

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/264387860>

# The effect of the 'Gait keeper' mutation in the DMRT3 gene on gaiting ability in Icelandic horses

Article in *Journal of Animal Breeding and Genetics* · July 2014

DOI: 10.1111/jbgs.12112

CITATIONS

39

READS

1,530

8 authors, including:



**T. Kristjánsson**

Agricultural Advisory Center

8 PUBLICATIONS 134 CITATIONS

[SEE PROFILE](#)



**Sigridur Bjornsdottir**

Icelandic Food and Veterinary Authority

45 PUBLICATIONS 635 CITATIONS

[SEE PROFILE](#)



**Agust Sigurdsson**

The Agricultural University of Iceland

23 PUBLICATIONS 385 CITATIONS

[SEE PROFILE](#)



**Lisa S Andersson**

Swedish University of Agricultural Sciences

87 PUBLICATIONS 1,388 CITATIONS

[SEE PROFILE](#)

Some of the authors of this publication are also working on these related projects:



Equine performance genomics [View project](#)



Genetics of size [View project](#)



ORIGINAL ARTICLE

## The effect of the 'Gait keeper' mutation in the *DMRT3* gene on gaiting ability in Icelandic horses

T. Kristjánsson<sup>1</sup>, S. Björnsdóttir<sup>2</sup>, A. Sigurdsson<sup>1</sup>, L.S. Andersson<sup>3</sup>, G. Lindgren<sup>4</sup>, S.J. Helyar<sup>5</sup>, A.M. Klonowski<sup>5</sup> & T. Arnason<sup>1</sup>

1 Agricultural University of Iceland, Hvanneyri Borgarnes, Iceland

2 Icelandic Food and Veterinary Authority, Selfoss, Iceland

3 Capilet Genetics AB, Öster Skogsta, Västerås, Sweden

4 Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden

5 Matís, Reykjavík, Iceland

### Keywords

Gaiting ability; genotype effect; genotype probability.

### Correspondence

T. Kristjánsson, Agricultural University of Iceland, IS-311 Borgarnes, Iceland.

Tel: 00345-8662199;

Fax: 00354-4335001;

E-mail: thorvaldurk@lbhi.is

Received: 22 April 2014;

accepted: 30 June 2014

### Summary

A nonsense mutation in *DMRT3* ('Gait keeper' mutation) has a predominant effect on gaiting ability in horses, being permissive for the ability to perform lateral gaits and having a favourable effect on speed capacity in *trot*. 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). The aim of this study was to evaluate further the effect of the *DMRT3* nonsense mutation on the gait quality and speed capacity in the multigaited Icelandic horse and demonstrate how the frequencies of the A- and C- alleles have changed in the Icelandic horse population in recent decades. It was confirmed that homozygosity for the *DMRT3* nonsense mutation relates to the ability to pace. It further had a favourable effect on scores in breeding field tests for the lateral gait *tölt*, demonstrated by better beat quality, speed capacity and suppleness. 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*. These results indicate that the AA genotype reinforces the coordination of ipsilateral legs, with the subsequent negative effect on the synchronized movement of diagonal legs compared with the CA genotype. The frequency of the A-allele has increased in recent decades with a corresponding decrease in the frequency of the C-allele. The estimated frequency of the A-allele in the Icelandic horse population in 2012 was 0.94. Selective breeding for lateral gaits in the Icelandic horse population has apparently altered the frequency of *DMRT3* genotypes with a predicted loss of the C-allele in relatively few years. The results have practical implications for breeding and training of Icelandic horses and other gaited horse breeds.

### Introduction

One of the major characteristics of horse breeds is their ability to perform specific gaits. A gait is a coordination pattern of the limbs identified by timing and

sequence of the footfalls. The gait chosen by a horse depends on speed, genotype and environmental factors (Alexander 1988; Clayton 2004). 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.

A nonsense mutation in *DMRT3* (*DMRT3\_Ser301-STOP*), also referred to as the 'Gait keeper' mutation, has been shown to have a great impact on gaiting ability in horses (Andersson *et al.* 2012). Previous work has indicated that the mutation is permissive for the ability to perform lateral gaits, such as *tölt* and *pace*, and homozygosity for the mutation is required although not sufficient for the ability to *pace*. Moreover, the mutation was reported to have a favourable effect on speed capacity in *trot* and seemed to inhibit the transition from *trot* to *gallop* in a study on Standardbred horses used in harness racing (Andersson *et al.* 2012). 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). Comparison of wild-type and *Dmrt3*-null mice showed that *DMRT3* is crucial for the normal development of a coordinated locomotor network that controls limb movement. It was concluded that *DMRT3* neurons are essential for left/right coordination as well as for coordinating the movement of fore- and hind legs (Andersson *et al.* 2012).

The Icelandic horse is bred for leisure riding as well as for sport competitions (Albertsdóttir *et al.* 2007; FIZO 2012), with the international breeding goal for the Icelandic horses promoting five-gaited horses. The breeding assessment system is based on breeding field tests for both riding qualities and conformation, where assessment of riding qualities includes judging of the five gaits (FIZO 2012). Scores are also given for *slow tölt* and *canter* although they are not weighed into the total score, but influence the scoring for *tölt* and *gallop*, respectively. The horses are judged on a scale from 5 (not presented) to 10 (best) with intervals of 0.5, the average being 7.5. The judges can also give standardized comments on the assessed traits that describe certain attributes of the traits and substantiate the scoring (listed in Table S1 for the five gaits). Horses can only receive scores above average if they present one or more of the listed advantages and horses below average have one or more of the listed

disadvantages. Horses can attend the breeding field tests from the age of four, the majority being five and 6 years old. Approximately 12.5% of the Icelandic horse population is presented based on a preselection by the breeders (Albertsdóttir *et al.* 2011).

