Animal Science Papers and Reports vol. 26 (2008) no. 3, 219-226
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

DNA polymorphism of the α^A-globin gene in domestic pigeon*

Andrzej Dybus[1,], Ming-Huang Chang[2], Yeong-Hsiang Cheng[3], Iwona Szatkowska[1]**

[1] Laboratory of Molecular Cytogenetics, Faculty of Biotechnology and Animal Science, Agricultural University of Szczecin, 71-460 Szczecin, Poland

[2] Department of Veterinary Medicine, National Chia-Yi University, Chia-Yi, Taiwan

[3] Institute of Biotechnology, Department of Animal Science, National I-Lan University, Taiwan

(Received March 15, 2008; accepted June 1, 2008)

The study aimed at identifying the polymorphism of domestic pigeon α^A-globin gene which can be a potential homing marker in selection of racing pigeons. A total of 329 domestic pigeons were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The PCR products were digested with 17 restriction endonucleases. One RFLP – detected with *Hph*I – was found in intron 1 and represented a C/T mutation. The second RFLP – detected with *Bse*LI – was a point mutation in the 3'UTR region of the gene (C/T mutation). The polymorphism in the 3'UTR region of the pigeon α^A-globin gene can potentially affect the stability of mRNA and modify the gene expression. The mechanisms of haemoglobin function reflecting variants of the α^A-globin gene remain unknown.

KEY WORDS: domestic pigeons / genetic markers / globin gene / PCR-RFLP

Human adult haemoglobin (Hb) is tetrameric and consists of two α and two β polypeptide chains. In human Hb the amino acid sequences of α and β chains determine the folding of each chain and influences the oxygen binding properties [Perutz 1983]. Degree of oxygen binding is associated with small changes in the tertiary structure of

*Supported by the Agricultural University of Szczecin, grant no. BW/HB/6/2004
**Corresponding author: andrzej.dybus@biot.ar.szczecin.pl

segments near the haems and a large shift in the quaternary structure from the T(ense) to the R(elaxed) state. Various ligands (protons, carbon dioxide, organic phosphates) bind preferentially to deoxygenated Hb, forming salt bridges within and between the chains that stabilize the T-structure, lowering its oxygen affinity and resulting in cooperative oxygen binding [Wells 1999].

Amino acid changes in Hb sequences near the haem-oxygen binding sites or at some other site which destabilizes the structure of the molecule, have the potential to interfere with the normal function of this protein in oxygen transport; some of the haemoglobinopathies arising as a single point mutation of one amino acid [Wells and Brennan 1992, Labie and Elion 1996]. Functionally important differences among animal species have evolved by only a few amino acid substitutions in key positions [Wells 1999, Weber and Fago 2004]. For instance, one amino acid substitution in its major Hb that is not found in other avian and mammalian species, distinguishes the bar-headed goose that crosses the Himalayas at altitudes near 10 km and has higher blood oxygen affinity than the greylag goose [Perutz 1983].

Adult pigeons possess only a single haemoglobin component, HbA [Sultana *et al.* 1989] coded by the α^A-globin gene [Ikehara *et al.* 1997]. In contrast, in most bird species HbA is accompanied by less abundant HbD [Takei *et al.* 1975, Hiebl *et al*. 1987, Eguchi *et al.* 1995]. It is possible, that the long-lasting and intensive selection of homing pigeons towards increased endurance of homeward-flight, have caused adaptive mutations in the pigeon's globin gene.

The aim of this paper was to study polymorphisms of the domestic pigeon (*Columba livia* var. *domestica*) α^A-globin gene (*AGLOB*) as potential homing markers for racing abilities.

Material and methods

A total of 329 domestic pigeons (252 homing and 77 non-homing) were genotyped.

Within the **homing** (H) group two subgroups were considered: 127 top-racing crossbreds (subdivided into short/middle distance and long distance birds) from sixteen lofts owned by Polish breeders (Northwestern Poland) and 125 purebreds from the *Natural Antwerp* Breeding Station (Belgium).

