

Single Nucleotide Polymorphism in the *LDHA* Gene as a Potential Marker for the Racing Performance of Pigeons

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The objective of the present study was to investigate the relationship between the g.2582481G>A, g.2583935G>A and g.2584057C>T single nucleotide polymorphisms (SNPs) within the lactate dehydrogenase A gene (*LDHA*) coding for lactate dehydrogenase isoform A and the racing performance of homing pigeons. As a measure of racing performance, we used the mean values of ace points won by individual birds during the whole season. The estimated heritability of the racing performance of pigeons was relatively low ($h^2=0.0596$; $SE=0.0249$). The analysis performed for all race reports together showed that the factors such as gender, weather conditions at the start and at the end of the race affect the analyzed trait. Of the 3 single nucleotide polymorphisms, only the effect of the g.2582481G>A genotype on the performance of racing pigeons was significant. Statistical analysis indicated the difference in the value of ace points between the animals of *GG* and *GA* genotypes for the g.2582481G>A SNP. The study showed that the genotype homozygous for g.2582481A is linked to the highest mean value of ace points. Consequently, the relationship between the genotype for g.2582481G>A and the racing performance was shown.

Key words: *Columba livia*, lactate dehydrogenase, *LDHA* gene, pigeon racing

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Introduction

The competitions of homing pigeons are becoming increasingly popular all over the world, and even, pigeon racing is considered a national sport in Taiwan. It is also very popular in Poland. The Polish Association of Racing Pigeon Breeders has approximately 50 000 members. Polish breeders can compete in numerous races at different distances in their sections through the racing season, and also, in many national one-loft races – the largest in this year is planned to include 4 000 pigeons and the total prize may reach €335 000. Nevertheless, the greatest competitions take place in China, e.g. Diamond Elite One-loft Race for 300 miles, in which the first prize is more than €1 million or The Golden Island One-loft Race in Qinhuangdo for 310 miles with €4 million in prize money. The prices for the best pigeons and their offspring reached even several

hundred thousand USD and are still increasing (Smith, 2012).

Many factors, such as weather, feeding and training method, have been considered as potential determinants of the racing performance of pigeons. However, the genetic predisposition of individual birds should also be taken into account. A better understanding of the genetic background of racing performance in the pigeon, especially the identification of genetic markers for this trait, might be useful for further progress in pigeon racing.

Potential genetic markers associated with physical efficiency have been investigated in human (Hruskovicova *et al.*, 2006) and in animals involved in racing such as dogs (Mosher *et al.*, 2007), horses (Park *et al.*, 2003; Hill *et al.*, 2010) and pigeons (Dybus *et al.*, 2008). Dybus *et al.* (2006) indicated the lactate dehydrogenase gene (*LDHA*) coding for lactate dehydrogenase isoform A as a candidate gene for the racing performance of pigeons. This enzyme plays an important role in the metabolism of pyruvate and lactate, which implies that its activity is crucial for the overall physical performance. It has been previously reported by Chaplin *et al.* (1997) that LDH activity is strongly influenced

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by the type of training. The highest muscle LDH activity was observed in sprint-trained birds and the lowest one in endurance-trained birds.

The aim of the present study was to analyze the relationship between the polymorphism within the pigeon lactate dehydrogenase A gene (*LDHA*) coding for lactate dehydrogenase isoform A and the racing performance of homing pigeons.

Materials and Methods

The study included a total of 123 homing pigeons (60 hens and 63 cocks) derived from the Polish champions' lofts (Eugeniusz Cichoń, $n=59$; Antoni Pawlina, $n=64$) located in the Lubuskie Province, Sulęcín County. In both lofts, the pigeons were trained and raced according to a total widowhood method. Blood samples were collected from the medial metatarsal vein to the test tubes containing anti-coagulant (K_3 EDTA) in September 2011. DNA isolation was carried out using the MasterPure™ DNA Purification Kit for Blood Version II (Epicentre, Madison, WI, USA). The PCR-RFLP-based assays were used for genotyping of the 2582481G>A, g.2583935G>A and g.2584057C>T single nucleotide polymorphisms (SNPs) as described previously (Table 1).