The aim of this study was to evaluate the effect of the *DMRT3* nonsense mutation on the gait quality and speed capacity in the multigaited Icelandic horse and demonstrate how the frequency of the A- and C-alleles has changed in the Icelandic horse population in recent decades.

## Material and methods

### *Estimation of DMRT3 genotype effect on gait traits*

#### *Selection of horses*

Horses were selected for genotyping on the basis of their scores for the different gaits in breeding field tests. For practical reasons, the selection was limited to horses judged in Iceland and Sweden, in the years 2000–2012, with a stored DNA sample according to the global database WorldFengur (<http://www.worldfengur.com>). The first criterion was the score for *pace* including both horses with scores below average (5.5–7.0) and higher performing (7.5–10). The number of horses in each score for *pace* was in accordance with the proportion of horses getting each score annually for the last 5 years in Iceland. This provided 390 five-gaited horses with a wide distribution of scores for the other gaits. In the next step, four-gaited horses (with the score 5.0 for *pace* and various scores for the other gaits) were added until at least 20 horses showed each score for each gait in the range of 7.0–9.0 and as many as possible in the range of 9.5–10. This added 243 horses and enabled comparison of four- and five-gaited horses of different gait quality with respect to the *DMRT3* genotype. Horses were selected at random when possible, but in the case of few available candidates, all were selected. Finally, 34 horses were selected on the basis of the judges' comments describing quality and speed capacity of the gaits (FIZO 2012) (Table 1). As comments are not necessarily recorded for each trait for all horses, comparison with the genotype data was limited to the following variables: good beat in *walk*; good speed capacity in *trot* versus lack of speed; clear beat in *trot* and good suspension in *trot* versus four-beated *trot*; good speed capacity in *gallop* versus lack of speed; clear beat in *gallop*; good suspension in *gallop* versus lack of suspension in *gallop*; good speed capacity in *tölt* versus lack of speed; good beat in *tölt* versus trotty *tölt*; and supple *tölt* versus stiff *tölt*.

**Table 1** Number of horses in the data set within scores for each gait assessed at breeding field tests for Icelandic breeding horses

Trait	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10.0	Total
Walk	0	9	24	47	114	140	176	105	29	2	1	647
Trot	1	0	2	19	71	95	194	172	87	25	1	667
Gallop	0	0	1	2	28	109	235	197	84	11	0	667
Canter	26	0	0	10	62	162	197	93	35	11	1	597
Tölt	0	0	2	4	21	71	182	217	112	55	3	667
Slow tölt	3	0	5	8	43	130	220	150	63	10	1	630
Pace	263	44	47	59	80	30	35	46	50	12	1	667

### Description of data

The data set consisted of 667 horses, of which 360 were stallions and 307 mares. Where a horse had been scored more than once at breeding field tests, only the record where the horse obtained its highest total score for riding ability was used. Scores for *tölt* and age at first evaluation at a breeding field test were also investigated. The age ranged from 4 to 14 years, with a mean of  $6.4 \pm 1.7$  years. The horses were born in 1986–2008 with a mean birth year  $2001 \pm 4.3$ . The total number of sires was 271, with an average of  $2.5 \pm 3.7$  offspring per sire (range: 1–43). The data set included 404 five-gaited horses (*pace* score  $\geq 5.5$ ) and 263 four-gaited horses (*pace* score = 5.0).

### SNP genotyping

Samples from the 667 horses were obtained from two repositories in Iceland and one in Sweden. DNA was extracted from nose swabs and blood using mag<sup>TM</sup> kit (AGOWA GmbH, Berlin, Germany) and Gentra Pure-gene Blood Core Kit (QIAGEN Inc., Venlo, Limburg, the Netherlands), respectively.

Custom TaqMan SNP Genotyping assays (Applied Biosystems, Inc., Foster City, CA, USA) were used to genotype the *DMRT3\_Ser301STOP* SNP with the following primers and probes: Forward primer: 5'-CCTC TCCAGCCGCTCCT-3'; reverse primer: TCAAAGATG TGCCCGTTGGA-3'; wild-type probe: 5'-CTGCCGAA GTTCG; mutant probe: 5'-CTCTGCCTAAGTTCG-3'. rt-PCRs were carried out on a 384-well ABI PRISM 7900 HT sequence detection system (Applied Biosystems) and a 96-well Stratagene Mx3005P.

### Statistical analyses

Statistical analyses were performed using SAS (SAS Institute Inc. 2009). The mean, standard deviation (SD), skewness and kurtosis were calculated to describe the variation of the gaits within the sample of 667 horses. To ascertain whether the distribution of the gaits deviated significantly from zero measure of skewness and kurtosis, the following calculations were made: Estimated skewness  $>1.96\sqrt{6/n}$  for

$p < 0.05$  and estimated skewness  $>2.33\sqrt{6/n}$  for  $p < 0.01$ ; and estimated kurtosis  $>1.96\sqrt{24/n}$  for  $p < 0.05$  and estimated kurtosis  $>2.33\sqrt{24/n}$  for  $p < 0.01$ .

