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The Cys83Gly amino acid substitution in feather keratin is associated with pigeon performance in long-distance races

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ABSTRACT: The aim of this study was to investigate the association of the g.710T>G polymorphism in the keratin gene, which results in a cysteine to glycine amino acid change at position 83 (Cys83Gly) in feather keratin, with homing pigeon racing performance. A total of 123 homing pigeons were investigated. The data set used in this study consisted of scores from 17 short races (less than 400 km) and 11 long races (greater than 500 km) that took place in the 2011 and 2012 racing seasons (2589 race records in total). The genotyping of the g.710T>G polymorphism was performed using the artificially created restriction site-PCR assay. The *T* allele and the *TT* genotype were prevalent with frequencies of 0.658 and 0.447, respectively. The *TT* pigeons had the highest mean of ace points in the long races and in all races overall, while the *GT* birds scored the best in the short races. Nevertheless, the effect of the polymorphism was significant only in the long races ($P = 0.0451$), in which the pigeons carrying the *TT* genotype showed better racing performance in comparison with those carrying the *GG* genotype ($P \leq 0.05$). In order to explain this phenomenon, several bioinformatics tools were employed to check for the possible consequences of the Cys83Gly substitution for feather keratin. The cysteine at position 83 was indicated to form a disulphide bond, while the Cys83Gly substitution was predicted to disturb the stability of the protein. However, the predictions preformed using the different tools were not entirely consistent. Nevertheless, the loss of the cysteine at position 83 of pigeon feather keratin may affect the structure of feathers, thus changing their biomechanical characteristics, and consequently, may influence the flying ability of pigeons.

Keywords: *Columba livia*; feather; keratin; pigeon racing; ACRS-PCR; *F-KER*; SNP; single nucleotide polymorphism

The main characteristic feature of domestic pigeons is their homing ability, which allows them to return to their home lofts from distances of up to several thousand kilometres. This feature is utilised by breeders in the numerous homing pigeon competitions that are organised over different distances almost worldwide.

Many factors are considered to influence the results achieved by pigeons in racing competitions. Besides environmental factors such as weather, feeding or the methods used for training, also the genetic predispositions of a particular bird have a significant impact on pigeon racing performance (Proskura et al. 2014; Proskura et al. 2015).

Homing pigeons have been bred selectively for a strong homing ability, which has probably resulted in the preservation of genetic variants favourable for this trait. For instance, Dybus and Haase (2011) observed genetic differences between homing and non-homing pigeons.

In pigeon racing, it is not only important to a breeder that a pigeon returns to a loft, but also that it does so quickly. Apart from numerous environmental factors, general physical efficiency and a properly developed locomotion system influence the overall time period in which a pigeon completes a race. The proper structure of feathers is pivotal for pigeon flying ability. Thus, even subtle differences in their construction may affect flight quality, and consequently have an effect on the racing performance of pigeons.

The main component of feathers is a protein called β-keratin (~10 kDa), which accounts for approxi-

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mately 90% of the feather rachis (Reddy and Yang 2015). A study conducted on chickens showed that mutation within the feather keratin gene may lead to an enormous change in feather structure (Ng et al. 2012). Dybus and Haase (2011) reported the presence of the g.710T>G single nucleotide polymorphism (SNP) in the pigeon feather keratine (*F-KER*) gene, which results in a Cys83Gly amino acid change in the protein. The replacement of cysteine with glycine may affect the structure of the keratin molecule. Although no visible differences have yet been detected between the feathers of birds with different g.710T>G genotypes, subtle changes in their quality may potentially affect plumage resistance, and thus racing performance (Dybus and Haase 2011).

The aim of this study was to investigate the association of the g.710T>G polymorphism in the *F-KER* gene, which results in the Cys83Gly amino acid change in feather keratin, with homing pigeon racing performance.

MATERIAL AND METHODS

The study included a total of 123 homing pigeons (60 hens and 63 cocks) derived from the two leading flocks (n = 59 and n = 64) of the Sulecin 085 section (Sulecin County, Lubuskie Province, Poland). The pigeons from both flocks were trained and raced according to the total widowhood method.

Blood samples were collected from the medial metatarsal vein into test tubes containing anticoagulant (K_3EDTA) in September 2011. DNA isolation was performed using the MasterPure™ DNA Purification Kit for Blood Version II (Epicentre Biotechnologies, Madison, USA) based on the salting-out procedure described by Miller et al. (1988). The genotyping of the g.710T>G SNP in the pigeon *F-KER* gene was performed using the artificially created restriction site-PCR assay as described previously (Dybus and Haase 2011). A 146-base pair product was amplified using the following primers: forward 5'-TGAAGGGGTACACATCATCG-3' (C was placed at position 19 instead of the complementary G in order to create a restriction site) and reverse 5'-CCTTCTGGATTCCCCAGAGT-3'. PCR was followed by digestion with the *Ava*I endonuclease and electrophoretic separation.

