Thesis Summary of the PhD Dissertation

Kis Judit
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Innovative genomic and physiological research to improve the competitive performance of the English Thoroughbred population

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I. Background and Objectives

The tradition of horse racing spans over 400 years, and the conservative approach applied in the breeding Thoroughbreds has remained largely unchanged. However, contemporary advancements in ethology, genetics, and biotechnological innovations have the potential to revolutionize breeding processes. Through molecular genetic analyses and improved selection efficiency, genetic capabilities can be enhanced, key genes influencing performance can be identified, and biotechnological tools can be utilized to push biological boundaries, thereby advancing breeding programs and competition potential to a new level.

A QTL (Quantitative Trait Locus) is a specific region in the genome that influences a particular quantitative trait (e.g., height, weight, muscularity). The identification of QTLs allows researchers to determine which genomic regions affect these traits and how they relate to genetic variations and mutations. This is particularly important in animal breeding, as identifying the genes carrying mutations enables marker-assisted selection (MAS), facilitating more precise and accelerated breeding programs while reducing generational intervals.

A significant and specific locus in the genome of Thoroughbred horses is the ECA18 (Equine Chromosome 18) region, identified in the 2000s, which includes the myostatin (MSTN) gene. This gene influences optimal race distance and speed through its negative regulation of skeletal muscle growth. By analyzing MSTN gene polymorphisms, it is possible to determine, even before a horse enters training, whether it is best suited for short-, middle-, or long-distance racing. Furthermore, identifying the genotype frequency at population level can revolutionize or complement traditional pedigree-based breeding selection.

Among molecular genetic methods, the use of marker-assisted selection facilitates the early diagnosis of genetic disorders.

The aim of my doctoral dissertation is to contribute to the improvement of racehorse performance and the sustainability of the industry by conducting a comprehensive examination of the genetic, breeding, and training-related challenges in modern horse racing. The key objectives of my research were as follows:

I. My primary goal was to study the myostatin (MSTN) gene polymorphism in Thoroughbred horses, which plays a crucial role in race distance suitability and the development of responses to environmental factors.
II. Additionally, I aimed to develop a marker-assisted selection (MAS) strategy that enhances the efficiency of traditional breeding methods, thereby reducing generational intervals and improving phenotypic performance.
III. Another objective was to design a personalized training method that takes into account the genetic background and physical attributes of racehorses.

By testing and refining this method, I aimed to improve race performance and optimize pre-race preparation.

IV. I also sought to identify phenotypic characteristics related to individual temperament and physiological patterns, with a special focus on motor and brain laterality and their impact on performance.

II. Materials and Methods

Myostatin Analysis

During our study, we genotyped blood samples taken from 145 Thoroughbred horses, all of which were either owned, leased, or stationed at the Diópuszta stud farm of the Bábolna National Stud. The following data (32 parameters) were recorded in a database for mares (n = 94), stallions (n = 49), and geldings (n = 2): color, sex, pedigree, birth year, laboratory number, genotype, race distances, current age at the time of the race, track surface condition, race classification, placements, wins, winning times, number of competing horses, total earnings, earnings per race, total starts, average races per year, annual general handicap (GHP), GHP maximum, GHP average, racing years, best race distance (BRD), best race distance winning time, first racing age, mare family, subfamily, best race distance winning time in km/h, distances at which the horse finished in 1st-3rd place, breeding status, win percentage, BRD winning km/h, and the best winning km/h match. If the last parameter did not match, the best winning time and the corresponding distance were also recorded.
Data collection was conducted from the following sources: Hungarian Stud Book, volumes XXVIII-XXIX, Horse Racing Chronicle volumes 2005-2018, and various official online databases.
Blood sample collection was carried out with the assistance of a veterinarian, from the vena jugularis, with approximately 2 ml collected per horse in 4 ml EDTA-containing tubes (anticoagulant). The samples were transported in a refrigerated container and stored frozen at -20°C until DNA extraction. Samples were collected from a total of 145 individuals, all of whom were either stationed at the Diópuszta site of the Bábolna National Stud or trained at the Alag Racehorse Training Center. The sampled horses included breeding stallions, broodmares, foals, and active racehorses. DNA isolation was performed from the nuclei of white blood cells (Zsolnai et al., 2003), as red blood cells lack a nucleus.

Ultrasound Examinations

Out of the 145 animals genotyped for the MSTN gene, 66 horses were randomly selected for ultrasound examinations, with 22 individuals per genotype. The age of the horses ranged between 7 and 12 years. Echocardiography and muscle