Effects of the age\*sex interaction (four age classes: 4, 5, 6 and  $\geq 7$  years old horses, two sex classes: mares and stallions) and the genotype of the horse (two classes: AA and CA genotypes) on the gait traits were estimated with analysis of variance using PROC GLM (SAS Institute Inc. 2009). The following model was assumed for each gait trait:

$$y_{ijn} = \mu + \text{age} - \text{sex}_i + \text{genotype}_j + \text{age} - \text{sex} * \text{genotype}_k + e_{ijn}$$

where  $y_{ijn}$  is a gait trait (six traits: *walk*, *trot*, *gallop*, *canter*, *tölt* and *slow tölt*) for the  $n$ th horse,  $\mu$  is the population mean, age-sex<sub>*i*</sub> is the combined effect of the  $i$ <sup>th</sup> age-sex group ( $i = 1, \dots, 8$ ), genotype<sub>*j*</sub> is the effect of the  $j$ <sup>th</sup> genotype ( $j = 1, 2$ ; 1 = AA, 2 = CA), age-sex\*genotype<sub>*k*</sub> is the effect of the interaction of  $k$ <sup>th</sup> age-sex by genotype ( $k = 1, \dots, 16$ ) and  $e_{ijn}$  is a random  $\sim \text{NID}(0, \sigma_e^2)$  residual effect. Because of their low number, horses with the CC genotype ( $n = 8$ ) were not included in this analysis. A Student's t-test was used to ascertain whether both scores for *tölt* and age at first evaluation differed significantly between horses with AA and CA genotypes. Then a chi-square test with 1 df was performed to study whether proportions of genotypes within a subgroup of 28 horses that had received scores of 9.0–9.5 for *tölt* as 4 years old deviated significantly from the proportion of the genotypes within the whole data set.

Discriminant analysis was performed using stepwise selection to obtain a subset of the gaits to be able to discriminate between the genotype classes (AA and C-). Only gaits that were significant in the stepwise discriminant function procedure and that had partial  $R^2$  values  $\geq 0.01$  were retained in the final model. These gaits were then included in a canonical discriminant analysis to find a linear combination of the gaits that best summarized the difference between the genotype classes (AA and C-). Mahalanobis distance between the class means was estimated. The analyses were

performed using the PROC STEPDISC and PROC CANDISC (SAS Institute Inc. 2009), respectively.

Chi-square tests with 1 df were performed to study whether proportions of genotypes within groups of horses receiving certain judges' comments describing the gaits deviated significantly from the proportion of the genotypes within the whole data set.

#### Change in allele frequency over time

When the mode of inheritance is known, genotype probabilities at individual loci in large animal populations can be estimated from genotyped data or phenotypic data on a part of the population. For this purpose, efficient computing algorithms have been created (van Arendonk *et al.* 1989; Fernando *et al.* 1993; Janss *et al.* 1995; Kerr & Kinghorn 1996).

WorldFengur (<http://www.worldfengur.com>) provided a pedigree file containing 410 285 horses for this study (birth years 1860–2012) where of 83% were born after 1989. The average pedigree depth for horses born 2009–2012 was 5.1 generations (max. value 15), and the corresponding five generations pedigree completeness index (PEC) was 85% according to the method of MacCluer *et al.* (1983).

So far, only a very small proportion of the population of Icelandic horses has been genotyped for the *DMRT3* mutation (706 genotyped horses were available for this study). However, recording of *pace* scores in breeding field tests may provide approximate information on the conditional probabilities of the genotypes for a larger number of horses. As a starting point, *pace* scores were extracted from the breeding field test records kept in WorldFengur based on the following conditions: a) *pace* score  $\geq 6.0$  (indication of a carrier of the A-allele), b) *pace* score = 5.0 (no *pace* shown) and *trot* score  $\geq 7.0$  (initial indication of a C-allele carrier). Data were excluded for horses receiving 5.5 for *pace* and for horses with no *pace* shown in combination with limited or bad *trot*. In total, 55 073 records on 33 036 horses fulfilled the required conditions. The highest *pace* score for horses with repeated observations was selected, and one record per horse was used.

Six phenotypic classes were formed based on the information content of the genotype data and the phenotypic data (first two columns in Table 2). The genotype data consisted of 521 AA, 177 CA and eight CC horses. The phenotypic *pace* scores  $\geq 7.0$  were taken to indicate the AA genotype (score 1), and *pace* scores 5.0 were used as a preliminary indication of the CA or CC (C-) genotype (score 4), while *pace* scores 6.0–6.5 were assumed to exclude

**Table 2** Phenotypic scores and corresponding possible genotypes for Icelandic horses included in the pedigree list. The distribution of the scores is shown before and after G-E updating, for the 410 285 Icelandic horses included in the pedigree list

Phenotypic scores	Genotypes	Initial scores		G-E updated scores	
		N	%	N	%
1	AA	17 284	4.21	67 019	16.33
2	CA	176	0.04	7910	1.93
3	CC	8	0.002	8	0.002
4	C-	8857	2.16	4661	1.14
5	A-	6620	1.61	160 633	39.15
9 (no score)	–	377 348	91.97	170 054	41.45

the CC genotype and leave score for CA or AA genotypes (score 5).

The present genotype data and earlier results (Andersson *et al.* 2012) have shown that horses with *pace* scores  $\geq 7.0$  are almost certainly of genotype AA, while a large part (>30%) of the horses receiving *pace* score of 5.0 (shown as four-gaited horse) are also AA although *pace* was not presented, for various reasons. By use of pedigree data and the laws of Mendelian inheritance, the preliminary phenotypic scores can be updated and improved. It is feasible to use the Genotype Elimination (G-E) algorithm of Lange (1997) to improve the phenotypic score data by creating a legal data set compatible with the pedigree and Mendelian models (Table 2). The G-E algorithm is an iterative procedure for eliminating genotypes where incompatibility is observed between any offspring–parent pairs in the pedigree list. The G-E algorithm was run repeatedly (seven times), and inconsistency was listed. Unlikely scores of offspring were adjusted to score 1 whenever both parents had genotype AA confirmed on the basis of genotype or phenotypic data. As many horses shown as four-gaiters are truly AA, this procedure of updating seems important (Andersson *et al.* 2012). The data with G-E updated scores were used as an input in the segregation analysis by the Geneprob Fortran program of Kerr & Kinghorn (1996). The Geneprob program is based on the concept of 'iterative peeling' and, as all such algorithms, which are based on probability equations, the method is sensitive to inconsistency in the data. The prior use of the G-E procedure is highly recommendable before segregation analysis in large data sets where errors in pedigree and/or data recording are inevitable.

