Indian Journal of Animal Sciences **85** (4): 411–413, April 2015/Article

Variability within *IGF1* and its linkage to body size and predisposition to hip dysplasia in dogs

ANNA FROST[1], W S PROSKURA[2], J BARNIK[3], I SZATKOWSKA[4] and A DYBUS[5]

West Pomeranian University of Technology, Szczecin Doktora Judyma 6, 71-466 Szczecin, Poland

Received: 29 August 2013; Accepted: 19 November 2014

ABSTRACT

Insulin-like growth factor (IGF) plays a very important role in development and functioning of joints, and is also considered as one which may be linked to body size in domestic dogs (*Canis familiaris*). The aim of this study was to establish if there is an association of g.41221438A>G SNP within insulin-like growth factor-1 gene (IGF1) with determination of body size and with predisposition to hip-dysplasia in domestic dogs. There was a significant difference of genotypes distribution between groups of small and large dogs ($P \leq 0.001$) and also between groups of individuals predisposed and non-predisposed to hip dysplasia ($P \leq 0.001$). On the basis of this research we propose g.41221438A>G single nucleotide polymorphism as a very interesting candidate for genetic marker of both body size and predisposition to hip-dysplasia in dogs.

Key words: Body size, *Canis familiaris*, Hip dysplasia, *IGF1* gene

The *IGF1* gene is considered as the one which may be linked to body size in dogs. The *IGF1* gene locus in dogs was mapped on 15 chromosome. Sutter *et al.* (2007) included an association analysis of 20 SNPs and body size in dogs and indicated g.44228468A>G SNP within *IGF1* gene (NC_006597.1, CanFam1.0, minus strand), which we annotated as g.41221438A>G (NC_006597.3), as the most promising genetic marker for that trait.

Insulin-like growth factor (IGF) plays a very important role in development and functioning of joints, and is strictly involved in the process of chondrogenesis (Hill and Logan 1992). Loeser *et al.* (2000) indicated that disorder in distribution and in functionality of IGF is linked to occurrence of degenerative joint disease. It is also known as osteoarthritis (OA) and is one of the most common causes of joints damage in mammals. OA leads to deterioration of cartilage resulting finally in painful joints dysfunction (Paradis *et al.* 2003). Osteoarthritis appears especially in aging dogs and cats but it very often remains undiagnosed. Currently, there is no therapy which could completely cure the joints affected by OA. But with appropriate treatment (Rychel 2010) progress of the disease can be slowed or even stopped and the damaged joints can be partially regenerated. In dogs, commonly OA occurs simultaneously with other joint diseases, such as hip dysplasia, elbow dysplasia or cranial cruciate ligament rupture (Clements *et al.* 2010).

The characteristic symptoms of hip-dysplasia in dogs are delayed onset of the capital femoral ossification (Madsen *et al.* 1991, Todhunter *et al.* 1997), incongruity between acetabulum and femoral head (Janutta *et al.* 2005) and laxity of the joints (Lust *et al.* 1993, Smith *et al.* 1993). The last one can lead to cartilage degeneration, especially in hip joint (LaFond *et al.* 2002). The appearance of canine hip-dysplasia is very common in large, small as well as mixed breeds (Guiliard 2003).

The hip-dysplasia occurrence in dogs is a heritable quantitative trait, and the degree of heritability in different dog breeds vary from 0.17 to 0.6 (Fisher 1979, Lingaas and Klementsdaal 1990, Tomlinson and Mclaughin 1996, Swenson *et al.* 1997). Of the environmental factors linked to predisposition for CHD occurrence, body size seems to be the most important. Whereas, it was established that size of body may be determined in some part by genetic factors, especially, *IGF1* gene and variability within it (Chase *et al.* 2009).

The main aim of the present study was to estimate allelic and genotypic frequencies of g.41221438A>G (NC_006597.3, CanFam3.1, minus strand) substitution within canine *IGF1* gene. Additionally, we investigated possible association of that SNP with body size and predispositions for hip-dysplasia in dogs.

MATERIALS AND METHODS

Dogs (188) of several breeds were analyzed in this study. Genomic DNA was isolated from blood samples (5 µl) using DNA purification kit.