Because the g.710T>G substitution leads to a change from cysteine (TGC) to glycine (GGC) at position 83 in the pigeon feather keratin protein, bioinformatic analyses were carried out to investigate its potential impact using the following tools: I-Mutant 2.0.7 (Capriotti et al. 2005), iStable (Chen et al. 2013), MUpro (Cheng et al. 2006), PMut (Ferrer-Costa et al. 2005), Provean 1.1 (Choi et al. 2012), SIFT (Kumar et al. 2009), and SNAP (Bromberg et al. 2008). Disulphide bond formation and localisation were predicted using CysState (Mucchielli-Giorgi et al. 2002), DiANNA 1.1 (Ferre and Clote 2006), DISULFIND (Ceroni et al. 2006), DBCP (Lin and Tseng 2010) and DINOSOLVE (Yaseen and Li 2013).

The data set used in this study consisted of scores from 17 short races (less than 400 km; 1463 race records) and 11 long races (greater than 500 km; 1126 race records) (2589 race records in total). Each pigeon participating in a particular race acquired ace points (AP) in the range from 0 to 100, where the maximum value was conferred for the first place. The method for calculating AP is described in detail in Proskura et al. (2014).

The effect of the F-KER genotype on the number of ace points won by individual pigeons was estimated using the linear model described by Proskura et al. (2014) extended by the addition of the effect of racing season. The following fixed effects were included in the statistical model: genotype (*GG*, *GT*, *TT*), sex (male, female), flock (1, 2), weather conditions at the start of a race (sunny, changeable), weather conditions at the end of a race (sunny, changeable, rainy, windy, cloudy), race category (short, long), racing season (2011, 2012). Scheffe's test was performed for multiple comparisons. All the statistical analyses were conducted using Statistica 10 software (StatSoft Inc., Tulsa, USA).

RESULTS

In the present study, we attempted to determine whether the g.710T>G SNP in the *F-KER* gene is associated with pigeon racing performance. In both groups, the *T* (wild-type) allele was prevalent. However, the frequency of the *G* allele in Flock 1 was more than two-fold higher than in Flock 2. In addition, the *GG* genotype was observed in almost a quarter of the pigeons in the first group, while it was extremely rare in the second group. A statistically significant difference in genotype distribution between the flocks was observed (χ^2 = 15.463, P = 0.0004). The data on allele and genotype frequencies are shown in Table 1. The statistical model used

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Table 1. Genotypic and allelic frequencies of the g.710T>G SNP in the *F-KER* gene

Flock	Genotype			Allele	
	TT	*GT*	*GG*	*T*	*G*
Total	0.447 (*n* = 55)	0.423 (*n* = 52)	0.130 (*n* = 16)	0.658	0.342
1	0.305 (*n* = 18)	0.458 (*n* = 27)	0.237 (*n* = 14)	0.534	0.466
2	0.578 (*n* = 37)	0.391 (*n* = 25)	0.031 (*n* = 2)	0.773	0.227
χ^2	*P* = 0.0014			*P* = 0.0001	

χ^2 test was performed to compare the genotypic distributions between the flocks

for the association analysis included fixed effects of genotype, sex, flock, weather conditions at the start of a race, weather conditions at the end of a race, race category and racing season. Weather and sex were significant in all analyses. Racing season was significant in analyses of all races and in long races, while *F-KER* was significant only in the latter (Table 2).

DISCUSSION

Dybus and Haase (2011) observed the following genotype frequencies in non-homing pigeons: 0.019, 0.107 and 0.874 (for *GG*, *GT* and *TT*, respectively). The respective values in homing pigeons were the following: 0.046, 0.348 and 0.606. The frequency of the *G* allele in the non-homing group (f = 0.073) was significantly lower in comparison with the homing group (f = 0.22), and in the present study the *G* allele was even more frequent (f = 0.342). Differences in genotype distribution were revealed between the group investigated in the present study and the groups analysed by Dybus and Hasse (2011): in both the non-homing and homing groups the chi-squared test gave the following result: χ^2 = 44.6, P = 0.

Numerous factors are considered to play a role in the results achieved by pigeons in racing competitions. Besides the genetic predispositions of individual birds, also environmental factors such as weather, feeding, and training methods play a significant part in pigeon racing performance. Of the fixed effects included in the statistical model, race category, racing season, weather, and sex were indicated to be strictly associated with pigeon scores (Table 2), which is mostly in accordance with the findings of Proskura et al. (2014). The effect of flock was not significant, which might be explained by the similar feeding systems or training methods used at both studied lofts.

Sex appeared to be a very important factor affecting the racing ability in pigeons. In all analysed races, hens achieved more ace points (AP = 33.4, SE = 1.03) than cocks (AP = 25.00, SE = 0.96), demonstrating a significantly better racing ability (P = 0). The same trend was observed (P < 0.01) when short and long races were analysed separately. The results obtained in the present study are in agreement with those reported by Proskura et al. (2014), where the same group of pigeons was investigated with respect to lactate dehydrogenase A gene polymorphism and racing ability. However, only data from one racing season were analysed in this study.