The resulting changes in the phenotypic scores are shown in Table 2. The increase in fully and partly informative scores was from 8.03% in the initial data to 58.55% in the G-E updated scores.

The frequency of the C-allele in the founder population,  $p(C)$ , was assumed to be either 0.13 as in the sample of 706 genotyped horses or 0.30, which may be a more probable value in the founder population (ca. 5 generations back) according to preliminary results. These values were used as priors in the genotype probability computations. Many horses in the population were slightly inbred (average inbreeding coefficient was 2.5%). The prior genotype probabilities for inbred animals are not exact as the algorithm in Geneprob does not account for the increased probability of inbred animals being homozygous. For animals with sufficient phenotypic or genotypic information, the effects of prior allelic frequency or inbreeding level on the posterior genotypic probabilities are negligible.

The accuracy of the genotype probability estimates was evaluated by the genotype probability index (GPI) developed by Kinghorn (1997) to indicate the information content from the segregation analysis.

Mean genotype probabilities within each year were the estimate for the genotype frequencies within each cohort, and from these, the annual development in the frequencies of the A- and C-alleles was plotted for the birth years 1980–2012. A chi-square test with 1 df was performed to evaluate whether the genotypes would conform to the Hardy–Weinberg proportions. The chi-square value for each year (cohort) was regressed on year for the period 1980–2012. These calculations included 146 763 horses with a GPI of  $\geq 30\%$  (Kinghorn 1997).

## Results

### *Effect of DMRT3 genotype on gait traits*

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 five-gaited 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.

The mean, range and variation of six gait traits are presented in Table 3. The distribution of the traits *walk*, *trot* and *canter* deviated significantly from zero measure of skewness and kurtosis.

The *DMRT3* genotype had a significant effect on all gaits except *slow tölt* (Table 4). Scores for *walk*, *trot*, *gallop* and *canter* were significantly higher among horses with the CA genotype compared with AA horses which had significantly higher scores for *tölt*.

The interaction term between the age–sex classes and genotype (two classes: AA genotype and CA genotype) proved to be non-significant for all gaits except for *tölt*. Stallions aged four and 5 years with the AA genotype had significantly higher scores for *tölt* than their contemporaries with the CA genotype. Mean scores of 4-year-old stallions with the AA and CA genotype were 8.55 and 7.90, respectively ( $p < 0.01$ ), and mean scores of 5-year-old AA and CA stallions were 8.48 and 8.18, respectively ( $p < 0.05$ ). Moreover, 6-year-old mares with the AA genotype had significantly higher scores for *tölt* (8.25) than 6-year-old mares with the CA genotype (7.93) ( $p < 0.05$ ). Mean scores for *tölt* at first evaluation in breeding field tests for CA and AA horses were 8.11 (mean age: 5.5 years) and 8.15 (mean age: 5.1 years),

**Table 3** The mean, range and variation of six gait traits of the 667 horses included in the data set

Trait	Mean	SD	Min	Max	Skewness	Kurtosis
Walk	7.66	0.73	6.00	9.50	−0.25*	−0.37
Trot	8.09	0.69	6.00	10.00	−0.18	−0.39*
Gallop	8.16	0.55	6.50	9.50	−0.14	0.07
Canter	7.89	0.60	6.50	10.00	0.29*	0.11
Tölt	8.36	0.64	6.00	10.00	−0.19	0.29
Slow tölt	8.05	0.59	6.00	10.00	−0.04	−0.13

Levels of significance: \* $p < 0.05$ .

**Table 4** Results of analysis of variance for the effect of *DMRT3* genotype on gait traits (667 horses). Least square means of six gait traits of homozygous mutant (AA) and heterozygous (CA) horses. The p-values indicate where there is significant difference between least square means

Trait	Number of AA	Number of CA	AA	CA	p-value
Walk	502	143	7.52	7.71	*
Trot	509	150	7.99	8.24	***
Gallop	509	150	8.08	8.36	***
Canter	474	119	7.61	8.32	***
Tölt	509	150	8.39	8.26	*
Slow tölt	488	136	8.01	8.04	NS

Levels of significance: \* $p < 0.05$ ; \*\*\* $p < 0.001$ .

respectively. CA horses were significantly older at first evaluation than AA horses, while the difference in mean score for *tölt* was not significantly different. Further, it was shown that significantly more horses had the AA genotype (93%) compared with the CA genotype (7%) within a subgroup of 28 horses that had received 9.0 or higher for *tölt* at the age of 4 years.

The selection procedure of STEPDISC was used to select the subset of the gaits that best reveals the difference between the genotype classes. All gaits were selected in the final model, and multivariate tests (Wilks' lambda and Pillai's trace) indicated highly significant ( $p < 0.001$ ) differences between horses with the AA and C- genotypes. However, *pace* followed by *canter*, *tölt*, *gallop* and *trot* had, according to their  $R^2$  and F-values, more discriminant power than *walk* and *slow tölt* (results not shown), so the latter were removed from the final model. In the canonical discriminant analysis, the canonical coefficients generated were significant ( $p < 0.001$ ). The adjusted canonical correlation between the resulting discriminant function and the classification variable of the AA or C-genotype was 0.58. The structure of the discriminant function is shown in Table 5. The traits with the highest absolute canonical coefficients or loadings contribute the most to the divergence between genotype classes. *Canter* had the highest positive coefficient, followed by *gallop* and *trot*; high scores for these traits indicated a C- genotype. *Pace* and *tölt* had negative coefficients, with *pace* having a higher loading value; high scores for these traits indicated an AA genotype. The mid-point between the group centroid scores, which may be used as a cutting point to assign a previously unclassified horse to a genotype group, was 0.52; a horse with a value  $>0.52$  would be classified as a C- horse and a horse with a value  $<0.52$  would be classified as an AA horse. The Mahalanobis distance ( $D^2$ ) was 3.23 and showed a significant difference between the genotype classes, indicating that horses would be correctly classified in 82% of all cases.

