DMRT3 gene mutation and higly linked SNP in gaitedness horse breeds

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Abstract

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The DMRT3 mutation has had a major effect on the diversification of the domestic horse, as the altered gait characteristics of a number of breeds, including this mutation. Doublesex and mab-3 related transcription factor 3 (DMRT3) gene shown that a mutation from cytosine (C) to adenine (A) has a major impact on harness racing performance of different breeds. The DMRT3 mutant allele (A) has been found in high frequency in gaited breeds and breeds bred for harness racing (trot or pace), while other horse breeds were homozygous for the wild-type allele (C). The heterozygous allele (CA) were found in horse breeds for harness racing and four- and five-gaited breeds. Sequncing at SNP BIEC2-620109 resulted in detection of both heterozygous (CT) and homozygous genotypes (CC, TT). Mutation in DMRT3 has a major impact on gaitedness in horses and is presented at a high frequency in gaited breeds and in horses breed for harness racing. The aim of the study is to describe the effect of the DMRT3 gene mutation and higly linked SNP in different gaited and harness horse breeds.

Keywords: horse; breeds; harness; gaits; DMRT3 mutation

Introduction

Based upon speed and footfall patterns, the horse has four major gait classifications. The fastest gait is the gallop, a four-beat asymmetrical gait with a moment of suspension at speeds of 9-20 m/s. A slower variation of the gallop is the canter, a three-beat asymmetrical gait with a moment of suspension at 2.9-9 m/s and considered a separate gait for competitions. The slowest gait is the walk, an even four-beat symmetrical gait with no suspension (two or three legs on the ground at any given time) at speeds of 1.2-1.8 m/s. Gaits other than the aforementioned gaits, performed at speeds generally faster than the walk, are known as the intermediate gaits; these include among others trot, pace, amble, tölt (or rack), running walk, foxtrot, and marcha batida, and they can be performed at speeds ranging from 2.8 to 16 m/s (Barey, 2013). Trot and pace range in speed and are defined by two moments of suspension when all four feet are off the ground simultaneously. Trot is diagonally symmetrical, and pace is laterally symmetrical. Both the trot and pace have a brief moment of suspension when all four feet are off the ground before one pair lands at the same time. Any horse able to perform any of the four-beat intermediate gaits is considered a 'gaited' horse (Staiger et al., 2017). The aim of the study is to describe the effect of the DMRT3 gene mutation and higly linked SNP in different gaited and harness horse breeds.

The DMRT3 mutation – a single base change with major impact on a complex trait and identification

In 2012, Andersson et al. reported a mutation in the DMRT3 (doublesex and mab-3 related transcription factor 3) gene that affects locomotion in horses. The authors show that a premature stop codon in the DMRT3 gene has a major effect on the pattern of locomotion in horses. The role of this

gene on a specific subset of spinal cord neurons was demonstrated in mice. A single base change (A/C) at nucleotide position 22999655 on horse chromosome 23 causes a premature stop codon and results in a truncated protein. The mutation is permissive for the ability to perform alternate gaits and has a favourable effect on harness racing performance. A recent study in fact demonstrated that a single-base substitution in the double-sex and mab-3-related transcription factor 3 (DMRT3) gene has a major impact on the ability of a horse to pace and amble, reve aling the strong genetic basis for this trait. The mutation (cytosine C to adenine A), initially discovered in Icelandic horses, causes a premature stop codon and thus a truncation of the DMRT3 protein (Andersson et al., 2012).

Characteristics and the frequency of the DMRT3 mutation in different horse breeds

The mutation (DMRT3_Ser301STOP) also was denoted „Gait keeper" due to the strong effect on the pattern of locomotion.The DMRT3 gene was first located by a GWAS (genome-wide association study) in the small Icelandic horse breed in which all horses with pacing ability, except one, were homozygous AA, while only 31% of the horses without pacing ability were homozygous AA (Ricard, 2015). The mutation has been found in all tested gaited breeds and in breeds used for harness racing, whereas it is absent in most of the three-gaited breeds, such as the Swedish Warmblood and Thoroughbreds (Jäderkvist et al., 2014).