All pigeons participated in racing competitions organized by the Sulęcín Section 085, a member of the Polish Association of Racing Pigeon Breeders (PZHGP). Our study included full racing season data that consisted of scores from 8 short races (<400 km) and 6 long races (>500 km). The final ranking of pigeons during the whole season was based on ace points (AP). These points are awarded to birds that completed a given race (a general rule in Poland is that 20% of starting pigeons win prizes). The first pigeon at finish is awarded 100 AP. The AP won by an individual bird was calculated by the following formula:

$$p = \frac{(a-b+1)}{a} * 100$$

where

p is the ace points awarded,

a is the number of pigeons on the prize list that is equal to

20% of pigeons on an entry list (or less than 20% if less than 20% of starting pigeons finish),

b is the position on the list.

The effect of the g.2582481G>A, g.2583935G>A and g.2584057C>T genotypes on the total number of ace points won by individual pigeons in all races was estimated using the following model:

$$y_{ijklmno} = \mu + g_i + s_j + h_k + ps_l + pp_m + k_n + obs_{ijklmno} + e_{ijklmno},$$

where

$y_{ijklmno}$ is the analyzed trait,

μ is the overall mean for the trait,

g_i is the effect of the i th genotype ($i=AA, AB, BB$),

s_j is the effect of the j th gender (males, females),

h_k is the effect of the k th breeder ($k=A, B$),

ps_m is the effect of the m th weather conditions at the start (sunny, changeable),

pp_n is the effect of the n th weather conditions at the end (sunny, changeable, rainy, windy, cloudy),

k_n is the effect of the n th race category (short, long),

$obs_{ijklmno}$ is the random non-genetic effect of the $ijklmno$ th observation,

$e_{ijklmno}$ is a random error.

The effect of genotype on the mean value of AP won by individual pigeons in short races (<400 km) and long races (>500 km) was estimated using the same statistical model as above, excluding the effect of race category (k_n). Scheffe's test was used for multiple comparisons.

Heritability (h^2) was estimated as a ratio of genetic and phenotypic variances using the computer package ASREML 2.0 (Gilmour *et al.*, 2008).

$$y = Xb + Za + Wobs + e,$$

where y is a vector of observations for an individual; X , Z , and W are known incidence matrices relating observations to fixed or random effects; b is a vector of fixed effects; a is a vector of random genetic effect, with $a \sim N(0, A\sigma_a^2)$; obs is a vector of random non-genetic effect used to correct for repeated observations for an individual, with $obs \sim N(0, I_{obs}\sigma_{obs}^2)$; e is a vector of residuals, with $e \sim N(0, I_e\sigma_e^2)$. I_c and I_e are identity matrices of appropriate dimensions, and A is a

Table 1. Primer sequences and restriction enzymes (RE) used for the PCR-RFLP genotyping of single nucleotide polymorphisms (SNPs) within the pigeon *LDHA* gene

SNP	Primer sequences	Ta	RE	Reference
g.2582481G>A intron 7	F 5' - TGAAGGGGTACACATCATGG -3' R 5' - CCTTCTGGATTCCCCAGAGT -3'	57°C	<i>Hae</i> III	Dybus <i>et al.</i> , 2006
g.2583935G>A intron 5	F 5' - GACCTATGTGGCCTGGAAGA -3' R 5' - CACCACCTGCTTATGGACCT -3'	61°C	<i>Tai</i> I	Dybus, 2009
g.2584057C>T exon 5	F 5' - GACCTATGTGGCCTGGAAGA -3' R 5' - CACCACCTGCTTATGGACCT -3'	61°C	<i>Nco</i> I	Dybus, 2009