**Table 5** Total canonical structure of the discriminating function separating horses of the AA and CA genotypes

Trait	Coefficient
Pace	-0.79
Canter	0.59
Gallop	0.40
Trot	0.27
Tölt	-0.11
F-value	62.72
p-value	***

Levels of significance: \*\*\* $p < 0.001$ .

The proportions of the AA and CA genotypes differed significantly within groups of horses receiving judges' comments describing beat in *trot*, *gallop* and *tölt*; suspension in *trot* and *gallop*; and speed capacity and suppleness in *tölt*. Horses with the CA genotype more often had good suspension in *trot* and *gallop*, better beat in *gallop* and were less likely to be four-beated in *trot* while AA horses were more likely to be supple in *tölt* and to possess good speed capacity in *tölt* (Table 6).

#### Change in allele frequency over time

The results of the segregation analyses with the two different prior allele frequencies ( $p(C) = 0.13$  and  $p(C) = 0.30$ ) were compared in terms of information content (Table 7). The true allele frequency for the C-allele is probably closer to 0.3 in the founder population (see Figure 1). The frequency of accurately estimated genotypes was slightly higher, and therefore, only the results from the analysis with  $p(C) = 0.3$  will be presented and discussed further.

The increase in exactly evaluated genotypes in the segregation analysis compared with the G-E procedure is shown in Table 8. The data included eight

**Table 6** The proportion of genotypes within groups of horses receiving certain judges' comments for the gaits. The p-values indicate where the proportions deviate significantly from the expected proportions of 0.76 for the homozygous mutant genotype (AA) and 0.24 for the heterozygous genotype (CA) according to a chi-square test with 1 degree of freedom

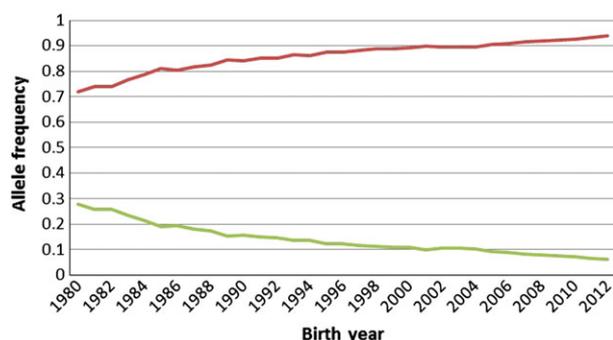
Trait	Number	AA	C/-	p-value
Walk				
Clear beat	132	0.73	0.27	NS
Trot				
Good speed capacity	123	0.79	0.21	NS
Lack of speed capacity	88	0.83	0.17	NS
Clear beat	131	0.74	0.26	NS
Four-beated	64	0.91	0.09	*
Good suspension	92	0.45	0.56	***
Gallop				
Good speed capacity	166	0.79	0.21	NS
Lack of speed capacity	35	0.83	0.17	NS
Clear beat	65	0.6	0.4	*
Good suspension	77	0.42	0.58	***
Lack of suspension	71	0.96	0.04	***
Tölt				
Good speed capacity	222	0.83	0.17	*
Lack of speed capacity	38	0.53	0.47	***
Clear beat	257	0.78	0.22	NS
Trotty beat	27	0.19	0.81	***
Supple	99	0.87	0.13	*
Stiff	33	0.82	0.18	NS

Levels of significance: \* $p < 0.05$ ; \*\*\* $p < 0.001$ .

**Table 7** Illustration of information content in the studied data for estimation of genotype probabilities. The genotype probabilities are derived from segregation analyses with two different prior allele frequencies ( $p(C) = 0.13$  and  $p(C) = 0.30$ )

GPI	P(C) = 0.13		P(C) = 0.30	
	N	%	N	%
100	75 059	18.29	75 232	18.34
90	84 214	20.53	85 106	20.74
80	94 623	23.06	98 536	24.02
70	109 773	26.76	118 732	28.94
60	119 944	29.23	135 489	33.38
50	134 662	32.82	161 567	39.38
40	183 269	44.67	199 211	48.55
30	254 340	61.99	226 410	55.18

GPI, Genotype Probability Index.



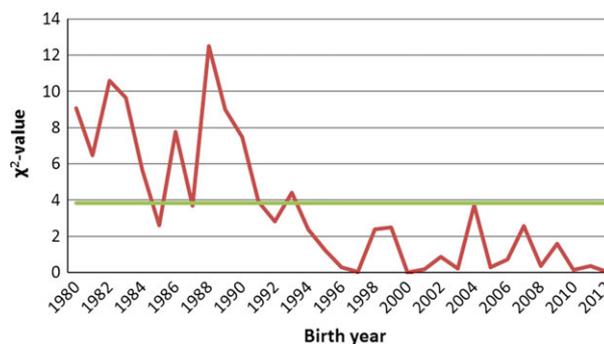
**Figure 1** Frequencies of the A- and C-alleles in *DMRT3* in the Icelandic horse population from 1980–2012; the red line refers to the A-allele, and the green line refers to the C-allele.