The mutation is permissive for the ability to perform alternate gaits and occurs at high frequency in the 'gaited' breeds (Promerová et al., 2014). As noted earlier, these breeds also have the ability to extend the speed of their intermediate gaits to those equivalent to, or exceeding, that of canter (Barrey, 2013). The mutation also occurs at a high frequency in harness racing trotters and pacers, and it allows them to extend the speed range of their symmetrical gait (trot or pace) instead of switching to the asymmetric canter/gallop (Andersson et al., 2012; Promerová et al., 2014). The mutation occurs at a low frequency in breeds typically considered 'non-gaited', and recent evidence suggests that the mutation may have a negative effect on the desired quality of trot and canter in some breeds (Kristjansson et al., 2014; Jäderkvist et al., 2015). Pace is a two-beat gait in which the horse moves the two legs on the same side of the body in a synchronized, lateral movement, trot, where the diagonal front and hind legs move forward and backward together (Andersson et al., 2012).

The DMRT3 mutant allele (A) has been found in high frequency in gaited breeds and breeds bred for harness racing, while other horse breeds were homozygous for the wild-type allele (C). Horses with the CA genotype had on the other hand significantly higher scores for walk, trot, canter and gallop, and they performed better beat and suspension in trot and gallop (Kristjansson et al., 2014). The wild-type allele C fixed in native breeds such as the Ardennes, Breton, Fjord, Fresian, Haflinger, Hucul etc. (Ricard, 2015). The mutant allele A is fixed in most breeds that have been selected for trot racing or for pace racing, such as the American Standardbred, whereas allele C is fixed in breeds that have been selected for gallop racing, such as the Thoroughbred (high-speed galloping breed), the Arabian (endurance riding and show breed) and Swedish warmblood horses (Promerová et al., 2014). Trot is not the most natural gait for some AA horses and unclear trot can be "normal" especially in AA horses (Björnsdóttir, 2018).

Promerová et al. (2014) tested horses from 141 horse breeds and found the A allele highly prevalent (frequency > 0.90) or fixed among breeds used for harness racing or selected for performance of 4-beat gaits including Tennessee Walking Horses, Missouri Foxtrotters, Kentucky Mountain Saddle Horse, Columbian and American Paso Fino, harness racing trotters of several breeds and harness racing pacers. American Saddlebred has identified as a breed in which some horses performed a 4-beat gait. The DMRT3 mutant allele (A) was found in high frequencies in gaited breeds and breeds bred for harness racing, while tested non-gaited horse breeds were found homozygous for the wild-type allele (C) (Andersson et al., 2012). The frequencies of the A allele are higher in study of Regatieri et al. (2016) (0.31 to 0.35) compared with the finding of 0.275 in the publication of Promerová et al. (2014). Research to date suggests that for a horse to be able to pace, homozygosity for the DMRT3 mutation (A) is required (Andersson et al., 2012; Kristjansson et al., 2014; Jäderkvist et al., 2015). Only around 70% of the Icelandic horses that are homozygous mutant (AA) are reported to pace (Andersson et al., 2012; Jäderkvist et al., 2015). Patterson et al. (2015) tested 81 horses of Mangalarga Marchador breed (MM) – the Brazilian horse breed known for a uniquely smooth gait. 41 of them are homozigous wild type CC (batida – a horse using the 'batida' gait places the feet in diagonally coupled footfalls more frequently than laterally, although moments of triple-limb support exist) and 21 horses are homozygous mutant AA (the gait picada – more footfalls are often laterally coupled, rather than diagonally, without loss of triple support moments) and 19 horses are heterozygous mutant CA. Homozygous AA genotypes are observed in both trotting and pacing Standardbred racehorses (Andersson et al., 2012), suggesting that the mutation likely controls transitioning gait into the canter or the ability to coordinate the three-beat diagonal-coupled footfall pattern

at high speeds (Patterson et al., 2015). Both batida- and picada-type MM horses can perform lateral gaits. Therefore, the DMRT3 mutation is not solely responsible for controlling the lateral gait pattern or for the ability to perform a four-beat gait in this breed (Patterson et al., 2015).