The descriptions of SNPs refer to their positions within the minus strand of Cliv_1.0 Primary Assembly (GenBank: NW_004973198.1). The SNPs were described in the reference articles as *LDHA/Hae*III (intron 6), *LDHA/Tai*I (intron 4) and *LDHA/Nco*I (exon 4), respectively. The discrepancy in the assignment of the SNP positions was caused by an update of the *LDHA* sequence - one non-coding exon was found. Ta: annealing temperature.

numerator relationship matrix calculated from a pedigree over 2 generations. Fixed effects in the model were: gender (males, females), breeder (k=A, B), weather conditions at the start (sunny, changeable), weather conditions at the end (sunny, changeable, rainy, windy, cloudy) and race category (short, long).

Results and Discussion

Three SNPs within the *LDHA* gene were investigated. The frequency distribution of genotypes for g.2583935G>A, g.2584057C>T, and g.2582481G>A is presented in Table 2. The g.2583935G>A SNP is located in intron 5 of the pigeon *LDHA* gene, while the g.2584057C>T SNP is a synonymous substitution within exon 5. The g.2582481G>A SNP is located in intron 7, very close to the GT splice donor site. The g.2583935G>A, g.2584057C>T SNP and g.2583935G>A SNPs were primarily described as *LDHA/TaiI* in intron 4, *LDHA/NcoI* in exon 4 (Dybus *et al.*, 2009), and *LDHA/HaeIII* in intron 6 (Dybus *et al.*, 2006), respectively. The discrepancy in the assignment of the SNP positions was caused by an update of the *LDHA* sequence – since an additional first non-coding exon was added (GenBank, Gene ID: 102094829).

The final position of a bird in a given race may be affected by numerous factors. This implies that the relationship between genotype and racing performance should not be considered for each race separately. Therefore, we used the mean values of ace points won by individual birds in a series of races as a determinant of racing performance. The analysis carried out for all race reports together indicated that

the factors such as gender, weather conditions at the start and weather conditions at the end of the race affect the racing performance of pigeons ($P<0.05$). However, among the three investigated polymorphic sites, only the effect of g.2582481G>A on the racing performance was significant (Table 3). The association of g.2583935G>A and g.2584057C>T with pigeon racing performance was not confirmed ($P=0.9002$ and $P=0.6208$, respectively).

Dybus *et al.* (2006) indicated that the frequencies of the *GA* and *GG* genotypes for g.2582481G>A differ between the group of racing pigeons (University loft, Poland) and that of top-racing pigeons from China and Taiwan ($P<0.001$). Moreover, the g.2582481A allele was more frequent in the top-racing pigeons ($P<0.001$). Based on the above-mentioned preliminary study, Dybus *et al.* (2006) suggested that the *AA* genotype for g.2582481G>A may be associated with racing performance. Moreover, on the basis of investigation of six other polymorphic loci within the pigeon *LDHA* gene, Ramadan *et al.* (2013) reported significant difference in genotype distribution between the groups of homing and non-homing pigeons.

The effect of the genotype for g.2582481G>A on the racing performance of pigeons was significant for all races together ($P=0.0107$) and the short races ($P=0.0186$). Statistical analysis revealed the difference in the AP values between the *GA* and *GG* genotypes for all races ($P=0.0157$) and short races ($P=0.0280$). The *AA* genotype was associated with the highest mean value of ace points considering all races and the short ones (Table 4). However, birds carrying this genotype are extremely rare. Therefore, we

Table 2. Genotypic frequencies of g.2582481G>A, g.2583935G>A and g.2584057C>T SNPs within the pigeon *LDHA* gene

SNP	n	Genotype			Allele	
		AA	GA	GG	G	A
g.2582481G>A	123	0.016	0.195	0.789	0.886	0.114
		0.090	0.317	0.593	0.752	0.248
g.2583935G>A		0.659	0.268	0.073	0.793	0.207

