Short Communication

Detection of Genetic Variations Using RAPD Markers in Siberian Huskies Affected with Swimming Puppy Syndrome

Siriwadee Chomdej [1,2] Arphaphorn Dokphut [1,2] Waranee Pradit [1,2] Korakot Nganvongpanit[2,3] *

Abstract

Swimming puppy syndrome (SPS) is a curious disease found in dogs. The causes and pathology of this disease are still unknown, although heredity is usually considered to be one of the underlying factors. The objective of this study was to investigate the association between genetics and SPS in a Siberian husky model. Four Siberian husky puppies diagnosed with SPS were subjects of this study, while three other healthy Siberian husky puppies served as controls. Blood samples were collected for DNA isolation, using random amplified polymorphic DNA (RAPD) technique with 16 random primers. No genetic variation was found between affected puppies and healthy puppies, which indicated that swimming puppy syndrome is not controlled by genetics.

Keywords: dog, RAPD, Swimming puppy syndrome

[1] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[2] *Materials Science Research Center, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand*
[3] *Animal Bone and Joint Research Laboratory, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand*
**Correspondence author E-mail: korakot.n@cmu.ac.th*

บทคัดย่อ

การตรวจสอบความหลากหลายทางพันธุกรรมของกลุ่มอาการขากางในลูกสุนัข ในสุนัขสายพันธุ์ไซบีเรียนฮัสกีด้วยเทคนิค RAPD marker

สิริวดี ชมเดช [1,2] อาภาพร ดอกพุฒ [1,2] วารณี ประดิษฐ์ [1,2] กรกฎ งานวงศ์พาณิชย์ [2,3]*

กลุ่มอาการขากางในลูกสุนัข (Swimming puppy syndrome; SPS) เป็นความผิดปกติที่น่าสนใจในสุนัข จนถึงปัจจุบันสาเหตุและกระบวนการเกิดโรคยังคงไม่ทราบแน่ชัด แต่เชื่อว่าพันธุกรรมอาจเป็นสาเหตุหนึ่งของความผิดปกตินี้ การศึกษาครั้งนี้ต้องการหาความสัมพันธ์ระหว่างพันธุกรรมและการเกิดกลุ่มอาการขากางในลูกสุนัขพันธุ์ไซบีเรียนฮัสกี้ เก็บตัวอย่างเลือดจากลูกสุนัขพันธุ์ไซบีเรียนฮัสกี้ จำนวน 7 ตัว แสดงอาการกลุ่มอาการขากางในลูกสุนัข จำนวน 4 ตัวและปกติ 3 ตัว ทำการสกัดดีเอ็นเอจากตัวอย่างเลือดเพื่อนำมาหายีนที่มีความสัมพันธ์กับกลุ่มอาการขากางในลูกสุนัขด้วยวิธี random amplified polymorphic DNA (RAPD) โดยใช้ไพรเมอร์แบบสุ่ม จำนวน 16 จากการศึกษาไม่พบความสัมพันธ์ของลักษณะพันธุกรรมและการเกิดกลุ่มอาการขากางในลูกสุนัขซึ่งเป็นไปได้ว่าการเกิดกลุ่มอาการขากางในลูกสุนัขไม่ได้มีความเกี่ยวข้องกับพันธุกรรม

คำสำคัญ: RAPD กลุ่มอาการขากางในลูกสุนัข สุนัข

[1] *ภาควิชาชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ อ.เมือง จ.เชียงใหม่ 50200*

[2] *ศูนย์วิจัยวัสดุศาสตร์ คณะวิทยาศาสตร์ มหาวิทยาลัยเชียงใหม่ อ.เมือง จ.เชียงใหม่ 50200*

[3] *ห้องปฏิบัติการวิจัยโรคกระดูกและข้อในสัตว์ ภาควิชาชีวศาสตร์ทางสัตวแพทย์และสัตวแพทย์สาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่ อ. เมือง จ. เชียงใหม่ 50100*

