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Are results obtained in the stallion performance tests of Norikers affected by the presence of the Glycogen Synthase 1 mutant H-allele?

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Summary

A recent study on Noriker horses reported a carrier frequency of 0.34 for the mutant H-allele of the glycogen synthase 1 (*GYS-1*) gene in a sample of mares and breeding stallions. The present study aimed to identify possible effects of the H-allele on exercise tolerance and performance parameters. In the annual stallion selection at the station in Stadl Paura (Upper Austria), the performance potential of twelve three-year-old Austrian Norikers (nine wildtype R/R, three heterozygous R/H) was assessed in driving, drafting and riding. The magnitude of exercise-induced stress was evaluated by the level of salivary cortisol. The exercise intensity was estimated based on maximal heart rate (HR_{max}) during riding and drafting. HR_{max} in the heterozygous horses (R/H) was 171±10 beats/min after riding and 169±20 beats/min after drafting. Salivary cortisol concentrations of these horses were 1.23±0.62 ng/ml after drafting and 3.21±0.82 ng/ml after riding. During riding, the wildtype horses (R/R) had HR_{max} of 163±19 beats/min and salivary cortisol concentrations of 2.40±1.40 ng/ml, while during drafting the HR_{max} was 160±32 beats/min and the salivary cortisol 1.02±0.35 ng/ml. We also examined the performance traits of 188 Noriker stallions. R/R (p=0.007) and R/H (p=0.006) horses had a significantly better exercise capacity than H/H Norikers. Noriker horses with two H-alleles had slightly but significantly worse gaits

Zusammenfassung

Beeinflusst das mutierte H-Allel der Gys-1-Synthetase die Ergebnisse in der Noriker-Hengstleistungsprüfung?

Erst kürzlich wurde veröffentlicht, dass in der österreichischen Norikerpopulation ein Trägertieranteil von 34 Prozent für das mutierte H-Allel der Glykogen-Synthase 1 (*Gys-1*), verantwortliche Mutation der Polysaccharid-Speichermyopathie Typ 1 des Pferdes, besteht. In der folgenden Studie wurden die Auswirkungen des H-Allels auf die Belastbarkeit der Pferde und die Leistung im Training zur Hengstleitungsprüfung untersucht. Zwölf dreijährige Norikerhengste (neun Wildtypen R/R, drei mischerbige Tiere R/H) wurden im Pferdezentrum Stadl Paura unter dem Sattel, im Fahren, sowie im Schwachholz- und Schwerzug ausgebildet. Das Ausmaß an Stress wurde anhand von Speichelkortisol bestimmt. Die Leistungsintensität wurde mit Hilfe von Herzfrequenzaufzeichnungen erhoben. Die drei mischerbigen (R/H) Hengste zeigten beim Reiten (HR_{max} 171±10 Schläge/min, Speichelkortisol 3,21±0,82 ng/ml) und im Schwerzugtraining (HR_{max} 169±20 Schläge/min, Speichelkortisol 1,23±0,62 ng/ml) statistisch nicht signifikant unterschiedliche maximale Herzfrequenzen (HR_{max}) und Speichelkortisolwerte als die neun Wildtypen (R/R) nach dem Reiten (HR_{max} 163±19 Schläge/min, Speichelkortisol 2,40±1,40 ng/ml) und

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than R/R (p=0.043) and R/H (p=0.009) colts. In conclusion, there are some indications that Norikers with an H-allele are less resilient and have a worse exercise capacity.