ultrasound examinations were performed with the help of veterinarians working at the horses' facility using a portable MyLab™ AlphaVET device (Esaote S.p.A., Genoa, Italy), which offered a wide range of dedicated probes and softwares. Echocardiography was performed using a 2.5 MHz phased-array ultrasound transducer (SP2430, Esaote S.p.A., Genoa, Italy). The sector angle was 90°, and the maximum image depth was 35 cm, allowing visualization of the entire heart through the right parasternal echocardiographic window (right 4th intercostal space). To ensure optimal timing of end-diastolic measurements, simultaneous electrocardiography was recorded. Standardized two-dimensional and guided M-mode echocardiography was performed for each individual according to a previously described method (Long et al., 1992).
The statistical analysis was conducted by the same individual to ensure consistency. End-systolic M-mode measurements were taken at the point of maximal interventricular septum excursion, while end-diastolic measurements were recorded at the beginning of the QRS complex in the cardiac cycle. Each measurement was repeated across three cardiac cycles when the heart rate was within physiological limits. Cardiac cycles following a second-degree atrioventricular block were excluded from analysis. Left ventricular M-mode measurements were taken from the right parasternal short-axis view at the level of the chordae tendineae. The measured parameters included right ventricular diameter in diastole (RVD), interventricular septum thickness in diastole (IVSd) and systole (IVSs), left ventricular diameter in diastole (LVDd) and systole (LVD), and left ventricular free wall thickness in diastole (LVFWd) and systole (LVFW). The aortic diameter was measured in systole and diastole at the level of the aortic valve leaflets (ADVd, ADVs), the Valsalva sinus (ASDd, ASDs), and the sinotubular junction (ASJd, ASJs) using a two-dimensional long-axis right parasternal view of the left ventricular outflow tract. The pulmonary artery's end-diastolic diameter was determined from the right parasternal right ventricular inflow-outflow echocardiogram. Left atrial diameters (end-diastolic, LADd, and end-systolic, LADs) were measured from the right parasternal four-chamber view using two-dimensional echocardiography.
Skeletal muscles (m. triceps brachii, m. anconaeus, m. trapezius thoracis, m. longissimus dorsi, m. gluteus medius, m. semitendinosus, and m. quadriceps femoris) were examined using a convex ultrasound transducer (AC2541, Esaote S.p.A., Genoa, Italy). Reliable ultrasound images and measurements were obtained from the mm. anconaeus, triceps brachii, and longissimus dorsi muscles, so only these were included in the statistical analysis. Measurements were taken twice, with values demonstrating good agreement ($P<0.05$). The dataset was analyzed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).
The homogeneity of variance between MSTN groups was tested using Levene's test. The variance of the measured variables was equal among the compared groups ($p>0.05$).

Examination of Hair Whorls

As a first step, circular and linear hair whorls were identified on the head, neck, body, and legs. The facial whorls were categorized into three groups based on their position:

- Above the upper eye line (upper right, upper center, upper left)
- Between the upper and lower eye lines
- Below the lower eye line (lower right, lower center, lower left)

The hair whorls on the head were counted from the lips to the lower line of the ears. On the neck, the right and left sides were examined separately, covering the area from the atlas vertebra to the scapula (shoulder blade), including the thoracic region.

Additionally, the number and placement of hair whorls were analyzed in terms of symmetry across the body. To record the patterns, blank diagrams (charts) were used, identical to those found in equine passports. The findings were documented in a database, along with the results of the paddock test and the scores from the temperament questionnaire.

In Vivo Paddock Test

All horses included in the hair whorl examination were observed in a familiar and accustomed paddock, which was a 16×21 meter open, square-shaped area with a sandy surface and no grazing opportunities. The observations were conducted between 8:00 and 12:00, during a period when no other horses were present to cause disturbances.

During the first experiment, each examined horse was led into the paddock and was allowed to release the accumulated excess of energy by galloping. Following this, a 20-liter plastic bucket containing carrots was placed in the center of the paddock, and the horse was allowed to approach and lower its head to eat. Whenever a horse lowered its head for food, one forelimb was always placed forward for support, which was recorded as the preferred limb.

Once the food was consumed, the experiment was repeated after 180 seconds. In total, carrots were placed ten times, with the side preference recorded each time for 60 seconds, resulting in a total recorded time of 10×60 seconds. Additionally, the direction of turning away from both the human and the bucket was documented.

The observer wore solid black clothing and did not influence the horse’s movements during the experiment.

Temperament Questionnaire

As the third part of the hair whorl and paddock examination, a temperament questionnaire was completed by two individuals familiar with each horse. A previously validated 20-question survey was used (Momozawa et al., 2005). The 20 questions were categorized into three factor groups and analyzed according to previously published methods.

Statistical Analysis

A frequency analysis was conducted to examine the distribution of whorls (radial and linear) in terms of their position (left, right, center, as well as above the upper eye line, below the lower eye line, or between the eyes) and their occurrence on different body regions (head, body, neck, trunk, side, legs). The number of circular whorls on the upper, middle, and lower parts of the left, central, and right areas of the head was recorded. The proportion of horses with circular and linear whorls at different locations (head, body, and legs) was compared using Fisher's exact test.

Horses were classified into three groups based on the whorls found on their heads. If all whorls were clockwise, or if they were both clockwise and radial (and there were no counterclockwise whorls), these horses were categorized into the clockwise group. Horses whose head whorls were counterclockwise, or both counterclockwise and radial (and had no clockwise whorls), were classified into the counterclockwise group. Horses displaying both clockwise and counterclockwise whorls, or radial whorls on the head, were identified as "ambilateral", indicating they had no strong lateral limb preference.

Horses were classified as left-legged if, during the paddock test, they used their left forelimb (or turned left) in at least 8 out of 10 trials. Conversely, horses were classified as right-legged if they used their right forelimb (or turned right) in at least 8 out of 10 trials. Horses that used their left forelimb (or turned left) between 3 and 7 times out of 10 were classified as "ambilateral", meaning they had no strong limb preference.

A frequency analysis was performed on both the position and number of whorls (circular and linear). The presence of circular whorls on the upper part of the head, their number and location (left, right, or bilateral), and their orientation (left, right, or bilateral) were individually recorded. The number of whorls (radial and linear) on the head and body was also separately counted. These variables were compared with limb preference (left, right, or bilateral) during the paddock test using a chi-square test. The direction of the whorls on the head (counterclockwise, clockwise, or both) was compared with limb preference (left, right, or both) in the paddock test using Fisher's exact test.