***ผู้รับผิดชอบบทความ E-mail:** korakot.n@cmu.ac.th*

Introduction

Swimming puppy syndrome (SPS) is an as-yet unexplained disease mostly occurring in some neonatal dogs. This syndrome is also known as swimmer syndrome, flat pup syndrome, splay leg (paraparesis), splay weak (tetraparesis), and myofibrillar hypoplasia. Physiological changes and pathogenesis of this disease are unclear, and the limited number of published reports is a major obstacle to increasing knowledge of this disorder. Although some publications have presented methods for the treatment of this disease (Verhoeven et al., 2006; Yardimci et al., 2009), only one research report in 2012 published the results of serum biochemistry analysis (Nganvongpanit, 2012). Ngavingpanit (2012) reported that complete blood counts and blood chemistry in four Siberian husky puppies diagnosed with swimming puppy syndrome, when compared with four healthy Siberian husky puppies, only serum creatine kinase in affected puppies was significantly higher than in normal puppies. In 1977, Lorenz and colleague identified certain underlying factors of this syndrome. In the early stage of the disease, newborn puppies seem normal: they gain weight quickly, suck well and appear to be completely healthy. Signs begin to appear when the puppy learns to walk (2^{nd}–3^{rd} week), exhibiting spread-out legs like a swimmer. Heredity has often been attributed to be the primary underlying factor of SPS. Thus, the important question is: is this syndrome controlled by genetics? So far, scientists and veterinarians have not been able to answer this question. This case report investigates

Table 1 Case and control puppy information

Puppies affected with swimming puppy syndrome	**Case 1**	**Case 2**	**Case 3**	**Case 4**
Sex	Male	Male	Male	Female
Age (weeks)	6	7	8	8
Weight (kg)	1.6	2.5	3.6	3.8
Limb affected	all limbs	hind limb	hind limb	hind limb
Number of puppies/litter	1	3	5	5
Control puppies	**Control 1**	**Control 2**	**Control 3**	
Sex	Male	Female	Female	
Age (weeks)	8	9	9	
Weight (kg)	2.5	2.5	2.2	
Number of puppies/litter	2	4	4	

the relationship between genetics and SPS in dogs, using random amplified polymorphic DNA (RAPD) technique. This technique is a simple and rapid technique which requires very small quantity of genomic DNA and no sequencing, cloning and hybridization representing distinct advantages over other molecular techniques generally used in genomic characterization. Presently, RAPD is wildly use as technique of choice to study genetic variations in many aspects (Ben Abdeljelil et al., 2011; Nathues et al., 2011; Dione et al., 2012; Taha, 2012).

Case history

Four Siberian husky puppies diagnosed with swimming puppy syndrome based on clinical signs were subjects in this study, while three other healthy Siberian husky puppies served as controls. The differential diagnosis of SPS was used 1) clinical signs; walrus-swimming movements on the belly, extended limbs and 2) time of disease presented; within 2 months old. Moreover, all puppies recovered within 1 month treatment program; limb bandage and physical therapy. Three SPS puppies were affected at both hind limbs, while the other was affected at the fore and hind limbs. All puppies recovered and walked normally within 1-2 months after being treated using bandage technique and rehabilitation program without any medication. This is a proof that all puppies included in this study were affected with SPS. Puppy information and pedigrees are presented in Table 1 and Fig 1. Two ml blood samples were collected from the cephalic vein for DNA isolation. The isolation of total genomic DNA for molecular marker analysis was carried out utilizing the phenol–chloroform method, as described in a previous work (Chomdej et al., 2011). Genomic DNA from each sample was diluted to a concentration of 10 ng/ml for use in RAPD technique with 16 random primers (Table 2).

PCR was performed in a total volume of 25 µl volume containing: 1x reaction buffer (500 mM KCl, 15 mM $MgCl_2$, 100 mM Tris-HCl, 1 mg/ml BSA, 100 mM $(NH_4)_2SO_4$, RBC Bioscience), 2 mM $MgCl_2$ (RBC Bioscience), 0.2 mM dNTP (Vivantis Technologies), 0.4 µM primers (Operon Technologies), 1 U *Taq* DNA polymerase (RBC Bioscience), 10 ng/ml genomic DNA and deionized distilled water. PCR was performed by MJ Mini Personal Thermal Cycler (Bio-Rad) with the cycling profile as follow: 95^{0}C for 5 min for 1 cycle, 94^{0}C for 30 sec, 45^{0}C for 60 sec and 72^{0}C for 90 sec for 35 cycles and 72^{0}C for 5 min. After the PCR completed, the amplified samples were evaluated using agarose gel electrophoresis.

