forster P, Levine MA, Oelke H, Hurler M, et al. (2002) Mitochondrial DNA and the origins of the domestic horse. *Proc Natl Acad Sci USA* 99(16): 10905-10910.
31. Ometto L, Glinka S, Lorenzo DD, Stephan W (2003) Inferring the Effects of Demography and Selection on *Drosophila melanogaster* Populations from a Chromosome-Wide Scan of DNA Variation. *Mol Biol Evol* 22(10): 2119-2130.
32. Gu J, Orr N, Park SD, Katz LM, Sulimova G, et al. (2009) A genome scan for positive selection in thoroughbred horses. *PLoS ONE* 4(6): e5767.
33. Petersen JL, Mickelson JR, Rendahl AK, Valberg SJ, Andersson LS, et al. (2013) Genome-Wide Analysis Reveals Selection for Important Traits in Domestic Horse Breeds. *PLoS Genet* 9(1): e1003211.
34. Evans DL (2007) Physiology of equine performance and associated tests of function. *Equine Vet J* 39(4): 373-383.
35. Rose RJ, Hodgson DR, Bayly WM, Gollnick PD (1990) Kinetics of VO<sub>2</sub> and VCO<sub>2</sub> in the horse and comparison of five methods for determination of maximum oxygen uptake. *Equine Vet J Suppl* (9): 39-42.
36. Hodgson DR, MCGowan CM, Mckeever KH (2014) Principles and Practice of Equine Sports Medicine. *The Athletic Horse* (2<sup>nd</sup> end), Elsevier, p. 10.
37. Troxell ML, Britton SL, Koch LG, Troxell ML, Britton SL, et al. (2003) Genetic Models in Applied Physiology: Selected Contribution: Variation and heritability for the adaptational response to exercise in genetically heterogeneous rats. *J Appl Physiol* 94: 1674-1681.
38. Dominique JE, Gérard C (2006) Myostatin regulation of muscle development: Molecular basis, natural mutations, physiopathological aspects. *Exp Cell Res* 312(13): 2401-2414.
39. Caiment F, Bouix J, Tobin J, Laville E, Larzul C, et al. (2006) A mutation creating a potential illegitimate microRNA target site in the myostatin gene affects muscularity in sheep. *Nat Genet* 38(7): 813-818.
40. Mosher DS, Quignon P, Bustamante CD, Sutter NB, Mellersh CS, et al. (2007) A mutation in the myostatin gene increases muscle mass and enhances racing performance in heterozygote dogs. *PLoS Genet* 3(5): e79.
41. Østbye TKK, Wetten OF, Klunderud TA, Jakobsen KS, Yafe A, et al. (2007) Myostatin (MSTN) gene duplications in Atlantic salmon (*Salmo salar*): Evidence for different selective pressure on teleost MSTN-1 and -2. *Gene* 403(1-2): 159-69.
42. Schuelke M, Wagner KR, Stolz LE, Hübner C, Riebel T, et al. (2004) Myostatin Mutation Associated with Gross Muscle Hypertrophy in a Child. *N Engl J Med* 350(26): 2682-2688.
43. Dallolio S, Fontanesi L, Costa LN, Tassinari M, Minieri L, et al. (2010) Analysis of horse myostatin gene and identification of single nucleotide polymorphisms in breeds of different morphological types. *J Biomed Biotechnol*
44. Thompson PD, Moyna N, Seip R, Price T, Clarkson P, et al. (2004) Functional polymorphisms associated with human muscle size and strength. *Med Sci Sports Exerc* 36(7): 1132-1139.
45. Harbo T, Brincks J, Andersen H (2012) Maximal isokinetic and isometric muscle strength of major muscle groups related to age, body mass, height and sex in 178 healthy subjects. *Eur J Appl Physiol* 112(1): 267-275.
46. Saunders MA, Good JM, Lawrence EC, Ferrell RE, Li W, et al. (2006) Human adaptive evolution at Myostatin (GDF8), a regulator of muscle growth. *PLoS Genet* 2(12): e1000477.
47. Harris RA, Kinley BMM, Huang B, Wu P (2002) Regulation of the activity of the pyruvate dehydrogenase complex. *Adv Enzyme Regul* 42: 249-259.
48. Buck MJ, Squire TL, Andrews MT (2002) Coordinate expression of the PDK4 gene: a means of regulating fuel selection in a hibernating mammal. *Physiol Genomics* 8(1): 5-13.
49. Cottin F, Metayer N, Goachet AG, Jullian V, Slawinski J, et al. (2010) Oxygen consumption and gait variables of Arabian endurance horses measured during a field exercise test. *Equine Vet J* 42(38): 1-5.
50. Kronfeld DS, Custalow SE, Ferrante PL, Taylor LE, Moll HD, et al. (2000) Determination of the lactate breakpoint during incremental exercise in horses adapted to dietary corn oil. *Am J Vet Res* 61(2): 144-151.
51. Lee JH, Kim EJ, Kim D, Lee JM, Park SB, et al. (2012) Hypoxia induces PDK4 gene expression through induction of the orphan nuclear receptor ERR $\gamma$ . *PLoS One* 7(9): e46324.
52. Pilegaard H, Keller C, Steensberg A, Helge JW, Pedersen BK, et al. (2002) Influence of pre-exercise muscle glycogen content on exercise-induced transcriptional regulation of metabolic genes. *J Physiol* 541(Pt 1): 261-271.
53. Shendure J, Ji H (2008) Next-generation DNA sequencing. *Nat Biotechnol* 26(10): 1135-1145.
54. Goodwin S, McPherson JD, McCombie WR (2016) Coming of age: ten years of next-generation sequencing technologies. *Nat Rev Genet* 17(6): 333-351.