The **non-homing** (NH) group was composed of Carrier, Polish Krymka, Danzing Highflyer, Pigeon polonais, German Barb, Fantail, German Magpie, German Highflyer, Strasser, King, Budapestian Highflier, Straslund Highflier, German Nun and Polish Musian pigeons kept at four lofts owned by Polish breeders (Szczecin city, Northwestern Poland).

The crude DNA was isolated from the 3 µl blood samples using the MasterPure™ kit (EPICENTRE TECHNOLOGIES). The PCR-RFLP method was used to identify the polymorphism. The PCR primers were designed to produce an 890-base pair amplification product, encompassing a part of exon 1 (80 bp), exons 2 and 3,

intervening introns, and 217 bp of the 3'UTR (GenBank AB001981) using Primer 3 software. The forward (F) and reverse (R) primers' sequences were:

AGLO-F: 5'- AACGACAAGAGCAACGTGAAG-3' and

AGLO-R: 5'- CAAGAGCCCATTTCACCTACA-3'.

The PCR mixture contained approximately 60 ng of genomic DNA, 10 pmol of each primer, 1×PCR buffer, 1.5 mM $MgCl_2$, 200 µM dNTP and 0.3 Units Taq-polymerase (Eur_X) in a total volume of 15 µl. The following cycles were applied: denaturation at 95ºC for 2 min followed by 33 cycles at 95ºC for 20 s, primer annealing at 61ºC for 40 s, and PCR products synthesis at 72ºC for 55 s, with final synthesis at 72ºC for 5 min. Amplified DNA was digested with seventeen restriction endonucleases: *Alu*I, *Apo*I, *Bse*LI, *Bsp*143II, *Bst*1107, *Cfr*13I, *Eco*47I, *Eco*RI, *Hae*III, *Hin*6I, *Hind*III, *Hinf*I, *Hpa*II, *Mva*I, *Pst*I, *Rsa*I, and *Vsp*I (MBI FERMENTAS). The digestion products were separated by horizontal electrophoresis (90 V, 50 min) using 2-4% agarose gels (PRONA) in 1 × TBE and 1.0 µM ethidium bromide. In the case of *Bse*LI (5'-CCNNNNN^NNGG-3') enzyme RFLP was applied.

The PCR products (*AA*, *AB* and *BB* genotypes) were sequenced in an ABI Prism Sequencer (PERKIN-ELMER) and analysed using the CHROMAS software. The sequencing was performed at the Polish Academy of Sciences Institute of Biochemistry and Biophysics, Warsaw. The nucleotide sequence of the new variant of the α^A-globin gene was submitted to the GeneBank database and registered under accession no. DQ629929.

Distributions of genotypes' frequencies were compared among analysed groups of pigeons using *chi* square test with Yates' correction.

Results and discussion

The following DNA restriction fragments were observed due to the *AGLOB/Bse*LI polymorphism: 524, 258, 77 and 31 bp for the $AGLOB^A$ and 524, 243, 77, 31 and 15 bp for the $AGLOB^B$ (Photo 1). The $AGLOB^{AA}$ genotype was detected only in two homing (H) pigeons. In the non-homing (NH) group, the $AGLOB^{AB}$ genotype (n=5) was detected in four Strasser and one King individual (Tab. 1).

The PCR products of H pigeons ($AGLOB^{AA}$, $AGLOB^{AB}$, $AGLOB^{BB}$) were isolated from agarose gels using DNA Gel-Out (A&A BIOTECHNOLOGY), sequenced in an ABI Prism Sequencer (PERKIN-ELMER) and analysed using CHOMAS software v.1.22. The sequencing was performed at the Polish Academy of Sciences Institute of Biochemistry and Biophysics, Warsaw. The sequences alignments were made for the two alleles using the BLAST2 software (http://www.ncbi.nlm.nih.gov/blast2).