**Table 8** Number of exactly estimated genotypes in the segregation analysis when prior  $p(C) = 0.3$

Genotypes	N	%	Increment compared with G–E updated scores
AA	67 273	16.40	254
CA	7950	1.94	40
CC	9	–	1

genotyped individuals with the CC genotype, and only one horse with exactly confirmed CC genotype was additionally revealed in the segregation analysis. Then, 24 horses were found to have estimated genotype probability  $>0.85$  for the CC genotype.

The trend from 1980 to 2012 in the frequency of the alleles A and C was estimated in the whole population through calculation of genotype probabilities. The frequency of the A-allele was estimated to be 0.72 in 1980 and 0.94 in 2012 (Figure 1).



**Figure 2** Development of chi-square values over time, testing Hardy–Weinberg equilibrium of *DMRT3* genotypes in the Icelandic horse population, with indicated 0.05 significance level for one degree of freedom (green line).

The chi-square test was used to evaluate whether the genotypes reflected Hardy–Weinberg proportions. This was performed for each birth year from 1980 to 2012, and the chi-square value regressed on birth year (Figure 2). For the years 1980–1993, the genotypes were not in Hardy–Weinberg equilibrium as the values were above 3.84, which is the 0.05 significance level for one df. In this period, the proportion of the CA genotype was higher than expected and the proportion of the homozygotes was subsequently lower. In the years 1994–2012, the genotypes were estimated to be in Hardy–Weinberg equilibrium in the population. The results showed that the proportion of the genotypes in the selected material of 667 horses conformed to the Hardy–Weinberg proportions. These findings therefore agree well with the fact that the majority of horses in the selected material are born in 1997–2005.

## Discussion

The population of the multigaited Icelandic horse allows for detailed estimation of the effects of the *DMRT3* nonsense mutation (*Ser301STOP*) on gaiting ability. The assessment of the different gaits is systematic and standardized (FIZO 2012), and the population is not fixed for the mutation (Andersson *et al.* 2012). In this study, the effect of the *DMRT3* nonsense mutation on the gaiting ability of the Icelandic horse was estimated using more detailed information and a larger sample of assessed breeding horses than previously published (Andersson *et al.* 2012). The horses in the data set were selected with regard to scores and judges' comments referring to the individual gaits to include as detailed information about both gait quality and speed capacity as possible. The mean scores for

the gaits (Table 3) were, however, higher in the selected material than in all Icelandic breeding horses presented for breeding assessment in a similar period (Albertsdóttir *et al.* 2008), except for *walk* and *pace*. No single sire is believed to have a great impact on the results as the average number of offspring per sire is low and it is assumed that the data set reflects the estimated proportion of genotypes in the population. The rider has been shown to have a significant effect on gait quality (Albertsdóttir *et al.* 2007). The rider effect was, however, not included in the model where the genotype effect on the gaits was estimated because of obvious risk of confounding effects of the rider and genotype, as 74% of riders rode only one or two horses. Kerr and Kinghorn's method (1996) of calculating genotype probabilities facilitated the estimation of the development in the frequency of the A- and C-alleles, which shows how breeding decisions have shaped the distribution of *DMRT3* genotypes over time.

#### Effect of the *DMRT3* nonsense mutation on gait traits

This study confirmed favourable effects of the *DMRT3* nonsense mutation on the lateral gaits *tölt* and *pace*. Almost all horses with a *pace* score of 5.5 or higher were homozygous for the *DMRT3* nonsense mutation, confirming that the AA genotype is a prerequisite for the ability to pace. The AA genotype is, however, not sufficient for the ability to perform *pace* as 45% of horses classified as four-gaited were homozygous mutants. This high proportion of AA horses presented as four-gaited (without *pace*) could thus be influenced by other genetic and environmental factors. Presenting all gaits at breeding field tests will give the possibility of highest total score and is therefore the main goal. However, the score for *tölt* is the most valuable trait for the marketing price of the horse, so *tölt* has the highest weight in the total score. Presenting four gaits is an alternative, preferable for horses that do not have outstanding performance in *pace*. Training of *tölt* receives the highest priority and it is well known that pace training can in some instances impair the *tölt* quality. Therefore, many horses are ridden as four-gaiters even if they could perform *pace* up to a certain level (Árnason & Sigurdsson 2004). The presence of few CA horses receiving a score of 5.5 or higher for *pace* (3.2% of the five-gaited horses), which all received scores below average for *pace*, is most likely a phenotypic misclassification or in some instances presumably resulting from training, conformation or other factors that can facilitate CA horses to perform low quality pace. *Tölt* and *pace* are very

similar gaits, both being in fact four-beat, lateral gaits (Wilson *et al.* 1998), and phenotypic misclassification is therefore not unexpected. The main features that separate them is the shorter time between ground contact of lateral legs in *pace* than *tölt* and a moment of suspension in *pace*, which should be non-existing in *tölt* (Zips *et al.* 2001).