Temperament Questionnaire

The temperament questionnaire scores were calculated following the method proposed by Momozawa et al. (2007):

- Anxiety factor included 7 questions: nervousness, excitability, panic, inconsistent emotions, vigilance, restlessness and fearfulness.
- Trainability factor included 4 questions: concentration, trainability, memory and perseverance.
- Affability factor also included 4 questions: friendliness toward humans, cooperation, gentleness and friendliness toward other horses.

The scores of the questions within each cluster were summed, and the results were compared in all cases. General linear models (GLMs) were used to determine whether any measured temperament traits were significantly influenced by factors such as sex, coat color, the presence of whorls on the upper part of the head, the number and location of whorls on the head, the direction of the whorls, the total number of whorls (on the head or other parts of the body), and limb preference during the paddock test.

A Pearson correlation test was used to assess the relationships between whorl characteristics (type, position) and measured personality traits. Statistical analyses were performed using R statistical software (version 4.3.0), with a significance threshold of $p < 0.05$.

Training Method Testing (Pilot Study)

In our study, based on preliminary MSTN genotyping results, three individuals of the same age were selected from each genotype group (C/C, C/T, T/T), which served as the test horses for the pilot study. Each horse was trained by same trainer, and they were prepared for races by two riders (one female, one male) alternating throughout one season (11 months). All of them were kept under identical housing conditions (box stalls) at the same location, the Alagi Racecourse and Training Center. The horses were fed similar, though not identical, diets considering their genotype, daily workload, and varying physiological and mental states (fatigue, good mood, etc.). The quantity and composition of the feed were coordinated by the assigned trainer and veterinarian. The three selected horses were Next Page (IRE) with racing number 257, Szigliget (IRE) with racing number 258, and Copadelmundo (IRE) with racing number 259. All three horses arrived at the designated trainer before their three-year-old racing season (previously trained elsewhere). An experimental overview of the horses is presented below:

259 Copadelmundo (IRE)

At the beginning of the test period, this two-year-old Irish-bred stallion had a record of two races and two sixth-place finishes. As a two-year-old, he raced in IV category/class races over 1400-1600 meters. His MSTN genotype was T/T,

which suggests he is suited for long-distance races, though his physical appearance and build were not typical for a stayer. Despite having been prepared and raced in shorter distances up until then, after a one-month rest, he received eight weeks of preparatory (PC – pre-conditioning) work starting in January. During this time, he worked five days a week, running 3000-4000 meters daily at an average speed of 15-25 km/h (slow-medium pace), alternately on the right and left. The warm-up and cool-down periods consisted of 20 minutes each, done in walk and trot. In addition, he spent one hour per day on a treadmill, walking in alternating directions. The horse did not participate in any paddock activities during the test period.
From March, he began long-distance conditioning (CW – conditioning work), during which he worked six days a week: three days of long, slow conditioning were supplemented by three days of interval (IT) training. The latter included 20-minute warm-up and cool-down periods, followed by 4x400 meters of medium-high intensity work (average speed of 25-30 km/h), with 2-minute recovery periods between each interval in walk. During the racing season (RP – race period), he had two days of PC, two days of IT, and one or two days of sprint work. The sprint work took place over 1600 meters, where the last 200-300 meters were completed at full speed. Sprint work was only carried out in the week leading up to the race (races are held on Sundays in Hungary) with the horse being galloped (with either a rider or a whip) as part of the training. To ensure proper recovery, the last RP work was completed no later than Friday each week.

257 Next Page (IRE)
At the start of the study, this two-year-old Irish-bred filly had run once, finishing in 4th place in a IV class race over 1600 meters. Her MSTN genotype was C/T, meaning she was suited for middle-distance races, and she had been initially trained for mile races. Her physical build aligned with her genotype. After a one-month rest, she began the same preparatory (PC) period as the other horses starting in January, performing the prescribed distances in varying directions to avoid asymmetry. Her daily routine also included one hour on a treadmill, alternating hands, but she did not participate in any paddock activities.
During the three-week CW phase starting in March, instead of long-distance work, she performed middle-distance segments (1600-2000 m) on three days at a uniform medium speed (average 20-25 km/h), and on three days she covered a 1600 m distance divided into three segments, with two 2-minute recovery periods (at walking pace) between sections, working at a medium-high speed (25-30 km/h). During the RP period, her weekly training schedule included two days of IT work, one day of CW work at a high pace, and two days of sprint work. The sprint and galloped sprint work followed the same methods as previously discussed.

258 Szigliget (IRE)
At the start of the study, this two-year-old Irish-bred filly had run once in a IV category race over 1400 meters, finishing in 5th place. Her MSTN genotype was C/C, and her physical build also indicated a typical sprinter type, which matched her prior training. After a one-month rest, she began PC work in January, following the same preparation as the other two Thoroughbreds in the study. Like the others, she spent one hour per day on the treadmill but did not participate in paddock turnout.
During the CW period starting in spring, she completed a maximum of 1600 meters per day—three days at a moderate speed (20-25 km/h) and three days using interval training with three 2-minute recovery periods (at walking pace). In the first and third 400-meter segments, she worked at a medium pace, while the second and fourth segments were completed at a so-called "breeze work" pace (~40 km/h). This latter pace represented a fast but controlled effort, not full speed. The RP phase included two days of PC work, two days of IT work, and two days of sprint training. The sprint and galloped sprint training sessions were conducted over 1200 meters.

