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Associations between polymorphisms in the myostatin, α^A-globin and lactate dehydrogenase B genes and racing performance in homing pigeons

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ABSTRACT: The aim of this study was to investigate the associations between variability in the myostatin, α^A-globin and lactate dehydrogenase B genes and racing performance in homing pigeons. The study included 123 animals (60 females and 63 males) participating in racing competitions. The data set used in this study consisted of scores from 17 short (≤ 400 km) and 11 long races (≥ 500 km) (2589 race records in total). Our study is the first study to analyse the associations between polymorphisms in the myostatin, α^A-globin and lactate dehydrogenase B genes and racing performance in pigeons. However, no associations were found between the SNPs analysed and the studied traits.

Keywords: *Columba livia*; MSTN; AGLOB; LDHB; pigeon racing; SNP

Homing pigeon races are very popular in some regions of the world. One type of contest is the so-called one loft race, where young pigeons compete under similar environmental and loft conditions. Identification of genetic markers in animals might be useful for understanding the genetic background of economically important traits (Kumar et al. 2017; Meena et al. 2017; Verma et al. 2017) and could also facilitate pigeon breeding (Proskura et al. 2014; Proskura et al. 2015a).

A large number of studies have been published regarding the associations between genetic markers and physical efficiency in humans (Ahmetov et al. 2015) and animals (McGivney et al. 2012), also in the form of an advanced GWAS analysis (Shin et al. 2015). The genes included in our study appear to be important in determining of physical performance. Myostatin (MSTN) is an essential protein responsible for regulating the growth and development of skeletal muscles. The work of a great many authors has gone into identifying the variability of the *MSTN* gene locus and comparing this with results achieved by sporting horses. Petersen et al. (2014) demonstrated the influence of a variant of the *MSTN* gene involving a T/C substitution in the first intron and its insertion into the promotor on the magnitude of muscle fibres in different breeds. This variant was found to have a direct effect on the sporting results achieved by the horses. Van den Hoven et al. (2015) showed strong association between an SNP in the *MSTN* gene and the ability of non-elite Thoroughbreds to race over short and long distances. In dogs, a functional mutation in the *MSTN* gene responsible for increased muscle mass and enhanced racing performance was reported (Mosher et al. 2007).

Liang et al. (2016), in turn, showed that increased physical activity induces expression of the *LDHB* gene, thereby possibly enhancing the activity of mitochondrial enzymes and increasing oxygen consumption during prolonged exertion, e.g., during active flight. Dybus et al. (2006) and Ramadan et al. (2013) reported significant differences in *LDHA* genotype distributions between groups of homing and non-homing pigeons. In previous papers, polymorphisms in the α^A-globin (Dybus et al. 2008), lactate dehydrogenase-B (Dybus 2009) and myostatin (Dybus et al. 2013) genes was reported.

The *HBB* gene also appears to have an important influence on the results achieved by pigeons dur-

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ing races. This is because mutations of this gene can lead to genetic disorders such as sickle-cell anaemia. The results of a study by Malczewska-Lenczowska et al. (2016), performed on endurance sportsmen in order to examine the effect of polymorphism in the *HBB* gene on the total mass of haemoglobin and respiratory volume, demonstrated statistically significant differences between persons with the *GG* genotype and those with the *CG* genotype.

The aim of this study was to examine the associations between four SNPs located in three genes (*MSTN*, *AGLOB* and *LDHB*) and racing performance of homing pigeons.

MATERIAL AND METHODS

The material for genetic study – blood samples taken from the metatarsal vein – was obtained in September 2011. Samples were withdrawn from 63 males and 60 females (n = 123) into collection tubes containing anticoagulant (K_3 EDTA). DNA was isolated using the Master Pure^{TM} DNA Purification Kit for Blood Version II (Epicentre Biotechnologies, Madison, USA). The presence of SNPs in the *AGLOB*, *MSTN* and *LDHB* genes was analysed using PCR-RFLP assays (Table 1).

The data for analysis consisted of the results achieved by homing pigeons participating in races over long (≥ 500 km) and short (≤ 400 km) distances in 2011–2012. All the birds came from district 085 in Sulecin, which is a member of the Polish Association of Homing Pigeon Breeders. Results were obtained from 2589 race records – 1463 short-distance ones and 1126 long-distance ones. Each bird participating in the race could acquire from 0 to 100 ace points (APs), which were awarded according to ranking. The winner of each race received the maximum number of points. The details are given in Proskura et al. (2014).