The results clearly showed a positive effect of the AA genotype on the *tölt* ability. This seems to depend both on superior speed capacity and suppleness of the AA horses compared with the CA horses. Speed capacity and suppleness greatly impact the scoring for *tölt* (FIZO 2012). The significant interaction between genotype and the age–sex classes in the analysis of variance indicates that AA horses have more natural ability to *tölt*. CA horses were also significantly older when presented at breeding field tests for the first time. This could indicate that they need longer training than AA horses to develop an acceptable *tölt* capacity, as the quality of *tölt* is one of the main criteria for the preselection of horses to the breeding field tests (Albertsdóttir *et al.* 2011). Moreover, AA horses are overrepresented in the group of 28 horses in the data set that had received a score of 9.0 or 9.5 for *tölt* at the age of four. Heterozygous horses had significantly higher scores for the basic gaits *walk*, *trot*, *gallop* and *canter*. A previous study (Andersson *et al.* 2012) has shown that Icelandic horses with the CA genotype had significantly higher scores for *trot* compared with homozygous mutant horses. This was confirmed in the current study and further related to correct beat and suspension. It was also revealed that CA horses had significantly higher scores for *gallop* and *canter* compared with AA horses, possessing more often correct beat and suspension in *canter/gallop*. Correct beat in *canter* (a pure three-beat) depends on synchronized movement of diagonal legs in much the same way as in *trot* (Clayton 2004). These results indicate that the AA genotype reinforces the coordination of ipsilateral legs, with the subsequent negative effect on the synchronized movement of diagonal legs. This agrees well with previous suggestions that *DMRT3* neurons play a critical role in left/right coordination, as well as in coordinating the movement of fore- and hind limbs (Andersson *et al.* 2012). The negative effect of the AA genotype on beat and suspension in *trot* as well as in *canter/gallop* has probably the same cause, suggesting a negative effect of the AA genotype on the synchronized movements of diagonal legs. The genotype effect on scores for canter is strong (Table 4), but high scores for canter demand correct beat and suspension (FIZO 2012). This is further supported by the higher proportion of horses with the CA genotype among

horses that received the judges' comment *trotty tölt*, which involves too much association of diagonal legs in *tölt*.

Standardbred trotters with the AA genotype have been reported to have significantly higher breeding values for racing performance compared with the CA genotype (Andersson *et al.* 2012). It was suggested that the AA genotype promotes speed capacity at *trot*. This was not supported in the current study probably because riders at breeding field tests for Icelandic horses are not always riding them to their limit in speed in *trot* to maintain correct beat, as correct beat counts more than high speed in the scoring for *trot* (FIZO 2012). It has been suggested that the transition from *trot* to *gallop* is triggered when musculoskeletal forces reach a critical level and that peak forces are reduced at a certain speed by the transition from *trot* to *gallop*, as *gallop* is a more compliant gait with the sequential ground contact of limbs (Farley & Taylor 1991). This critical level could be avoided at high speed in *trot* by dissociating diagonal legs (become four-beated) and therefore placing the legs more sequentially on the ground. It has, indeed, been shown that the magnitude of diagonal dissociation increases with speed (Drevemo *et al.* 1980). Therefore, the superiority of AA horses in *trot* racing could be explained by their 'ability' to be four-beated in *trot* as shown in the current study. That could be an advantage when high speed in *trot* is required but a disadvantage when qualities such as correct beat and suspension are required.

The results of the canonical discriminant analysis supported the findings of the analysis of variance for the effect of the *DMRT3* genotypes on the gaits. Based on scores for *trot*, *canter*, *gallop*, *tölt* and *pace*, it was possible to discriminate between AA and CA horses with high confidence. *Pace* had the highest negative loading and, along with a high score for *tölt*, suggested an AA genotype. High scores for *trot* *canter* and *gallop* suggested a CA genotype, with *canter* having the greatest discriminating power of the basic gaits.

#### Change in allele frequency over time

The change in the frequency of the A- and C-alleles indicated a selection in favour of the A-allele in the Icelandic horse population over the last decades. This result must be interpreted in the light of the effect of the AA genotype on *tölt* and its crucial role for the ability to *pace*. Since the definition of the official breeding objective for the Icelandic horse in 1950 and until now, an excellent five-gaited horse has been the main aim (Hugasón 1994). This entailed a heavy

emphasis for many years on the selection of Icelandic breeding horses with good capacity for both *tölt* and *pace*. Breeding value estimations for Icelandic horses are based on breeding field test scores, where *tölt* has the highest weight and *pace* a relatively high weight. In addition, both traits have high genetic variation compared with other assessed traits, especially *pace* (Albertsdóttir *et al.* 2008). The quality of horses with respect to these traits therefore greatly impacts their ranking, and breeders have to a greater extent based their selection on breeding values since 1984 (Árnason & Van Vleck 2001). The observed trend in the genotype frequencies implies that the C-allele may be lost in the Icelandic horse population around year 2030. In the light of the favourable effect the C-allele seems to have on the basic gaits, the breeding goal for the Icelandic horse should perhaps be redefined. Competitions for four-gaited horses, where the basic gaits and *tölt* have equal weights, have become more popular resulting in high market value of high-class four-gaited horses (Albertsdóttir *et al.* 2007). The current study indicates how probability calculations can be used to estimate the genotype of an individual using genotype and phenotypic information combined with prior knowledge about genotype effect. The results demonstrate the strength of genomic methods in monitoring the effect of breeding decisions on genetic variability.

In the years 1980–1993, the *DMRT3* genotypes were not in Hardy–Weinberg equilibrium. The observation of a higher proportion of CA horses than expected from the gene frequencies suggests compensatory mating, where the mating of two four-gaited horses has probably been avoided over these years. The reason for possible reduction in compensatory mating according to *DMRT3* genotypes and a consequent Hardy–Weinberg equilibrium in the population in the years 1994–2012 can probably be explained by decreased selection intensity on the mares' side due to expansion in population size (Sigurdardóttir 2011), combined with growing popularity of four-gaited horses among which the AA genotype is more common.

#### Conclusions

Homozygosity for the *DMRT3* nonsense mutation is permissive for *pace* and has a major effect on the quality of *tölt*, *trot* and *canter/gallop*, and speed capacity in *tölt*. Selective breeding for lateral gaits in the Icelandic horse population has altered the frequency of *DMRT3* genotypes with a predicted loss of the C-allele in relatively few years. The results have practical

implications for breeding and training of Icelandic horses and other gaited horse breeds.