According to Kristjansson et al. (2014) the Icelandic horse is a multigaited horse breed showing the standard gaits of all domestic horse breeds that are walk, trot, canter and gallop. In addition, it has tölt and pace. Tölt is a four-beat running gait with lateral sequence of footfalls and without suspension. Pace is considered a two-beat gait with a moment of suspension where lateral legs move almost synchronously back and forth and is optimally a very fast gait. Icelandic horses that possess walk, trot, canter, gallop and tölt are referred to as four-gaited horses, whereas horses that additionally have the ability to perform pace are called five-gaited horses. In the Icelandic horse, the pace has a tendency for significant asymmetry between subsequent flight phases, resulting in a broader distribution of footfall ratios (Robilliard et al., 2007). For this reason, the fifth gait of the Icelandic is often called the 'flying pace' Five Natural Gaits. As it can be a broken two-beat gait where one foot lands before the other and not together, as occurs in a true pace. Both four- and five-gaited Icelandic horses exhibit the 'tölt', a four-beat lateral gait (Ziegler, 2005). Only 31% of the four-gaited horses possess a homozygous AA genotype at DMRT3 (Andersson et al., 2012). Homozygous AA genotypes are observed in both trotting and pacing Standardbred racehorses (Andersson et al., 2012), suggesting that the mutation likely controls transitioning gait into the canter or the ability to coordinate the three-beat diagonal-coupled footfall pattern at high speeds. This hypothesis is supported by the observation by Andersson et al. (2012*)* that Icelandic horse homozygous mutants had inferior scores for gallop.

Han et al. (2015) tested 14 Chinese local horse breeds and collected 506 samples of them. Nine [Chaidamu (CDM), Chakouyi (CKY), Datong (DT), Yanji (YJ), Balikun (BLK), Kazakh (KZK), Yushu (YS), Hequ (HQ) and Ningqiang (NQ)] of 14 breeds exhibited the DMRT3 gene polymorphism with the frequency of the mutant allele (A) ranging from 2.17% to 81.89%. Four of these breeds (CKY, YJ, DT and YS) have been recorded to have the ability to pace. The CKY breed, well known in China for its talent for pacing, has the highest AA frequency (67.24%). All 20 of the CKY horses exhibiting pacing had the AA genotype. The five breeds carrying only the CC genotype are unable to pace. The DMRT3 nonsense mutation occurs mainly in Northwestern China, where horses having a natural talent for pacing, with the frequency of the DMRT3 mutation decreasing from northwest to southwest.

In the study of Jäderkvist et al. (2014b) investigated if there is a connection between the genotype in DMRT3 and gaitedness in Morgan and American Curly horses. Previous study of the same authors showed that the frequency of the mutated A allele in these breeds was 14.0% and 16.7% respectively. They are observed 59 Morgan and 101 American Curly horses in different countries, together with material for hair sampling. The frequency of the mutation in this study was 14.4% in the Morgan horses and 70.3% in the American Curly, 55% of the American Curly horses were homozygous AA. The results show that there is a strong association between the genotype in DMRT3 and gaitedness in both Morgan and American Curly horses (P = 8.6E-8, P = 2.2E-16 respectively). Also, how often the American Curly horses performed trot was influenced by the DMRT3 genotype (P = 1.4E-6). This study concludes that the genotype in DMRT3 has a significantly strong impact on gaitedness of Morgan and American Curly horses. The authors identified a few horses that are multigaited CC, which might be due to phenotypic misclassifications or that there are additional genes that influence the ability to perform alternate gaits in horses.

Staiger et al. (2017) used target-capture sequences from 89 horses, representing 26 breeds, for a 686-kb region overlapping the DMRT3 gene to reveal sequence polymorphisms showing strong association with the DMRT3 stop mutation and to precisely define the haplotype block associated with different gait phenotypes. A large number of sequence variants associated with the DMRT3 stop mutation were identified. The authors calculated the nucleotide diversity in sliding Windows of 10 kb in horses homozygous for either the DMRT3 stop mutation or the wild-type allele in this region and revealed an extended haplotype within a 295-kb region associated with the stop mutation. This 295-kb region showed a nearly 20-fold reduction in nucleotide diversity in horses homozygous for the stop codon compared with horses homozygous for the wild-type allele. Nucleotide diversity among wild-type chromosomes was similar to the genome average (Wade et al., 2009). This result shows that the Gait keeper haplotype recently underwent a selective sweep.

According to authors the standard gaits of the domestic horse and wild equids include the (flat) walk, trot, canter, and gallop. However, instead of the two-beat contralateral gait of the trot, some horses perform the pace, a two-beat ipsilateral gait. Other natural variations in movement include four-beat ambling gaits characteristic of the Tennessee Walking Horse, Peruvian Paso, Paso Fino, and others, with unique variations in rhythm between breeds. Two genes annotated in Ecab2.0, doublesex and mab-3 related transcription factors 2 and 3 (DMRT2, DMRT3), are found in the ECA23