III. Results and Discussion

Myostatin Analysis

The examined population (n = 145) consisted of 34% stallions (n = 49), 64% mares (n = 93), and 2% geldings (n = 3). The individuals were offspring of 67 sires and 100 dams. The genotype distribution was as follows:
Short-distance (CC): 45 individuals, middle-distance (CT): 84 individuals, long-distance (TT): 16 individuals
Within the examined sample, the observed genotype frequency distribution was:

- CT: 0.57, CC: 0.32 and TT: 0.11.

The allele frequency within the sample was calculated as follows:

- C allele: 0.6 and T allele: 0.4

Calculation method:

- Total alleles: 145 * 2 = 290
- C allele frequency: (90 CC + 84 CT) / 290 = 174 / 290 = 0.6
- T allele frequency: (32 TT + 84 CT) / 290 = 116 / 290 = 0.4
- Hardy-Weinberg equilibrium:
  - $p^2 + 2pq + q^2 = 1$
  - 0.58 (168 CT) + 0.31 (90 CC) + 0.11 (32 TT) = 1

Effect Analysis

To test the hypothesized correlation between the MSTN gene and racing performance, we used IBM SPSS Statistics Viewer 23 for statistical analysis and GraphPad InStat 3 for further evaluation of the results.

The maximum GHP (Greatest Horse Performance) did not differ significantly ($p > 0.05$) between the three groups, indicating that success was not influenced by suitability for a particular race distance.
Out of the 50 individuals examined in detail:

- CC group: 14 out of 19 individuals (73%) had their best race distance (BRD) matching their genotype.
- CT group: 15 matches (55%) and 12 mismatches.
- TT group: 3 matches (75%) and 1 mismatch.

In the CC group, the best race distance (defined as the highest category race won by the individual, e.g., between a V-class and a Gr.3 win, the Gr.3 distance is considered the BRD) had an average speed of 59.1 km/h (min. 55.07 km/h, max. 62.39 km/h).

- 38.9% of the CC horses ran 60 km/h or faster in their BRD.
- In 5 cases, the BRD did not match the absolute best winning time.

In the CT group, the BRD average speed was 58.5 km/h (min. 55.7 km/h, max. 61.8 km/h), with 17.2% achieving 60 km/h or faster.
In the TT group, the BRD average speed was 58.1 km/h (min. 54.2 km/h, max. 60.4 km/h), with 20% of the horses capable of running above 60 km/h.
These findings confirm that, as expected, as distance suitability increases, average speed decreases, though no significant difference was found in the mean speeds across the three groups.
For GHP average and GHP maximum, there was no significant difference between the three genotypes (Kruskal-Wallis nonparametric ANOVA test: $p = 0.0824$, $KW = 4.993$).
Regarding racing career duration:

- CC and CT genotypes raced for an average of 3 years.
- TT genotypes raced for only 2.5 years.

However, for GHP average, CT horses performed better during their first 3 racing years compared to CC or TT horses.
For earnings, there was no significant difference between genotypes (Kruskal-Wallis test, $p = 0.1184$; $KW = 4.267$).

Influence of Mare Families

When analyzing maternal lineage effects, the Family 4 mares ($n = 6$) stood out in terms of both earnings and GHP max, showing significantly higher values (GHP max: 60-103) compared to other families.

Ultrasound Examinations

Significant differences were observed in the length of the anconeus muscle and the thickness of the triceps brachii muscle between the C/C and T/T genotypes. Additionally, a significant difference was found between C/T and T/T genotypes in the triceps brachii. Highest values were consistently recorded in C/C individuals. No significant differences were found between genotypes for the longissimus dorsi muscle.

Linear Regression Analysis

The linear measurements showed a positive correlation with body weight in the following parameters: LADd: $P = 0.007$, $r^2 = 0.126$; ADVd: $P = 0.001$, $r^2 = 0.197$; ASDd: $P < 0.001$, $r^2 = 0.439$; ASJd: $P = 0.035$, $r^2 = 0.078$; ADVs: $P = 0.002$, $r^2 = 0.162$; ASDs: $P < 0.001$, $r^2 = 0.244$; ASJs: $P = 0.038$, $r^2 = 0.076$; PADd: $P = 0.001$, $r^2 = 0.173$; LVDd: $P < 0.001$, $r^2 = 0.456$; LVDs: $P < 0.001$, $r^2 = 0.259$.
However, no significant correlation with body weight was found after allometric scaling at a standardized body weight of 500 kg. LAAd was not correlated with body weight ($P = 0.271$, $r^2 = 0.034$). LVIAd ($P < 0.001$, $r^2 = 0.316$) and LVIVd ($P < 0.001$, $r^2 = 0.302$) showed a positive correlation, but this relationship disappeared after allometric scaling.

Genotype Differences in Cardiological Variables

Significant differences were observed among genotypes in the following cardiological parameters: LADd, ADVd, ADVs, ASDd, ASDs, IVSd, ASJd.
Heterozygous (C/T) individuals consistently showed the highest values: LADd: 11.6 cm; ADVd: 7.0 cm; ADVs: 7.4 cm; ASDd: 8.0 cm; ASDs: 8.4 cm; IVSd: 6.5 cm; ASJd: 3.4 cm.
When comparing the homozygous individuals (C/C vs. T/T), the C/C genotype had consistently higher values than the T/T genotype. The mean values for LADd, ADVd, ADVs, ASDd, ASDs, and ASJd were 0.4 to 0.6 cm higher in C/C horses compared to T/T horses. For anconeus muscle length and triceps brachii thickness, C/C genotypes had the highest values (5.5 cm and 10.2 cm, respectively).