Present address: [1,2]PhD student (afrost@zut.edu.pl, wproskura@zut.edu.pl), [3]Student (fred86@poczta.onet.pl), [4]Professor (iszatkowska@zut.edu.pl), [5]Assistant Professor, (adybus@zut.edu.pl), Laboratory of Molecular Cytogenetics.

Genotypes of g.41221438A>G SNP were determined using PCR-RFLP method. PCR primers were designed as previously described (Sutter *et al.* 2007). The PCR mixture contained ~40 ng of DNA template, 25 pmol of each primer, 1×PCR buffer, 2mM $MgCl_2$, 0.2mM dNTP and 0.5 units of recombinant *Taq*-polymerase in a total volume of 15 ìl. The following temperature cycles were applied: denaturation at 92°C/5 min, followed by 42 cycles at 92°C/30 sec, primer annealing at 52°C/30 sec, amplicons synthesis at 72°C/30 sec and final synthesis at 72°C/5 min. After amplification, specificity and efficiency of PCR products (5 µl) was evaluated by electrophoresis in 1.5% agarose gels (PRONA) in 1×TBE and digested (10 µl) with 3 units of *Msp*I restriction endonuclease (NEB). Restriction fragments were separated through 2% agarose gels (PRONA). Gels were stained with ethidium bromide.

Differences in allelic and genotypic distribution between the groups of small (<9 kg) and large (>30 kg) dogs, as well as between the groups of dogs predisposed and non-predisposed to CHD, were estimated using the chi-square test.

RESULTS AND DISCUSSION

The DNA fragment of 642 base pairs was amplified. After digestion with *Msp*I restriction enzyme 3 genotypes were observed (Fig. 1), as follows: *AA* (642 bp), *AG* (642, 344, 298 bp) and *GG* (344, 298 bp).

The distribution of alleles and genotypes of g.41221438A>G SNP within *IGF1* gene was estimated for small and large dogs (Table 1) and also for groups of dogs predisposed and non-predisposed to hip-dysplasia (Table 2).

The population studied was divided according to body weight into groups of small (<9 kg) and large dogs (>30 kg). Allele *A* was predominant in small dogs, while it was totally opposite in the large dogs in which the *G* allele was prevalent (Table 1). Statistical analysis showed the difference in genotype distribution between analysed groups

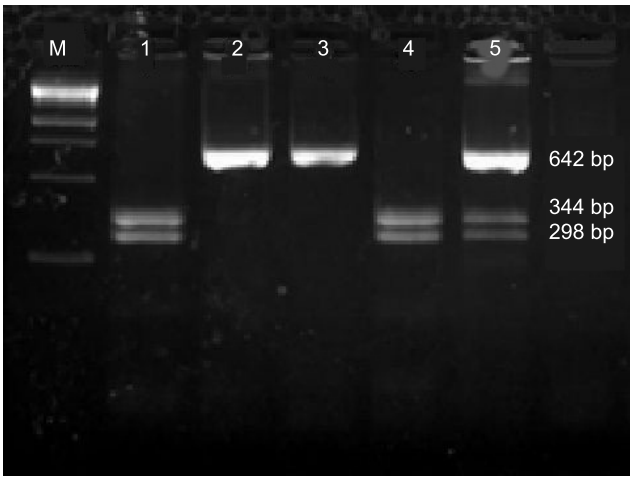

Fig. 1. Genotyping of g.41221438A>G SNP in canine *IGF1* gene. Lane M, DNA marker; lanes 1, 4, *GG*; lanes 2, 3, *AA*; lane 5, *AG*.