The effect of the g.710T>G genotype appeared to be significant only in the case of long races (P = 0.0451). Scheffe's test revealed a significant difference in the mean of ace points in long races between the *TT* and *GG* genotypes (P = 0.0243). Detailed results are given in Table 3.

The g.710T>G SNP in the *F-KER* gene coding for pigeon feather keratin leads to a change from a polar

Table 2. The effects of factors included in the statistical model used for the association study between the g.710T>G SNP genotype and pigeon racing performance

Factor	All races		Short races (< 400 km)		Long races (> 500 km)	
	F	*P*	*F*	*P*	*F*	*P*
g.710T>G	1.3794	0.2560	0.4857	0.6166	3.1958	0.0451
Sex	15.9131	0.0001	14.6236	0.0002	7.8285	0.0061
Flock	1.3984	0.2395	1.0202	0.3147	0.9725	0.3263
Racing season	10.8407	0.0010	3.2396	0.0721	11.0747	0.0009
Weather at the start	46.9158	0.0000	61.1311	0.0000	6.2638	0.0125
Weather at the end	11.5809	0.0000	15.0649	0.0000	6.0255	0.0005
Race category	5.2643	0.0218	–	–	–	–

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Table 3. Mean values of the number of ace points in association with the g.710T>G SNP genotypes

Genotype	All races		Short races (< 400 km)		Long races (> 500 km)	
	RR	AP ± SE	RR	AP ± SE	RR	AP ± SE
TT	1125	30.63 ± 1.09	629	29.47 ± 1.44	496	32.10[a] ± 1.65
GT	1127	29.22 ± 1.08	645	30.45 ± 1.46	482	27.58 ± 1.60
GG	337	25.09 ± 1.86	189	26.50 ± 2.52	148	23.30[b] ± 2.75
Total	2589	29.30 ± 0.71	1463	29.52 ± 0.95	1126	29.01 ± 1.07

AP = mean of ace points, RR = number of race records, SE = standard error of the mean

[a,b]Statistically significant differences ($P \leq 0.05$)

cysteine to a nonpolar glycine (Cys83Gly), an amino acid with markedly different characteristics. Cysteine residues are very important for the stability of a protein due to their ability to form disulphide bonds. Therefore, the loss of cysteine involved in bond formation, and thus the loss of a bond, may hold serious structural and functional consequences for a protein (Li et al. 2011). There are 13 cysteine residues in pigeon feather keratin (GenBank: BAA33472.1). Using the DIANNA 1.1 web server which is based on artificial neural networks (Ferre and Clote 2006), and the DBCP web server based on amino acid sequence alignments (Lin and Tseng 2010), we predicted the cysteine at position 83 to form a disulphide bond with the cysteine at position 101 of pigeon feather keratin. Additionally, analysis carried out on the DINOSOLVE web server (Yaseen and Li 2013), indicated with high confidence that the Cys83 forms a disulphide bond ($P = 0.7919$), but in this case, it was predicted to most likely pair with Cys30 ($P = 0.7971$). In contrast, the output from the DISULFIND web server (Ceroni et al. 2006) showed no involvement of Cys83 in forming intramolecular bonds, but with only a moderate confidence level of six (nine indicates maximum confidence). As it is well-established that disulphide bonds are also formed between cysteine residues from different protein chains (Niu et al. 2013), the loss of cysteine could drastically influence the complicated three-dimensional structure of structural proteins such as feather keratin.

The potential effect of the Cys83Gly amino acid change was previously investigated using different bioinformatics tools. I-Mutant 2.0.7 (Capriotti et al. 2005) predicted the Cys83Gly amino acid change to decrease the Gibbs free energy of unfolding of the protein and to disrupt its stability with a high reliability index of nine, which is the maximum value. MUpro (Cheng et al. 2006) and iStable (Chen et al. 2013) also indicated that the Cys83Gly amino acid change decreases the stability of the protein, with confidence levels of –0.71 (maximum confidence = –1) and 0.75 (maximum confidence = 1), respectively. PMut (Ferrer-Costa et al. 2005) predicted the Cys83Gly amino acid change to be pathological (pathogenicity index = 0.7526, maximum value = 1) for feather keratin, but with only a moderate confidence index of five (maximum value = 9). SNAP (Bromberg et al. 2008) indicated that the substitution was non-neutral with an expected accuracy of 58%. In contrast, Provean 1.1 (Choi et al. 2012) predicted the Cys83Gly amino acid change to be neutral, while SIFT (Kumar et al. 2009) indicated it to be tolerated with a maximum score (1) based on the alignment of 111 sequences.

In this study, we have demonstrated that pigeons carrying the *TT* genotype for the g.710T>G SNP in the *F-KER* gene showed better racing performance in comparison with those carrying *GG*. However, the significance of this phenomenon was confirmed only for long-distance races. We propose that the loss of cysteine at position 83 of the pigeon feather keratin protein may affect the structure of feathers, thus changing their biomechanical characteristics, and, consequently, influencing the flying ability of pigeons.

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