Table 3. The effects of factors included in the statistical model used for the association study between the g.2582481G>A genotype and the pigeon racing performance

Factor	All races		Short races (<400 km)		Long races (>500 km)	
	F	P	F	P	F	P
g.2582481G>A	4.5541	0.0107	4.1090	0.0168	1.4935	0.2253
Gender	26.2962	0.0000	15.7668	0.0001	11.0607	0.0009
Breeder	4.1280	0.0424	0.5622	0.4536	5.1304	0.0238
Weather at the start	7.9489	0.0049	2.0626	0.1514	7.8628	0.0052
Weather at the end	5.0610	0.0005	7.4903	0.0006	1.5972	0.1887
Race category	0.5913	0.4421	—	—	—	—

Table 4. Mean values of ace points in association with the genotypes for g.2582481G>A and the race category

Genotype	All races			Short races (<400 km)			Long races (>500 km)		
	RR	AP	SE	RR	AP	SE	RR	AP	SE
<i>AA</i>	19	37.70	9.67	10	46.52	13.41	9	27.90	14.03
<i>GA</i>	271	37.02 ^a	2.41	129	37.23 ^a	3.36	142	36.83	3.43
<i>GG</i>	1090	29.96 ^b	1.09	528	29.44 ^b	1.58	562	30.45	1.50

RR: number of race records. AP: mean of ace points. SE: standard error of the mean. Different superscript letters indicate statistically significant differences: $P \leq 0.05$.

obtained only 19 race records. Nevertheless, our hypothesis was confirmed and the association of the genotype for g.2582481G>A with racing performance was delineated.

It still remains an open question how the g.2582481G>A substitution affects the racing performance of pigeons, but some hypotheses can be raised. As the g.2582481G>A SNP is located very close to the GT splice donor site, it is possible that this substitution affects the pre-mRNA splicing process, and thus influences the *LDHA* gene expression. The other possibility is that the g.2582481G>A SNP is in linkage disequilibrium with some unknown functional SNP. An in-depth investigation is necessary in order to establish the exact background of an association between the g.2582481G>A substitution and the analyzed trait.

In addition, we tested the relationship between gender and racing performance. Hens appeared to have significantly better racing ability ($P=0$), as they acquired more ace points throughout the season (AP=35.78, SE=1.38), in comparison with cocks (AP=26.99, SE=1.41). The same tendency was observed in short and long races separately ($P<0.05$). Unfortunately, there is no available data to discuss with, but according to the one author who is an experienced pigeon breeder, this is typical of pigeon racing that hens are better in racing competitions.

The estimated heritability of the racing performance of pigeons was relatively low ($h^2=0.0596$, SE=0.0249). Nevertheless it should be emphasised that the h^2 value depends on many factors, i.e. animals species, target populations, trait type, and the phenotyping strategies for the traits. Röhe *et al.* (2001) and Sobczyńska and Łukaszewicz (2003) reported very different h^2 values (0.05–0.29 and 0.12–0.25, respectively) for performance in horses but they utilized different performance measures. Furthermore, Tozaki *et al.* (2012) showed that the heritability estimated by various methods may differ significantly (0.25 obtained from a non-linear model and 0.11 obtained from a linear model). The relatively low level of heritability of racing performance estimated in the present study for pigeons is in agreement with the results obtained for horses in the aforementioned studies and with those obtained for dogs (Täubert *et al.*, 2007). However, it was the first attempt at determining the heritability of this trait in pigeons. Despite the estimated heritability being very low, it cannot be concluded that the racing performance of pigeons is the trait depending only on

environmental factors. In order to fairly determine the contribution of genetic variance to the analyzed trait, the estimation should be performed on a larger population.

Our work is the first so comprehensive association study of the racing performance of pigeons and genetic variability. We indicated the g.2582481G>A SNP as a possible genetic marker that can be applied in Marker Assisted Selection.

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