Table 1. Genotypic and allelic frequencies of g.41221438A>G SNP within *IGF1* gene in groups of dogs belonging to breeds classified as small (<9 kg) or large (>30 kg)

Group	Genotype			Allele		χ^2
	AA	AG	GG	A	G	
<9 kg (n=41)	0.537 (n=22)	0.293 (n=12)	0.170 (n=7)	0.680	0.320	39.86
>30 kg (n=121)	0.107 (n=13)	0.24 (n=29)	0.653 (n=79)	0.227	0.773	P≤0.001

Table 2. Genotypic and allelic frequencies of g.41221438A>G SNP within *IGF1* gene in groups of dogs belonging to breeds classified as predisposed or as non-predisposed to hip dysplasia

Group	Genotype			Allele		χ^2
	AA	AG	GG	A	G	
Predisposed (n = 94)	0.128 (n=12)	0.213 (n=20)	0.659 (n=62)	0.234	0.766	25.43
Non-predisposed (n = 94)	0.400 (n=38)	0.280 (n=26)	0.320 (n=30)	0.540	0.460	P≤0.001
Total (n = 188)	0.266 (n=50)	0.245 (n=46)	0.489 (n=92)	0.388	0.612	

(P≤0.001) and confirmed the possible association of g.41221438A>G SNP with body size in dogs.

IGF1 gene is a strong genetic factor which determines body weight in mammals. Baker *et al.* (1993) indicated that knockout mice with a complete deficiency of that gene were characterized by 40% less birth weight in comparison to normal mice. Whereas, partial deletion of *IGF1* gene cause intrauterine growth retardation and severe postnatal growth disorders (Woods *et al.* 1996, 1997).

Sutter *et al.* (2007) performed an analysis of haplotypes containing 20 SNPs within canine *IGF1* gene and reported one common haplotype for 14 small and 3 giant dog breeds. Of the 20 SNPs studied, g.41221438A>G SNP was indicated as the most promising genetic marker for body size in dogs. The *A* allele was predominant in all small dog breeds, while the *G* allele was predominant in all giant breeds (Sutter *et al.* 2007) and in the present study similar tendency was observed. On the basis of previous studies (Sutter *et al.* 2007) and our study, it may be assumed that g.41221438A>G SNP is strictly linked to body size in dogs.

It is well-known that breed size is correlated with occurrence of some orthopedic diseases found in many dog breeds. According to that fact, Chase *et al.* (2009) used g.41221438A allele frequency for defining dog breeds, as follows: the breeds with a frequency of more than 0.85 was classified as small, while the breeds with a frequency of less than 0.15 was classified as large. They estimated a correlation of IGF1 with hip dysplasia, patella luxation, and pancreatitis (P<0.01) and assumed that selection for size has influenced frequency of these diseases.

Finding the genetic marker for predisposition of hip-

dysplasia occurrence in dogs could be very helpful in indicating susceptible individuals. Thus, specimens predisposed to CHD could be diagnosed in initial phase of disease progress, until the joints will be damaged significantly.

IGF plays a very important role in the overall process of chondrogenesis in dogs, as well as in humans. For that reason, it is considered that the polymorphism within *IGF1* gene coding for that factor, could be crucial in development of hip dysplasia. We analysed the g.41221438A>G polymorphism within *IGF1* gene in dogs. In the group of individuals predisposed to hip dysplasia, the *GG* genotype was the most frequent (0.659) and the predominant allele was G (0.766), while in the group of non-predisposed dogs the most frequent was *AA* variant (0.400) and the predominant allele was A (0.540). The differences in genotype distribution between the 2 analysed groups were observed ($P \leq 0.001$). We found that the SNP analysed in the present study could be linked to inborn predisposition for hip-dysplasia in dogs.

On the basis of our study we propose that the g.41221438A>G single nucleotide polymorphism as a very interesting candidate for genetic marker of both body size and predisposition to hip-dysplasia in dogs. Nevertheless, the genetic background of determination of body size in dogs and other mammals seems to be good subject for further research.

REFERENCES

Baker J, Liu J P, Robertson E J and Efstratiadis A. 1993. Role of insulin-like growth factors in embryonic and postnatal growth. *Cell* **75**: 73–82.

Chase K, Jones P, Martin A, Ostrander E A and Lark K G. 2009. Genetic mapping of fixed phenotypes: disease frequency as a breed characteristic. *Journal of Heredity* **100**: 537–41.

Clements D N, Short A D, Barnes A, Kennedy L J, Ferguson J F, Butterworth S J, Fitzpatrick N, Pead M, Bennett D, Innes J F, Carter S D and Ollier W E. 2010. A candidate gene study of canine joint diseases. *Journal of Heredity* **101**: 54–60.