Abbreviations: *GYS-1* = Glycogen Synthase Type 1; H-allele = Gene, with G-to-A substitution in *GYS-1* (mutated gene); H/H = homozygous for mutated *GYS-1* gene; HPA axis = hypothalamic-pituitary-adrenal axis; HR = heart rate; PSSM = Polysaccharide Storage Myopathy; R-allele = wildtype *GYS-1* gene; R/H = heterozygous for mutated *GYS-1* gene; R/R = homozygous for wildtype gene

Zugtraining (HR_{max} 160±32 Schläge/min, Speichelkortisol 1,02±0,35 ng/ml). Ferner wurden Leistungsparameter von 188 Norikerhengsten ausgewertet. R/R (p=0,007) und R/H (p=0,006) Pferde zeigten eine signifikant bessere Arbeitsleistung als H/H Tiere. H/H Pferde wiesen einen signifikant schlechteren Bewegungsablauf im Trab unter dem Sattel auf als R/R (p=0,043) und R/H (p=0,009) Hengste. Es gibt Hinweise, dass Pferde mit H-Allel möglicherweise weniger belastbar sind und eine schlechtere Arbeitsleistung erbringen. In Zukunft ist ein bewusster und gezielter Einsatz mischerbiger Tiere mit Prüfung der Nachzucht erforderlich, bevor Tradition und Brauchtum aufgegeben werden.

Introduction

The Noriker horse, also known as the Pinzgauer, is a traditional Austrian horse breed with a recorded history dating back to the year 1575 (DRUML, 2006). It has even been suggested that the roots of the breed go back to the period of the Roman colonization of the alpine region. Norikers are categorized as a coldblood breed. Historically the breed was used as a pack horse, carrying loads up to 130 kg. More recently it has been used in forestry and agriculture (DRUML, 2006). It was generally believed that the breed was free of the ingression of genetic material from other heavy horses and thus should be free of the glycogen synthase type 1 mutation (the *Gys-1* H-Allele). However, DRUML et al. (2017) recently found this mutation in a Noriker foal cohort (n=1,553) with a carrier frequency of 0.33 and in stud horses (n=517) with a carrier frequency of 0.34. Previously, SCHWARZ et al. (2011) found a *Gys-1* H-allele carrier frequency of 0.18 in 50 Austrian Haflingers. The clinical significance of the mutation was difficult to quantify in the latter breed. Standard exercise tests did not reveal abnormally increased levels of the muscle-specific creatine kinase (E.C. 2.7.3.2.) activity (SCHWARZ et al., 2011).

An attack of exertional rhabdomyolysis in a carrier of the *GYS-1* H-allele is a complex event and cannot be predicted by genotype alone. The relative high frequency of the *GYS-1* H-allele might be explained by competitive advantages or a more desired conformation in gaits and other traits in H allele carriers, associated with easy keeping and drafting power. It seems possible that *GYS-1* H allele carriers are better movers but more susceptible to stress. To test this hypothesis, we analysed the horses' performances in the annual stallion station tests, the national tests for selection of potential sires. We measured salivary cortisol concentration (PALME and MÖSTL, 1997) to study the effects of the testing programme on the hypothalamic-pituitary-adrenal (HPA) axis of the Norikers. We also quantified exercise intensity by measuring the heart rate (EVANS and ROSE, 1988; COTTIN et al., 2006; DYBDAL et al., 1980; BECKER-BIRCK et al., 2013).

Materials and Methods

Horses and exercise

This study was approved by the ethics committee of the Vetmeduni Vienna (ETK-06/10/2015).

Twelve three-year-old Noriker stallions that participated in the one-month station training and selection process were studied on two occasions during their final tests. All horses were kept under the same management, feeding and training conditions during the entire training and subsequent trait testing. The stallions were mainly kept in boxes but had regular access to paddocks if possible. Feeding with hay (up to twenty kilograms) and grain diet (three kilograms oats and Pegus Classic premixed horse muesli at a ratio of 1:1) was performed three times daily, whereby the energy levels were adapted to the intensity of work. Horses were exercised five to six times per week and were ridden, driven or had to draft. Each training session lasted for 15 to 20 minutes. All horses were familiar with their riders and drivers. The final test included riding and driving on the first day and wood logging and heavy drafting on the second day. The potential breeding stallions included three heterozygotes for the *GYS-1* mutation (R/H) and nine wildtypes (R/R).