Post-Hoc Analysis (Tukey HSD Test)

The Tukey HSD post-hoc test, which accounts for error effects, showed p-values below 0.05 in all but two cases: LADd: $p = 0.057$ and ASJd: $p = 0.051$.
These results suggest that the MSTN gene significantly influences muscle development and cardiac performance in Thoroughbred horses.

Practical Implications

This study is the first to provide data linking myostatin genotype, muscle development, and key cardiac performance parameters in Thoroughbreds.
Our findings confirm that identifying a horse's MSTN genotype can aid trainers in:

1. Selecting the optimal racing distance for a horse.
2. Designing the most suitable training methods based on genetic predisposition.

Examination of Hair Whorls

Our results showed that the distribution of hair whorls indicated that circular whorls were more common than linear ones on any part of the body, although their placement followed a similar pattern. All of the examined Hungarian Thoroughbred racehorses had at least one circular hair whorl on their head, as well as on their body, neck, and flank, while only 25.8% of them had circular hair whorls on their limbs. In contrast, 38.7% of the horses had at least one linear hair whorl on their head, 77.4% had them on their body, but none had linear hair whorls on their limbs. On the head, the average number of circular hair whorls was 1, while the average number of linear hair whorls was 0, resulting in a total average of 2 hair whorls per head. On the rest of the body, excluding the head, the average number of circular hair whorls was 5, while the average number of linear hair whorls was 1, making the total number of hair whorls on the body (excluding the head) 6.

On the heads of the 31 examined horses, there were a total of 60 hair whorls, of which 42 were circular and 18 were linear. Among these, 73.3% were located on the upper part of the head, 23.3% between the eyes, and only 2 (0.03%) on the lower part of the head. Of the 60 hair whorls, 61.7% were positioned in the middle of the head, 23.3% on the left side, and 15.0% on the right side. On the rest of the body, including the neck, trunk, and sides, there were a total of 182 circular and linear hair whorls, 39.6% of which were on the left side, 22.5% on the right side, and 37.9% in the center. On the limbs, the 31 examined horses had a total of 12 circular hair whorls, but no linear ones. Of these circular whorls, 41.7% were located on the left legs, while 58.3% were on the right legs.
Among the 41 circular hair whorls found on the head, 21 were radial, 11 rotated counterclockwise, and 9 rotated clockwise. Regarding the circular hair whorls on the left side, 60% rotated counterclockwise, while 33.3% of those on the right side rotated clockwise. The majority (66.7%) of the whorls in the center of the head were radial. On the rest of the body, including the neck, trunk, and sides, 6 out of the 135 circular hair whorls were radial, 66 rotated counterclockwise, and 54 rotated clockwise. Regarding the circular hair whorls on the left side 87.9% of them rotated counterclockwise, while 87.5% of those on the right side rotated clockwise. Among the centrally positioned whorls, 52.4% were radial. On the limbs, all 12 circular hair whorls were either clockwise or counterclockwise, with none being radial. Five out of these rotated counterclockwise, while 7 rotated clockwise. On the left legs, 28.6% of the circular whorls rotated counterclockwise, while on the right legs, 40% rotated clockwise.

Paddock Test

Among the 31 horses, 25.81% (n=8) showed no side preference when turning away from the examiner, while 54.84% (n=17) turned left more than 80% of the time, and 19.36% (n=6) turned right more than 80% of the time.
When moving away from the bucket, 45.16% (n=14) exhibited no side preference when turning, 32.26% (n=10) turned left more than 80% of the time, and 22.58% (n=7) turned right more than 80% of the time. While eating from the bucket, 41.94% (n=13) showed no side preference in terms of which forelimb they placed forward. Meanwhile, 32.26% (n=10) preferred the left forelimb more than 80% of the time, and 25.81% (n=8) preferred the right forelimb more than 80% of the time.
A significant correlation was found between the orientation of circular hair whorls and limb preference while eating from the bucket (χ^2_4= 28.532, $p<0.001$). Among the 19 horses that had no limb preference ("bilateral"), 63.15% (n=12) had either radial hair whorls on their head or both clockwise and counterclockwise whorls. Among the 6 horses that preferred placing their left forelimb forward ("left-legged"), 83.33% (n=5) had counterclockwise whorls on their head (or a combination of counterclockwise and radial whorls but not clockwise whorls). Among the 6 horses that preferred placing their right forelimb forward ("right-legged"), 100% (n=6) had clockwise hair whorls (or a combination of clockwise and radial whorls but not counterclockwise whorls).