Associations between APs and SNPs were analysed with the following mixed model using lmekin function in the coxme package for R software:

$$y = \mu + g + s + h + k + ps + pp + k + i + a + e$$

where: y = analysed trait; μ = overall mean; g = effect of genotype (*AA*, *AB*, *BB*); s = sex effect (males, females); h = breeder effect (A, B); ps = weather at the start effect (sunny, changeable); pp = weather at the end effect (sunny, changeable, rainy, windy, cloudy); k = race category effect (short, long); i = effect of individual accounting for repeated observations (1–123); a = a random polygenic component accounting for all known pedigree relationships (three generations); e = random error

Pedigree data was handled using Pedigree Viewer 6.5b. The additive relationship matrix was based on a three-generation pedigree using the kinship2 R package.

Table 1. Primer sequences and restriction enzymes used for SNP genotyping

SNP[1]	Location	Primer sequence	T_a[1]	Length	RE[2]
AGLOB g.2899462C>T NW_004973488.1	66 bp downstream	F: CATGGCTAGAGCTGGACACA R: AGCCCATTTCACCTACATGC	61 °C	890 bp	*Bse*LI
LDHB g.564756A>G NW_004973421.1	exon 8 p.(Ile283Val) ATT → GTT	F: AAGTGAGGGGTTTGGAGCTG R: GAAGGCTCAAGAAGACATCATTGT	60 °C	272 bp	*Hpy166*II
LDHB g.564102A>G NW_004973421.1	intron 7	F: AAGGGATACACAAACTGGGCTA R: GATGTACAGTGAATAAACCCCACA	62.5 °C	995 bp	*Hind*III
MSTN g.11440232C>T NW_004973256.1	exon 3 ACC → ACT p.(=)	F: GCAGAGATTTTGGCCTTGAC R: GAGGTGAGTGTGCGGGTATT	61 °C	185 bp	*Btg*I

PCR-RFLP assay for genotyping *AGLOB*:g.2899462C>T (Dybus et al. 2008)

PCR-RFLP assay for genotyping *LDHB*:g.564102A>G (Dybus 2009)

PCR-RFLP assay for genotyping *MSTN*:g.11440232C>T (Dybus et al. 2013)

[1]Annealing temperature

[2]Restriction enzyme

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Table 2. Genotypes and allele frequencies of the studied SNPs

SNP	Genotype			Allele	
*LDHB/ Hind*III	*AA* 0.415 (*n* = 51)	*AG* 0.504 (*n* = 62)	*GG* 0.081 (*n* = 10)	*A* 0.667	*G* 0.333
*LDHB/ Hpy166*II	*AA* 0.203 (*n* = 25)	*AG* 0.740 (*n* = 91)	*GG* 0.057 (*n* = 7)	*A* 0.573	*G* 0.427
*MSTN/ Btg*I	*TT* – –	*CT* 0.195 (*n* = 24)	*CC* 0.805 (*n* = 99)	*T* 0.098	*C* 0.902
*AGLOB/ Bse*LI	*TT* – –	*CT* 0.154 (*n* = 19)	*CC* 0.846 (*n* = 104)	*T* 0.077	*C* 0.923

RESULTS

The genotypes and allele frequencies obtained in the study are presented in Table 2. Three genotypes (*AA*, *AG* and *GG*) were identified for the *LDHB/Hind*III and *LDHB/Hpy166*II genes, while for the *MSTN/Btg*I and *AGLOB/BseL*I genes two genotypes were found (*CT* and *CC*). The association between four SNPs (in three genes) and the racing performance of pigeons was analysed. The effect of all the SNPs studied on the racing performance of pigeons was neither significant for all races together (Table 3) nor for short/long races separately (Tables 4 and 5). The largest difference in APs was found for the *MSTN* gene. Individuals with the *CC* genotype were on average awarded 5.44 points more than those with the *CT* genotype. However, these differences were not statistically significant.