Experimental design

Horses were examined during stationary training at Stadl-Paura, Austria's national equestrian centre. Before exercise, a physical examination was performed in the stable to detect possible signs of muscular disorder. Riding was performed in a riding hall and logging and heavy load drafting in another arena. For logging, the horses had to pull a tree stem of six to seven meters through six staggered passes (Fig. 1b,c). For drafting, horses had to pull loads of 20 % of their body weight over a distance of 500 meters within seven minutes (Fig. 1a, ARGE Noriker). The burden of driving a light marathon wagon could not be assessed due to organizational constraints.

Cortisol analysis

Salivary samples for the determination of baseline cortisol concentrations were taken from horses at rest (60 min before starting the equestrian training; sample 1), immediately after training (sample 2) and at rest 60 minutes after finishing the training (sample 3). Saliva was collected with cotton rolls (Salivette Code Blau, Sarstedt, Germany). A surgical arterial clamp helped to fix the cotton roll loosely to the tongue for about one minute, until the swab was well soaked. The rolls were marked individually and frozen at -18 °C until analysis. Saliva was diluted 1:10 with assay buffer before measurement with a cortisol enzyme immunoassay as described by PALME and MÖSTL (1997). For details of the salivary cortisol assay see von LEWINSKI et al. (2013).

Heart Rate

Heart rate was monitored with a mobile recording system (POLAR, Equine M400, Finland; Fig. 2). Recordings were taken according to the manufacturer's recommendations. After moistening the coat, two electrode pads were fixed under the saddle by plastic straps and an accompanying sensor device was attached to the rack (Fig. 2) or saddle. The heart rate was displayed instantaneously on the screen of the watch-receivers, which were worn by the riders or drivers. The data were transferred to a laptop and saved in excel files. The start and end of exercise activities and the duration of various paces were precisely recorded in a notebook by the first author. Initial data were processed by the software of the heart rate monitoring system (Polar Flow, POLAR, Finland). Further analysis was performed with IBM SPSS Statistics 23 (IBM Corporation, Armonk, New York, USA).

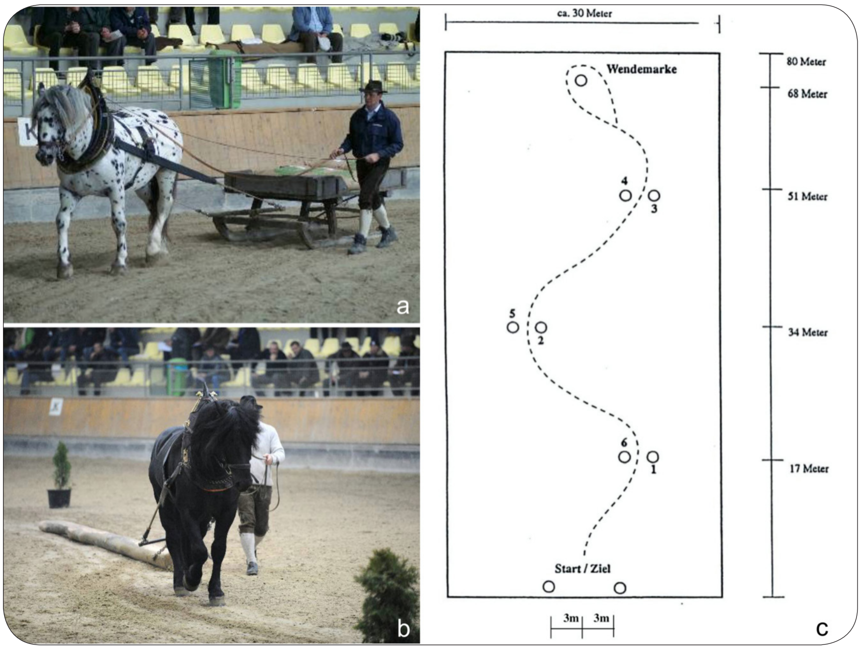

Fig. 1: Heavy load drafting (a; picture by ARGE Noriker); wood logging (b; picture by ARGE Noriker); the track of wood logging (c; drawing by ARGE Noriker). / Schwerzug (a; Bild ARGE Noriker); Schwachholzzug (b; Bild ARGE Noriker); Parcour Schwachholzzug (c; Zeichnung ARGE Noriker).