### Acknowledgements

This work is supported by a funding grant from the Foundation for the Preservation of the Icelandic Horse. It was also partially supported by funds from The Swedish Research Council Formas and the Swedish Research Council. The authors would like to acknowledge Jón H. Hallsson and Áslaug Helgadóttir for helpful comments on the text and Vilhjálmur Svansson for contribution of DNA samples.

### Competing interests

Lisa S. Andersson and Gabriella Lindgren are co-inventors on a patent application concerning commercial testing of the *DMRT3* mutation. Other authors do not have any actual or potential competing interests.

### References

- Albertsdóttir E., Eriksson S., Näsholm A., Strandberg E., Árnason T. (2007) Genetic analysis of competition data on Icelandic horses. *Livest. Sci.*, **110**, 242–250.
- Albertsdóttir E., Eriksson S., Näsholm A., Strandberg E., Árnason T. (2008) Genetic correlations between competition traits and traits scored at breeding field-tests in Icelandic horses. *Livest. Sci.*, **114**, 181–187.
- Albertsdóttir E., Eriksson S., Sigurdsson Á., Árnason T. (2011) Genetic analysis of 'breeding field test status' in Icelandic horses. *J. Anim. Breed. Genet.*, **128**, 124–132.
- Alexander R.M. (1988) Why mammals gallop. *Am. Zool.*, **28**, 237–245.
- Andersson S.L., Larhammar M., Memic F., Wootz H., Schwochow D., Rubin C.J., Patra K., Árnason T., Wellbring L., Hjälms G., Imsland F., Petersen J.L., McCue M.E., Mickelson J.R., Cothran G., Ahituv N., Roepstorff L., Mikko S., Vallstedt A., Lindgren G., Andersson L., Kullander K. (2012) Mutations in *DMRT3* affect locomotion in horses and spinal circuit function in mice. *Nature*, **488**, 642–646.
- van Arendonk J.A.M., Smith C., Kennedy B.W. (1989) Method to estimate genotype probabilities at individual loci in farm livestock. *Theor. Appl. Genet.*, **78**, 735–740.
- Árnason T., Sigurdsson Á. (2004) International genetic evaluations of the Icelandic horse. In: I. der van Honing (ed), Book of Abstracts (No.10) of the 55th Annual Meeting of the European Association for Animal Production. Wageningen Academic Publishers, Bled, Slovenia, pp. 327.
- Árnason T., Van Vleck L.D. (2001) Genetic improvement of the horse. In: A.T. Bowling, A. Ruvinsky (eds), *The Genetics of the Horse*. CABI publishing, Massachusetts, pp. 473–497.
- Clayton H.M. (2004) *The dynamic Horse*. Sport Horse Publications, Michigan, USA.
- Drevemo S., Fredricson I., Dalin G., Bjorne K. (1980) Equine locomotion: 2. The analysis of coordination between limbs of trotting standardbreds. *Equine Vet. J.*, **12**, 66–70.
- Farley C.T., Taylor C.R. (1991) A mechanical trigger for the trot–gallop transition in horses. *Science*, **253**, 306–308.
- Fernando R.L., Stricker C., Elston R.C. (1993) An efficient algorithm to compute the posterior genotypic distribution for every member of a pedigree without loops. *Theor. Appl. Genet.*, **88**, 573–580.
- FIZO. (2012) English FEIF Rules for Icelandic Horse Breeding. (available at: <http://www.feiffengur.com/documents/fizo12e.pdf>. last accessed 01 February 2014).
- Hugason K. (1994) Breeding of Icelandic toelter horses: an overview. *Livest. Prod. Sci.*, **40**, 21–29.
- Janss L.L.G., van der Werf J.H.J., van Arendonck J.A.M. (1995) Computing approximate monogenetic model likelihoods in large pedigree with loops. *Genet. Sel. Evol.*, **27**, 567–579.
- Kerr R.J., Kinghorn B.P. (1996) An efficient algorithm for segregation analysis in large populations. *J. Anim. Breed. Genet.*, **113**, 457–469.
- Kinghorn B.P. (1997) An index of information content for genotype probabilities derived from segregation analysis. *Genetics*, **145**, 479–483.
- Lange K. (1997) *Mathematical and Statistical Methods for Genetic Analysis*. Springer-Verlag, New York, USA.
- MacCluer J.W., Boyce A.J., Dyke B., Weitkamp L.R., Pfennig D.W., Parson C.J. (1983) Inbreeding and pedigree structure in Standardbred horses. *J. Hered.*, **74**, 394–399.
- SAS Institute Inc. (2009) *SAS/STAT 9.2 User's Guide*, 2nd edn. SAS Institute Inc, Cary, NC.
- Sigurdardóttir H. (2011) Athugun á úrvalsstyrkleika og erfðafráfarum í einstökum eiginleikum í íslenskri hrossarækt [Investigation of selection intensity and genetic progress within breeding field test traits in Icelandic horse breeding]. BSc dissertation, Agricultural University of Iceland, Department of Land and Animal resources.
- Wilson B.D., Neal R.J., Howard A., Groenendyk S. (1998) The gait of pacers 2: factors influencing pacing speed. *Equine Vet. J.*, **20**, 347–351.
- Zips S., Peham C., Scheidl M., Licka T., Girtler D. (2001) Motion pattern of the toelt of Icelandic horses at different speeds. *Equine Vet. J. Suppl.*, **33**, 109–111.

### Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Attributes assessed in the scoring of gait traits in breeding field tests for Icelandic horses and their weight in the total score.