Two polymorphic sites were identified: one in intron 1 and another in the 3'untranslated region (3'UTR). The first is either cytosine or thymine. In the case of cytosine the additional *Hph*I recognition site is observed. The second polymorphic site is either C**C**AAACAGGG in the $AGLOB^B$ (recognition site for *Bse*LI enzyme) or C**T**AAACAGGG (no digestion for this sequence) in the $AGLOB^A$ (Fig. 1 and 2).

Table 1. Frequencies of genotypes and alleles of the *AGLOB/Bse*LI

Group	Genotype			Alleles	
	AGLOB^{AA}	*AGLOB*^{AB}	*AGLOB*^{BB}	*AGLOB*^{A}	*AGLOB*^{B}
H – homing pigeons (n=252)	0.008 (n=2)	0.143 (n=36)	0.849 (n=214)	0.079	0.921
NH – non homing pigeons (n=77)	- -	0.065 (n=5)	0.935 (n=72)	0.032	0.968
Total (n=329)	0.006 (n=2)	0.125 (n=41)	0.869 (n=286)	0.068	0.932

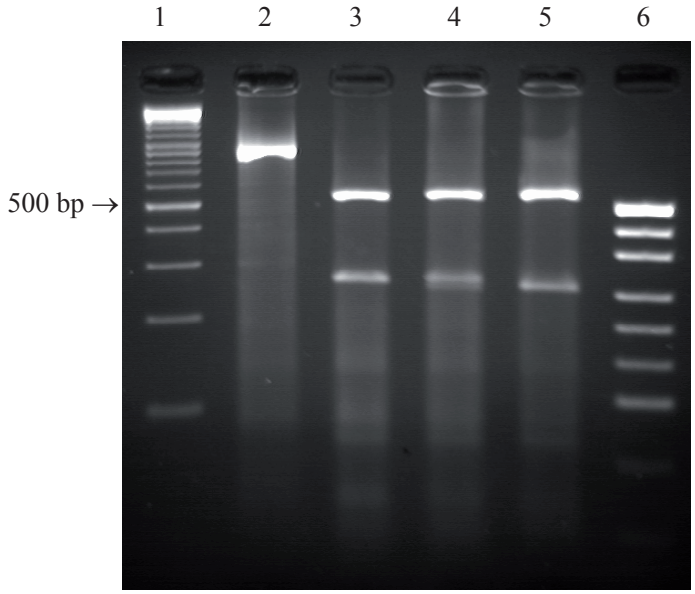

Photo 1. The PCR-RFLP analysis of *AGLOB/Bse*LI polymorphism by restriction fragments. Lane 1 – 100bp DNA Ladder Plus (MBI Fermentas), lane 2 – PCR product, lane 3 – *AGLOB*^{AA}, lane 4 – *AGLOB*^{AB}, lane 5 – *AGLOB*^{BB}, lane 6 – pUC19/*Msp*I (MBI Fermentas).

Half-lives of globin mRNAs are relatively long and range from 24 to 60 h [Lodish and Small 1976, Ross and Sullivan 1985]. The 3'UTRs are regulators of gene activity and play a crucial role in the post-transcriptional stage of gene expression [Hilleren and Parker 1999, Mitchell and Tollervey 2000] mainly through the modulation of mRNA stability by trans-acting proteins [Liebhaber and Russell 1998] or non-coding RNA [Moss 2000]. Within its 3'UTR the α-globin mRNA contains a stabilizing C-rich element (CRE) – Wang *et al*. [1995], Wang and Kiledjian [2000] – termed "the α-complex". This specific ribonucleoprotein complex correlates with mRNA stability by binding to the α-globin 3'UTR [Weiss and Liebhaber 1995, Wang *et al*. 1999].