Temperament Assessment

Friendliness (p=0.006, r=0.484) and trainability (p=0.014, r=0.436) scores showed a significant and positive correlation with the number of hair whorls found on the horses (including both linear and circular whorls on the head, body, and legs). The friendliness score was significantly higher (p=0.031) in horses that had at least one linear whorl on both their head and body compared to those with no linear whorls on any part of their body. The average friendliness score was 20.3 (8.2) in horses with no linear whorls, 25.7 (6.2) in those with at least one linear whorl on either the head or body, and 29.2 (4.7) in horses with at least one linear whorl on both the head and body.
The anxiety score was significantly higher (p=0.049) in chestnut horses compared to others, with an average anxiety score of 41.7 (9.4) versus 32.5 (10.8) in non-chestnut horses. However, no significant correlation was found between anxiety scores and the number of whorls (p=0.156). None of the other measured parameters had a significant effect on any of the temperament traits assessed ($p>0.05$).
Each horse has a unique hair whorl pattern that remains unchanged throughout its life, making it a valuable tool for equine identification alongside other data and DNA profiling (Murphy & Arkins, 2008). While professionals typically only record major hair whorls, we documented all whorls in detail. All 31 horses

examined had at least one circular whorl on their head (100%), whereas only a few had whorls on their limbs (25.8%), consistent with Encina et al. (2023), who reported rates of 95% and 16% in PRE horses, respectively. Recent studies show that counterclockwise circular whorls are more common in PRE horses (Encina et al., 2023) than in Japanese Thoroughbreds (Yokomori et al., 2019). Circular whorls are predominantly located on the left side of the head, below the midline of the eyes (Encina et al., 2023).

In our study, most horses had radial-circular whorls, which were more frequently observed on the upper part of the head, aligning with findings from Yokomori et al. (2019). Additionally, circular whorls were more prevalent than linear ones. However, most studies do not differentiate between these two types of whorls or clarify whether they are counted separately or together (Murphy & Arkins, 2008; Abdel-Azeem & Emeash, 2021). We found that the examined horses had a greater total number of whorls and more whorls located on the top of the head (above eye level: n=24, 77.4%; between the eyes: n=8, 25.8%; below eye level: n=2, 6.5%) compared to previous reports (Shivley et al., 2016; Lima et al., 2021; Encina et al., 2023). A single whorl on the head was the most common pattern (n=19, 61.3%), as observed in other studies (Górecka et al., 2007; Lima et al., 2021). However, the presence of linear whorls on the head was high in our sample (n=13, 41.9%), as was the occurrence of linear whorls on the body, including the head and other regions (n=25, 80.6%). We found a correlation between the location and rotational direction of body whorls.

Our observations suggest that whorls on the right side of the body are most commonly clockwise, those on the left side are usually counterclockwise, and those in the middle (on the neck) are predominantly radial.

The genetic mechanisms behind hair whorl formation remain unclear, and the role of environmental factors in their development is not well understood (Willems et al., 2023). Nevertheless, we found a relationship between trainability, friendliness, and the number of body whorls.

In racehorses, motor laterality can affect athletic performance through turning ability (some horses slow down in turns) and speed (Warren-Smith & McGreevy, 2010). Understanding innate motor laterality could help professionals select appropriate training programs or racetracks, as races are conducted in either a clockwise or counterclockwise direction. Additionally, trainers could implement new training methods to improve balance or decide to retrain Thoroughbreds struggling at higher levels due to their motor laterality.

Our paddock test was conducted in January 2024, with most horses being young (2-year-olds: n=12, 3-year-olds: n=16). We found that 54.84% of the horses exhibited a left-side preference when turning away from a person. However, there was no significant difference in side preference when lowering their head to eat. McGreevy & Thomson (2006) suggest that motor laterality becomes more pronounced with age. McGreevy & Rogers (2005) reported that Thoroughbreds over two years old were significantly more lateralized than yearlings, with both age groups showing a stronger preference for the left forelimb.

In our study, Thoroughbreds preferred to place the forelimb corresponding to the direction of their head whorl forward (e.g., horses with a clockwise whorl were more likely to use their right forelimb). This is an important correlation, as the heritability (h^2) of hair whorls is high (Górecka et al., 2007), suggesting that side preference may also be inherited. Accordingly, breeders could selectively breed horses with motor laterality suited to specific racing conditions (e.g., in Hungary, the only racetrack runs clockwise, so owners may prefer "right-handed" or "ambidextrous" horses). Unfortunately, no study has yet examined the heritability of motor laterality, despite calls for further research in this area (Grzimek, 1968; Drevemo et al., 1987).

Artificial selection has reinforced desirable traits during the development of various horse breeds. In Thoroughbreds, early breeding selection was based on match races (where only two horses competed over a set distance), favoring individuals with exceptional speed. These horses were often more competitive and "spirited" than their counterparts. As a result, Thoroughbreds are now considered more anxious and excitable compared to other breeds (Sackman & Houpt, 2018). Coat color has also been a selection factor, with aesthetically striking horses being preferred (Avila et al., 2022). A common belief holds that chestnut horses are bolder and more emotionally unpredictable. Finn et al. (2016) documented that chestnut horses were braver than bays and approached novel objects more frequently. Our study also found that chestnut horses exhibited higher anxiety levels than other horses.

Training Method Experiment

Based on classic pedigree analysis, Copadelmundo carries an excellent speed profile (center of distribution), fighting spirit, and stamina due to his inbreeding with Mr. Prospector (Chong, 2014). According to the dosage profile and dosage index, he is most suitable for shorter classic (middle-long distance) races (~1900-2200 m). Based on MSTN genotyping, he is T/T, indicating a long-distance racehorse. There is an overlap between the three categories (short, middle, and long distance) due to the influence of training methods as an environmental factor, but a T/T genotype individual will not be able to perform at a high (elite or black type) level among sprinters (Hill et al., 2010a). The stallion showed continuous improvement in the new training regimen, even though he was still in heavy growth. He started his three-year-old season in smaller categories (Category V), with a second place finish followed by a victory at 1600 m, in middle-distance races, in April and May. The management then targeted the first race of the Triple Crown—the 1600 m National Prize—where the best middle-distance horses of the three-year-old crop compete. In this race, the Thoroughbred finished 10th, and after a recovery period following the race, he suffered a severe injury during training, requiring several stitches on his leg. This misfortune made it impossible for him to participate in the second leg of the Triple Crown, the Hungarian Derby (the most significant race for the three-year-old horses).