To verify the association of RAPD bands with SPS, the presence and absence of bands from all primers were statistically calculated using a chi-square test. The appearances of polymorphic fragments were scored as 0 and 1 for the occurrence and the absence, respectively. The data were used to calculate the *p*-value to receive *p*-value at < 0.05.

Table 2 Sixteen random primer sequences used in this study

Primer ID	Sequences (5´→3´)	G+C percentage
A4	GCATCAATCT	40
AP42	AACGCGCAAC	60
OPB04	GGACTGGAGT	60
OPB05	TGCGCCCTTC	70
OPB06	TGCTCTGCCC	70
OPB07	GGTGACGCAG	70
OPB08	GTCCACACGG	70
OPB10	CTGCTGGGAC	70
OPB17	AGGGAACGAG	60
OPB18	CCACAGCAGT	60
OPS11	AGTCGGGTGG	70
OPS16	AGGGGGTTCC	70
OPW09	GTGACCGAGT	60
R37	GAGTCACTCG	60
R55	CGCATTCCGC	70
R105	GCACCGAACG	70

Results and Discussion

In this study, only one breed of dog was used to eliminate the genetic variation among breeds. However, no significant difference was observed between the genetics of SPS and healthy puppies. This result correlated with the pedigrees of the five Siberian husky families in the study, which had not previously presented any indications of heredity to this syndrome, as shown in Fig 1. Thus, the results from this study, both RAPD result and pedigrees, appear to indicate that SPS in dogs is not controlled via genetics. Nevertheless, this study is only a brief report with a low number of samples, especially SPS samples. In addition, the lack of data for the grandparent generation is one of the limitations for pedigree analysis in this study. Furthermore, RAPD is not the only detection technique for genetic variation. Other techniques to identify the DNA fingerprint such as restriction fragment length polymorphism (RFLP) and amplified fragment length polymorphism (AFLP) (Vos et al., 1995) could be applied for analysis of the relationship of heredity and SPS. However, RAPD technique was chosen for use in this study for several reasons: we are more familiar with this well-established technique; and, using RAPD, we have recently found an association between genes and patellar luxation in dogs (data not yet published).

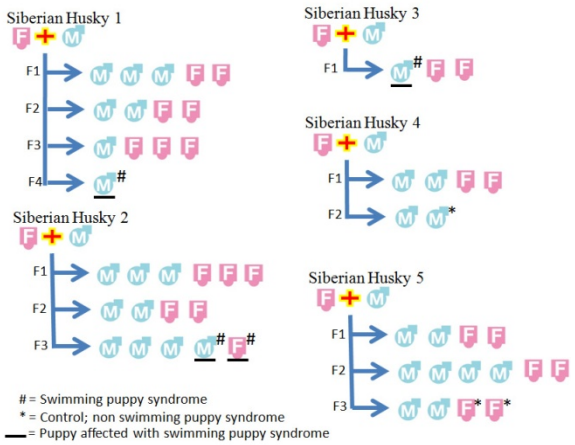

Figure 1 Pedigrees of 7 puppies participating in the study (4 puppies with SPS and 3 unaffected controls). Blood samples were collected from dogs from five Siberian husky families. F1–F4 = litter number, male/female puppies per litter, and SPS characteristics.

Other risk factors, including environment and nutrition, and especially musculoskeletal abnormalities as mentioned in the studies of Nganvongpanit (2012) and Lorenz (1997), should be evaluated to clarify the cause(s) of this syndrome, if possible. Increasing the number of affected puppies studied would increase confidence in the results; but this is very difficult because SPS cases in dogs are rare. Data from Nganvongpanit in 2013 from a 6-year retrospective study of 2,443 puppies (1,183 males and 1,260 females) in Thailand, from October 2006 through September 2012, found that only 2.13% of puppies were affected with this disease. Finally, finding the answer to the question of whether SPS is controlled by genetics remains an exciting prospect; but so far all data and knowledge support that SPS is not a genetic disorder.

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