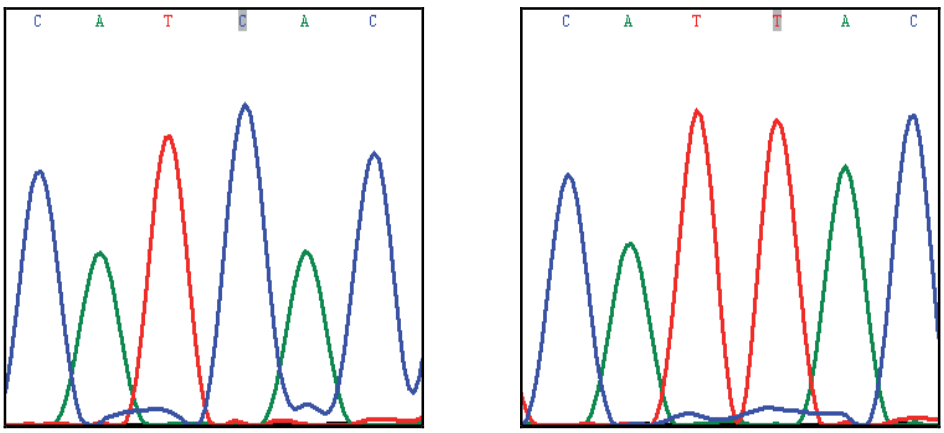

Fig. 1. Results of sequencing analysis (forward primer) – C/T substitution in intron 1.

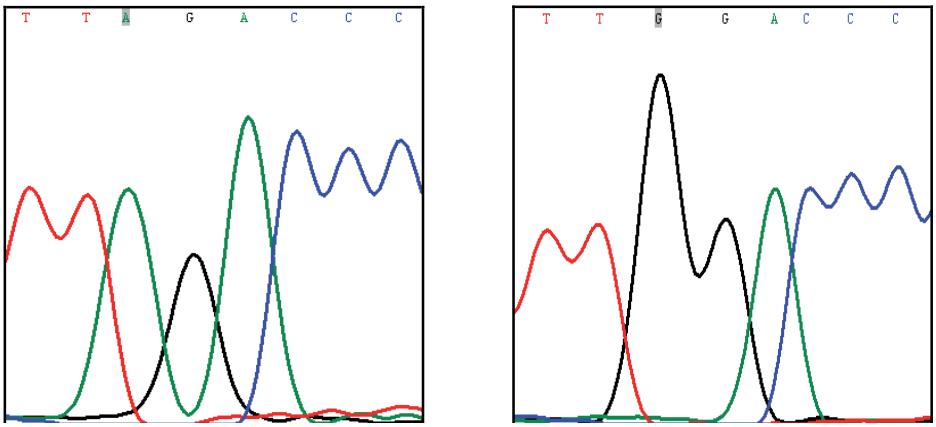

Fig. 2. Results of sequencing analysis (reverse primer) – C/T substitution in 3'UTR.

Cai *et al*. [1992] and Basak *et al*. [1993] reported mutations near the 5' region of the polyadenylation site considered to interfere with mRNA stability. Maragoudaki *et al*. [1998] reported the β-gene C→G mutation at 6 bp 3' to the termination codon. In the cited study an analysis of gene expression demonstrated a 20-34% reduction in mRNA levels associated with this +1480 C→G mutation compared to normal β-globin gene alleles.

The frequency of the *AGLOB*^{A} allele appeared low (0.068). Frequencies of genotypes in the H and NH pigeons were similar (*chi*-square = 3.36, P = 0.067) – Table 1. No differences were observed between the genotype distributions in short-middle and long distance H pigeons (*chi*-square = 1.92, P = 0.165). It is remarkable that in the group of long-distance H pigeons the frequency of *AGLOB*^{A} was fourfold higher (0.138) than in NH group (0.032) – Table 2.