Fisher T M. 1979. The inheritance of canine hip-dysplasia. *Modern Veterinary Practice* **60**: 897–900.

Guilliard M J. 2003. Hip dysplasia in dogs. *Veterinary Record* **152**: 120.

Hill D J and Logan A. 1992. Peptide growth factors and their interactions during chondrogenesis. *Progress in Growth Factor Research* **4**: 45–68.

Janutta V, Hamann H and Distl O. 2005. Complex segregation analysis of canine hip dysplasia in German shepherd dogs. *Journal of Heredity* **97**: 13–20.

LaFond E, Breur G J and Austin C C. 2002. Breed susceptibility for developmental orthopedic diseases. *Journal of the American Animal Hospital Association* **38**: 467–77.

Lingaas F and Klemetsdaal G. 1990. Breeding values and genetic trend for hip-dysplasia in the Norwegian Golden retriever population. *Journal of Animal Breeding and Genetics* **107**: 437–43.

Loeser R F, Shanker G, Carlson C S, Gardin J F, Shelton B J and Sonntag W E. 2000. Reduction in the chondrocyte response to insulin-like growth factor 1 in aging and osteoarthritis: Studies in a non-human primate model of naturally occurring disease. *Arthritis and Rheumatis* **9**: 2110–20.

Lust G, Williams A J, Burton-Wurster N, Pijanowski G J, Beck K A, Rubin G and Smith G K. 1993. Joint laxity and its association with hip dysplasia in Labrador retrievers. *American Journal of Veterinary Research* **54**: 1990–99.

Madsen J S, Reimann I and Svalastoga E. 1991. Delayed ossification of the femoral head in dogs with hip dysplasia. *Journal of Small Animal Practice* **32**: 351–54.

Paradis M, Sauve F, Charest J, Refsal K R, Moreau M and Dupuis J. 2003. Effects of moderate to severe osteoarthritis on canine thyroid function. *Canadian Veterinary Journal* **44**: 407–12.

Rychel J K. 2010. Diagnosis and Treatment of Osteoarthritis. *Topics in Companion Animal Medicine* **25**: 20–25.

Smith G K, Gregor T P, Rhodes W H and Biery D N. 1993. Coxofemoral joint laxity from distraction radiography and its contemporaneous and prospective correlation with laxity, subjective score, and evidence of degenerative joint disease from conventional hip-extended radiography in dogs. *American Journal of Veterinary Research* **54**: 1021–42.

Sutter N B, Bustamente C D, Chase K, Gray M M, Zhao K, Zhu L, Padhukasahasram B, Karlins E, Davis S, Jones P G, Quignon P, Johnson G S, Parker H G, Fretwell N, Mosher D S, Lawler D F, Satyaraj E, Nordborg M, Lark K G, Wayne R K and Ostrander E A. 2007. A single IGF1 allele is a major determinant of small size in dogs. *Science* **316**: 112–15.

Swenson L, Audell, L and Hedhammar, A Ê. 1997. Prevalence, inheritance and selection for hip-dysplasia in seven breeds of dogs in Sweden and a cost/benefit analysis of a screening and control program. *Journal of the American Veterinary Medical Association* **210**: 207–14.

Todhunter R J, Zachos T A, Gilbert R O, Erb H N, Williams A J, Burton-Wurster N and Lust G. 1997. Onset of epiphyseal mineralization and growth plate closure in radiographically normal and dysplastic Labrador retrievers. *Journal of the American Veterinary Medical Association* **210**: 1458–62.

Tomlinson J and Mclaughlin R. 1996. Canine hip-dysplasia: developmental factors, clinical signs, and initial examination steps. *Veterinary Medicine* **91**: 26–33.

Woods K A, Camacho-Hübner C, Savage M O and Clark A J. 1996. Intrauterine growth retardation and postnatal growth failure associated with deletion of the insulin-like growth factor I gene. *The New England Journal of Medicine* **335**: 1363–67.

Woods K A, Camacho-Hübner C, Barter D, Clark A J and Savage M O. 1997. Insulin-like growth factor I gene deletion causing intrauterine growth retardation and severe short stature. *Acta Paediatrica. Supplement* **423**: 39–45.