Table 3. The association of the studied SNPs with racing performance of pigeons in all races

SNP	Genotype	RRs	APs	SE	*P*
*LDHB/ Hpy166*II	*AA*	473	28.18	1.67	0.640
	AG	1947	29.65	0.82	
	GG	169	28.37	2.71	
*LDHB/ Hind*III	*AA*	1065	29.24	1.12	0.908
	AG	1297	29.39	0.99	
	GG	227	29.03	2.40	
*MSTN/ Btg*I	*CC*	2096	29.88	0.79	0.978
	CT	493	26.80	1.56	
*AGLOB/ Bse*LI	*CC*	2199	29.61	1.83	0.989
	CT	390	27.50	0.77	

APs = ace points; RRs = race records; SE = standard error

Table 4. The effect of the studied SNPs on racing performance of pigeons in 100–400 km races

SNP	Genotype	RRs	APs	SE	*P*
*LDHB/ Hpy166*II	*AA*	270	26.69	2.19	0.388
	AG	1095	30.19	1.10	
	GG	98	29.83	3.61	
*LDHB/ Hind*III	*AA*	602	29.17	1.50	0.729
	AG	733	29.74	1.33	
	GG	128	29.88	3.22	
*MSTN/ Btg*I	*CC*	1180	29.78	1.06	0.942
	CT	283	28.45	2.12	
*AGLOB/ Bse*LI	*CC*	1241	29.92	2.42	0.917
	CT	222	27.29	1.03	

APs = ace points; RRs = race records; SE = standard error

DISCUSSION

Analysing the influence of genetic variation on phenotypic traits is a typical approach to explaining the diversity between individuals. Two types of genetic markers are most important – STRs and SNPs (Guang-Xin et al. 2016; Gupta et al. 2016). The majority of results concerning the influence of genetic variability on physical performance have come from different studies of human genes. Nowadays, a very popular method is whole genome sequencing (WGS), which is a very powerful technique for the detection of chromosomal regions responsible for the studied trait. The most popular animal sport is horse racing, so many research teams seek quantitative trait nucleotides in equine genomes (Weller and Ron 2011) that are respon-

Table 5. The effect of the studied SNPs on racing performance of pigeons in 500–800 km races

SNP	Genotype	RRs	APs	SE	*P*
*LDHB/ Hpy166*II	*AA*	203	30.15	2.59	0.943
	AG	852	28.95	1.22	
	GG	71	26.35	4.13	
*LDHB/ Hind*III	*AA*	463	29.34	1.70	0.967
	AG	564	28.92	1.48	
	GG	99	27.94	3.61	
*MSTN/ Btg*I	*CC*	916	30.02	1.20	0.541
	CT	210	24.58	2.30	
*AGLOB/ Bse*LI	*CC*	958	29.22	2.82	0.998
	CT	168	27.78	1.15	

APs = ace points; RRs = race records; SE = standard error

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sible for the observed variation in racing horse performance (Moon et al. 2015; Van den Hoven et al. 2015).

Associations between gene polymorphism and racing performance of pigeons have been analysed mainly by Polish researchers. Proskura et al. (2014) demonstrated a strong association between an SNP in the *LDHA* gene (g.2582481G>A) and the results achieved by old pigeons in races. A later study (Proskura et al. 2015b), relating to the *LDHA* gene and carried out on a larger group of birds (n = 313), failed to demonstrate any association between polymorphism in this gene ($P \leq 0.07$) and the results achieved by racing pigeons. It is worth mentioning, however, that birds of the highly unique $LDHA^{AA}$ genotype achieved an average of 47.21 APs, whereas birds of the $LDHA^{GG}$ and $LDHA^{AG}$ genotypes obtained nearly 20 points fewer (27.75 and 26.01 points, respectively). There are some very interesting results in the paper by Proskura et al. (2015b), which demonstrated a relationship between an SNP in the *DRD4* gene and the racing performance of homing pigeons. Recently, that same research team (Proskura et al. 2017) performed a study analysing mutation at position g.710T>C of the keratin gene, which causes cysteine to be replaced by glycine. The results of these papers established an association ($P \leq 0.045$) between the genotype of a bird and the number of APs awarded for long-distance flights. Birds of the *TT* genotype received an average of 32.1 APs, whereas those of the *GG* genotype were awarded 23.3 APs on average. In contrast, genotype was not found to have any effect on the results of races over distances shorter than 400 km.

The genotypes and alleles frequencies obtained in this study were similar to those published previously (Dybus et al. 2008; Dybus 2009; Dybus et al. 2013). In this study, we have reported the first analysis of the association of racing performance and genetic variability in the *LDHB*, *MSTN* and *AGLOB* genes in domestic pigeons. However, there were no associations between the analysed SNPs and racing performance.

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