haplotype shared across gaited breeds. This 'gait' haplotype was also found at low frequency in the Mangalarga Paulista (3.0%), Morgan (8.0%), and Saddlebred (30%). The presence of the same ECA23 haplotype in the gaited breeds within the Mangalarga Paulista, Saddlebred, and Morgan is not surprising. Certain individuals within these breeds have the ability to gait, although alternate gait is not a breed-defining characteristic. Conversely the 'gait' haplotype is not found exclusively in populations that are gaited; this was also true for the DMRT3 mutation that is presumably driving this signature of selection (Andersson et al., 2012). For example, the French Trotters and Standardbreds included in the calculation do not display alternative gaits beyond the walk, trot, canter, and gallop, but are bred to race at a trot (Petersen et al., 2013). In this study population, the 'gait' haplotype also segregates (54% presence) in the Finnhorse, which is divergently selected for light draft, riding, or trotting types (Hendricks, 2007). There is evidence that trotting performance is heritable (Thiruvenkadan et al., 2009) and haplotypic evidence, as well as that reported in (Andersson et al., 2012), indicates that an effect of this locus on trotting cannot be ruled out.

The gaited populations in which this haplotype is found (Icelandic, Tennessee Walking Horse, Peruvian Paso, Puerto Rican Paso Fino) have various types of alternative gaits. Unless there are several variants captured within this haplotype, it appears that this locus does not itself explain the entirety of the variation in gait present in domestic horses. We therefore hypothesize that gait is a polygenic trait, and while a major locus on ECA23 may be permissive for gaitedness, variations among breeds are determined by modifying loci (Petersen et al., 2013).

Kristjansson et al. (2014) are tested 667 horses (360 stallions and 307 mares) with the age ranged from 4 to 14 years. The horses were born in 1986-2008. The majority of the 667 horses genotyped for the DMRT3_Ser301STOP mutation, or 509 (76.3%), were homozygous for the A-allele (AA) and 150 (22.5%) were heterozygous (CA) while only 8 (1.2%) were found homozygous for the wild type (CC). Accordingly, the frequency of the A-allele was 0.88 and of the C-allele 0.12 in this data set and the genotypes conform to the Hardy-Weinberg proportions. Among the four-gaited horses, 118 of 263 (45.0%) were homozygous AA, 137 (52.0%) heterozygous CA and 8 (3.0%) homozygous CC, while 391 of 404 (96.8%) five-gaited horses were homozygous AA and 13 were heterozygous CA (3.2%). The 13 fivegaited horses with the CA genotype had scores from 5.5-7.0 for pace with a mean score of 5.92, compared with a mean score of 7.30 for horses of the AA genotype.

According to Jäderkvist et al. (2014a), the effect of the

mutation was not as obvious in Coldblood trotters (CBT). Most notably, homozygous A, CBT were significantly more successful (i.e. higher prize money earned) at 3 years of age, but as the horses matured the association weakened. By contrast, studies of both Standardbreds and Finnhorses, a Finnish CBT, clearly demonstrated the superiority of AA horses for all traits regardless of age (Jäderkvist et al., 2014a; Jäderkvist et al., 2015). This juxtaposition regarding the superiority of AA horses is an important distinction to make given its potential for significantly altering the genetic structure of the CBT breed (Jäderkvist et al., 2017a). The aim of the last study of Jäderkvist et al. (2017b) was to investigate the suggested precociousness of AA horses, as well as the association between the DMRT3 mutation and racing performance at older ages, in a randomly selected horse material. They are tested hair and blood samples of first 770 horses listed (485 raced, 285 unraced) from the Animal Genetics Laboratory at the Swedish University of Agricultural Sciences, Uppsala, Sweden and the Norwegian University of Life Sciences, Oslo, Norway. The total sample consisted of 90 stallions, 332 geldings and 347 mares from 128 sires, with an average of 6 offspring per sire. Performance data for the years 2003-2015 was provided by the Swedish Trotting Association. To investigate the effect of DMRT3 at different ages, the authors were defined three different age intervals: 3 years, 3 to 6 years, and 7 to 10 years of age. The age interval 3 to 6 years was chosen as the genetic evaluation of CBTs is based on performance results and racing status between the ages 3 to 6 years (Ärnason, 1999). The following performance traits were analyzed: rankings, racing times, earnings, disqualifications and estimated breeding values (EBVs). Total of 769 horses were successfully genotyped for the DMRT3 SNP. The frequency of the AA genotype was significantly higher ($P = 0.05$) in the group of unraced horses than in the group of raced horses. The genotypes for the raced and unraced horses deviated significantly from HWE ($P = 0.004$, respectively $P = 0.05$), while the genotypes for all horses combined (n = 769) did not deviate from HWE ($P = 0.35$). Fifty-five percent of the raced horses started their first race at 3 years of age and there were no significant differences between the genotypes. The average age for the first race was 3.8 years and it did not differ between the genotypes. At 3 years of age, there were two significant differences between the genotypes; the race time where the AA horses were significantly faster than the CC horses and the disqualifications where the CC horses had the highest number. For the age interval 3 to 6 years, the CA horses performed significantly better than the CC horses for the majority of the traits. Also, the AA horses had significantly more placings and fewer disqualifications than the CC horses, but the lowest earnings. At 7 to 10 years of age, the