Performance traits

Performance parameters scored by the colts at the Central Stallion selection of the years 2006 to 2015 were analysed. Data were included from the thirteen stallions in which cortisol concentrations and heart rates were studied. The *GYS-1* genotypes of all 188 Noriker stallions were recently determined from the stud book (ARGE Noriker). All data on traits were analysed for association with the *GYS-1* genotypes. Phenotypic traits included quality of walk, trot and canter under the saddle and in harness. Rideability, driving manners by pulling a marathon phaeton and drafting techniques of logs and heavy loads were also included. All traits were scored on a scale from one (worst) to ten (best).

Fig. 2: POLAR equine M400 heart rate recording system fixed to a horse. / POLAR equine M400 Trainingscomputer angebracht an einem Pferd mittels Deckengurt.

Statistical analysis

Descriptive statistics (mean ± SD) were used to present heart rates, cortisol concentrations and trait ranking. Normal distribution was tested using the Kolmogorov-Smirnov-test in SPSS. We used mixed linear models; groups (R/R, R/H, H/H) were differentiated with ANOVA for repeated measurements. The level of significance was set at $p<0.05$. We calculated Kendall-Tau rank correlation coefficients to differentiate scores for basic gaits at riding and drafting and to note the judges' scoring system. Trait scores were analysed with the Kruskal-Wallis test. Significant effects were further analysed pairwise with the Kruskal-Wallis test. For better readability, data are displayed as mean ± SD.

Results

Physical Examinations

During the entire testing period, no horse showed any muscular impairment or other clinical signs of rhabdomyolysis. One horse of the R/R genotype was excluded from logging and drafting training on the second day due to an oro-labial injury.

Salivary cortisol concentrations

Mean (± SD) salivary cortisol concentrations (ng/ml) after drafting and riding are shown in Figure 3. Riding appeared to release more cortisol in Norikers than drafting. Genotypes did not differ in salivary cortisol concentration (ng/ml) after riding (p=0.482) and drafting (p=0.630). In drafting, salivary cortisol concentrations were lower and continuously increased from the first sample to the last sample one hour after completion in both genotypes. However, cortisol levels were not significantly different between the *GYS-1* genotypes at any sampling time.

Maximal Heart rates (HR_{max})

The intensity of exercise was estimated by the maximal heart rate (HR_{max}) attained by each horse. Maximal heart rates (mean ± SD) at riding in trot and canter and after drafting logs or heavy loads are shown in Figure 4 for R/R and R/H genotypes. Unexpectedly, the overall highest values (165±17 bpm) for all horses were found after trotting when ridden. However, they were not significantly (p=0.207) different from those during the other three forms of exertion (drafting heavy loads: 162±29 bpm; logging: 148±45 bpm; riding trot: 165±17 bpm). R/H genotypes did not have significantly higher heart rates than R/R genotypes (p=0.327).

Traits

Scores of basic gaits during riding and drafting are given in Table 1. Homozygous horses (H/H) appeared to be worse at walking and trotting in harness and trotting when ridden than the other genotypes.

The scores for walk at riding and walk at drafting were weakly (τ 0.35; p=0.008) correlated, while the scores for trot at riding and trot at drafting were not correlated (τ 0.04; p=0.589). The scores for walk at riding were correlated with those of trot at riding (τ 0.34; p<0.001) and walk at drafting was weakly correlated with trot at drafting (τ 0.25; p<0.001).