Table 2. Frequencies of genotypes and alleles of the *AGLOB/Bse*LI in different purebred lines of homing (H) pigeons

Group	Genotype			Allele	
	$AGLOB^{AA}$	$AGLOB^{AB}$	$AGLOB^{BB}$	$AGLOB^{A}$	$AGLOB^{B}$
Short/middle distance					
Janssens	-	0.125	0.875	0.063	0.937
	-	(n=6)	(n=42)		
Van Dyck	-	0.250	0.750	0.125	0.875
	-	(n=3)	(n=9)		
total (n=60)	-	0.150	0.850	0.075	0.925
	-	(n=9)	(n=51)		
Long distance					
Bricoux	-	0.333	0.667	0.167	0.833
	-	(n=2)	(n=4)		
De Smet-Matthys	-	0.083	0.917	0.042	0.958
	-	(n=1)	(n=11)		
Stichelbaut	-	0.167	0.833	0.083	0.917
	-	(n=2)	(n=10)		
Thone	-	0.364	0.636	0.182	0.818
	-	(n=8)	(n=14)		
Wanroy	0.083	0.167	0.750	0.167	0.833
	(n=1)	(n=2)	(n=9)		
Aarden	-	1.000	-	0.500	0.500
	-	(n=1)	-		
total (n=65)	0.016	0.246	0.738	0.138	0.862
	(n=1)	(n=16)	(n=48)		
Natural Antwerp (n=125)	0.008 (n=1)	0.200 (n=25)	0.792 (n=99)	0.108	0.892

The polymorphism identified in the 3'UTR region of the pigeon α^{A}-globin gene may influence the stability of the mRNA and differentiate gene expression. Thus, the effect of different variants in the α^{A}-globin upon gene expression and biochemical parametres of blood oxygen-carrying ability may prove useful for genetic improvement of racing pigeons. However, the effect of the rare $AGLOB^{A}$ allele on homing performance should be verified in further, more advanced studies.

Acknowledgement. *The authors are grateful to Dr. Andrzej Oreńczak for help in collecting the biological samples.*

REFERENCES

1. BASAK A.N., OZER A., KINDAR B., AKAR N., 1993 – A novel 13 bp deletion in the 3'UTR of the β globin gene causes β thalassemia in a Turkish patient. ***Hemoglobin*** 17, 551-555.
2. CAI S.P., ENG B., FRANCOMBE W.H., KENDALL A.G., WAYE J.S., CHUI D.H., 1992 – Two novel β thalassemia mutations in the 5' and 3' noncoding regions of the β globin gene. ***Blood*** 79, 1342-1346.
3. EGUCHI Y., IKEHARA T., KAYO S., EGUCHI T., TAKEI H., 1995 – Amino acid sequence of alpha- and beta-polypeptide chains of turkey (*Meleagris gallopavo*) hemoglobin. ***Biological Chemistry Hoppe-Seyler*** 376, 437-440.
4. HIEBL I., KOSTERS J., BRAUNITZER G., 1987 – High-altitude respiration of birds. The primary structures of the major and minor hemoglobin component of adult goshawk (*Accipiter gentilis*, *Accipitrinae*). ***Biological Chemistry Hoppe-Seyler*** 368, 333-342.
5. HILLEREN P., PARKER R., 1999 – Mechanisms of mRNA surveillance in eukaryotes. ***Annual Review of Genetics*** 33, 229-260.
6. IKEHARA T., EGUCHI Y., KAYO S., TAKEI H., 1997 – Isolation and sequencing of two alpha-globin genes alpha(A) and alpha(D) in pigeon and evidence for embryo-specific expression of the alpha(D)-globin gene. ***Biochemical and Biophysical Research Communications*** 234, 450-453.
7. LABIE D., ELION J., 1996 – Sequence polymorphisms of potential functional relevance in the beta-globin gene locus. ***Hemoglobin*** 20, 85-101.
8. LIEBHABER S.A., RUSSELL J.E., 1998 – Expression and developmental control of the human alpha-globin gene cluster. ***Annals of the New York Academy of Sciences*** 850, 54-63.
9. LODISH H.F., SMALL B., 1976 – Different lifetimes of reticulocyte messenger RNA. ***Cell*** 7, 59-65.
10. MARAGOUDAKI E., VRETTOU C., KANAVAKIS E., TRAEGER-SYNODINOS J.A., METAXOTOU-MAVROMMATI A., KATTAMIS C., 1998 – Molecular, haematological and clinical studies of a silent β-gene C→G mutation at 6 bp 3' to the termination codon (+1480 C→G) in twelve Greek families. ***British Journal of Haematology*** 103, 45-51.
11. MITCHELL P., TOLLERVEY D., 2000 – mRNA stability in eukaryotes. ***Current Opinion in Genetics & Development*** 10, 193-198.
12. MOSS E.G., 2000 – Non-coding RNAs: lightning strikes twice. ***Current Biology: CB*** 10, 436-439.
13. PERUTZ M.F., 1983 – Species adaptation in a protein molecule. ***Molecular Biology and Evolution*** 1, 1-28.
14. ROSS J., SULLIVAN T.D., 1985 – Half-lives of beta and gamma globin messenger RNAs and of protein synthetic capacity in cultured human reticulocytes. ***Blood*** 66, 1149-1154.
15. SULTANA C., ABBASI A., ZAIDI Z.H., 1989 – Primary structure of hemoglobin α-chain of *Columba livia* (Grey Wild Pigeon). ***Journal of Protein Chemistry*** 8, 629-646.
16. TAKEI H., OTA Y., WU K., KIYOHARA T., MATSUDA G., 1975 – Amino acid sequence of the alpha chain of chicken AI hemoglobin. ***The Journal of Biochemistry*** 77, 1345-1347.
17. WANG X., KILEDJIAN M., WEISS I.M., LIEBHABER S.A., 1995 – Detection and characterization of a 3' untranslated region ribonucleoprotein complex associated with human alpha-globin mRNA stability. ***Molecular and Cellular Biology*** 15, 1769-1777.
18. WANG Z., DAY N., TRIFILLIS P., KILEDJIAN M., 1999 – An mRNA stability complex functions with poly(A)-binding protein to stabilize mRNA in vitro. ***Molecular and Cellular Biology*** 19, 4552-4560.
19. WANG Z., KILEDJIAN M., 2000 – The poly (A)-binding protein and an mRNA stability protein jointly regulate an endoribonuclease activity. ***Molecular and Cellular Biology*** 20, 6334-6341.