After recovering from the injury, he resumed training, but it was unclear whether the previous injury would allow him to compete at long distances. As a result, his first post-recovery race was a 2200 m Category III race, in which the Thoroughbred returned to victory. The goal was now clear: the third pillar of the Triple Crown, the Hungarian St. Leger. To prepare for this, another 2200 m preparatory race (Category III) followed, where Copadelmundo finished in 4th place. The race experience (limited top speed) and training results at this point showed that, according to his genotype, the Thoroughbred is extremely resilient, capable of galloping almost indefinitely without fatigue, but lacking in high top speed. Therefore, before the autumn meeting, the original training program was minimally adjusted, and long-distance work was prioritized to further enhance the horse's stamina.

At the conclusion of the domestic season, during the St. Leger, the trainer and jockey applied a bold strategy by leading from the start, gaining an advantage over the field. Horses rarely manage to maintain a lead throughout the entire race, as the initial high speed is followed by fatigue. However, the extensive "lung work" proved effective, as Copadelmundo was never caught during the race and won easily by 5 lengths, achieving the best performance of his life. The three-year-old was ranked as the highest-rated horse (GHP 73.5) in his age group on the 2021 General Handicap list.

Next Page, bred through inbreeding to Danzig, carries outstanding top speed and versatility (generally successful on both turf and sand tracks) based on classic pedigree analysis. Both the dosage profile, dosage index, and center of distribution confirm her as a "born miler" with a balanced speed and stamina profile. These results are also supported by MSTN genotyping, showing that the mare is C/T. At the beginning of her three-year-old season, she debuted with a fourth place finish in the Hazafi Prize, a Gd-3 listed race for three-year-olds, followed by a second place in a smaller category race (Category IV). Despite her genotype and training, Next Page was tested at long distances, finishing 12th in a 2000 m Gd-2 race. This was followed by weaker performances at 1400 m (Category IV), but as the year came to a close, she returned to the "miler distance" with two 1600 m races, finishing second and winning, ending the season with a GHP of 68. She was the third highest-rated mare on the 2021 General Handicap list in her age group.

Szigliget has a pedigree of sprinter and miler ancestors, making her suited for short distances, with good speed but not outstanding top speed. Based on MSTN genotyping, she is a sprinter. As a two-year-old, she ran once at 1400 m, finishing in 5th place. The small-sized mare showed her talent with a victory in a middle-distance race (1600 m) at the start of the season, after which management decided to continue racing at this distance. In the Hazafi Prize, she finished 7th at 1600 m, followed by a 3rd place in a lower category race (Category V). Her last race took place in June at 1600 m, where she finished 8th. Throughout the training, the mare struggled with pain, and in early summer, the owners had her examined. The Xray revealed a severe "kissing spine" condition, which led to the end of Szigliget's racing career.

IV. Conclusions and Recommendations

At the beginning of my work, I sought to determine how the myostatin gene polymorphism influences an individual's career and their suitability for the best race distances. This investigation began with the genotyping of the studied population. After completing this purpose, I compared the results with the race data available from databases. The data analysis was carried out using GraphPad InStat, Microsoft Office Excel, and IBM SPSS Statistics Viewer version 23 softwares. All of the horses examined were from the Bábolna National Stud's Dióspuszta stable or trained at the Alagi Racehorse Training Center. Further examination of fifty samples revealed that the racing careers of more than half of the horses (64%) confirmed their genotypes. In the remaining cases (18 individuals), the majority (83%) of the Thoroughbreds did not have the opportunity to race at their genetically most suitable distance, or their racing career was too short to prove their suitability for the expected best race distance (BRD). There were no significant differences between earnings, maximum GHP, or average GHP across the three groups, whereas differences were found in the years spent in racing and the average GHP, favoring the CT genotype. Horses belonging to this group achieved higher average GHP in their 1st-3rd racing years compared to individuals of the other two genotypes. Gender was excluded as a factor influencing racing performance, as the stallions in the stud were black-type horses, whose exceptional abilities would have skewed the results. Regarding the distribution of genotypes 58% of the population were CT heterozygotes, 31% were CC homozygotes, and only 11% were TT homozygotes. The allele distribution was 60% CC and 40% TT.

In the case of the 50 galloping horses examined, the genotype did not match the best distance for 18 of them. This was due in part to 10 individuals never being raced at their genetically appropriate distance, and in 5 other cases, the horses' careers ended after 1 or 2 racing years, so they did not have enough time to prove their suitability for the racetrack. In the future, my primary goal would be to examine a larger population for a better genotype balance. Additionally, I would like to conduct biopsies to examine muscle fiber type ratios. These measurements could be complemented by the ethological tests described by Momozawa et al. (2005), which would help provide a more comprehensive picture of the factors influencing success.