Table 2 shows the performance as scored by the judges. On average, the H/H genotypes appeared to score better for logging and driving

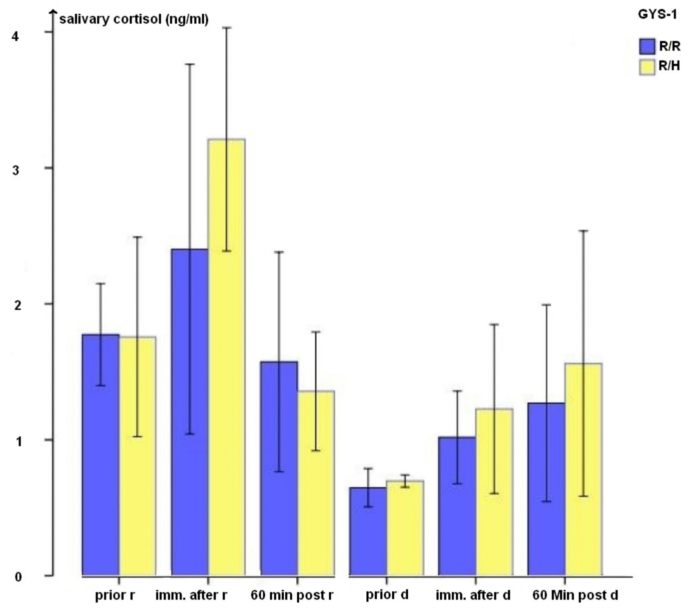

Fig. 3: Salivary cortisol values (mean ± SD) in Noriker horses at rest, immediately after training and 60 minutes after the end of riding (r) and drafting (d) wood logs and heavy loads. Riding and drafting took place on two different dates. There were no statistically significant differences between genotypes immediately after riding (p=0.482) and drafting (p=0.630). / Speichelkortisolkonzentrationen von Norikern (Mittelwert ± Standardabweichung) in Ruhe, unmittelbar nach dem Training sowie 60 Minuten nach Trainingsende von (r) Reiten und (d) Zug von Schwachholz und schweren Lasten. Reiten und Zug wurde an unterschiedlichen Tagen durchgeführt. Innerhalb der Genotypen konnten keine statistisch signifikanten Unterschiede unmittelbar nach dem Reittraining (p=0,482) und Zugtraining (p=0,630) gefunden werden.

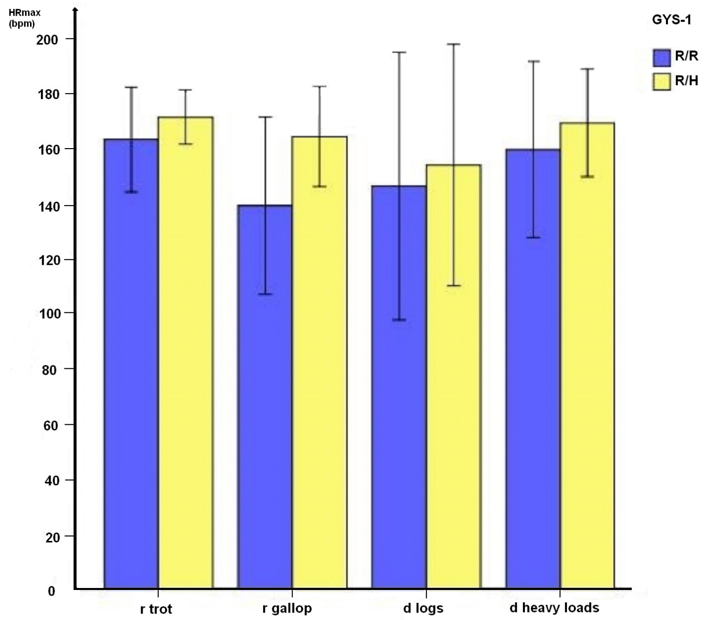

Fig. 4: Maximal heart rates (mean ± SD) in Noriker horses at riding (r) trot and canter and drafting (d) logs and heavy loads. There were no statistically significant differences in maximal heart rates during riding or drafting (p=0.207). Genotypes did not differ in maximal heart rates (p=0.327). / Maximale Herzfrequenzen von Norikern (Mittelwert ± Standardabweichung) beim Reiten (r) in Trab und Galopp, sowie beim Zug (d) von Schwachholz und schweren Lasten. Es bestanden keine signifikanten Unterschiede in den maximalen Herzfrequenzen während des Reit- oder Zugtrainings (p=0,207). Die Genotypen unterschieden sich auch nicht in den maximalen Herzfrequenzen (p=0,327).

and worse for drafting, while the R/R genotypes scored better for riding and drafting. None of the scores differed significantly between the three genotypes.