20. WEBER R.E., FAGO A., 2004 – Functional adaptation and its molecular basis in vertebrate hemoglobins, neuroglobins and cytoglobins. ***Respiratory Physiology & Neurobiology*** 144, 141-159.
21. WEISS I.M., LIEBHABER S.A., 1995 – Erythroid cell-specific mRNA stability elements in the alpha 2-globin 3' nontranslated region. ***Molecular and Cellular Biology*** 15, 2457-2465.
22. WELLS R.M., 1999 – Evolution of haemoglobin function: molecular adaptations to environment. ***Clinical and Experimental Pharmacology & Physiology*** 26, 591-595.
23. WELLS R.M., BRENNAN S.O., 1992 – The detection and importance of functionally abnormal hemoglobins. ***The New Zealand Medical Journal*** 105, 329-330.

Andrzej Dybus, Ming-Huang Chang,
Yeong-Hsiang Cheng, Iwona Szatkowska

Polimorfizm DNA w genie α^A-globiny gołębia domowego

S t r e s z c z e n i e

Celem pracy była identyfikacja polimorfizmu w genie α^A-*globiny* gołębia domowego, który może być rozpatrywany jako potencjalny marker dla cech wydolności powrotnolotnej w selekcji gołębi pocztowych (sportowych). Badaniami objęto 329 gołębi domowych, wykorzystano technikę PCR-RFLP. W poszukiwaniu zmienności sekwencji nukleotydowej zastosowano 17 enzymów restrykcyjnych. Zidentyfikowano dwa miejsca polimorficzne: pierwsze dla enzymu *Hph*I zlokalizowane w 1 intronie genu – substytucja C/T – oraz drugie dla *Bse*LI w rejonie 3'UTR genu (C/T). Polimorfizm w rejonie 3'UTR genu α^A-*globiny* gołębia może potencjalnie wpływć na stabilność mRNA, modyfikując przez to ekspresję genu. Wpływ opisanej mutacji w rejonie 3'UTR na funkcje hemoglobiny pozostaje nieznany.