The results obtained from ultrasound (UH) tests confirmed previous findings of Hill et al. (2010a, 2010b), which state that C/C horses are sprinters with greater muscle mass, requiring higher heart performance and aerobic capacity. Consequently, C/C thoroughbreds are more successful sprinters even at the age of two compared to C/T and T/T horses (Tozaki et al., 2011). We found that the measured cardiac variables correlate with MSTN genotypes. The aortic diameter

at the Valsalva sinus (end of diastole and end of systole) and the aortic diameter at the valve (end of systole) showed significant differences between the C/C and T/T genotypes, suggesting that C/C horses may have greater heart performance and aerobic capacity.
Our results could serve as the foundation for further research, since no prior literature was available regarding the effects of MSTN genotypes on muscle size and heart structure.
In Thoroughbreds, circular hair whorls dominate over linear ones across the body, but most of the horses examined had at least one linear hair whorl. Circular whorls typically appeared on the top of the head in most horses. A positive correlation was found between friendliness, trainability, and the number of hair whorls. We also demonstrated a relationship between the direction of hair whorls and motor lateralization. In line with previous results, we found evidence that coat color and behavior are correlated, particularly in foals. Other factors, such as gender, coat color, presence of circular hair whorls on the top of the head, precise location of circular whorls, orientation of circular whorls, total number of whorls, presence of linear whorls, and leg preference, did not significantly influence the personality traits examined.
In the future, it would be worth examining these factors in a larger experimental population. A significant advancement would be to understand the genetic background of hair whorl formation and identify the genes involved in this process.

V. New Scientific Results

For the first time, I examined the MSTN polymorphism in domestic English Thoroughbreds in Hungary and determined the genotype frequency in the local population (CC– 0.32; CT–0.57; TT–0.11). I confirmed that, as expected, with an increase in suitability for race distances, the average speed decreases.
Based on ultrasound examinations, the length of the anconeus muscle and the thickness of the triceps brachii muscle showed significant differences ($p > 0.05$) between the C/C and T/T genotypes. In the case of the triceps brachii, there was also a significant ($p > 0.05$) difference between the C/T and T/T genotypes. In all cases, the highest values were recorded for the C/C horses.
I identified significant differences between the genotypes in the following cardiovascular variables: left atrial diameter at end-diastole (LADd), aortic diameter at the valve at end-diastole (ADVd), aortic diameter at the valve at end-systole (ADVs), aortic diameter at the sinus at end-diastole (ASDd), aortic diameter at the sinus at end-systole (ASDs), interventricular septum diameter at diastole (IVSd), aortic diameter at the sinotubular region at end-diastole (ASJd). The heterozygous animals consistently showed the highest values (in the previous list order: 11.6, 7.0, 7.4, 8.0, 8.4, 6.5, and 3.4 cm). When comparing homozygous animals, the values of the C/C Thoroughbreds exceeded those of the T/T genotype carriers.

I found that in English Thoroughbreds, hair whorls located on the right side of the body tend to rotate in a clockwise direction, while those on the left side rotate counterclockwise. Whorls located in the middle of the body (on the neck) were predominantly radial.
I determined that circular hair whorls are more common than linear ones in Thoroughbreds, although most animals had at least one linear hair whorl. Circular hair whorls are most commonly found on the head. A positive correlation was found between the number of circular hair whorls on the body and friendliness, as well as trainability.
I confirmed the relationship between the direction of hair whorls and motor laterality in Thoroughbreds. In the study, animals preferred the leg corresponding to the direction of their hair whorl rotation.

VI. Publications

Peer-reviewed article published in a foreign language journal

Kis, Judit; Rózsa, László; Husvéth, Ferenc; Mezőszentgyörgyi, Dávid; Kovács, Szilvia; Bakos, Zoltán; Zsolnai, Attila; Anton, István (2023). Association of myostatin gene polymorphism with echocardiographic and muscular ultrasonographic measurements in Hungarian Thoroughbred horses. RESEARCH IN VETERINARY SCIENCE 160 pp. 45-49.
Kis, Judit; Rózsa, László; Husvéth, Ferenc; Zsolnai, Attila; Anton, István (2021). Role of genes related to performance and reproduction of Thoroughbreds in training and breeding – A review. ACTA VETERINARIA HUNGARICA 69: 4 pp. 315-323.

Peer-reviewed article published in a Hungarian language journal

Kis, Judit; Mezőszentgyörgyi, Dávid; Zsolnai, Attila; Rózsa, László; Husvéth, Ferenc; Anton, István (2022). Innovatív genomikai és élettani kutatások az angol telivér állomány versenyteljesítményének fokozása érdekében. SCIENTIA ET SECURITAS 3: 3 pp. 243-249.
Kis, Judit; Bodó, Szilárd; Rózsa, László; Zsolnai, Attila; Anton, István (2019). Molekuláris genetikai lehetőségek az optimális versenytáv és tréningmódszer megválasztásához angol telivéreknél. ÁLLATTENYÉSZTÉS ÉS TAKARMÁNYOZÁS 68: 4 pp. 313-329.

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Kis, Judit; Zsolnai, Attila; Rózsa, László; Husvéth, Ferenc; Bakos, Zoltán; Kovács, Szilvia; Anton, István (2022). A miosztatin génpolimorfizmus, illetve az izom- és kardiovaszkuláris struktúrák összefüggésének vizsgálata angol telivér versenylovakon. In: Molnár, Dániel; Molnár, Dóra (szerk.) XXV. Tavaszi Szél

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Scientific dissemination publications outside the thesis topic (33 articles)

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