Wildtype colts (p=0.007) and colts with only one H-allele (p=0.006) had a significantly better exercise capacity than homozygous colts, based on the mean scores given by the judges. Homozygous horses (H/H) received significantly worse scores for trot quality than the wildtypes (p=0.043) and the heterozygous horses (p=0.009). There were no significant differences between the R/R genotype and the group carrying one or two H-alleles for any other gait scores, or for the final rank.

Tab. 1: Scores (mean ± SD) given by the judges to twelve three-year-old Noriker stallions (nine wildtypes R/R, three heterozygous R/H) for basic gaits during stationary training / Notenvergabe der Richter (Mittelwert ± Standardabweichung) für die Basis-Gangarten an zwölf dreijährige Norikerhengste (neun Wildtypen R/R, drei mischerbige Tiere R/H) während der Hengstleitungsprüfung

	R/R (n=120)	R/H (n=61)	H/H (n=7)
Walk in harness	7.3 ± 0.6	7.4 ± 0.5	7.0 ± 0.5
Trot in harness	7.5 ± 0.5	7.5 ± 0.4	7.1 ± 0.4
Walk at riding	7.3 ± 0.7	7.4 ± 0.8	7.3 ± 0.9
Trot at riding	7.5 ± 0.6	7.4 ± 0.5	7.1 ± 0.6
Canter at riding	7.5 ± 0.6	7.3 ± 0.6	7.4 ± 0.6

Tab. 2: Scores (mean ± SD) given by the judges to twelve three-year-old Noriker stallions (nine wildtypes R/R, three heterozygous R/H) for performance in driving, drafting wood logs and heavy loads and riding during stationary training / Notenvergabe der Richter (Mittelwert ± Standardabweichung) an zwölf dreijährige Norikerhengste (neun Wildtypen R/R, drei mischerbige Tiere R/H) für die Gesamtleistung im Fahren, Schwachholzzug, Schwerzug und Reiten während der Hengstleitungsprüfung

	R/R (n=120)	R/H (n=61)	H/H (n=7)
Driving	7.6 ± 0.4	7.6 ± 0.4	7.8 ± 0.5
Logging	7.8 ± 0.5	7.7 ± 0.6	8.0 ± 0.4
Drafting	7.8 ± 0.5	7.7 ± 0.6	7.4 ± 0.5
Riding	8.0 ± 0.6	7.9 ± 0.5	7.8 ± 0.5

Discussion

We investigated possible differences in exercise tolerance and gait score in horses with a mutation in the gene encoding skeletal muscle glycogen synthase 1 (*Gys-1*), which causes equine polysaccharide storage myopathy, and horses without the mutation. Previous studies (MCCUE et al., 2008; SCHWARZ et al., 2011) have given no clear indication of muscular impairment, which was observed in only one of three Gys-1 heterozygous horses investigated. We also found no abnormalities. The absence of even subtle clinical symptoms might result from the optimal training, feeding and housing management at the testing station.

Riding a Noriker colt in trot appears to induce the highest rise of salivary cortisol, suggesting that riding a trot may be a relative strong stressor. This idea may be explained by the fact that draft horses generate most power at slow walk (DRUML and GRILZ-SEGER, 2010), so drafting a load might be less uncomfortable than bearing the weight of a rider. Riding may also be stressful due to the head-neck position of the horses (BECKER-BIRCK et al., 2012; ZEBISCH et al., 2014). We found salivary cortisol levels of 2.60±1.27 ng/ml for both genotypes immediately after riding and levels of 1.07±0.41 ng/ml after drafting. The former value is slightly higher than the 2.2±0.4 ng/ml immediately after dressage or jumping reported by BECKER-BIRCK et al. (2013). The effect of the riders' proficiency on the horse could also be a stress factor but was not investigated in our study. SCHMIDT et al. (2010) showed the most pronounced reaction of the HPA axis (2.0 to 3.0 ng/ml salivary cortisol) immediately after mounting. The observed increase of salivary cortisol over time in our study is a physiological reaction to exercise (FREESTONE et al., 1991).