CA and CC horses had significantly more starts than the AA horses. The AA horses also had the lowest number of placings and earnings. Additionally, the heterozygous horses had significantly better race times compared to the other genotypes. The CC horses had the highest number of disqualifications. The average EBV of the raced horses was 108.5 and it did not differ between genotypes (P > 0.05). Genotype CC had an extremely negative effect on all racing performance criteria, from qualification and earnings to the proportion of finished races, although the effect was not always significant due to the small number of horses with racing performances that carried genotype CC (Ricard, 2015).

Jäderkvist et al. (2017a) finded only two significant differences (race time for voltstart and number of disqualifications) between the genotypes and traits associated with precocity (i.e. performance at 3 years of age). Additionally, the proportion of AA horses that raced for the first time at 3 years of age and the average age for the first race did not differ significantly from the other genotypes. If AA horses were truly more precocious, a clear difference would be apparent, with a greater proportion of AA horses racing at 3 years of age. Perhaps a more interesting observation is the rather low proportion of raced AA horses. As the ability to race is the most important factor for a successful racehorse, the large proportion of unraced AA horses suggests a possible unfavorable association of the genotype with racing performance. While the genotypes for the whole population did not deviate from HWE, the genotypes for the raced and unraced horses did deviate from HWE when analyzed separately. Although successful at young ages, the AA horses did not perform as well for the older ages, where the median values for earnings and victories were the lowest of the three genotypes (Jäderkvist et al., 2017a). All AA horses had the lowest number of starts for all ages except 3 years of age. This has also been seen in Finnhorses, where the AA horses, despite the superior effect of the homozygous genotype, had the lowest number of starts for all ages above four years of age (Jäderkvist et al., 2015a). Although the low number of starts for AA Finnhorses may be explained by their superior performance, as successful racehorses tend to compete less often, the low number of starts for both AA Finnhorses and CBTs may also indicate an association between the AA genotype and poor durability (Jäderkvist et al., 2017a). Albeit still uncharted and possibly controversial, the disparate effect of the AA genotype on performance in CBTs is potentially influenced by an increased preference of AA CBTs for the gait pace (Jäderkvist et al., 2014a). Pacing in a race will lead to disqualification and therefore horses are prevented from pacing by the use of different weights and shoes to balance the horse. Heavy balancing of young horses could potentially increase the risk for injuries, which in turn may have a detrimental impact on performance. Although a potential reason for the low frequency of the AA genotype in the CBT population, not all AA CBTs have a propensity for pace. Some AA horses possess a conformation that makes them more prone to pace, but not all AA horses naturally pace, as demonstrated in Icelandic horses (Kristjansson et al., 2016). At the last study Jäderkvist et al. (2017a), the CC CBTs were the least successful prior to 6 years of age, but their performance improved as they matured. CC CBTs reportedly display an inferior trotting technique compared to the other genotypes (Jäderkvist et al., 2014a), which is often indicative of a horse that prefers to gallop rather than extend the trot when prompted for an increase in speed. This is supported by the high frequency of the CC genotype in breeds traditionally used for gallop races or recreation as well as the high numbers of disqualifications for the CC horses in the study (Andersson et al., 2012; Jäderkvist et al., 2014a; Promerová et al., 2014; Jäderkvist et al., 2017a). Moreover, previous studies have demonstrated an unfavorable association between the CC genotype and harness racing performance in other breeds (Jäderkvist et al., 2014a; Jäderkvist et al., 2015a; Ricard, 2015).

While the exact reasons for this are unknown, it is possible that this poor performance is rooted in a propensity to gallop. Despite this possible gait preference in CC CBTs, the frequency of the CC genotype is still relatively high in the CBT population (Jäderkvist et al., 2014a; Promerová et al., 2014). Ricard (2015) measured the effect of genotype CC on the criteria used for trot racing performances in France and demonstrated that it is extremely negative for all criteria (qualification, annual earnings, proportion of finished races) both for harness and under saddle races.