We found higher maximal heart rates in trotting (R/R horses: 163±19 bpm, R/H horses: 171±10 bpm), than in horses at canter (R/R horses: 139±32 bpm, R/H horses: 164±18 bpm) but there were no significant differences (p=0.207). In warmbloods and thoroughbreds, canter at submaximal speed causes a linear increase in heart rate (von ENGELHARDT et al., 2005). In canter, the front leg is coupled 1:1 to respiration (HERMANSON et al., 2008) but this is different, probably less efficient, in the trot. For the Noriker horse, canter might be either mechanically or metabolically more effective than trotting, possibly due to a greater body mass in relation to the magnitude of pulmonary ventilation at the two paces.

Horses without the *Gys-1* mutation and heterozygous horses had a significant better subjectively determined exercise capacity than homozygous horses

(H/H). Colts with two H-alleles had a significantly lower quality of trot than the wildtype and heterozygous horses. Subtle muscle pain or muscle atrophy may have a role in these findings. On average, judges gave H/H genotypes better scores for logging and driving, whereas R/R horses received better scores for riding and drafting. However, differences were minimal, and none were significant. Unfortunately, homozygous horses were not available for the clinical part of our study.

In most European countries, direct and indirect breeding measures include performance testing of sires, mares and even geldings between three and seven years of age over a variable period ranging from one to 365 days. For example, in Great Britain, Hungary and Sweden performance tests take eight days, in Germany stallions are generally examined for 50 days and in Belgium, France and Ireland the results of competitions for one year are used. Gaits, rideability and jumping ability are usually judged subjectively but the character and behavioural traits are also important (THORÉN-HELLSTEN et al., 2006). The Noriker stallion performance test in Austria lasts only 30 days, which is perceived as a suboptimal training period for some horses. Colts are usually exercised by their owners or professional riders and drivers, but some may not have been ridden at all before. GERBER OLSSON et al. (2000) suggest that heritabilities for traits do not depend on the length of the performance test, because results of an eight-day test are in the same range as those from German 50-day tests. The same group has summarized studies of genetic correlations between traits recorded at station performance tests of stallions in Europe (THORÉN-HELLSTEN et al., 2006). Most traits have moderate to high heritabilities but show little correlation with performance, except with jumping. In our study, Noriker horses that had a good walk at riding also had a good trot at riding, but the correlation did not hold for drafting. The walk is the most important trait of a cold-blood horse like the Noriker. In the past, the breed had been largely used as a packhorse. By putting too much emphasis on riding and trot, the genuine traits of the Noriker horse may become lost. Although the scoring system provides a scale from one to ten, judges seem not to exploit the entire scale and score all horses between five and nine, with most scores between six and seven. This does not provide sufficient discriminatory power and may account for our inability to detect significant differences between the traits of the three genotypes.

A major limitation of the present study is the small number of horses investigated. A larger number of *Gys-1* allele positive horses should be studied to obtain more significant results, which would be helpful in improving the Noriker selection programme.

Fazit für die Praxis:
Die Verwendung von Norikerpferden mit dem *Gys-1* H-Allel für die Zucht hat möglicherweise Pferde mit schlechterer Arbeitsleistung und schlechterem Bewegungsablauf zur Folge. Allerdings sollten Pferde mit H-Allel, nach bestandenem Leistungs- und Belastungstest, zur Erhaltung von Linie und Farbe weiterhin zur Zucht eingesetzt werden dürfen, bis weitere Untersuchungen diese Empfehlung widerlegen.

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