SNP BIEC2-620109

The effect of the DMRT3 gene was analyzed based on the same genopypes and genotypes at the SNP BIEC2-620109 of Ricard (2015), since its C and T alleles correlate to the phenotypes of the C and A alleles of the DMRT3 mutation, respectively. The frequency of the C allele at SNP BIEC2-620109 was 24.2% for the 630 genotyped horses. The frequencies for the three genotypes were 56% (355) for TT, 39% (245) for TC and 5% (30) for CC, which did not deviate significantly from Hardy-Weinberg equilibrium. Most of the 630 horses were born between 2002 and 2008 (87%) and 41% were females. The frequency of allele C did not depend on year of birth, except for a significant drop observed in 2008 (frequency of 15%). Selection intensity for French trotter horses with genotype CA at an early age is higher than for horses with genotype AA (Ricard, 2015). The French trotter

breed is one of the rare horse breeds that is selected for harness racing (trot or pace) and that carries a polymorphism at the DMRT3 gene (Andersson et al., 2012; Promerová et al., 2014). Modeling the selection process over generations may explain the polymorphism. The selection process was modeled using a 2-step selection process, i.e. (1) after qualification (Q) and (2) after mature racing performances (RP). According to the results of Ricard (2015), the disqualification showed low heritabilities (0.06 to 0.09) and average repeatabilities (0.16 to 0.25). The trait "best-recorded time" was less heritable (0.17) than earnings and was strongly and favorably correlated with earnings (from -0.79 for earnings at 5 years of age or more to -0.92 for earnings at 3 years of age. Qualification had the highest heritability. Genetic correlations between qualification and annual earnings were positive but low. Therefore, qualification and annual earnings are different traits. Annual earnings and ability to finish a race without disqualification are also two different traits, whereas the abilities of a horse to race under harness and under saddle are genetically closely related.

The 630 French trotters were including in research of Ricard (2015). The horses were retained based on birth year because performances were only available for horses born between 1996 and 2008. Among the 630 horses included in the analysis, 496 (79%) were qualified, which is a much higher proportion than in the overall French trotter population during this time period (37%) and average earnings per finished race were approximately three-quarters of a phenotypic standard deviation higher than the average of the overall French trotter population. The genotypes were obtained using the Equine SNP50 BeadChip supplied by Illumina. This chip includes the single nucleotide polymorphism (SNP) BIEC2-620109 on chromosome 23 at position chr23:22967656 bp that was used to map the DMRT3 gene by GWAS (Andersson et al., 2012) (Table 1).

Although of lesser importance, author also demonstrated the negative effect of genotype CT on qualification and early performances in French trotter horses. The DMRT3 mutation affects mainly ambling ability, which suggests that the abil-

ity to perform this gait may also be a factor of disqualification in trotting races (Ricard, 2015).

Conclusion

To conclude, this review showed that the DMRT3 "Gait keeper" mutation and SNP variation have observed in breeds grown throughout the world. The mutation appears in harness and gaited breeds. The mutation is rarely seen in other breeds. May be it's a responisibility to pace and other alternative gaits in horses. The search for gene variation and its phenotypic expression will continue with the aim to understand genetics mechanism for locomotion pattern and harness racing performance in horses.

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Table 1
Used primers by DMRT3 and SNP genotyping

	Forward primer	Reverse primer	Wild-type probe	Mutant probe
DMRT3_Ser301STOP SNP/ Kristjansson et al., 2014	5`-CCTCTCCAGCC-GCTCCT-3`	TCAAAGATGTGCCC-GTTGGA-3`	5`-CTGCCGAAGTTCG	5`-CTCTGCCTA-AGTTCG-3`
DMRT3_Ser301STOP/ Negro Rama et al., 2016; Jäderkvist et al., 2017b	5`AATCTTCCCCAAC-CAGAAGCCCACG3`	5`ACAGGGTGACAT-CATTGGGCAGG3`		
DMRT3_Ser301STOP/ BIEC2_620109 SNP Promerová et al., 2014	5′-GCAAAGTG-CAGAAATAGTCTTTTG-GA-3′	5′-CACTCTTTTG-GAATGGTTCACATTA-AGG-3	5′- TAGTGCAAACGG-TACGTT-3	5′-AAATAGTGCAAA-CAGTACGTT-3′

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